CHEMICAL PROPERTIES OF TREATED NEEM (*Azadirachta indica* A. Juss) SEED AND PRESERVATIVE POTENTIALS OF ITS OIL ON ONIONS, SELECTED GRAINS AND FERMENTED LOCUST BEANS

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DEDICATION

This research work is dedicated to Almighty Allah for His mercy, safety and protection, to my children Hanif, Halim and Hazim, to all the MUSLIM UMMAH and also to my late parent Alhaji and Alhaja Riskiat Salami for their passion in seeking for knowledge.

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ABSTRACT

Neem seed is known to serve as antimicrobial agent on agricultural commodities. However, the consumption of its high dosage pose a health risk due to presence of other antinutritional factors. There is limited information on appropriate treatments for reduction of the antinutrients in neem seed to safe consumption level. There is need to further determine effects of some treatments on neem seed chemical properties to ensure safety for consumption. Thus, this study was designed to investigate the effects of steeping in water, alkaline solution and roasting on neem seed and toxicity of using its oil as a food preservative.

Neem seeds were subjected to three treatments based on preliminary studies such as; steeping in water, and 40% NaOH solution for 24 h, and roasting for 10 min at 60^{o}C. Proximate (crude protein, oil, crude fibre, ash, moisture and carbohydrate contents), minerals (Ca, Na, Mg, P, K, Cu, Zn, Fe, Mn and Se) and anti-nutrients contents (cyanide, nitrate, oxalate, phytate, tannin and azadirachtin) ofun-treated and treated seeds were determined using AOAC methods. Oil was extracted from raw and roasted seeds using hydraulic press.Fatty acid profile and bioactive compounds of the oil were determined using standard method. Weevil infested grains (maize, beans and rice), Fermented Locust Beans (FLB), and mouldy onions were treated with different concentrations (0.6. 0.7, 0.8, 1.0, 2.0 and 3.0 mL/200g) of oil extracted from roasted seed. The samples were stored at ambient conditions (26.0±2.0^{o}C, RH 70%) for 45 days.Weevils population in grains and microbial growth on FLB and onions were determined weekly, using established procedure. Toxicity of roasted seed oilwas conducted using male and female rats (100.0±5.0g) in a group of three (n = 5), fed with FLB (control, 1.0, 2.0 and 3.0µL/100g) for 13 days. Haematology and histopathology of kidney, liver, heart, testes and ovaries for rats were done. Data were analysed usingANOVA at $\alpha_{0.05.}$

Crude protein, oil, crude fibre, ash, moisture and carbohydrate contents of untreated seed were 18.6±0.07, 41.2±0.03, 3.6±0.04, 2.6±0.05, 6.0±0.02 and 28.1±0.02%; while treated were 16.9-19.1, 40.4-41.8, 3.5-3.6, 2.4-2.6, 5.9-12.1 and 24.4-28.1%, respectively. The seeds had high contents (mg/100g) of Fe (16364.0-16482.0), Zn (2463.0-2398.0) and Mn (1164.3-1182.0). Steeping in water significantly reduced cyanide (45.0%), nitrate (12.5%), tannin (50.0%) and Azadirachtin (16.7%) contents. Major fatty acids in neem oil were oleic (39.10±0.03), linoleic (18.60±0.03), stearic (17.70±0.02) and palmitic (14.5±0.01%) acids. Roasting significantly affected the oil profiles. Fourteen bioactive compounds were detected with dominants being n-Hexadecanoic acid (28.62%), 9,12, 15-Octadecatrienoic acid (17.1%), Neophytadiene (13.7%), Hexadecanoic acid (10.0) and 9,12-Octadecadienoic (8.3%). Highest percentage weevils' mortality of 59.2% in maize, 41.25% in beans and 36.67% in rice were recorded at 0.6, 0.7 and 0.8 mL/200g, respectively. *E-coli*, *Pseudomonas*, *Staphylococcusaureus*and *Bacillus sp* were the organisms detected in stored FLB. The microbe populations were significantly reduced by the oil. No spoilage was recorded from treated onions. Reduced erythrocytes and degradation of hepatocyte were observed in rat tissues fed with 3 µL/100g dose, an indication that the concentration was outside the safe limit for rat.

The treatments reduced antinutritional factors in neem seed. Oil from roasted neem seed reduced weevils in grains and retarded microbial multiplication on fermented locust beans and onion. Low oil dosage is recommended.

Keywords: Treated neem seeds, Neem seed oil properties, Natural preservative, Food safety
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CHAPTER ONE

INTRODUCTION

1.1 Background

Neem (*Azadirachta indica*) tree, a multipurpose tree and a source of numerous compounds with distinct chemical systems and biological consequences (Baswa *et al.*, 2002). Its fruit is thought to possess anti microbial characteristics against different pathogenic microorganisms with antiviral houses which include antioxidant, anti-malaria, anti-mutagenic, anti-carcinogenic, anti- inflammatory (Maragathavali *et al.*, 2012). Food preservation with chemicals are regularly restrained by way of possibility of bioaccumulation and the consequent toxicity to man, as a consequence, the look for natural preservatives of plant foundation. Fungi motive spoilage of numerous meals commodities which includes cereals, pulses, fruit and vegetable via the manufacturing of mycotoxin which makes meals dangerous for human consumption and adversely influences their dietary fee (Pandey *et al.,* 2016). The micro organism species accountable for many illnesses and pronounced loss of many crops in countrywide and worldwide significance mduring garage. Food spoilage occurs as a critical trouble in tropical hot and humid climates. Food borne pathogens and their poisonous metabolites produce losses up to (25%) in excellent and amount of overall agricultural food commodities via out the world, in step with FAO (2005).

Neem plant and its components play roles in in-hibitiing the increase in numerous microbes consisting of viruses, bacteria and pathogenic fungi. Its seed oil is powerful as antibacterial and antifungal marketers on spoilage of food. Extract of better plant commonly consists of big quantity of increase inhibiting phenolic acid which act as useful resistance towards the sicknesses of plant (Akujobi *et al*., 2006). Little awareness of oil from neem seeed disrupts synthesis of cell membrane and this enhances its utility

in clinical, Agricultural and household products (Kumar *et al.* 2010). Active components of the oil are nimbin, nimbidine, and nimbinin (Baswa *et al.*, 2002). Neem it is referred to as dongoyaro in some parts of Nigeria.

The neem tree has unique great of drought resistance as the plant commonly flourishes in an area with annual rainfall beneath 400mm. It grows in exclusive styles of soil and might tolerate high to very excessive temperature however can not survive in temperature underneath 4^{o} C (39^{o} F). All parts of this tree have distinct biological activities and the important economic component of neem tree is oil content availabe seed kernel (Noorul and Gayathri, 2016). This plant oil has been hired within the manufacturing of numerous objects such as soap, lubricants and pesticides.

Soaking, cooking, toasting, autoclaving, microwave heating, sprouting, chemical remedy improve legumes traits through the elimination and inactivation of some anti-nutritional elements (Farran *et al.*, 2001). It is, consequently, crucial lessen the anti-dietary factors and toxins in neem a good way to boom its nutritional satisfactory and to allow effective usage of neem seeds. Application of insecticides entails the use of pricey system and also necessitates education earlier than use, notwithstanding being hazardous to customers. These make it tough for grain farmers in Africa to use pesticides and, are consequently now not the answer to the garage insects. Oil obtained from neem seed is of extremely good fitness significance because it has an anti-germ property which changed into proven from its insecticidal cleaning soap (Kovo, 2006).

Neem plant is beneficial for production of client merchandise, along with insecticides, lubricans, gas for oil lamp, e.t.c. In India and Africa (Vierge, 2001). Oil from neem plant suggests no threat to human and animals in the course of exposure in the system of its remedies due to the fact its merchandise are useful for human consumption and medication. In addition, neem is not dangerous to useful insects, however can best have an effect on the ones pests feeding on the dealt with plant (Khanna, 2001). Regardless of all the numerous blessings, there are constrained records on its software as bio-preservative in garage of meals grains and onions and locust beans.

The studies work is achieved to justify using neem seed oil as preservative in storage of grains as well as to check its antimicrobial effects on preservation of fermented African locust beans and onion bulbs in the direction of extending their shelf lifestyles.

1.2 Problem Statement

Neem tree products including seed are reported to possess scientific and economic benefits (USDA GRIN, 2003). However, there is sparse literature on safe human consumption of neem seed, and its oil utilisation in preservation of rice, maize, beans, locust bean and onion despite challenges in domestic preservation. Food safety and preservation continue to attract attention which includes application of plant products properties in alternative to synthetic chemical preservatives. Some plant oils have been reported to be effective in antimicrobials, antioxidants, ant mutagenic and anti carcinogenic properties (Sánchez-González *et al.,* 2011). Neem oil was observed to inhibit growth and sporulation of *Penicillium verrucosum* and *Penicillium brevicompactum* which produce mycotoxins (Ochratoxin A.) in dry-cured meat products (Mossini *et al.,* 2009), and possesses intense antimicrobial properties against bacteria (Rawami and Goutam, 2011). Neem seed with about 40% oil content contains anti nutrient such as azadirachtin, meliacin, gedunin, salanin, nimbin and valassin (Lale, 2002). Appropriate anti nutrient reduction methods, and safe oil application in rice, maize, beans, locust bean and onion preservation are of research interest.

1.3 Justification

Neem grows on a wide range of soils and can survive extreme weather conditions which make its products readily available in commercial quantity (Rojas-Sandoval *et al.,* 2014). Evidence based information on effect of some pretreatment techniques on chemical properties of neem seed add value to its utilisation. Microbial food spoilage is also an area of immense concern for food industry. It has negative impact on the shelf life, textural characteristics, and overall quality of finished products which affect the customer choices, and results in significant commercial losses. Prevention or inhibition of microbial growth in food is of outmost importance for the current global food production. There is need for new processing methods to be used either alone or in combination with the already existing ones in order to reduce or eliminate food borne

pathogens and spoilage bacteria. Chemical additives have been used extensively to prevent the survival and proliferation of microorganism, but its safety and impact on human health are of public health concern.

1.4 Objectives

The research work was designed to investigate effective methods of removing anti nutrients factors in neem seed and utilization of its oil as preservative. The specific objectives were;

1. To examine effect of roasting, alkaline treatment, and soaking of neem seed on its chemical properties.
2. To extract and estimate chemical components of neem seed oil.
3. To investigate potentials of neem seed oil in preservation of rice, maize, beans, locust bean and onion.
4. To determine safety of the preserved foods.

CHAPTER TWO

LITERATURE REVIEW

2.1 Neem Plant

Neem tree (*Azadirachta indica)* popularly know as *dogon yaro* in Hausa, of the family Maliaceae, traceable to the South-East Asia and found in most tropical coutries. It is on record that it was brought to Africa, and has since been cultivated in many countries. The genus Azadirachta consists of species which is associated with and on occasion careworn with that of the genus Melia. It trees grow effectively in many countries.

2.1.1 Description of Neem Plant

Neem plant is about 15 to 30 m tall, and propagated by seed and by using neem seedlings of nine to twelve months old which can be transplanted. Yield of fruit per each tree is between 37 and 50 kg per year. Fruit yields of 40 kg produce 24 kg of dry fruit (60%) with other components of 48% pulp, 4.5% seed coat, 25% husk and kernel (23%). The kernel contains about 45% neem oil and 55% neem cake (Subbalakhmi *et al.*,2012). *Neem plant begins to* fruit around 5 years of age, while its economic fruit yield starts between 10 to 12 years. Fruit ripening occursin rainy season during which it falls to the ground and germinates within 15 days under natural conditions. In season of extreme drought, *the plant* becomes leafless. Each structure of the tree has medicinal properties. It serves as a natural herb, commonly available, throughout the year andhas benefits that only a few natural products can provide.

2.1.2 Neem Plantation in Nigeria

Neem plantation startred in early 19th Century in Africa and its introduction to Nigeria by Colonial authority in 1928 was reported by Okrikata and Oruonye (2013). Due to characteristic resistance to harsh weather, it grows well in savanaah regions in Nigeria and is matures after three years. Thus people in the location take advanatage. Noticeable

in some major cities such as Markurdi, n eem is planted along major streets in Makurdi metropolis and its suburbs, where it shields people walking inside the scorching sun during dry season period. During fruiting period, many fruits littered the ground. Investigation of factors responsible for its fruit yield is necessary in order to recognize the growing importance of neem. Maximum yieldof 169m^3 fuel wood per hectare after a rotation of 8 yearswas reported from northern Nigeria, while between 108 and 137 m^3 of fuel wood per hectare were recorded from Ghana within the same time (Neem Foundation 2016). Production of neem on a large scale via seed propagation is vital, in order to fulfill the significant potentials of its industrial and environmental uses.

2.2 The Volatile/ Essential Oils

Volatile oils are aromatic liquid oils that possess radical scavenging characteristics normally residein plant materials such as buds, plant life, leaves, bark, twigs, seeds, herbs, wooden, end result and roots). Community demands safe and high great, meals which might be free from preservative and have prolonged shelf-existence in new decade. Safety of ingredients has won more attention due to elevated new food-borne ailment prevalence. Also there are new results from makes use of of natural merchandise which have extra practical properties instead of artificial chemical preservatives (Sánchez-González *et al.,* 2011). Essential oils were broadly investigated inside the midst of intrinsic antimicrobials because of its potentials.

Method of steam distillation, among diverse methods of crucial oils production together with fermentation, urgent, enfleurage or extraction, are generally used for commercial production (Burt, 2004). The flavonoid composite, that are responsible chemical for flavour and aroma, exist at numerous awareness tiers (ranging from ppt to pph) in their resources. The excellent and quantity of the crucial oil can be motivated via environmental elements of booming the plant like favour climate, abundant soil traits, and viable plant parts. Being a especially volatile fabric, the molecular weights of important oil are commonly much less than 300 and the oil has a few not unusual physical properties which includes optical hobby, refractive index, immiscibility with water, enough solubility to impart aroma to water and solubility in most organic solvents which includes alcohol and ether (Mukhopadhyay, 2000).

Also this oil, as a excessive cost high volume product, is favorable to exploitation with supercritical (CO_2 and other suitables) fluid technology. The sorts of foods processed are taste, perfume, spice extracts, while essential oils from vegetation, animals sources, hop extraction with alpha and beta acids yield essential oils with purification and fractionation of aroma constituents (Khosravi-Darani, 2010).

2.2.1 Food preservation using essential oil

Consumption of meals infected with pathogenic micro organism and their pollution leads meals-borne illnesses which creates a main problem in public fitness. Fresh fruits and veggies can be handled with antifungal outcomes of natural antimicrobials a good way to enhance their excellent and nutritional price (Khosravi-Darani,. 2010.). Bioactive molecules obtained from critical oilare used directly in food merchandise for meals cleansing (Solórzano and Miranda., 2012). Innate antimicrobial sellers may be used by myself or together with different maintenance technology. For instance, a synergism among NaCl and mint oil changed into observed towards *Salmonella enteritidis* and *Listeria monocytogenes* (Tassou *et al.*,1995). The combination of essential oils from thyme and nisin confirmed a synergistic hobby in opposition to L. Monocytogenes in minced beef at some stage in refrigerated storage (Solomakos *et al.,* 2008). Mixtures of oregano crucial oil and sodium nitrite showed their outcomes on growth and toxin manufacturing via C. Botulinum through appearing synergistically to inhibit its increase in broth, whereas oregano oil implemented by myself had no full-size inhibitive impact on the increase (Mahin and Klanoush, 2013).

Effectiveness of plant antimicrobials relies upon on elements together with genetic, subculture, post harvest condition, the approach of extracting vital oils,type of solvent, the extent of inoculums and increase phase. It additionally depends on the intrinsic or extrinsic homes of that food inclusive of pH, antioxidants, water content, preservatives, fat/oil content, incubation time/temperature, packaging/material manner, and bodily shape of meals in addition to awareness and availability oxygen (Burt, 2004).

Effect of oregano and thyme oils in opposition to micro organism which includes S. *Typhimurium* and *S. Aureus* increases with low oxygen levels. Use of vacuum packaging for vital oil pruduces an excellent nutrients renovation approach (Solomakos *et al.*,

2008). Its effectiveness increases at low temperatures which permit the cell membrane and extra solubility in lipid membrane (Frangos *et al.*,2010). Synergistic impact of thymol with carvacrol in high hydrostatic pressure (HHP) has been suggested. The feasible count of *Listeria monocytogenes* (mid-exponential section) have been considerably reduced via mixed HHP treatment at 300 MPa in present of three mmol/l thymolor carvacrol in compare to individual treatments due to damage of HHP to the cell membrane (Karatzas *et al*., 2002).

Combinational application of different essential oils has been shown to cause synergism. Gutierrez *et al.,* (2008) showed greater impact of combined application of oregano and thyme more than when assessed individually. Oregano combination with sage or thyme cause elongation of lag phase of *Escherichia coli*, while the combined use of oregano and rosemary did not induce synergistic effects in compare to single usage of essential oil. The Oil is needed in higher concentration to achieve the same effect in food (Gill *et al*., 2002). Presence of more nutrients in foods than simple media enable fasterrepair of damaged cells in bacteria (Gill *et al.,* 2002). Low water activity in food as well as its fat content causes limitation of antibacterial activities. Power of hydrogen (pH) has impact on essential oil antimicrobial activity. Increased hydrophobicity of the oil, with low pH, makes it clearly dissolved with essential oil.

2.2.2. Potentials of Volatile Oils as Antioxidant

Interaction of oxygen moleculewith polyunsaturated fatty acids (PUFA), brings about deterioration of food, aging of organisms and cancer promotion due to peroxidation of lipid. Radicals involved can be used as food additives because of protective role of antioxidants. Back from ancient time, Spices in foods possess antioxidant capacities that improve flavors (Wang, 2008). Recently, the essential oils from various plantextracts are more attractive asnatural source for effective and prolong stability storage of foods.

Food safety and the potential impacts of synthetic additives on health are the major agitation of consumers and government. Addition of essential oil compounds including carvacrol and thymol, to food may lead to inhibition of the microbial and chemical deterioration (Ultee*et al., ;*1999 Burt, 2004). Main composition of thyme (i.e. thymol and carvacrol) have antioxidant activity (Nakatani, 2008, Miura *et al.,* 2002).

The antimicrobial and antioxidant effects of phenolic compounds increase when combined with other natural preservatives (Yamazaki *et al.*,2004). The antioxidant activity of *Citrus sinensis* is related to the presence of phenolic compounds and flavonoids. Phenolics antioxidant compounds show defense mechanisms in plant by nuetralizing reactive oxygen species (ROS) (Wollgast and Anklam, 2000). The phenolic content and composition depend on environmental factors, genetic and after-harvestprocessing conditions (Cowan, 1999). The antioxidant activities of phenolics are due to donation of hydrogen, free radical scavenging metal ion chelation, with single oxygen quenching that acts as a substrate.

2.2.3 Activities of Volatile oils against storage fungi

Fungi destroy food commoditiessuch as cereals, pulses, fruits, and vegetables, by producing toxins thereby rendering it unhealthy for human consumption and adversely affect its nutritional value (Pandey *et al*., 2016). Spoilage of food commodities during storage is a chronic problem in tropical hot and humid climates. According to Food and Agricultural Orgnisation (2005), pathogenic fungi in food affect quality of one-quuater of most agraicultural products in food storage. Food contaminat moulds are of economic importance with resultant hazards. Hence, use of essential oils to combat food lossesis a cost-effective approach during storage and transit by prevention of fungal growth. Recently, anti-fungal potential of volatile oils is very significant throughout the world (Bosquez-Molina *et al*., 2010).

Antifungal activity of essential oils involvesdestruction of fungal hyphae through sesquiterpene compounds present in the oils. Moreover, essential oils increase membrane permeability by swelling and dissolving the cell membranes (Cox *et al*., 2000).

2.2.3.1 Challenges and prospects in using essential oils in food: Limitation in use of essential oils for food preservation occur as a results of its concentrations required to achieve sufficient antimicrobial activity. Hydrophobic constituents of essential oil affect food matrix components and impairesuchfood products, such as fat, starch, and proteins (Kyung, 2011). Antimicrobial potential of essential oil constituents depends on pH, temperature, and the level of microbial contamination in food (Somolinos *et al*., 2010).

Impacts of essential oils constituents on or their constituents and food matrixis observed by investigating the growth of microorganisms in culture medium that contains various composition of fat, protein, or starch as well as the antimicrobial compound of interest. This is investigated by using a food model media (Gutierrez *et al*., 2009), that provide quick answers for which kind of food products the compound in question can be applied.The pungent odour and taste of some essential oils resulted to negative organoleptic effects on the consumers beyond the acceptable verge, even at low concentrations, (Lv *et al*., 2011). The oils may be packed in edible polymers with breakable sachets which permit slow diffussion to the food surface and the head space of foods packages such as whole or milled grain.

Diffusion price of agents in critical oils is be reduced by way of incorporating the components in movies and fit for human consumption coatings outside the food product on the way to hold the lively compounds on the product floor for prolonged durations of time (Sánchez-González *et al.*, 2011). Essential oils is encapsulated into nanoemulsions (Hyldgaard *et al.*,2012) to prevent addition, of the sensory consequences of the oils, to the matrix of a meals product. This technique increases balance of unstable additives, protects from meals interaction, and additionally will increase antimicrobial activity through passive cell expansion and absorbtion (Donsí *et al*., 2011).

Essential oils can be reduced in awareness via their mixed application with other antimicrobial constituents that offer a symbiotic effect with out affecting their antimicrobial interest (Nguefack *et al*., 2012). Combinations of crucial oil in synergies, offer potent antimicrobial blends, as a key for its makes use of in meals maintenance without simultaneous sensory outcomes.

2.2.3.2 Efficacy of risky oils in food preservation: Essential oils is implemented as meals preservative in a good way to sell shelf-life of meals commodities. This may be utilized in natural and method paperwork, in various storage bins like, tin, glass, polyethylene, or natural fabrics to enhance the stableness of meals commodities (Pandey *et al.*, 2014). Componenets of volatile oil acquired from, citronella, citronellol, eugenol, farnesol, and nerol have blanketed chili seeds and culmination in opposition to fungal contamination for about 6 months (Tripathi *et al.*, 1984). For example, vital oil in

Ageratum conyzoides correctly managed rotting of mandarins from blue mould and also elevatedits shelf-life through as much as 30 days (Dixit *et al.*, 1995) at the same time as oilin *Cymbopogon nardus, C. Flexuosus,* and *Ocimum basilicum* has managed anthracnose infection of banana and elevatedits shelf-existence via up to 21 days (Anthony *et al.*, 2003). *Cymbopogon flexuosus* oil is able to shield *Malus pumilo* end resultagainst rotting for about 3 weeks (Shahi *et al.*, 2003).

In addition, Putranjiva roxburghii oil function as an effectivefumigant, against *Aspergillus flavus* and *Aspergillus niger* that cause biodeterioration of groundnuts throughout garage, and also improved its shelf-lifestyles of groundnut for about 6 months (Tripathi and Kumar, 2007). The use of Cymbopogon pendulous vital oil as a fumigant elevated groundnut shelf-existence by using 6–one year and has proved to be more powerful than P. Roxburghii important oil (Shukla, 2009). Thus, efficacy of essential oils is primarily based on use of oils from one of a kind plant species, their chemical composition, dose degree, and form of storage container.

Essential oils from thyme have retarded ailment prevalence in papaya fruit (Bosquez-Molina *et al.,* 2010), even as cinnamon oil is beneficial to increase garage existence of banana through up to twenty-eight days through reducing fungal infection of banana (Maqbool *et al.,* 2010). Formulations of *C. Pentaphylla and C. Ambrosioides* oils preserveseeds from pigeon pea for up to six months (Pandey *et al.*, 2014). *Artemisia nilagirica* oil used as a fumigant inside cardboard elevated the shelf-lifestyles of desk grapes (Sonker *et al*., 2015). Oil from *Lippia alba* had been reported to retard fungal proliferation and aflatoxin manufacturing in inexperienced gram (*Vigna radiata*) and additionally more advantageous to its shelf-lifestyles with the aid of up to six months.(Pandey *et al.*, 2016).

2.3 Neem Oil for controlling Meat spoilage

Application of antimicrobial agent, received from plant, is an alternative technique to contemporary use of antibiotics on the grounds that vegetation with their agro-industrial waste, and also acts as biologically-active substances compared to these synthetic antibiotics. Plant resources provide best occasion to manipulate microorganisms in food and another option to synthetic preservatives (Palanappian and Holley, 2010). Bacteria

meat spoilage via development of sour flavor, off-taste, discoloration, fuel manufacturing, slime production and low pH. These organisms are normally associated with gram-tremendous, gram-terrible, anaerobic and facultative genera and their bad consequences are resulted from impact of extracellular compounds, along with lipases and proteases, released from dominating desructive microorganisms.

Neem seed is many of the bushes of 21st century due to its excellent capability of pest management, environmental safety and medicine (National Research Council, 1992). Neem oil is the most commercially applicable among many different merchandise acquired from the seeds. The pastime of neem oil pleasant as an antimicrobial has already been investigated (SaiRam *et al.,* 2002). The efficacy of neem to oppose micro organism affecting meat exceptional changed into already investigated the usage of neem cake extract of ethyl ethanoate NCE (EtOAc, CH3COOCH2CH3) (Del Serrone and Nicoletti, 2013).

Neem oil has higher antimicrobial interest towards spoilage bacteria in comparison to that of neem cake extract, NCE. Oil extract of neem leaf inhibits production of *Penicillium verrucosum* and *P. Brevicompactum* boom, sporulation and mycotoxin (ochratoxin A) in dry-cured meat products (Mossini *et al.,* 2009). In addition, neem plant possesses excessive antimicrobial properties in opposition to bacteria of human hobby (Rawani and Goutam, 2011).

2.4 Nutritional Attributes of Neem

There are variations in dietary aspect of neem depending on a few factors (feedipedi.Org). For instance, its seed desserts received from complete seeds have particularly low protein content material and a excessive fibre content, crude fibre (Ismael *et al.,* 2009). Neem cake, received from decorticated seeds, has high protein content material and low fibre content. Neem leaf has high protein content material, mild nutritional and antinutritional dietary fibre with a high degree of lignin. It also contains high amounts of calcium with low quantities of phosphorus (Gowda *et al*., 2000). Oil content material of the seed varies, relying on technique of extraction normally between (1-10%) DM. In addition, cakes obtained from decorticated seeds and partly de-pulped

seeds vary, based on record of decortication and de-pulping of the seeds (Dutta *et al*., 2012).

2.5 Components of Anti-dietary Factors

The anti-dietary factors are compounds launched from herbal feed stuffs via everyday metabolism of substances inside plants because of different mechanisms which includes inactivation of a few vitamins, reduction in the digestive manner withsystematic usage of feed that exertsnegative results to most fulfilling vitamins. Plants exist collectively in the presence ofpopulations of bacteria, insects, fungi and grazing animals as predators, and hence, need to develop defense mechanisms for continue existence.

Many vegetation release chemicals, as secondary compounds, that are not immediately worried inside the manner of plant increase however only act as protectors against insects and fungal attack. These compounds additionally have an effect on humans and the nutritive contents of forages. The antinutrients are categorical compounds, which are commonly, not lethal, but may affect productiveness of animals by using generated toxins at some point of intervals of storage and during confinement when large quantity of such feeding material is fed on through animals (Uzombah *et al.,* 2019).

Those antinutritional elements have influences on useful shrub and tree forages by way of affecting their additives along with non-proteins, alkaloids, glycosides, oxalic acid phytohemagglutinins, , triterpenes and polyphenolics as proven in (Table 2.1).

Table 2.1: Anti nutritional factors found in feeding ingredients

Plant-derived nutrient	Anti-nutrients present
Soy bean paste	Lectins, protease inhibitors, phyto-estrogens, phytic acid, antivitamins, allergens, and saponins,
Rape seed paste	Glucosinolates, Protease inhibitors, tannins, and phytic acid,
Lupin seed paste	Saponins, , Protease inhibitors, alkaloids, and phytoestrogens
Sunflower paste	Protease inhibitors, saponins, and arginase inhibitor
Cotton seed paste	Phytic acid, phytoestrogens, gossypol, antivitamins, and cyclopropenoic acid
Leucaena leaf paste	Mimosine
Alfalfa leaf paste	Saponins, antivitamins, phytoestrogens, protease inhibitors,
Mustard paste	Tannins, and glucosinolates,
Sesame paste	protease inhibitors, and Phytic acid,

(D'Mello, 2000)

2.5.1 Effect of Processing on Reduction of Anti-nutritive Substance

Heat treatment is an important procedure in feed manufacture to improve safety and nutritive value of animal feeds. It is an effective method for control and elimination *Salmonella*, *E.coli* and *Campylobacter* contamination of poultry litters (Jeffrey *et al*., 1998). Thermal processing also cause denaturation lectins, cyanogens and protein inhibitors. However, alien proteins require complex proceduresthat involve using hot aqueous ethanol extracts (D'Mello, 1991). Ammoniation is an effective method for processing of aflatoxin-contaminated oilseeds destined for animal feed. It involves treatment of feedstuff with ammonium hydroxide solution. A complete process of ammoniation reaction allowed irreversible detoxification that ensures total elimination of aflatoxin contaminants.

Sun drying of animal feeds can beviable and cheap mehod of detoxification of aflatoxins especially in tropicalcountries like India. Nutritionally inert adsorbents like bentonite or activated charcoal can be used in feeds at low levels to make milk free from AFM1 or under prescribed limits. Effectiveness of decontamination helps to decrease remnants AFM1 in the milk of dairy cows. Some components of plant have endowment topose adverse effects resulted from productivity of farm livestock (D'Mello, 2000). These components occur in the foliageandseeds of almost every plant used inanimal feeding.

2.6 Toxins in Plants

Plant toxins are poisonous secondary plant metabolites that occur naturally in food, feed, weeds and ornamental crops. It is secreted by plants as a means of defence against various threats such as bacteria, fungi, insects and predators. Toxic substances are potentially harmful to human health when ingestedwhich usually cause food poisoning. Various concentrations for some important toxins are presented in Table 2.2.

These are grouped into a heat-labile type, that are sensitive to standard processing temperatures; such as lectins, protease inhibitors and cyanogens and a heat-stable type

Table 2.2: Principal sources and concentrations of Toxins in Plant

Toxins	Principal sources	Concentrations
Phospholipid	jackbean	73 units/miligram protein
	winged beans	40-320 units/miligram
	lima beans	59 units/miligram protein
trypsin inhibitors	Soybean	88 units/milligram
antigenic proteins	soybean	-
cyanogens	cassava root	186 mg HCN/kilogram
condensed tannins	acacia spp.	65g/ kilogram
	lotus spp.	30-40g/ kilogram
quinolizidine alkaloids	lupin	10-20g/ kilogram
glucosinolates	rapeseed	100 mmol/ kilogram
gossypol	cottonseed	0.6-12g/ kilogram (free)
Steroidal	brachiaria,decumbens;	
	panicum spp.	-
s-methylcysteine		
sulphoxide	kale	40-60g/ kilogram
mimosine	leucaena leucocephala	145g/ kilogram (seed)
		25g/ kilogram (leaf)
Phyto-oestrogens	Clover; Lucerne, soybean	-

Source: (D'Mello,1995)

which are not sensitive such as proteins, antigens, condensed tannins, quinolizidine alkaloids, glucosinolates, gossypol, saponins, some amino acids like S-methyl cysteine sulphoxide and mimosine, as well as phyto-estrogens. The chemical diversity are numerous and are described as follows:

2.6.1 Saponins: Saponins exist numerously in plant kingdom and about 500 species are identified which belong to more than 80 different families. Some common forageswith saponins are white clover (*Trifolium repens*), red clover (*T. prateuse*) and soybean (*Glycinemax*)(Elizabeth *et al.,* 2005). These are plant glycosides which yield sugars, such aspentoses, hexoses and uronic acids, on hydrolysis. The aglycones obtained from polycyclic ring systems are known as sapogenins. Two main groups of saponins, on chemical nature basis, are steroids (C_{27}) and triterpenoides (C_{30}) (Narayanasamy, 2017). With the triterpenoid, sapogenins further divided into three classes based on the compound namely ursane, oleanane or lupine.Saponins possess significant characteristics like bitter taste, foaming inaqueous solution and hemolysis of red blood cells. Saponins have characteristicproperties which determine their biological activity.

Structurally saponinscontain fat soluble aglycone and water soluble glycone part which jointlyconfers lower surface tension and thus form stable foam when dissolved in water. Saponins readily combine with cholesterol resulting in minimization of the activity of original saponin. Saponins contribute permeability changes incell wall, thereby exerts general toxicity on many organs and tissues. Saponins occur in all parts of the plant scuh as plants stems (Yehudith, 1969), roots and blossoms of the plant in varying amounts (2-3%). The levels vary due to stage of cutting (first cut Lucerne have more than second or third cuts). The levels are inversely correlated with crude fibre and hay yield.

There is also an indication that leaves contain twice the amounts of saponins as that of stems and saponin contents decline as the plant gets older (Sen *et al.,* 1998). Whenever animals consume saponin rich diets, it alters the structure of their ruminal contents as a result of entrapping numerous bubbles of fermentation gases throughout the ingesta. This condition is described as *frothy bloat* and gases would be difficult to expel out. Animal

becomes restless and respiration rate increases. At ruminal level, saponins also exert antiprotozoal effects (defaunation), antimethanogenic and selective action on rumen bacteria. Rumen ammonia level may tend to reduce due to indirectresultant decreased in protozoa level caused by the added saponin. The fundamental resultsfrom saponins in fermentation of rumen have been extended share of propanoatewith reduced in level of acetate to propanoate ratio (Elizabeth *et al.,* 2005). The higher propanoate percentage brings about decrease ratio of acetate and butyrateas the most important fermentation give up products from protozoa. Therefore, as the range of protozoa become reduced by way of saponins, a considerable increase in the percentage of propanoate would be predicted (Leng*et al.,* 1992). The performance in vitro of microbial protein synthesis become improved with the addition of Quilaja saponins to a hay substrate (Makkar and Becker, 1996).

Therefore, saponins are discovered to penetrate nutrients in such a way that a better percentage of the digested substrate are involvedin the formation of microbial mass, the same time, as a lower proportion to short chain fatty acids and gasoline (Elizabeth *et al.,* 2005). In addition, it was stated that saponins caused anenomoursereduction in apparent and proper digestibility of substrate in an in vitro fermentation study. At intestinal level, these saponins cause inhibition of certaindigestive enzymes such as a -chymotrypsin. Levels of Lucerne meal (above 7%) in poultry caused depression in weight gain in chickens and also depress egg production which can partially altered by feeding of cholesterol and cotton seed oil in the diet for the saponins to get bound.

Extraction of saponins from quinoa (*Chenopodium quinoa)* by washing and abrasion was attempted (Ridout *et al.,* 1991). Soaking in water and rinsing remove these componentsin the feedstuffs. The remedial measures for bloat include feeding of dry roughage prior to feeding saponin rich fodder and spraying of oil.

2.6.2 Mimosine: Mimosine, also known as ‘leucenine’ is a toxic amino acid found in genus *Leucaena* such as subabul. There are concerns about importance of *L. leucocephala*, with mimosine content of 2-6% in its leaf which varies with seasons and maturity. Mimosine cannot be degraded after absorption In

mechanism of its action, mimosine may acts as an antagonist to amino acid as it forms complexes. This toxin is degradedextensively to dihydroxy pyridine (DHP) in rumen of livestock. Excess dihydroxy pyridine DHP is absorbed into the blood stream from in which it reaches thyroid gland and inhibits biosynthesis of thyroxin hormone. It additionally has big deleterious properties along through disruption of reproductive strategies and teratogenic results in animals (D'Mello, 1991).

Livestock such as, sheep, pigs and even rabbits are quite sensitive to mimosine and for that reason its feed ought to no longer be fed with the eating regimen as sole feed. The predominant signs are excessive salivation, decrease in weight, eroded gums, enlarged thyroid gland lack of hair, and reduction in reproductive performances. The principal symptoms of toxicity in ruminants embody horrible boom, hair and wool absence, swollen raw coronets above the hooves, lameness, mouth and oesophageal lesions, goiter and depressed serum thyroxine degre. These symptoms indicate effects of mimosine while some are due to its metabolite present in rumen. Signs of toxicity also include skin pores and lesions, that resemble Zn deficiency, and reduction in calving percent due to leucaena feeding (Jones *et al*., 1989). A method of lessen the mimosine hassle could be through improvement in use of low mimosine cultivars. Thus, poisonous effects of mimosine can be reduced through process of heat remedy by using supplementation feed added to amino acids or (Ivan, 2003) or with steel ion s along with iron II ions, aluminium III ions and zinc II ions (Ivan, 2003).

It also occurs on every part of plants such as leaves, root, barks, tuber, fruit and seed. Common saponins containing plants are listed in Table 2.3 (Wina *et al.,* 2005)

Table 2.3: Saponin containing forages commonly used as livestock feed

Family and species	Plant part	Saponin or sapogenin name
Fabaceae		
Acacia auriculoformis	fruit	Acaciaside
Albezia lebbek	pods, bark	Albiziasaponin
Earpodtree (*Enterolobium cyclocarpum)*	leaf, fruit	Saponin content (3.9mg/g)
Glyricidia sepium	root, fruit	Hederagenin
Glycine maxima (soybean)	seed	Soyasapogenol
Lupines spp. (lupin)	seed	Soyasapogenol
Medicago sativa (alfalfa and Lucerne)	leaf, root, seed	Medicagenic (aglycone) Soyasapogenol
Meliotus alba (white sweet clover)	leaf, flower, root	Melitoniin
Pueraria Montana var lobata	root	Kudzusaponins (soyasapogenol)
Sesbania sesban	leaf , seed	Glucuronide-oleanolic acid stigmasta galactopyranoside
Sesbania pachycarpa	leaf	Saponin
Ladino clover (*Trifolium repens*)	leaf	Cloversaponins (soyasapogenol)
Red Clover (*Trifolium pretense*)	leaf	Soyasapogenin
Fenugreek (*Trigonella foenum-graecum*)	leaf, seed	Steroid saponin
Moringa oleifera	leaf	80g/kilo diosgenin equivalent
Oat (*Avena sativa*)	leaf, root, seed	Avenacin
Signal grass (*Brachiara documbens*)	leaf	Dioscin, diosgenin, yamogenin
(signal grass)		

Source: Wina *et al*., 2005

2.6.3 Tannins: Feedstuffs containing tannins are broadly used specially at some point of dry seasons in India whilst greater nutritious feeds are in quick supply. Tannins in plant kingdom are grouped into condensed-hydrolysable tannins which usually contain chemical compounds with different chemical structures. Its anti-dietary effects involve interference with digestive techniques by binding with enzymes or with feed components such as proteins and minerals (Liener, 1989). Hydrolysable group undergo degradation in body structures to shape tiny compounds which can enter the blood circle to effect toxicity on critical organs such as liver and kidney over a period of time. Recommended strategiesto eliminate condensed tannins are by dehulling the seeds to put off the tannin wealthy outer layer, autoclaving and treatment with alkali (Griffiths, 1991). Polyethylene glycol is used as a tannin blocking off agent to enhance the consumption of tannin wealthy flora and relieve the negative effects of tannin on ruminants (Silanikove *et al.*, 2006).

In popular, superb effects had been obtained whilst tannin content material inside the eating regimen of feed was above 50g/kgDM (Makkar, 2003) and a single supply of tannins become provided to baby ruminants (Silanikove *et al.,* 2006) with regards to progressed intake. Negative outcomes of tannins have been overcomed correctly by drenching 5 g of PEG per day in goats browsing on Prosopis cineraria shrub foliage for a length of 90 days. The located beneficial results in PEG group as compared to tannin institution are advanced protein digestibility, blood parameters, rumen ammonia nitrogen, overall risky fattyacid concentrations and growth performance. Further addition of PEG (50 g per kg DM of concentrate aggregate) ought to alternative high protein pay attention aggregate with low protein pay attention mixtures for goat youngsters surfing on Prosopis cineraria flora (Kinga *et al.*, 2018). Thus, dietary techniques can be efficiently devised for ruminants that have get entry to to tannin rich forages.

2.6.4 Cyanogen (Prussic acid): Cyanogens are glucosides of sugar and cyanidethat contain glycone. Intact cyanogens are harmless. Cyanogens are hydrolyzed by enzyme to yield hydrocyanic acid (HCN) which is poisonous. The hydrolysis response takes place inside rumen by microbial hobby. In non ruminant, such as pig and horse, enzyme liable

for launch of HCN is destroyed by way of the gastric HCl. Thus, ruminants are more at risk of cyanide than non ruminant animals.

Hydrogen cyanides (HCN) are rapidly absorbed whilst some are removed through lungs and the greater part is unexpectedly detoxified inside liver. Excess cyanide ion cause anoxia through inactivation of cytochrome oxidase systemwhich leads to loss of life within some seconds. Animals display symptoms such as anxiety, extraordinary inhalation, trembling of muscle mass, blue coloration of mouth lining, convulsions and respiratory failure depending on intensity of the anoxia (Harris and Shearer, 2003).

Large content of cyanogen is found in sure grasses inclusive of sorghum, Sudan grass, linseed and cassava root. It is non-toxic in intact plant tissues however when the flora are broken, hydrolytic enzyme is released from identical plant and hydrocyanic acid is liberated. This pastime takes area inside the rumen by using microbial agents. Forage sorghum, sorghum-Sudan hybrids, Sudan grass, and Johnson grass can collect cyanide under drought conditions if rainfall is scanty or irrigation isn't lots. During wilting of plants, its inner structures rupture and the HCN is free of the sugar molecule. The loose HCN is with no trouble absorbed into the blood circulation whilst ate up through grazing cattle where it prevents the absorption oxygen via the animals' blood. Moreso, chewing and digestion of un-wilted plant can release toxic stage of HCN inside the rumen.

Other conditions including extended cloudiness, soil acidity, abnormally high or low temperature (mainly unexpected changes in temperature), herbicide treatments, and occasional soil phosphorous adversely affect the ordinary growing features of a plant and also can result in accumulation of HCN. Proper curing or ensiling of forages that incorporate high ranges of HCN enables to reduce or remove the hazard as unfastened HCN volatilizes effortlessly after being launched (Harris and Shearer, 2003).

2.6.5 Nitrates: Nitrate is non poisonous to animals, however at extended levels can purpose a disease known as nitrate poisoning. Nitrates usually determined in forages are transformed by the digestion procedure to nitrite, and in turn the nitrite is converted to ammonia. Accumulation of nitrates happens in plant with the ability to grow swiftly and use soil nitrogen efficiently. Nitrate is absorbed beneath everyday condition by means of

plant roots within the soil, transported thru the stems, and transformed in the leaves to proteins and other materials which are beneficial for the animal.

Nitrate normally is utilized by the plant immediattly after its absorption from the soil. Its poisonous impact takes place when extra nitrate is ate up and transformed to nitrite quicker than the animal can use it. Free nitrite within the rumen is without difficulty absorbed into the blood circulate, in which it destroys potential of blood potential to take in and deliver oxygen. This ended in conversion of ammonia to protein via micro organism within the rumen. If farm animals swiftly ingest massive quantities of plants that comprise high levels of nitrate, nitrite could be accumulated in the rumen.

Nitrite is ten instances as poisonous to cattle as nitrateas it ends in acute toxicity in cattleas an end result of production of methaemoglobin,which cannot transport oxygen. Signs found in acute toxicity consist of worked breathing (dyspnoea), teeth grinding, uneasiness and immoderate salivation. Preserved forages have a small impact on nitrate levels. Hays and silage from excessive-threat plant life may additionally stay poisonous. Nitrate poisoning is commonly treated by intravenous injection of methylene blue and also large content material of concentrates material in the every day diet with adequate feeding of Vitamin A to enhance a protective impact (Harris and Shearer, 2003).

2.6.6 Moulds and its toxin production: Mycotoxins are the secondary metabolites produced by fungi with adverse effect of impairing productivity, growth, agility and health of animal (D'Mello and Macdonald, 1998). Contamination of forages and cereals with mycotoxin typically takes place through plants destruction with precise pathogenic fungi and symbiotic endophytes inside the field. It might also rise upin processing and transportation of harvested merchandise and feed, under environmental situations that are favourable to grow of spoilage fungi.

The key determinants of fungal colonization and mycotoxin manufacturing are moisture content material fabric and ambient temperature. Species of Mycotoxins become outstanding at the idea of geographical prevalenceand provide precise atmospheric conditions for growth and secondary metabolism. Moulds such as *Aspergillus flavus*, *A.*

Parasiticus and *A. Ochraceus* conveniently proliferate below warm, humid conditions, while *Penicillium expansum* and P. *Verrucosum* are basically temperate-fungi.

Moreover, the Aspergillus mycotoxins preside in plant products evolving from the tropics and exceptional heat regions, on the equal time because its mycotoxins arise notably in temperate meals, specificallyin grains. Fusarium fungi are more ubiquitous, because this genus carries toxigenic species that are solely related to cereals from heat international locations. A new supply of neutraceuticals that has some abilities of antifungal and cytotoxic homes were recognized (Mokhlesi *et al.,* 2012) from sea cucumber (*Holothuria leucospilota*). The usual organisms are at risk of *Aspergillus niger, Candida albicans, Pseudomonas aeruginosa, Staphylococcusaureus and Escherichia coli* and many others. Methanolic extract has shown capability cytotoxic effect of LC50 0.in 4μg/mL.

2.7 Toxicity consequences in Neem

Products from processed raw neem plants parts are nearly not poisonous to flora, birds, bees and mammals. Oil from neem plant is barely toxic to fish and different aquatic habitants. It is essential to recollect that bugs fed on neem treated plants are easily killed while other pollinators such as bees are not probable to be harmed. Absence of Azadirachtin in neem oil gives a product of fatty acids and glycerides which are broken down when entered the frame, useful for strength, and included into cells. Neem oil irritates eyes and skin and its Azadirachtin content is disturbing to skin pores and stomach. In other nations, neem oil is useful for manipulation of flea on cats in other countries.

2.7.1 The Intense toxicity: Intense toxicity is the dangerous impact pose by a toxic substance usually from a single publicity and from more than one exposures in quick time body (less than 24 hours). Adverse outcomes of acute toxicity arise inside 14 days of the management of substance.

2.7.2 The Intense oral toxicity: White albino rats became infertile after being fed for eleven weeks with neem leaves which contain Azadirachtin as the major element in a confirmed pesticide.Though the fertility is 100% reversible. Thus, neem products including neem leaves, neem oil and neem tea should notbe consumed by pregnant women, ladies trying to conceive, or youngsters. Neem seed oil toxicity additionally

produce occasional diarrhea, nausea and popular ache. Injection of sodium nimbidinate to humans did no longer produce any trendy side effects while use of neem seed oil for minor illnesses causes sharp poisoning in babies in Malaysia. The advanced symptoms and signs include encephalopathy, drowsiness, polymorph nuclear leukocytosis and metabolic acidosis, (Mishra and Nikhil, 2013). Symptoms including respiration problem, seizures cause weigh down of children but only to rise after 1.5hr of last dose. Also infected liver and death of toddler occurred after 12 days of ingestion of oil (Yun-Xia*et al.,* 2013). These signs may be because of mixed consequences from aflatoxin and other poisonous additives availablein the oil (Santosh *et al.,* 2020).

An oral LD50 (Lethal dose, 50%) of acute toxicity changed into positioned in mice fed with inorganic solvent extracts of neem leaf and bark. The mice confirmed signs and symptoms that accompany the ill health are pain, gaintestinal spasm, apathy, lack of apetite, hypothermia and died underneath terminal convulsions with no gross microscopic lesion observed from post-mortem (Okpanyi and Ezeukwv, 1981). Rats and rabbits fed with oral dose ofneem seed oil showed acute oral toxicity LD50 within 24h with poisonous consequences at the critical worried device and lungs (Mossini *et a., l* 2009).

2.7.3 Processes of removing toxicity in Neem

Neem seed cake (NSC) incorporates poisonous ideas called triterpenoids and bitter flavor which restriction its usage as meals. As an end result of the toxic additives in neem seed cake, many processes have beenimplemented to reduce its anti-nutritional impact on cattle. To detoxify these pollutants, a mixture of physical and chemical strategies are employed. The best approach in removing toxins in order toimprove the bioactive standards of the cake for palatability become reported to be water washing amongst others, while 22% dry matter was lost (Aruwayo and Maigandi, 2013).

2.8 Utilization of Neem

Neem is versatile tree as its culmination may be fed on in a natural state even as its younger twig, flora and greens are cooked before eating.Its leaves, bark, and seed extracts have been used for hundreds of years for ethnomedia and enthnoveterinary

remedies. Neem seeds are appropriate supply of azadirachtin, a triterpeniod compound, which might be also determined from other parts of the plant. The compound acts as an insect repellent by inhibiting them from feeding and disrupting their increase reproduction. Extracts of crude neem parts are regularly mixed with stored grains such as maize, rice and beans, in order to protect against insects. Important mycotoxin producing fungi and their major toxin are shown in Table 2.4.

Table 2.4: Mycotoxin producing fungi and its toxins*

Fungal species	Mycotoxins
Aspergillus flavus; A. parasiticus	Aflatoxins
A. Flavus	Cyclopiazonic acid
A. ochraceus;	Ochratoxin A.
Penicillium viridicatum; P.cylopium	
P.citrinum; P.expansum	Citrinin
P.expansum	Patulin
P.citreo-viride	Citreoviridin
Fusarium culmorum; F.graminearum	Deoxynivalenol
F.sporotrichioides; F.poae	T-2 toxin
F.sporotrichioides;F.graminearum;	Diacetoxyscirpenol
F.poae	
F.culmorum;	Zearalennone
F.graminearum; F.sporotrichioides	
F.moniliforme	
Alternaria alternate	*Coenophialum neotyphodium*

(D'Mello and Macdonald, 1998)

Extracts from neem plant protect plant life from foliage-consuming insects withno effect on pollinating bugs including honeybees. Limonoid compoundsalso act as antifeedants for bugs while others such as nimbin and nimbindin that constitue the sour componenets showcase antriviral quality. Oil, extracted from the seed, has commercial makes use of and also beneficial for ethnomedicine in India.

Neem affords a valuable firewood which makes an amazing coals and provides numerous environmental offerings (Orwa *et al.,* 2009). Benefit of neem plant for animal feeding remains limited. This is due to the factits leaves are useful as occasional forage for ruminants and rabbits while neem seed cake, obtained from oil extraction of entire seeds kernel, is not fit for human consumption. The resultant cake is useful as natural nitrogen soil improver (Ramachandran *et al*., 2007).

Neem seed cake is a reasonable amount protein substance and it has useful records by farmers.Also, its leaves are dried in India and located in cabinets to prevent insects from eating cloth, and inside tins in which rice is stored (Anna Porter, 2006). The leavesare burnt inside the tropical region to preserve against mosquitoes. Neem plants are also used in lots of Indian pageantsas an extensively utilized in baths as an ayurveric herb.

2.8.1 Uses of Neem in Industries

Industries such as drugs pharmacy, soaps and fabric industries use neem oil (Jattan *etal.,*1995). During the world neem conference held in 2002 as an effort to promote neem towards an “industrial plant”, it became recommend (Kumar, 2003) given that neem is incredibly exploited in India by way of Ayurvedic capsules industries. Neem oil and its leaf powder are employed in preparation of diverse cosmetics including nail oil, face creams, shampoo, conditioners and nail-polish (Jattan *et al.,* 1995). Onenew shampoo, made from seed extract of neem becomes highly effective against head lice beneath in vitro situations, greater than permethrin- primarily based product. Cake extract, as a derivative of neem oil enterprise, is useful in manufactureof farm animal meal, fertilizer and natural pesticide. Its oil is generally used in cleaning soap manufacturing. Medicated neem soaps also gain reputation and many of these neem-based totally industrial products are available presently (Khanna, 1992).

Toothpaste manufactured from neem is broadly applied in India and Eurpoean nations. It is a supply for plenty oral-hygiene arrangements and dental care products in India and Eurpoean international locations. Its bark yield gum and tannins which are used in tannings, dyeing e.t.c. Also pulp from its fruits serves as a wealthy sugar source in fermentation as well as methane fule production industries (Anintida, 2021). Thus, employment offer and profit making are assured through cultivation of neem and processing of its products. Also collection of neem seeds by rural ladies and supply to industries provides vital means of living for the negative families. The first neem production was in India who produces approximately 540,000 tons of seeds yearly that yield tons of neem oil and lots of neem cake. The quantity of azadirachtin present is predicted to be approximately 1600tons in step with annum, imparting large amount of uncooked materials for pesticide enterprise (Kumar, 2003).

Neem oil is important for polymenic resins properties as its resins is useful in syntheses of monoglyceride route and for polyurethane coatings. Theseresins are organized from reaction of traditional divalent acidic materials like phthalic and maleic anhydrides with MG of neem oil (Chaudhari *et al.,* 2014).

2.8.2 Veterinary makes use of neem

Neem plant is useful in supplying health help to live stock as wll as for animal feed in various paperwork in India. The epic get up in 3000 B.C., whilst pandava brothers, Nakul and Sahadeva, applied neem oil and leaves' arrangements to deal with wounds in horses and elephants. Extracts form neem provide effective treatments for stomach worms, ulcers, cutaneous sicknesses, intestinal helminthiasis due to its antiulcer, antibacterial, antiviral residences. Other components of thia plant such as gum, bark, leaf, culmination seeds are beneficial to animals. Its leaves were used specifically as antiviral sellers against viruses inclusive of foulfox and Newcastle sickness. Hot infusion from the leaves is useful in treatment of swollen glands, bruises and sprains. Neem plant bark is effective in opposition to cutaneous illnesses. Seed and kernel oils are used as antiseptic, antifungal and antibacterial by retailers.

Neem oil has anti-hyperglycaemic impact at the same time as alcohol and aqueous extracts of its plants show off deadly impact against parasite *Setaria cervi* (Mishra *et al.,* 2005). Farm animal pests together with flies, blow flies and biting flies are historically

managed by the usage of neem. Its leaves, oil and de-oiled seed cakes are useful as animal feed. Therefore farm animals are fed withneem as it contains beneficialamount of proteins, minerals and carotene. The leaves additionally have considerable levela of digestible crude proteins and overall digestible vitamins.

Oil obtained from neem seed contains long chain fatty acids useful for fowl feed. Cake extracted by applied pressure on neem seed is reported by Odunsi *et al.*,(2008) to be contain quality protein, valuable essential amino acids, sulphur, fiber contents, and nitrogen. The processed cake is useful as poultry feed due to its excellent appetizer and wormicidal sports. In the chicken enterprise, *Aspergillus flavus*that cause aflatoxicosis in infected hen is managed by means of using neem leaves. Neem leaf extract inhibits aflatoxin production via *Aspergillus parasiticus* and patulin production via *Penicillium expansum* (Mossini *et al.,* 2004).

2.8.3 Neem as Vegetable

Neem is applied in additives of mainland South-east Asia, particularly in cambodia aka sdov (Phyllypo Tum) Laos in which its miles referred to as Kadao, Thailand in which its miles called is Sadao, Myanmar, in which it's miles a ways known as tamar, and Vietnam wherein it is far called sau dau and is used to put together dinner the salad goi sau dau. When cooked lightly, neem taste is pretty sour and the meals is not continually appealing to all population, although it is believed to be properly for one's health. Neem gum is a rich source of protein.

2.8.4 Neem products and their Uses

Neem products that can be obtained from neem plant as confirmed in plate 2.Three under include the following:

Seeds: yields oil and cake. Its bitters are used for controlling pests as discipline trials on rice and cotton in Pakistan. It is safe and cheap to use as environmental biopesticide (Siddiqui, 1995).

Neem oil: useful for medical purposes such as bactericidal, analgesic, contraceptives, antihistaminic, anticholinergic, antihelminthic, antiprotozoal, insect repellents antipyretic, pesticides, antiviral, fungicides, and veterinary drugs. It is also beneficial for technical purpose such along with cosmetics, propellants, shampoos, lubricants, hair oils soaps and pastes for tooth.

Neem cake: Soil improver, animal feed, soil moisturizer, soil protectant. soil neutralizer,

Leaves: serve as antiviral, antidermatic, contraceptive, anticlotting agent, antihelminthic, antituberculosis, antifungal, soil enhancer, antitumour, cosmetics, insecticides, nematicides, antiseptic and insect repellents. It is likewise useful as a pot-herb, and are mixed with different greens in the coaching of soups and curries where it offers a slightly aromatic and sour flavor (Lemmens *et al*., 1995).

Branch Twigs: are useful in production of mouth deodorant, toothache reliever, tooth cleaners.

Bark: useful as antiallergenic, antifungal, antidermatic, antitumor, deodorant, antiprotozoal, e.t.c

Wood: used for production of boats, furniture, building carts, articlesidols, tools, farm implements

Flowers: have benefits as analgesic, curries, nectaries, soaps, stimulant. Dehydrated plant are suitable for eating and a vital oil has also been extracted from it. Supplemeaentary use include adhesives, as culmination, resin, fuel wooden, honey, pulp biogas, glue, wind breaker, tannin, wood preservatives (Sateesh,1998). Neem tree, as a tough timber, is proof against termites, borers and fungi. Neem tree is taken into consideration as appropriate for motive plywood for blockboard. Also beneficial for making doors, home windows, agricultural implements, carts, axles, yokes, packing instances, ornamental ceilings, fence posts, in deliver and boat building, and in furnishings, with awesome blessings of insect repelling homes (Tewari, 1992).

Neem fruits neem kernel neem seed

Plate 2.1 neem products

2.8.5 Chemical compounds in neem

Neem contains approximately a hundred thirty five compounds that may be isolated from particular elements of its structure. The compounds are categorized into essential corporations, mainly isoprenoids and others. Some of the bioactive compounds in neem are targeted inside the seed kernels. Seeds are continuously available in some regions, whilst seed production is restricted to 3 instances a 365 days in awesome regions. Storage of the seed, or fruit that includes the seed, is a tough exercising due to assemble-up of the powerful carcinogen, aflatoxin B1 (Chourasia and Roy, 1991) and remarkable problems (Sacande *et al*., 2000). One viable possibility is long time garage of neem kernel extract. However, it loses overall performance under everyday subsistence farming storage conditions is not regarded. Peasant farmers in Africa may be able to put together neem kernel extract at their comfort and preserve it for prolonged periods if severe degradation does now not rise up. The plant consists of several lots of chemical factors. The terpenoidsforms special components of the neem plant due to its biological materials the maximum active and nicely studied Azadirachtin compounds.

Various varieties of azadirachtins (A to K) had been isolated, the most giant of this is Azadirachtin. The neem terpenoids are present in all elements of the plant, inside the residing tissues. Recently, the website of synthesis and accumulation of the neem chemical compounds were diagnosed as secretory cells. Secretory cells are the most considerable inside the seed kernels. The secretory cells can be visible with iodine answer. Besides the terpenoids, neem additionally carries more than 20 sulphurous compounds accountable for the characteristic smell of crushed seeds and neem oil. Composition of non-isoprenoids embody amino acids, polysaccharides, sulphurous compounds, polyphenolics together with flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, phenolic acids, and lots of others. (Singh, 2005).

Bioactivities of some compounds in neem plant are confirmed underneath (Table 2.5).

Table 2.5: Compounds in the Plant that are bioactive

Compound	Source	Biological activity
Nimbidin	seed oil	anti-inflammatory, antiarthritic, antipyretic, hypoglycemic antigastric ulcer, spermicidal antifungal, antibacteria diuretic
Sodium nimbidate		anti- inflammatory
Nimbin	seed oil	Spermicidal antibacterial
Nimbolide	seed oil	antimalarial antifungal
Gedunin	seed oil	antimalarial
Azadirachtin	seed	Antimalarial
Mahmoodin	seed oil	Antibacterial
Gallicacid,(-)epicatechin andcatechin	bark	anti-inflammatory immunomodulatory
Margolone,margolononeand isomargolonone	bark	antibacterial
Cyclictrisulphide andcylic tetrasulphide	leaf	antifungal
Polysaccharides		anti-inflammatory
Polysaccharides Gla, Glb	bark	antitumour
Polysaccharides Glla, Gllla	bark	anti-inflammatory
NB-II peptidoglycan	bark	immunomodulatory

(Biswa *et a*l., 2002)

2.8.6 Socio-Economics Uses of neem

Neem plant is endowed with microclimatic ability because it has capacity to reduce the temperature of its environment by means of 10^0C less than the outside ambient temperature. Reports from World Neem Foundation indicated that its tree has functionality to do job of 10 air conditioners. Socially, it performs numerous social functions which includes provision of colour for avenue skills, aesthetic and protecting offerings round houses and number high ways, pasture tree for housed cattle, also it has benefits of food and provision of gas wood, poles and covering. Industrial packages of neem as clinical, agrochemical, soap, pharmaceutical and woods generate large sales to all contributors inside fee chain (Muhammad *et al.,* 2020).

Moreso, *Azadirachta indica* tree offers anticipated monetary and ecological offerings worth thousands of dollars in its life span (Saxena, 2012). It has become defined as a tree with manner to all global issues and moreover as a tree of the 21st century (World neem Conference, 2012). Neem, being multipurpose tree, is appreciably used inside the decision of physical, social and monetary issues (Muhammad, 2016). Its high-quality brought about environmental control methods such as task of reforestation and afforestation, stabilization of sand, erosion prevention and control, re-greening metropolis and rural settlements, control of draught, desertification in extensive form of agro-forestry initiatives. These attest to its excessive bodily potentials.

Occurrence of bioactive compounds in neem is because of its excessive edition to a large range of ecological situations that won't prefer maximum xyrophytic species within the identical familyof malieaciea (NAS, 2010).The cost of other bye-merchandise of neem, together with fuelwood, timber, and many others.In addition to its ability, advantages to the surroundings via reduction within the use of chemical pesticide has not been taken into account. This is because of assessment of direct value-gain ratio in the use of neem insecticides with usage from subsidised artificial pesticides. Thus, using neem bye-merchandise is considered uneconomical to the rural areas. This dictates the motive of investigatingentomological and monetary factorsin substitution of artificial pesticides with neem seed extracts (Marz, 1992).

2.9 Cash Crop Benefits of Neem

Neem plant generates family earnings in phrases of its processed and un processed bye merchandise. Trade in neem takes place a number of the seed collectors who add little cost to the goods. Its Prices vary across the season. In 1998, the fee for seed changed into low at the start of the season (US $zero.05 per kg) but due to horrible harvest of the neem seed in India, it became US $0.12 in line with kg (Chamberlain, 1999). Higher prices are paid for neem seed kernels.

Labour costs also have relation to the financial manufacturing of neem insecticides due to depth of labour during neem seed processing whichis not eqiuvalent to the earnings paid to unskilled labour. During Dominican Republic, a quite immoderate labour price means that a neem oil processing plant imports seeds from Haiti with inexpensive labour fees. Due to labour fee and amount of neem materal available in Ghana, an industry based totally in Ghana opt to import neem seed from Nigeria in the direction of their effort in putting in a manufacturing plant for neem merchandise (M. Ansare-Ansah, pers. Comm.19.

2.10 Medicinal Qualities of Neem

Some neem products which include seed and leaves incorporate compounds that own health advantages for human in phrases of their being antiseptic, antiviral, and antifungal pastime. There also are tips that the products also have inflammatory, hypotensive and anti-ulcer effect, similarly to oblique advantages to health (Kumar, 2010). Its leaves contain ingredient that inactives fungiwhich produce aflatoxin on mouldy peanuts, corn, and different foods thereby leaving the fungi alive, but switches off their ability to release afflatoxin as the most powerful recognized carcinogen. Neem is likewise treasured for dental hygiene due to presence of strongly antiseptic compounds in its bark. Also, neem extracthas been proyed to prevent enamel decay, as wellas each preventing and restoration inflammations of the gum ((Saxena, 1998; Riar, 1996) and thus useful as a lively ingredient in positive popular toothpaste in Germany and India. Traditional doctors added the antihelmintic and antimicrobial components of neem tree to the attention of herbal merchandise chemist. The techniques of neem oil extraction involves using petrol ether, ethyl acetate and dilute alcohol to remove water - insoluble additives such as nimbin, a sulphur loose crystalline product that has melting point of 205^{o}C and

empirical composition $C_7H_{10}O_2$; nimbidin, a cream-coloured containing amorphous sulphur, melting at 90-100^{O}C. Neem leaves and seeds extracts the usage of ethanol have been found to be powerful in opposition to chloroquine touchywithresistant strains of Plasmodium falciparum (Rheman *et al.,* 2021). Growing problem of resistance to the conventional treatments dictates needs for exploring neem fuction in malaria control in many Asian nations. Nimbdin is identified as the principle lively antibacterial aspect, and the highestcontributor to bitter factor within neem oil. It is a stable compound foundin massive quantities within neem.Additionally, it servesas natural insecticide (Sidhu *et al*., 2004).

2.10.1 Impact of neem as antimicrobial: Neem plays function in inhibiting growth of severa microbes consisting of viruses, bacteria, and pathogenic fungi. Some lively additives of neem have exhibited excessive efficacy, in opposition to maximum number of pathogens (Amal, 2007). Application of neem as fungicides is sensitive to common fungi species such as genera trichophyton (athletes foot), epidermophyton (ringworm of skin and nail), microsporum (ringworm of pores and skin and hair) and candida (thrush). Use of NIM-seventy six protects against systemic candidiasis (*Candida albican*).

2.10.2. Antibacterial interest in neem: Leaf extract of neem are effctive in control of foodborn pathogens and spoilage organisms. Antibacterial activities of neem extracts from bark, leaf, seeds and fruit extracts of *Azadirachta indica*evaluated on bacteria isolated from a person's mouth confirmed that bark and leaf extracts exerted antibacterial interest towards all of the bacteria isolated (Tiwari *et al*., 2010). Higher concentration of seed and fruit extracts additionally exhibited antibacterial pastime.

2.10.3 Antiviral hobby of neem: Neem Bark Extract (NBE) has proven a significantly blockage of virus entry into cells at various concentrations of fifty to 100 mg/mL (Tiwari *et al.,*2010). The extract exhibited a right away antiviral belongings via its blocking activity whilst pre-incubated with the virus. Leave extract of neem (*Azadirachta indica*) additionally exerted antiviral activity thru inactivation of virus and reduction of yield assay and additionally by interfere with its replication cycle at an early stage (Badam *et al*., 1999).

2.10.4. Antifungal hobby of neem: Neem leaf extract exhibited antifungal hobby towards fungi Aspergillus and Rhizopus inflamed seed. These fungal growth become considerably managed with the aid of alcoholic and water extracts. The alcoholic extract of neem changed into greater effective in reducing growth of the fungal species when compared with aqueous extract. While aqueous extract of neem cake inhibit spore germination of sporulating fungi. Also methanol and ethanol extract of Azadirachta indica exerted increased inhibition towards Aspergillus flavus, Alternaria solani, and cladosporium (Shrivastava and Swarnkar, 2014). It has been associated with aqueous extracts of diverse components in neem which include its oil confirmed antifungal activities in opposition to Alternaria solani Sorauer (Rafiullah *et al.,* 2020).

2.10.5 Antioxidant hobby of neem: Neem tree possesses antioxidant activity (Rahmani and Aly, 2015). These antioxidants deactivate free radicals, in biological cells regularly before they assault goals (Nunes *et al.,* 2012) and alsooppose activity of antioxidative enzyme which manage damages as a result of unfastened radicals or recative oxygen species. Free radicals are among the most important culprits within the prevalence of diverse sicknesses. However, illness are stopped through neutralization of diverse radicals. Plants components which includesleaves, seeds, fruits, bark and roots exhibit roles in prevention of disorder as a result of their wealthy supply of antioxidant (Islam *et al.,* 2016).

Extracts of neem leaf, flower, stem and bark have strong antioxidant potentials. Chloroform crude extract of neem leaves is also useful as a herbal antioxidant (Hossain *et al.,* 2013). Further greater, total antioxidant interest of leaves, flower and stem bark extract from neem tree had been discovered to be 0.959, 0.988 and 1.064mM respectively of general trolox (Sithisarn *et al.,* 2005).

2.10.6. Anticancerous hobby of neem: Cancer is multifactorial disease and important fitness trouble international. The alteration of molecular and genetic pathways plays functionin improvement and progression of cancer. Allopathic mode of treatment is powerful but with negative impact on the normal cell. Vegetation and their ingredients exhibit anticancerous effects on growth of malignant cells through methods of cell proliferation, apoptosis, tumour suppressor gene, and different molecular pathways (Rahmani *et al.,* 2014). Flavonoids and other elements in neem plant that play roles in

inhibition of cancer development. Excessive intake of flavonoid is correlated with a reduced danger of most cancers (Marchand, 2002). Diverse limonoids, present in neem oil, prevent mutagenic consequences through its use of 7,12- dimethylbenzene -a-anthracene (Kumar *et al.*, 2010). Nimbolide content of leaves and flower exhibit cytotoxic impacts onhuman choriocarcinoma cells through inhibition of the cells with LD50 of approximately dose and timevalues of 2.01 and 1.19NM for 7 and 24h, respectively, as reported in epidemiological research (Harish Kumar., 2009).

A check to evaluate chemo-preventive capacity of the limonoids, azadirachtin and nimbolide showed that azadirachtin and nimbolide inhibited the development of DMBA added on HBP carcinomas by influencing a couple of mechanism. This mechanism helps to prevent activation of procarcinogen and oxidative DNA damage, regulate antioxidant and carcinogen detoxifying enzymes, as well as inhibit tumor invasion and angiogenesis (Priyadarsini *et al.*, 2009).

Azardirachta indica and their active compounds performmajor rolethrough prevention of cancer improvement and progression. Based on test, it was considered that neem and its factor is discovered to play position in modulating various cellular signaling pathways. It additionally holds diverse ingredients and those elements set off the tumor inhibitortraits and inactivate their interest and phosphoinositol pathway inhibitors. Moreover it activates apoptosis suppression NF-KB signaling, and cyclooxygenase pathway. Neem and its additives play function inprevention of malignancies through modulatingits molecular pathways

2. 11 Technology of Neem Seed Advancemet

Neem fruits may be processed in one of a kind ways:

2.11.1 Hydrated processing on a small scale: This is performed by first depulping fruit with hand, through squeezing and smoothing in water using sieves or hand-driven changed espresso de-pulper. Uses of changed espresso de-pulpers purpose too much damaged to kernel which lead to subsequent fungal contamination. After de-pulping the seed is then washed in water, dried for four-five hours using solar which relies upon the climatic conditions. The seeds must be dried to acceptable moisture content of less than

7%. A higher moisture content material leads fungal infection and ultimately, a discount in azadirachtin content material fabric.

2.11.2 Hydrated technique in a de-pulping plant: here the fruit is deposited right into a processing plant where it's far sorted into ripe and unripe ones. This technique is much like that of small scale. The ripe ones are de-pulped in de-pulping curler (cylinders) with a capability of three hundred-500 fruit/hour. The de-pulped seeds are washed manually or through electric powered mill washing machine and later spread on drying sieves.

Absence of on-line blanketed roof for ripening the unripe fruits and for drying the seeds pose a major restriction on use of wet processing devices. Also problem faced in small scale and semi-commercial processing is the shortage of drying devices for the seeds in order to avoid fungal infection and reduce the moisture content of material. In arid location, the seed are wiped clean via rubbing them with sand due to lack of water.

The seeds are disinfected before garage by means of using chemical and later dried inside the sun or via manner of heating gadgets to attain moisture content material decrease than 7%. The seed needs to be stored in a shady, ethereal area. This is one of the most critical problems, in particular, this constitute critical problem on village degree, because of dearth of space inside the huts full of terrible lenders. There has been accurate experience with entrepreneurs sponsoring drying and storing centers to attain higher outstanding seeds and build a close dating among the collectors and themselves. This encompass receiving credit score rating to procedure the inferior first rate seeds into neem oil for the village.

2.12 Oil from Neem Seed

Oil obtained from neem plant is a form of vegetable oil usually extracted from seeds and kernelin the neem (*Azadirachta indica*), an evergreen tree. It is of high commercial importance when merchandise in organic farming and medication. Neem oil has variable colours such asgolden yellow, yellowish brown, reddish brown, dark brown, greenish brown, and bright red (Kaura *et al.*, 1998). Presence of strong scent that is related to mix odours of peanut and garlicand also consists mainly of triterpeniods, which might be chargeable for thesour flavor (Muhammed *et al.*, 2021). Neem oil composition is shown from the table below.

Table 2.6: Average composition of fatty acids in Neem oil

S/N	Common Name	Acidic Name	Composition Range
1	Omega-6	Linoleic Acid	6-16%
2	Omega-9	Oleic Acid	25-54%
3	palmitic Acid	Hexadecanoic acid	16-33%
4	Stearic acid	Octadecanoic acid	9-24%
5	palmitoleic acid	9-Hexadecanioc acid	_____

(Kaushik and Vir, 2000)

It is hydrophobic in nature and should be formulated with suitable sufractants so one can mix it in water for application purposes.

Neem seed oil as a major important medicine made from *Azadirachta indica* specie, is antiseptic and extensively utilized in treatment ofpores and skin ailments, rheumatism and sprains. It saponified without troubles, also beneficial within the manufacture of medicinal cleansing soap due to its antiseptic houses.Its soap could be very powerful in washing sores and additionally, in popular makes use of just like the ones of carbolic cleansing soap (Santosh *et al.,* 2021).

Azadirachtin is the maximum widely recognized triterpenoid in neem oil. Its content material cloth in neem oil ranges from 300PPM - 2500PPM depending on the extraction method and the splendid of the neem seeds used. The oil also contain numerous sterols, such as campesterol, beta-sotosterol, stigma sterol e.t.c. while its fatty acid composition suggests high quality to the oil. Four main fatty acids that represent the neem oil embody palmitic, stearic as saturated, oleic as monounsaturated and linoleic as polyunsaturated fatty acids (Kaushik and Vir, 2000).

2.12.1 Neem Oil Processing Method and Extrusion Technology

There are extraordinary technologies to be had for extracting lively substances of neem and those strategies depend in particular on the great reqired from the very last product. Neem seeds are treasured uncooked substances that comprise up to forty eight% oil in addition to its insecticidal and fungicidal properties. The extracted neem cake used as fertilizer also has consequences on soil pest. The price of the extracting plant varies as a consequence, high level of Azadirachtin content dictate high cost the extracted plant. Only polar solvents can be used for extraction because of solubility of azadirachtin, as a main neem factor. Regardless of this technique, the cold pressed neem oil should include as much as 0.6% azadirachtin A.

The composition of oil is also laid low with method of processing , because the strategies used, along with urgent (expelling) or solvent extraction are not going to cast off precisely the identical aggregate of additives inside the identical proportion (Usman *et al.*, 2014). The neem seed kernel produce oil yield that varies widely between 25-45%.

This oil is acquired via crushing of the seed kernel additionally, via bloodless urgent or a way incorporating temperature controls forty to 50^{o}C

2.13 Methods of Removing Anti-Nutritional Factors in Legume seeds

2.13.1 Thermal processing: This is a technique of using warmth to spoil heat sensitive antinutrients in food (Adeboyejo *et al.,* 2020). Nutritive excellent of legume grainsis advanced with the useful resource of heat remedy. Moreso, antinutrient degrees decreased during controlled heating at a temperature a great deal less than boiling for about 15 minutes (Udouzoro and Akpan, 2014). Cooking is a common manner to make legumes match for human consumption before used in the course of digestion. This improves protein quality by means of the use of activated anti-physiological elements, specially trypsin inhibitor and haemagglutinins and alsoby unfolding the protein shape, thereby enable them prone to assault through manner of digestive enzymes. Thermal processing also includes the usage of moist warm temperature in removing antinutrients in jack beans. Use of moist warmth is superior to anhydrous heat as a way of treatmentof jackbean seeds as said with the aid of Carlini and Udedibie (1997).

2.13.2 Boiling: Boiling lets in to inactivate warmness touchy anti-nutritients which can be launched into boiling water. The boiling water that includes some soluble compounds will be discarded (Adeleke *et al.,* 2017). Boiling of bambara groundnut seeds reduced the antinutrient content and improved protein digestibility of the seeds.

2.13.2.1 Two-stage cooking: This technique became utilized in Nigeria to cook dinner of some poisonous neighborhood foodstuffs, and additionally completed to jackbean. This system includes cooking the beans internal an hour, doing away with the preliminary water used and cooking over again with sparkling water for approximately 40 min (Udedibie *et al.,* 1996).

2.13.3 Autoclaving: This technique entails cooking below stress so one can shorting the time of cooking. Autoclaving jackbeans allow thermo-labile inhibitory substances such as cyanogenic glycosides, saponins, terpenoids and alkaloids to be detected (Udedibie and Nwaiwu, 1988). Thenutritional first-class of legumes is superior via this method by removing haemagglutinins and increasingother inhibitory substances.

Initial soaking, previous to autoclaving, is requiredfor total removal of toxicity in kidney bean and subject beans. Autoclaving is sufficient to get rid of the toxicity from finely ground navy bean meal. There became little dietary advantage in autoclaving for extra

than 1/2 an hour (Kessler *et al.,* 1990). It was stated that autoclaving of jackbeans changed into incredible approach that makes certain survival birds survive when received jackbean diets, as confirmed from the report of D'Mello *et al.,* (1985).

2.13.4 Soaking: This is one of the bodily processes that can be used to dispose of soluble antinutritional factors in meals. Additionally, itdecreases cooking time andimproves release of enzymes, collectively with endogenous phytases, found in plantmaterials such as almonds, other nuts and grains (Samtiya *et al.,* 2020). Soaking usually increase the hydration diploma of legumes and cereals, thereby making it mild and activate endogenous enzyme such as phytase which decorate ease of additional processes. It is usually recommended that wheat an barley have to be soaked for a time frame (Gupta *et al.,* 2015) earlier than intake, generally 12 to 24 hours (Ertas and Turker 2014). This is due to benefits of soaking in reducing content material of anti-nutrients and phytochemicals like phytate , tannins e.t.c.

Steeping using distilled water, 1% $NaHCO_3$ and combined salt solu-tions reduced universal phenols, ortho-dihydroxyphenols, tannins and phytates by 33, 41, 35 and 21 possibilities respectively (Devi *et al.,* 2018). Howevere,it reduces the whole protein, soluble sugar and tannins, in soybean flour (Agume *et al.,* 2017). Steeping soybeans in water at 22^{o}C for twenty-four h had no impact at the trypsin inhibitor pastime as determined by using Liu and Markakis (1987). Soaking of navy and crimson kidney beans in water for 16h at ambient temperature also confirmed insignificant decreases in trypsin inhibitor interest in military and crimson kidney as suggested by way of Dhurandhar and Chang (1990). No modifications inside the inhibitor hobby was determined while black beans were soaked in water for 16 h, as found by way of Trugo *et al.,* (1990). Soaking lentil seeds for in distilled waterfor 24h, but, ended in about fifty eight-66% reduction in trypsin inhibitor hobby (Batra *et al*., 1986).

2.13.5 Genomic era: This includes the use of genomic assets for pathways to RNA interference with a purpose to cast off the antinutritional factors (Pedrosa, 2004). Zinc-finger nucleases assemble turned into designed by means of Shukla *et al.,* (2009) to mutate the IPK1 gene, one of the phytic acid biosynthesis genes in maize. This is because corn incorporates excessive tiers of phosphorus saved within the shape of phytic

acid. Genome editing era can increase crop viability through genetically change of variety (Kim *et al.*, 2015).

2.13.6 Milling: milling may be used to put off antinutritional factors along with lectins, phytic acids, tannins found in bran of grains. It is a conventional method for separation of bran layer of grains when ground into flour. However, milling can also get rid of vital minerals in grains (Gupta*et al.,* 2015). During making of chappati, milling and heating method reduced phytic acid and polyphenol contents with further improvements in starch and protein digestion (Chowdhury and Punia, 1997). Moreover, low contents of phytate and oxalate were discovered in semi-sensitive flour in contrast with entire flour as a result of removing bran fraction (Suma and Urooj, 2014).

2.13.7 Germination: Germination is a way for decreasing the anti-dietary factors in plant-primarily based totally food through activation of phytase enzyme, in germinating seeds, that breaks down phytate, thereby decreasing phytic acid awareness in the samples. Germination leads toimproved nutritional and reduced anti-nutrients content material in plant-based totally ingredients (Chauhan, 2018).

Germinated cereals confirmed improved hobby of phytase-degrading enzyme in the same level with un-germinated cereals while a discounted endogenous hobby of phytase enzyme changed into located (Vashishth *et al.,* 2017). Malting of millet samples reduced its phytic acid content (Coulibaly *et al.,* 2011). Germination moreover adjust the isoflavone profile of isoflavonein soybean through activation of β-glucosidases thereby improving nutritional price because of the fact isoflavones show off chelating houses (de Camargo *et al.,* 2019). Processing of millets through germination creates discounts in polyphenol contents (as a whole lot as 75%) which had been found at the same time as in comparison to soaking, microwave remedy and fermentation.

Decrease in anti-nutrientssuch as tannin and phytic acid in germinated cereals boom the bioavailability of several minerals, which approximately increased nutritional cost of such meals products (Kokate *et al*., 2010).

2.13.8 Toasting: Toasting improves the nutritional best of jackbeans as first suggested by using Borchers and Ackerson (1950). However, toasting by itself should enhance the nutritive price of the beans for broiler chickens best in volume of up to 100 kg dietary

inclusion. Toasting as a sole method jackbean seeds processing does not appreciably lessen the volume of poisonous factors in jackbeans (Esonu *et al.,* 1998).

2.13.8.1 Microwave treatment: In the experiment with winged bean (*Psophocarpus tetragonolobus*) meal, water activity in winged bean meal was adjusted to 15% and it changed into face within 24hr at room temperature. It turned into suggested that trypsin inhibitor interest and haemagglutinating interest in winged bean meal have been not suffering from microwave treatment (Kadam *et al.,* 1987). Also microwave cooking of jackbeans becomes less effective than its autoclaving (D'Mello and Walker, 1991). The report contrasts with the paintings of Kadam *et al.,* (1987) who demonstrated marked superiority of autoclaving tactics over microwave treatment of winged beans. It is diagnosed that further research is required to examine the outcomes of pattern size and heating instances to be able to competently evaluate the efficacy of microwave treatment within the detoxing of jackbeans (Alajaji, 2006).

2.13.8.2 Extrusion cooking: Partial cleansing of jack bean seeds by means of extrusion cooking has been reported by several worker. Extrusion cooking of jackbean to assert entire inactivation of haemagglutinins, indicates only partial cleansing since it nevertheless led to depression of the experimental cockerels in a trial feeding with the product (Melcion *et al.,* 1991). It consequently showed that increase retarding impact of haemagglutinis had been nonetheless gift in the jackbean so handled. This method does not cause absolute inhibition of trypsin when used on malted soybean seed (Pinto *et al.,* 1997) and thus has little impact on lectins (Carlini and Udedibie, 1997). The nutritional qualtiy of jackbeans, when extruded, is similar to that of discipline beans (Lacassague *et al.,* 1988).

2.13.8.3 Pressure cooking: A strain cooker is commonly usedfor cooking most legume grains to short time and cost. Carlini and Udedibie (1997) determined how lengthy it takes the strain cooker to absolutely inactivate the concanavalin A and trypsin hibitors in *Canavalia ensiformis*. These beans had been subjected to four exclusive stress cooking instances: 15, 30, forty five and 60 min, and later dried, milled, extracted andexamined for haemagglutinating and trypsin inhibitor activities. It took 30 min of pressure cooking to absolutely inactivate the trypsin inhibitors in *Canavalia ensiformis* at same time as concanavalin, organising the fact that concanavalin A become more proof against heat

remedy than trypsin inhibitors. Poultry has been suggested to reveal some bad reaction to 2 hundred-three hundred g/kg nutritional including Canavalia ensiformis which has been stress-cooked for 30 min (D'Mello *et al.,* 1985; Udedibie and Nwaiwu, 1988; Udedibie and Madubuike, 1988). Despite its usefulness for a few domestic purposes, software of pressure cooking of the Canavalia seeds in big-scale business operation cannot be recommended with enthusiasm because of problems relating to the gadget required (Carlini and Udedibie, 1997).

2.14 Neem as a Commercial Products

Neem plant serves as lively element in non-pesticidal control (NPM) in an effort to provide herbal alternative of synthetic insecticides. Its dried seeds are floor into powdery form, soaked in water overnight and sprayed onto the crop. This is finished time and again, as a minimum every ten days, to decorate its effectiveness. Neem product does not kill crop bugs immediately, but functions as anti-feedant, repellent, egg-laying deterrent, and crop protectant against damage. It starves insects which later die after a few days. Additionally, it suppresses the hatching of eggs by insect pests. Neem cake is useful as fertilizer (Neem Archived, 2013). After extraction and purification of azadirachtin, neem is able to be used at various concentrations to supply azadirachtin manufactured from known and strong insecticides. Also, neem formulations comprise some of components which boom shelf-life, ease dealing with and scaling up of manufacturing manner. Sunscreens and para-amino benzoic acid are brought to reduce photo-oxidation of azadirachtin due to mild ultraviolet.

Acceptable trendy is to be had in many nations for the registration and next use of industrial insecticides on a commercial scale (Zubkoff, 1999).

2.14.1 Gum extracts from neem: Gum from neem tree has a clear, brown shade typically acquired from the trunk of the tree because of positive metabolic mechanism of the plant life. It is a multipurpose through product that is soluble in water and absorbs water to form a viscous solution.

2.14.2 Neem Honey: Neem honey includes water, fructose and glucose (22.88%), sucrose (7.46%), ash (0.06%), free acid (20.8 mg/kg). The honey is moderate amber in color with low viscousity. In India, neem trees are a top supply of honey bee forage. Honey acquired from the neem tree has more medicinal qualities swith precise but barely bitter flavor. Neem honey improves eye sight and is harmless for diabetic sufferers. It is

also used to deal with eye disease via applying as netrranjan (eye-liner). It may be very beneficial in care of burning sensation of the frame. Neem honey is noticeably valued because of the fact its miles believed to be a high-quality blood purifier and unique for the eyes. Neem bushes are a major provider of honey bee forage in India.

2.15 Neems Contributions in the National Economy

Neem manufacturing contributes to nearby and country wide economic system. As a potential agricultural country, seventy five% of overall land area in Nigeria supports this activities. The country is also endowed with beneficial agro ecology for cultivation of food crops and farm animal production (Polycarp, 2009) coupled with ample water resources for agricultural irrigation (Bichi, 2009). Since olden days, those potentials made the rural zone the primary source of country wide economy through its reation of rural employment, provision of meals and export income (CBN, 2006).

However, the rural areas are deprioritized due to economic opportunities in using oil reserves from which its sales accounted for greater than 90% of the country`s forex profits (CBN, 2006). Hence, Nigeria is put in a tough and a dangerous state of affairs being over dependent on oil as its most important forex earner. Oil is not only a non-renewable aid, but also has increased the number of becoming much less attractive, specifically with introduction of biofuel era.

Moreover, crude oil marketplace is extraordinarily unreliable because of unforeseen instances such as bad oil sales resulting from economic meltdown of 2009 which led to 10.33 reduction in budget allocated to Local, States and Federal Governments. This leads to economic recession in the country. A drastic discount from social and monetary offerings to the country that occured from every case result in the motive to diversify the economy (Benjamin, 2017). Additionally, Northern state of Nigeria enrichedin favorable agro-ecology for neem *(Azadirachta indica)* assets do not forget huge-scale funding of plantation and reforestation tasksfor beneficial reasons of prenevting desertification and increasing income to allow funding of social, financial and bodily services. Application of neem to reap each goals pose a proper approach, but, there may be a need for crucial analysis of economics of neem manufacturing, severally reiterated in tree choice and usage standards with recognized success from the set objectives, (Wilkinson and

Elevitch, 2012). Selected neem species from different tens is obtained based totally on findings of Food and Agriculture Organization (2008) that the plant prospers well in all elements of the State and additionally has couple of merchandise of high marketplace price and may therefore function every other supply of revenue.

2. 16 Safety, Toxicities, and Ld50 Values of Neem.

Oil from neem seed causes toxic encephalopathy and ophthalmophathy when fed on in big quantities (Bhastara *et al.,* 2010). It works eco-friendly and low-priced agent which suppresses termite attack (YashRoy and Gupta, 2000). Determination of toxicities of herbal compound is important earlier than their software in health control. Scientific on aniimal consumption and others confirmed that neem is safe at certain dose while high concentration had some adverse impacts. Neem oil poisoning in children cause hepatic toxicity, vomiting, encephalopathy and metabolic acidosis (Lai *et al.,*1990) and also, administration of leaf sap on rat prompted an anti anxiety effect at low doses, while excessive doses did no longer show toxicity (Raizada *et al.,* 2001). Examination based totally on rabbit became completed to check the toxicological evaluation and end result of the study showed an end result of modern growth in body weight in eachaside from control animals, while in course of the whole period of the administration of the neem extract, no sign of toxicity was observed from both organization (Boadu *et al.,* 2011). Also have a look at showed that, during acute toxicity check, LD50 values of neem oil were observed (Deng and Shi *et al*., 2013). Another take a look at become finished to estimate the toxicity in chook and it become observed that acute toxicity observe from neem leaf aqueous extract found out intraperitoneal LD50 of 4800 mg/kg and scientific signs and symptoms based on dose structured (Biu *et al*., 2010).

Fatal median doses LD50 recorded from neem leaf and steam bark extract were 31.60 and 489.90 mg/kg body weight, respectively (Akin-Osanaiya and Ibrahim, 2013). Water extracts of its leaves and seedshave LD50 of 6.2 and 9.4 mL/kgl, respectively (Barkr, 2013). Lethal dose values are calculated with probit assessment of LD50and LD90 estimates to be 8.4 and 169.8 µg/f/g of neem extract, respectively (Khan and Ahmed, 2000). Acute oral toxicity experiment of mice revealed LD50 of about 13g/kg body weight (Okpanyi and Ezeukwu, 1996).

2.18 Active Componets of *Azardirachta indica* l. (neem)

Azardirachtin is the maximum important active constituent of (Fatima *et al.*, 2017) *Azardirachta indica* (neem). Other constituents include nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinte, gedunin, salanrain, and querole. Neem leaves encompass substances which incorporates nimbin, nimbanene, g-desacetylnim Biene, nimbandiol, nimbolide, ascorbic acid in-hexacosanol and amino acid, 7-desacefy -7-benzoylazadiradione, and nimboil (Mazlin *et al.*, 2022).

Quercetin and f-sitosterol are polyphenol flavonoids such as Quercetin and f-sitosterol, obtained from clean neem leaves possess antibacterial and antifungal residences (Santoz *et al*., 2020)) even as its seeds incorporate precious constituents along with gedunin and azardirachtin.

2.18.1 Biotechnology and mechanisms of motion of active additives in neem

Extract from neem tree seeds consists of limonoids which can be used for generating biopesticides. Its extraction approach has risks such infection from fungi and heterogenicity within the content material of limonoidsas a result of its genetic, climatic and geographical model. However, use of bioreactors in manufacturing of limonoids from plant cell suspension and furry root cultures helped to overcome the ones problems (Bharat and Ram, 2020). The improvement of a -degree bioreactor method also enhances growth and production of limonoids with cell suspension way of life of Asadirachta indica.

Neem tree has therapeutics influences iin the diseases prevention and treatment. It reveals recovery characteristic due to its wealthy supply of antioxidant and specific treasured lively compounds which include azardirachta, nimbolina, nimbin, salannin and Quercetin. Mode of action of *Azardirachta indica* indicated that neem flowers parts exhibited its anti-microbial function thru inhibitory interest on breaking down of microbial increase potentially of mobile wall. Azardirachtinas a complicated tetrinortriterpenoid limonoidfound in seeds, is a crucialconstituent responsible for antifeedant and toxic consequences in insects. Ethanol extract of neem leaves showed in vitro antibacterial pastime towards*Staphylococcus aureus* and MRSA with best

inhibition zone mentioned at one hundred % concentration. Neem performs role as an unfastened radical scavenging as aresult ofits rich content of antioxidant. Azardirachtin and nimbolide confirmed attention-dependent in their antiradical scavenging interest and reductive functionality within the order of nimbolide - azardirachtin –ascorbate (Hossain *et al.,* 2013).

Neem issue shows effective function inside the manipulation of maximum cancers through regulation of mobile signaling pathways. Neem modulates the hobby of numerous tumor suppressor gene angiogenesis transcription factors and apoptosis. It additionally plays roles as anti inflammatory through regulative of the seasoned-inflammatory enzyme sports activities that consist of cyclooxygenase (COX) and lipoxygenic (LOX) enzyme.

2.18.2 Unsteadiness of neem products

Neem seed kernel cake produced via farmers should be used straight away after coaching because it undergoes photo-oxidation when exposed to ultraviolet mild, (Jarvis *et al*., 1997) which reduces its efficacy as pest manipulate marketers over the years. The number one triterpenoids that accompanying azadirachtin as a product of neem seed extractsinclude nimbin and salaninwhich are photo-oxidisedby ultraviolet slight regardless of their ability to oppose a number of insect pests (Jarvis *et al.*,1997). Thus, these products should be stored in cool, darkish conditions being sensitive to light and excessive temperature (Stark and Walter, 1995).

2.18. 3 Grains and pest infestation

Maize, as a cereal grain associated with wheat, rice, oat and barley is rankedsecond next to wheat in terms of global grain production as it thrives in various climates situations and is grown by using way of small holder families in many nations of the area. It is considered as flexible plant with many uses and a top source of meals for both human and animals.

Addtionally, maize is processed into numerous food and commercial products which incorporates starches, sweeteners, oil, drinks, commercial alcohol and gasoline ethanol. Everyday gadgets including toothpaste, cosmetics, adhesives, shoe polish, ceramics, explosives, creation materials, metal molds, paints, paper items garb, packaging,

carpeting, leisure system and meals utensils of renewable useful useful resource and textiles, contain corn as their essential additives. Thus, maize products are unexpectedly changing petroleum in masses of commercial packages. However, maize is susceptible to maize weevil (Sitophilus zeamais) as its main pest by using discipline and storage attacks within tropics and temperate regions of the arena (Bamaiyi *et al.*, 2006). The assault takes location even as moisture content of the grain lies among 18-20%. Subsequent infestations prevent end end result from the transfer of infested grain into shop and from the pest flying into garage facilities on the identical time as attracted through manner of the odour of the stored grain. Heavy infestation results in weight loss of as a bargain as 30-40% in saved grains.

Also, pest infests one in every of a type stored cereal grains as opportunity hosts, which embody secondary hosts of wheat, rice, sorghum and barley every in issue, in advance than harvest and throughout storage. According to FAO (2015) the anticipated worldwide annual losses because of pest's hobby within the challenge and storage are valued greater than $a hundred billion. In order to reduce intense losses experiencedin the course of garage, numerous strategies and manage methods were advanced and more are nonetheless being advanced. Destruction of meals commodities by the usage of bugs and distinct storage pests were competently checked by using the usage of artificial chemical control strategies which includes treatments of the stored commodity with carbon disulphide, phosphine or dusting with malathion, carbaryl, pirimiphosmethyl or permethrin. However, there are problems associated with the use of those synthetic chemical compounds. Such troubles embody immoderate endurance, bad informationon application method, developing costs of software program, pest resurgence genetic resistance with the aid of the insect and deadly results on non-targeted organisms as well as direct toxicity to clients.

Use of plant products, is these days evolved cheap, renewable and ecologically safe means of controlling insect pest infestations of stored cereal and grains particularly inside the tropics in which farmers are determined to use. Such plant substances encompass powders from components of the neem tree (*Azadirachta indica* A. Juss). Neem is widely recognized in its insecticidal homes and its miles very effective towards

a huge range of insect pests. For instancedried neem leaves are combined with grain in garage through farmers. Important insecticidal materials are present typically in the seeds, leaves and different factors of the neem (Ghimeray *et al.,* 2009). Thus, its additives are used to control home insects pests in stored grains, crop and as a source of human and cattle remedy.

Another vital grain is cowpea, an herbaceous legume that is substantially tormented by insect pests, both inside the field and whilst stored. Reduction of approximately 95% in its yield is because of insects which ususally rely upon region, year, and cultivar (Carlos, 2000). Aphids are developing pest of cowpea on discipline while bruchids are its storage pest. Loss of saved cowpeas in West Africa is induced majorly by means of cowpea weevil, *Callosobruchus maculatus*. Cowpea infestation starts offevolved from the sector and keeps at some stage in garage which leads to finish damage of the grains (Bamaiyi *et al.,* 2006). *Bruchidius atrolineatus* is a species of cowpea pest which does not reproduce in storage however cause damage to the crop all through harvest even as a single girl weevil can reproduces herself 20-fold to reason infestation each three-4 weeks which bring about general loss and destruction of the grain.

Harvested cowpea grains generally have heavy infestation within 2-three months (Carlos, 2000). Insecticides available, require highly-priced device and training for their use, no matter being polluting, and doubtlessly dangerous to users. For instance, phostoxin is a fumigant that can kill people and animals and this makes the cowpea growers to desist from its use.Insecticides, mainly the dirt and gaseous bureaucracy are endorsed for short-time period storage. The product Actellic (2%) or Actellic incredible and Phostoxin gas are very beneficial to the farmer, but they are highly-priced and may not be to be had everywhere. Phostoxin is a fumigant that could kill human beings and animals (Ntoukam, 2000). Green flora function a reservoir for inexhaustible source of harmless insecticides, whichcan be mammalian, trustworthy, and effortlessly biodegradable than artificial chemicals (Palanappian and Holley, 2010).

2.19 Neem for Post-Harvest Crop Protection: The greatest capability of neem's benefit is achieved within subsistence agricultural systems of the tropical regions, where

its tree grows quite simply as a plentiful multipurpose resource. Neem extracts are powerful in protection of crops. However, the rapid picture degradation of the bioactive compounds in neem kernel extract relegates its handiest usefulnessin postharvest storage, during which exposure to light is commonly minimized. Effectiveness of neem for protecting saved merchandise, particularly cowpeas as a vital supply of protein in lots of West African countries such as Mali has been recorded. Commercial neem has no offensive taste and aroma in historically prepared dealt with legumes (Dunkel *et al.,* 1995).

Post-harvest loss of saved grain is a primary constraint to poverty relief in developing nations (Belmain, 1999), as highlighted through the World Food Summit. For instance, some farmers in Ghana use botanical and artificial chemicals to protect saved grains at the same time as maximum farmers do not because they are now not aware of botanicals and can not come up with the money for chemical preservatives. In addition, meals grains are stored within house to makes fumigation fallacious for use (Saxena, 1995).
Before the advent of pesticides, a not unusual exercise among the rural Indian farmers is to combine grains with dried neem leaves earlier than storage as the maximum commonplace form of using neem to defend grains (Chukwuma *et al*., 2019). Neem oil is also appropriate for put up-harvest treatment of cowpea and lots of other crops earlier than storage. The performance of neem oil and groundnut oil in protection of tree seed stocks of Acacia nilotica, Tamarindus indica and A.Raddiana have been studied and the outcomes revealed that neem oil become greater powerful than groundnut oil in shielding the seeds from predation.

2.20 Neem as a Natural Preservative: Food conservation is executed by the usage of chemical agent known as preservative which destroyes or inhibits micro-organism in finished formulations. Food merchandise ought to be be protected with appropriate preservative to ensure protection in used and adequate shelf lifestyles that forestalls microbial contamination at the give up user degree (Mayank, 2021). Spoilage takes place in food and feeds due to presence and increase fungi which reduces its great. Fungi produce mycotoxins as entire secondary metabolites products of their metabolism, which have deleterious outcomes on one-of-a-kind organism. Ochratoxin A (OTA) is an

effective mycotoxin produced with the aid of the use of sure species of Aspergillus and Penicillium. Originally, it is far related to mouldy (Mossini *et al.,* 2009) of legumes, fruits, meat and cereal merchandise which at gift calls for hobby for its nephrotoxic consequences and its potenetial carcinogenic hobby (Bragulat *et al.,* 2001). Extract of Neem plant is an important plant merchandise which inhibit mycotoxin manufacturing. It contains of several additives, present in fruit, seed, leaf and oil, with several energetic compounds. (Martinez, 2002).

The predominant components of neem oil with antifungal sports are a mixture of triterpenoidal and tetranor triterpenoid compounds, 6-deacetyl-nimbin (Kaura *et al* 1998). Azadirachtin, 6-deacetly-nimbin, azadirachtin, nimbin/salannin and epoxyazadiradione had been the principal compounds obtained from chemical fractions of neem oil. When examined on my own, they did not have any considerable interest, however at the same time as combined they confirmed antifungal hobby, indicating viable additive/ synergistic consequences (Ghorbanian *et al.,* 2008). In the element of chemical preservatives that prevent or inhibit microbial boom, common training of chemical compounds used include acids, fragrant alcohol, n-methylo containing compounds, halogenated compounds, Isothiazolinones, quarternary nitrogen compounds, and 1,2 diols.

2.20.1 Preservation and safety of Foods

Food preservation against pathogens and spoilage organisms has been historically finished by chemical strategies. However, sooner or later of new years increase in client interest in growing onions which contain a low level chemical preservatives has developed (Xu and Lee, 2000). The advent of pathogens which can be immuned to classical preservatives (Orpin, 2017) has created a pressing opportunity to locate antimicrobial dealers (Grohs and Kunz, 2000). Consequently, food enterprise is interested in natural components for the partial alternative to artificial antimicrobials (Nava *et al.,* 2006). Onions are available all year when stored properly. Neem products as preservatives are shown in table 2.7 below.

Table 2.7: Limitations and benefits of neem plants products

Product	Advantages	Disadvantages
Commercial Formulations	It requires minimum labour, formulation is stable under UV,	Poor distribution, Poor quality, Registration necessary, Expensive in price, Risk of resistance in a single compound formulation
Neem Leaf Extracts	Continuous production of leaves through out the year, Minimum labour requirements, Simple and cheap preparationmethod	Less effective, contains no azadirachtin, the active ingredient,whichdegrades quickly
Neem Seed Extracts	Storage of seed for future purpose, Simple and cheap preparation method	Quick degradation of active ingredients, variable quality, seasonal Seed production
Neem Oil	Storage of seed for future purpose, simple and cheap preparation method	Production of seed is seasonal
Neem Cake	For manure and in nematode control. Neem oil as its by-product, is cheap and readily produced.	Requires high labour, production of seed is seasonal

(Jenkin *et al.*, 2003)

2.21 Preservation and storage of onions

Onions are sprayed the usage of maleic hydrazide at 500, 1,000 and 1,500 ppm at 2, 3 or four weeks earlier than harvest (Kale *et al.,* 1987). Among distinctive concentrations, maleic hydrazide at 1,000 and 1,500 ppm carried out three weeks earlier than harvest recorded maximum percentage of marketable bulbs after garage length at ambient temperature. Storage of onion bulbs in single layer on a floor for twelve months produced maximum in keeping with cent of healthful bulbs from Bombay White and Poona White cultivars (Gujar, 1976).

There was observation that Hisar-2 and ARka Niketan cultivars had been discovered 66.6, 65.7 in keeping with cent marketable bulbs, respectively, after 120 days of storage compared to 48.6 in VL-2 and 49.9 according to cent in VL-1. The percentage of marketable bulbs turned into decrease when onions were kept in ventilated store with humidified air than with dry air. The cultivar Locol Asomia Piaz confirmed better storability in phrases of maintaining higher consistent with cent of marketable bulbs (71.73%) after 150 days of storage length accompanied by Arka Niketan (48.38%) and Agrifound Light Red (43.35%). Also, pre-harvest spraying with maleic hydrazide decreased sicknesses and loss in weight in the course of storage at the same time as shoot root increase have been completely prevented (Kale *et al.,* 1987) .

2.21.1 Biochemical adjustments at some stages in storage of onion

At harvest, higher soluble sugar content material shows a terrific retaining first-class of onion bulbs. Cultivars with higher dry depend content have for longer garage duration than people with lower dry remember content. Onion sorts with high dry be counted content cloth keeps longer and retained their aroma better than the ones with low drycover. Also, large bulbs have more moisture content material than smaller ones regardless of storage strategies. Removal of tops surface from onions gives the bulbs a tendency to have lots much less dry count number quantity than the ones on which the tops have been retained. Varieties of onions with a low percentage of dry count number and general soluble strong content are extra vulnerable to sprouting losses (Sandhu *et al.*, 1976). During garage of onions, changes in storage temperatue bring about changes in sugar tiers of onion bulbs, as lower garage temperature leads to hydrolysis of sucrose and

fructose even as higher storage temperature causes better degree of sucrose within the bulbs (Darbyshire, 1978). Onion bulbs raised from onion sets contain better content material of dry matter than bulbs evolved from onion seed. Moreover, bulbs with better degrees of dry matter, general soluble solids, non-reducing sugar and phenolic contents exhibited longer storage lifestyles (Bajaj et al., 1980). Pre-harvest spraying of onions with maleic hydrazide at 1,000 ppm prompted maximum overall soluble solids within the bulbs, while cycocel treated bulbspossessed higher reducing sugar at decrease awareness (500 ppm) (Mishra and Pande 1979).

Red cultivars of onions that contain high level of dry matter, non-decreasing sugars and phenolic parts are appropriate for better garage than white cultivars (Bajaj *et al.,* 1980). Any growth in dry weight of onions also will increase the dry depend content material of the bulb (Bajaj *et al.,* 1980). As the in keeping with cent dry weight of onion bulbs accelerated, the fructose content additionally improved at some point of storage (Darbyshire and Henry 1981). The most vital biochemical modifications that occur all through onion garage grown in temperate regions are modifications in the quantities of carbohydrate constituents of the bulbs. The low content of decreasing sugar improves shelf lifestyles of onion (Yemane, 2013).

Physical and chemical composition of bulbs in terms of dry remember, overall soluble sugars, general soluble solids, reducing and non-lowering sugars are directly related to its storage method. High ascorbic acid content material turned into recorded with multiplied degree of sulphur application in chilli. The general sugar content of the bulb and the refractive index of onion juice additionally elevated in percent after 30 days of storage. Therefore, better content material of dry matter and total soluble sugar had been related to higher preserving fine of onion bubs (Kale *et al.,* 1987). Bulbs with high overall soluble solid, excessive dry rely, non-reducing sugar, thin neck and medium length of the bulbs have been proper garage exceptional of onion (Shaha *et al.,* 1992). Chemical properties of bulbs reduced with increase in its storage period. It changed into observed that carbohydrate content of onion bulbs remained regular during the garage length of 180 days and therefore, garage length had no full-size impact at the ascorbic acid content of onion bulbs.

Maximum retention of moisture became discovered in two tier device of storage structure compared with conventional storage approach (Maini *et al.,* 1997). Cultivars with a high dry matter percentage and large quantity of dry scales confirmed ability for longer garage length. Thisworks in line with drymatter and overall soluble solids improved at some point of garage from while reducing sugar content material decreased. However, non-reducing sugar content of onion bulbs increased throughout six months of garage length (Kale *et al.,* 1987). Depending at the duration of the garage length, the dry depend content material and its composition differed in onion bulbs (Hanson, 1999).

2.21.2. Microbiological Quality of Onions

Microorganisms specifically fungi, are a commonplace cause of onion loss at some point of storage through rotten of the bulbs (Narayana *et al.,* 2007). The black mould disorder as a result of *Aspergillus niger* is a restricting element in global production of onions (Orpin, 2017). The microorganisms survive between onion vegetation as a soil saprophyte and also on bulbs in area garage due to its unbiquitous in nature.It invades bulbs of onions by producing various enzymes or pollutants on injured onion tissues infield or during storage (Samuel and Ifeanyi, 2016). *Aspergillus niger*is commonly present with onion seeds produced in warm climates and its transmission from soil and naturally-infected seeds to onion seedlings leads to 30-80% loss or spoilage of onion bulbs. When onion suffering from microbes or infected by means of microbes is ate up, it caused very intense fitness troubles.

Microorganisms involved in spoilage are *Streptococcus pyrogenes, Esherishia coli, Pseudomonas aeroginosa, Morgorella morgonii, Staphylococcus aureus, Staphylococcus saprophyticus, Bacillus pumilis, Bacillus lensus, Staphylococcus capilis, Streptococcus sciuri, Bacilus lensus, bacillus antracis, bacillus subtilis, streptococcus equines, Mucor, Yeast, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Penicillium digitatum e.t.c.*

2.22 African Locust Beans

Parkia biglobosa belongs to the family of leguminosea, which include timber and shrubs which are hardly ever herbaceous. Other species which might be of monetary importance

consist of speces that yield wood, that are cultivated for ornamental purposes and that yield gum. African locust bean, a perennial deciduous tree has potential to withstand drought. Its seedis embedded in a mealy pulp that is high in power price as it contains about 29% crude protein and 60% saccharose. It is likewise rich invitamin C and high in oil content (Orwa *et al.,* 2009). It provides a good source of protein to man as well as for animal feeds, chick and fish. Studies by using Esenwah and Ikenebomeh, (2008) and Omafuvbe *et al.*, (2004) show some modifications inside the dietary and the anti-dietary ingredients of the processed samples of this vital meals supplement.

The acceptance of a substance as meals is based totally on numerous attributes which the substance should possesses. Such substance ought to be attractive in appearance and flavor, possesses correct flavouring and colouring homes. Flavour also increases the nutritional first-rate of any meals to which its miles brought. Locust beans are leguminous seeds which account for up to 80% of dietary protein and can act as the only source of protein for a few companies. Cooked locust beans are eaten as meals and are commonly used in fermented shape as condiments, in fermented shape, to enhance the flavours of ingredients (Achi, 2008). Its tree has been used both domestically and the world over in drug manufacturing in addition to for cosmetics manufacturing (Ojewumi, 2016).

Fermented African locust beans also received exclusive names in special nations such as kinda in Sierra Leone, Iru or dawadawa in Nigeria and Ghana, Afintin and sonru in Benin republic, and natto in Japan. Processing of locust beans is a traditional art; and the fermentation is executed by means of indigenous microflora derived from the on the spot environment (Olabiwonninu *et al.,* 2017).

2.22.1 Preservation and storage of Locust Beans

Locustbans ought to be preserved in order to maintain its freshness and avoid spoilage. Preservation of food as a technique of treating and handling food helps to prevent and substantially slow down spoilage (lack of pleasant, edibility or nutritive cost) brought about by micro-organisms (Modupe *et al.,* 2016). It also entails stopping microbial growth i.e fungi and other micro-organisms, as well as retarding the oxidation of fatsthat

causes rancidity. In addition, its procedures involve inhibition of microbes from growing old and discoloration due to enzymatic browning reactionwhich ariseduring food practices.

Preservation, is a system that inhibit and control the activities of organisms that cause meals spoilage. Growth and metabolic activities of Spoilage inflicting organisms produce through products which trade the texture, taste, flavor and the aroma of the food. Preservatives by using the use of their nature are supposed to preserve the food devoid of these adjustments. Preservatives act on each Gram fantastic and Gram terrible food spoilage organisms. The widespread idea of merchandise that include preservatives is to growth shelf lifestyles and prevent objects from spoilage. The extra shelf lifestyles a product has, the more marketable it will become. The use of preservatives makes merchandise live extra energizing, longer and deliver greater time for merchandise to be used.

Locust bean, iru in Yoruba, dawadawa in Hausa and ogiri `igala in Igbo, is also called kinda in Sierra-Leone and kpalugu in Ghana. Shelf life is the time period for the duration of which the meals product remains secure, retains preferred microbiology, physical, chemical and sensory traits even as nonetheless on shelf (Ademola *et al.*, 2013). Poor shelf existence is a chief thing affecting conventional fermented meals (Sarkar and Tamang 1995). Fermentation must, therefore be controlled or stopped after someday to preventfurther microbial growth thatleads to spoilage. This is achieved by retaining the seed after processing to reduce its moisture content material that brings about deterioration. Drying also helps to enhance its shelfand stability.

Without using additives, locust beans has shelf life of two to 3days and this means that it has to be disposed off after three days. Microorganisms, including bacteria, yeast and molds, are accountable for its spoilage and theyrequire moisture for their metabolism. Thus, moisture content have to be removed definitely so as to get rid of the organisms. This facilitates preserving the product for a protracted duration of time.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Neem seeds samples were obtained from the Faculty of Education in University of Ibadan. Care was taken to ensure absence of foreign materials and removal of defected seeds. Sampling method involved the use of matured ripened neem (yellowish) fruits, de-pulped sundried for 72 hours in accordance with EU regulation (2022).These seeds were stored in hermetic bins at room temperature till analyses were carried out.

3.1.1 Preparation of sample

The whole neem seeds was cleaned to remove sand particles and other foreign matters and later sundried for three days to remove the hard outer covering of the seed to obtain the kernel. The kernel of weight 1.5kg were made in triplicate. These samples were milled using electrical model 5 blender to allow for easy extraction of oil. The milled kernel samples were packaged in tightly covered plastic and kept in refrigerator below $4^{o}C$ to be used for further analysis.

3.1.2 Experimental Design

Research study was designed based on the plans of 1 by 3 in which seed sample was subjected to three pretreatments, 1 by 6 to determine soaking duration, 1 by 4 and 3 by 5 To determine pest reduction and antimicrobial action of neem oil as well as 1 by 3 to determine toxicity of the oil and determine variables obtained during observation.

3.1.3 Pre-treatment of the seed

Pre-treatment of seeds were carried out in batches of four groups based on the methods alkaline soaking (chemical treatment), water soaking, roasting while raw neem seed sample was used as control of which each batch was made with 250g weight of seed.

3.1.3.1 Soaking: A measured weight 250g of neem seeds was steeped in 250mL of water. The soaking hour was for 24 hours after which its water was discarded (Meseret *et al.,* 2019). The seeds weresundried and milled, aseptically sealed for further analysis.

3.1.3.2 Roasting: Roasting was carried out by using microwave oven. This was carried out at a temperature of 60ºC for period of 10 mins.

3.1.3.3 Chemical Treatment: This was carried out by soaking 250g of the seed in alkaline solution (40% sodium hydroxide) using 40 g of seed, for 24h. The seeds were sundried, milled into powdery form packedand covered in containers for further analysis.

3.2 Chemical Analysis of Neem Seed

Analysis of Moisture: Misture content of neem seed was analysed through the use of AOAC method 934.01 (2005) as described by Abiodun and Akinoso (2014).

About 5g of samples were weighed in the dried moisture can. The cans were placed in oven for 22-24 hours at 105^0C. The sample cans were removed from the oven and cooled in the dessicator for 45 min and weighed. It was re-heated and weighed continuously until a constant weight was obtained.

$$\%MC = \frac{\textit{initial weight of can + sample – final weight of can + sample}}{\textit{Sample weight}} \times 100 \ldots\ldots\ldots\ldots 3.1$$

Analysis of Protein Content: Analysis of protein in neem seed become finished using kjeldahl method as described within the technique of Tecator digsetor consumer guide, (2006) and Kjeltec 2200 auto distillation unit consumer guide. The pattern (5g) changed into weighed into the digestion tubes, Kjeldahl catalyst and 15 cm^3 of well known sulphuric acid was added. The combination was digested at 420ºC for forty five minutes in tecator TM digestor till the solution turned colourless. The digest become distilled with 50 cm^3 40 % sodium hydroxide solution further to good enough extent of distilled water in a kjeltc 2200 car distillation unit. 100cm^3 of the distillate turned into collected into 25cm^3 of boric acid, 14cm^3 and 20 cm^3 of zero.02g methyl pink and zero.02 g bromocresol inexperienced respectively, all made upto1000cm^3 with distilled water. Titration happened among the ammonium borate produced and trendy 0.1N hydrochloric acid solution until the green coloration disappeared and gray color became seen.

Calculation

%crude protein = % Total Nitrogen x conversion factor (6.25)…… 3.3

Determination Crude Fibre content : Method of AOAC, 2005 was used to analyse the content of crude fibre. Trichloroacetic acid (TCA) 1000 cm^3composed of 500 cm^3acetic acid, 450 cm3 distilled water 50cm^3 of concentrated HNO_3 and 20 g of TCA was added to the solution. To the defatted sample (5 g) was added to 100cm^3 of the TCA solution. The suspension was boiledunder reflux for 40 min, and then filtered using filter paper of known weight. The residue was then washed with hot distilled water and alcohol (ethanol). The filter paper and its contents were transferred into a previously weighed porcelain dish and dried in an oven at 100^oC for 8 hours. Then cooled and weighed (A). The residue was ashed at 600 ^oC for 5 hours, then cooled in a dessicator and weighed (B)

Calculation

$$\% \text{ Fibre} = \frac{(\text{weight A} - \text{weight B}) \; X \; 100}{\text{Sample weight}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

Fat Analysis: Content of Crude fat content in neem seed become decided by using AOAC, 2005 approach. Samples of approximately 5g have been weighed into the extraction thimble of a Foss 2955 soxhlet fat extraction gadget, included with cotton wool as described by Abiodun and Akinoso (2014). The thimble became inserted into the holder inside the system. The extraction cups had been weighed and 50cm3 of 25% diethyl ether changed into poured into it. The cup become inserted into the device. The extraction became began with boiling for 15 mins then rinsing for forty five minutes. The solvent changed into then evaporated and recovered. The cup as launched, and further dried to put off excess solvent, then weighed.

Calculation

$$\% \text{ Crude Fat} = \frac{(W_3 - W_2)}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

Where :W_3 is weight of cup with extracted oil, W_2 is Weight of empty cup

W_1 is Weight of sample

Carbohydrate content of the seed was estimated by differences and energy content calculated using Atwaters factor.

3.2.1 Determination of Mineral Elements

3.2.2 Analysis of Calcium, Potassium and Sodium: Calcium (Ca), potassium (K) and sodium (Na) contents of milled neem seed pattern were carriedout consistent with AOAC, 975.11 (2005), as described by Bamigboye *et al.,* (2020) using apparatus along with heating mantle, crucible, glass rod, flame photometer, a 100 mL volumetric flask, whatman No1 filter out paper, wash bottle 19 mL pipette, funnel and reagent 2MHCl.

Milled neem sample 5.0 g was weighed into an easy ceramic crucible. An empty crucible was used as blank for manage pattern. The crucibles were inside a muffle furnace at 500^{o}Cfor 4h. neem sample was allowed to cool down within the oven after being removed from the oven. Ash received from every sample become digested with addition of 5ml of molar (2M) hydrochloric acid to the ash within the crucible and heated to dryness on a heating mantle. Five mL of 2M HCl was added once more, boiled, allowed to cool and filtered into a 100ml volumetric flask. The filtrate was made up to mark with distilled water stoppered and Jenway Digital Flame Photometer (PFP7 Model) was placed inside the filtrate to obtain reading concentration of calcium, potassium and sodium using the standards similar to each mineral element.

The concentration of each of the element was calculated using the formula: %Ca r %K or%Na $= \frac{Meter\ Reading\ (MR)\ x\ Slope\ x\ Dilution\ factor}{10000} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$

Division with 10,000 gave the concentration in percentage from part per million (ppm or mg/kg).

3.2.3 Analysis of Se, Mg, Cu, Zn, Fe, Mn: Ashes acquired from each pattern of neem as defined above had been digested and washed into 100ml volumetric flask with distilled water made as much as markaccording to AOAC, 2005 (975.23). Using the tool placing for atomic absorption spectrophotometry model Buck 2HVGP, made by Buck clinical, the diluents had been aspirated into the Buck 2 hundred Atomic absorption Spectrophotometer (AAS) via the suction tube. Each of the trace mineral element were study at exclusive wavelength (Ca-422.7nm, K-766.5nm, Na- 589.0nm, Se- 196.1nm, Mg-202.6nm, Cu-324.7nm, Zn-213.9nm, Fe-372.0nm, Mn- 279.5nm.

3.2.4 Determination of Phosphorus

Phosphorus content was obtained by way of the use of the Vanado-molybdate (yellow) calorimetric approach defined through James (1995) using dry ash acid extraction. The pattern was weighed (5g) into an easy dry warmth resistant field incinerated in a muffle furnace for four hours. Resultant product was dissolved in 10cm3 of 2M HCl and diluted to 100cm3 distilled water in a volumetric flask. The solution become filtered and used for evaluation. About 2 mL part of the extract become mixed with identical extent of the vanado-molybdate reagent in 50 mL volumetric flask. Then a standard phosphorus answer prepared with the same reagents. A blank reagent turned into additionally organized and set apart. The solutions have been made as much as the $50cm^3$ with distilled water. Then, absorbances of the diverse solutions within the numerous flasks have been decided in a Cecil CE-1021 UV seen spectrophotometer at 420nm wave duration. The clean solution was used to set the spectrophotometer to 0.

Calculation: $p = \frac{1000\ x\ au\ x\ vf\ x\ c\ xD}{W}$ ……..…..……. 3.7

Where W is Weight of sample au is Absorbance of sample

as is Absorbance of standard P solution c is concentration of standard (g/mL)

vf is total extract volume va is volume of extract analyzed.

3.3 Determination of Some Antinutritional Factors

3.3.1 Determination of phytates: Phytate was determined from the method of AOAC (2005) described by Onwuka, (2010). Weighed samples (5.0g) were respectively extracted by mixing in 50 mL of 0.2N HCl solution for 30 minutes. These were filtered through a whatman No1 filter paper to obtain the extract. About 0.5mL portion of the extract (0.5 mL) were mixed with 1mL of acidified ammonium sulphate solution in a test tube and boiledfor 30 minutes in ashaking Uniscope SM 101 water bath. The solutions were cooledfor 15minutes.in iced. About 2mL of 2,2-bipyridinsSolution (2 mL) was added and mixed. A standard solution of phytate was prepared and treated the same way as above. The absorbances of the samples and standard were read in Cecil CE-1021 UV visible spectrophotometer at 510nm with blank solution used to set the instrument at zero.

Calculation

$$\%Phytate = \frac{(100)}{W} \times \frac{au}{as} \times \frac{c}{1000va} \times \frac{vf}{}$$ ……..3.8

Where

W was weight of sample as was absorbance of standard au was absorbance of sample

c was concentration of standard (g/dm^3) vf was total extract volume

va was volume of extract analysed.

3.3.2 Determination of hydrogen cyanide content of neem seed

Hydrogen cyanide content of neem seed was determined using the alkaline picric colorimetric procedure (AOAC, 2005). A measured weight (5g) of the sample was dispersed in $50cm^3$ of distilled water (1:10w/v) and allowed to stand over night at room temperature. It was filtered and the filtrate (extract) was used for the analysis. About $1cm^3$ portion of the filtrate was mixed with $4cm^3$ of alkaline picrate solution and heated with boiling water for 5 minutes. The absorbance of the developed colour (reddish brown) was read in a Cecil CE-102 UV visible spectrophotometer at 490nm wave length.

3.3.3 Determination of tannins content of neem seed

Tannins content material of neem seed changed into determined via the method described via Wahab and Elabor (2016). About 5g of sample became measured right into a 50mL beaker and 50% of methanol was added and protected with paraffin. The beaker turned into located in a water bathtub at 77-80^oC for 1h, stirred with a pitcher rod to prevent lumping. It become then filtered quantitatively using a double layered Whatman No.1 clear out paper into a 100mL volumetric flask and rinsed with 50% methanol. The filtrate became then made as much as mark with distilled water and mixed together.

About 10mL of sample became then pipetted into a volumetric flask. About 20mL distilled water, 2.5mL Foli-Denis reagent and 10mL of 17% Na_2CO_3 were delivered into the flask and mixed well. The combination changed into made up with distilled water, blended well and allowed to face for 20min to allow development of bluish-green colouration. Standard tannic acid solutions of variety 0 – 10 ppm were treated because the sample above. The absorbances of the usual tannic acid solutions and other samples were study after color improvement on a spectrophotometer at a wavelength of 760nm.

Percentage tannin turned into calculated using the equation:

$$Tannin\ \% = \frac{absorbance\ of\ sample\ x average\ gradient\ x\ Dilution\ factor}{Weight\ of\ sample\ x100} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

3.3.4 Determination of oxalate content of neem seeds

Oxalate content of seed become decided from the approach of AOAC, (2005). The sample (5.0g) became extracted three times by means of warming it, adding 20 ml of 0.3 N HCl at temperature of 50^{o}C and stirring it with a magnetic stirrer for 1h. The extracts acquired had been diluted to 100ml with water and these had been used for estimation of general oxalate estimation. About five ml of every extract became made alkaline with 1 ml of 5N NH_4OH. This become then acidified with glacial acetic acid and phenolphthalein indicator (2 drops) become brought. Decolouration confirmed the quit factor.

About 1 ml of 5% calcium chloride became brought and the aggregate become allowed to stand for three h and then it become centrifuged (IEC Centra GP8) at 140.868 g for 15 minutes. The supernatant became discarded while the precipitate was washed thrice with hot water, with persisted thorough blending and centrifuging every time. About 2 ml of three N H_2SO_4 turned into added to the mixture and precipitate was warmed in a water bathtub at 70^{o}C – 80^{o}C within 30 min to allow dissolution. The content material of every tube turned into titrated with freshly prepared 0.01N potassium permanganate solution. Titration turned into performed at room temperature (29^{o}C) till the first red color appeared at some stage in the answer. The solution changed into allowed to face until it changed to colourless. This was warmed to 70^{o}C – 80^{o}C and titration continued until a purple coloration endured for at least 30 s.

$$\%\ \boldsymbol{oxalate} = \frac{\mathbf{w\ x\ 100}}{\mathbf{5}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where w was weight of the sample

3.3.5 Determination of azadirachtin content of neem seed

Azadirachtin content of neem was determined according to method described by Nutan, (2002). Standard azadirachtin of concentration stages of 5mg/ml had been prepared from

100mg/L inventory Azadirachtin answer. Milled neem seed pattern of 10g turned into weighed right inside a 50 mL Conical Flask and 20ml of 2:1 Chloroform- Methanol combination became delivered and shaked thoroughly. This changed into allowed to stand for 15mins and centrifuged again for 15 mins. Resultant supernatant was removed and its precipitate washed with 20ml Chloroform-Methanol aggregate for further centrifugation. The final precipitatewas dissolved using 40ml Sodium Deodocyl Sulphate solution and 1 mL of 0.01M Ferric Chloride answer was introduced atrate of 30seconds, shaked and allowed to stand for half-hour.

Absorbances of the sample and that ofstandard concentrations of azadirachtin were examined using digital spectrophotometer at a wavelength of 510nm. The percentage azadirachtin turned into calculated the usage of the components:

:

$$\% \text{ Azardirahctin} = \frac{\textit{Absorbance X Dilution Factor}}{\textit{Weight of Sample X 10,000}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \ 3.4$$

3.4 Expression of Neem Oil by Hydraulic Press

The oil was expressed from 1.5kg of neem kernel after sun-dried for two weeks, using hydraulic srew press. Oil extracted from the seed passed inside a caged barrel-like cavity. Friction and continuous pressure, exerted by screw drives within the machine, was used to roll and compress the seed kernel material covered with a net fibre cloth. The liquid extract seeped out from small openings from which seed fiber could not escapeas it was being covered net. The extracted neem oil was collected in a bottle and covered tightly for further analysis and the pressed seed kernels became hardened cake whenremoved from the machine.

$$\text{Percentage oil Yield } (\%) = \frac{\textit{mass of oil extracted}}{\textit{Mass of sample}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.4.1 Analysis of fatty acids profile in neem oil

Fatty acids profile of neem oil was analysed according to method of AOAC (2005) as described using a CP-Select3900 fuel chromatograph ready with a FID (flame ionization detector) and a fused silica capillary column. The pattern oil was carefully introduce through the injection hollow the usage of hydrogen fuel (H_2) as the provider gasoline

with the flow charge of one.2 mL/min; break up ratio became 1:100. The programming temperatures ranged from 185°C to 250°C at a charge of 15°C/min. detector and injector temperatures were both at 250°C. Fatty acids were identified through comparison of their retention instances with those of pure reference standards (NIST, 2012).

3.4.2 Physical and chemical analysis of neem oil

3.4.3 Analysis to Determine saponification value: Saponification value was analysed according to the method of AOAC (2005). About 5.00ml of the oil sample weighed in a conical flask, dissolved with 5 cm^3 of chloroform to which 0.5M KOH alcohol was added. This was corked and refluxed for 30 minutes. It was carefully poured into a conical flask where few drops of phenolphthalein indicator were added. The solution was titrated against 0.5M HCl until pink colour disappeared which indicate end point. The saponification value was calculated thus:

$$SaponificationValue = \frac{(b-a) \times M \times 56.1}{W} \times 100 \quad \text{……..3.5}$$

where a is sample titre value, bis blank titre value, M is molar concentration of the HCl and 56.1 is molecular weight of KOH.

3.4.4 Determination of free fatty acid content: Free fatty acid (FFA) content neem oil was analysed using method described by according to AOAC (2005). About 10.0ml of samplewas measured into 250 mLflask that contained liquid alcohol of 25 mL and 1.0mL of phenolphthalein indicator was added to it. The mixture was titrated against 0.5N NaOH solution. The amount of NaOH used during the titration corresponded to the percentage of free fatty acid and this was calculated by using the equation:

$$\% FA = \frac{Titer\ value(mL) \times Normality\ of\ NaOH \times 5.61}{Wtof\ Sample\ used} \quad \text{……. 3.6}$$

3.4.5 Analysis of peroxide value of neem oil: The peroxide value was analysed using a method described by (AOAC, 2005). About 5.0g oil samplewas weighted into the 200 mL conical flask that contained 20 mL of petroleum ether and warmed in a water bath for 30 seconds. About 20 mL solution of potassium iodide was mixed with 25 mL of distilled water and titrated against 0.002*m* sodium thiosulphate solutions. White participate was formed while disappearance to colourless showed the end point of

titration. The peroxide value of the sample oil was estimated on the basis of the equation below. The same procedure was repeated for the blank solution.

Peroxide-Value= $\frac{100\ x\ S\ x\ M}{Weight\ of\ sample\ oil}$ ……. 3.7

Where:M is molar concentration of thiosulphate; S is volume of thiosulphate used

3.4.6 Acid Value Determination: Determination of acid value involved uses method described by AOAC (2005). About 10ml of the sample oil was weighed into a 250 mL conical flask. About 50ml of hot neutralized alcohol was added into the flask. The mixture was boiled in a water bath, after which 2 drops of phenolphthalein indicator were added into the content of the flask. This was then titrated against 0.1M sodium hydroxide until a pink coloured solution was observed, indicating the end point.

$$Acid\ Value\ = \frac{M\ (TS-TB)x\ 56.1}{weight\ of\ the\ sample} \quad \text{……3.8}$$

M is molar concentration of sodium hydroxide TS is Sample Titre value

TB is blank solution titre Value

3.4.7 Analysis of iodine value: Analysis of iodine value was carried out according to the method of AOAC (2005). About 5.0mloil sample waspoured into a flask containing carbon tetrachloride and Wijs solution (composed of iodine monochloride in glacial acet`ic acid) was also added. Blank solution was prepared. The mixture was stored in a dark place for 30 minutes at temperature of 25^{o}C after which 15ml potassium iodide solution was added with 100ml of distilled water. The mixture obtained was titrated against 0.1M sodium thiosulphate solution using 2ml of 1% starch as indicator. The titration was continued until the blue blackcolour disappeared, showing the end point. The iodine value was calculated from the equation**:**

Iodine Value$= \frac{12.692\ (TB-TS)\ x\ M}{Weight\ of\ the\ sample\ oil}$ ……..……3.9

M is Molar concentration of the solution TS is Titre value of the sample, TB is Titre value of the Blank

3.4.8 Determination of unsaponifiable matter in neem oil: Unsaponifiable matter was determined using method of AOAC (2005). About 300ml mixed solventsthat contained 70% ethanol and 25% toluene were added to 5ml oil stored in a glass column after saponification of the neem oil. The oil was poured inside washed column atrate of 12ml/

minute. Rotary evaporator was used to concentrate the mixture to 25ml and transferred to the tarred dish for evaporation in oven at 105^{o}C within 15 mins. The dried sample turned into weighed and tested for the last acids; the weight turned into calculated for the unsaponifiable be counted.

3.4.9 Analysis of Specific Gravity of neem oil: Specific gravity of neem oil s determined using a method of (AOAC, 2005). About 40ml of neem oil sample stirred uniformly were pouredinside a 250mlconical flask. The temperature was controlled to avoid drifting in the temperature value. Hydrometer was dipped inside the oil and its readingswere recorded.

3.4.10 Analysis of neem oil viscosity: Viscousity of neem oil was obtained bya method described by Akusu and Kiin-Kabari (2013) using rotary digital viscometer (NDJ-85, China) with spindle number 3 at 30 revolution per minutes.

3.4.11 Analysis of refractive index: Refractive index was determined by using refractometerwas used to determine refractive index of neem oil as described by AOAC (2005). Neem oil was concentrated by heating, according to Bankole *et al.*, (2013), in a water bath at temperature of 80^{o}C. The concentratewas placed onlower prism of the equipment and it was closed up. Water passed through a jacket at 45^{o}C, and the temperature was adjusted to 40^{o}C. Light was also adjusted while compensator was moved until a dark border line was observed on the cross wire. Readings from the equipment were recorded. This was carried out in triplicate and mean of the values was obtained as the refractive index of neem oil.

3.4.12 Flash point and fire point Analysis: Sample oil was poured into a tester cup, to a marked point, which was placed with its left hand pointing towards left front corner of the test compartment. Stirrer and thermometer probe were properly connected to the tester. Flaming and pilot light were carried out by lighting after closing the drought. Tester was put on while heater temperature was regulated. The stirrer and tester were switched on simultaneouslyto achieve homogeneity.

Flashoccurred when large flame was observed on the cup and the temperature at which it happened was recorded as the flash point for neem oil sample. Fire point was recorded at temperature during which combustion of oil was sustained after flash point of the sample oil was recorded. (AOAC, 2005).

3.4.13 Analysis of pour point of neem oil: This analysis concerned uses of technique described by AOAC (2005). About 5ml of homogenized oil sample poured into a check jar that contained thermometer placed at 3mm below the oil and was closed with a cork. The jarwas placed in jacket. The take a look at jar was then located within the jacket. The oil was allowed to cool to avoid error while carefully removing the jar from the jacket. It was tilted to examine whether there was any movement of the oil. The methodwas repeated until a factor was reached at which the oil in check jar showed no movementwhen the test jarwas held in a horizontal rolefor five mins.

3.4.14 Determination of cloud factor: Cloud factor of neem oil changed into determined with the useful resource of very accurate cloud-meter (manual sensor preferred-reflected photograph kind). The cloud meter consists of a wave manual sensor that has an incidence channel, emergence channel, and a detector surface which intersect alongside the detection floor. A prevalence optical fibre changed into associated with the exit of emergence channel. Heating and cooling of the wave manual sensor have to be completed within a preferred temperature variety. The sample oil was poured on the detection ground and moderately delivered into the prevalence optical fibre.Emergence slight from the optical fibre became detected. The guide sensor was changed into heated and cooled as a way to warmth and later cool the pattern. The temperature at which there has been usual reflection of slight within the emergence optical fibre showed the cloud aspect of the pattern oil (Kruka *et al.,* 1995).

3.4.15 GC-MS analysis of bioactive compounds in neem seed oil

GC-MS analysis was initiated by injecting oil sample into an injection portinside a gaschromatography device. After injection, components of the oil was evaporated and seperated one by one until the equipment finally showed various components present in the corresponding sample. Specific spectral pack was produced for each component which was recorded on a paper chart electronically as described by Momoh *et al,.* (2017). Two neem oil samples were analysed. Sample A contained oil from roasted kernel while sample B contained oil from un-roasted kernel. The samples were subjected to chromatographic analysis with the aid of a varian 3800/4000 gas chromatograph mass spectrometer equipped with an Agilentthat contained a splitter.

Capillary column of thickness BP5 (30 m × 0.25 mm × 0.25 microns) was under the chromatographic conditions of nitrogen gas carrier obtained by electron impact fragments to a power of 70 eV rate of 1.2 mL/min, 1:50 split flow and the volume of injected sample of 1 µL. programmed oven temperature, initial temperature was 70˚C with a heating ramp of 10˚C/min to 300˚C which remained stable at thesame temperature for 10 minutes. Subsequently the temperature was increased at a rate of 10˚C/minute to 300˚C for a total time of 30 minutes with an injector temperature 250˚C and the interface temperature 300˚C. Mass range was scanned between 50–500 m/z, and the detector voltage was set at 1150 V.

Compounds obtained were identified by comparing their retention times with those of authentic compounds and with the spectral data obtained from data library of the corresponding compounds (NIST, 2012). Quantities of the compounds were indicated as relative area percentage as derived from the integrator. Phytochemical components were idntified using the database of National Institute Standard and Technology MS library (NIST- MS library) comparing the spectrum obtained through GC – MS. Compounds present in the samples were identified.

3.5 Neem Oil Concentrations used for the Samples

The concentration of neem oil used in the study was prepared for onion sample by dilution of one litre of extracted neem oil, using a syringe, in 1000cm^3 of distilled water measured in a volumetric flask by using a measuring cylinder. This dilution was made to obtain percentage concentration of 10, 20, 30 and 40% using neem oil of volume 0.1, 0.2, 0.3 and 0.4 litres respectively. The onion samples of weight 270g were dip into the dilute solutions of each prepared neem oil concentration and removed. The onion samples were allowed to dry without exposure to sunlight and later packaged inside fibre cloth for futher studies. These samples were made in triplicate. Neem oil concentration for fermented locust beans samples was also prepared by dilution of 0.1 liter of neem oil, taken with syringe, in 1000cm^3 of distilled water measured in volumetric flask to obtain one micro litre as described above. The rneemoil concentrations of 1, 2, 3 and 4µL were obtained for treatmentof the locust beans samples of weight 45g and packaged in an air tight plastics for further study. The locust beans samples were also made in triplicate.

3.5.1 Storage of infected grains treated with neem oil: Storage study took effect after addition of extracted neem oil for preserving grains including rice, cowpea and maize. The grains were stored in polyethylene nylon. The oil concentrations were varied from 0.5, 0.6, 0.8, to 1.0mL/200g of the grains respectively to control the grain pests such as weevils and Aphids. The storage was carried within 29 days. The populations of pest were critically counted, while the numbers of mortality of the pests were recorded and sued in calculating the percentage pest mortality.

3.5.2 Collection of onion bulbs sample: The onion bulbs were selected randomly. About 200g of onion samples were weighed, using electronic weighing balance, into sterile net bags in microbiology laboratory at the Institute of Agricultural Research and Training, Moore Plantation, in Apata, for analysis.

3.5.3 Preservation of onion samples with neem oil: A healthy sample of one hundred onion bulbs were selected at random and weighed into five groups of 200g and treated with different neem oil concentrations of 10, 20, 30 and 40and 0% as the control sample. The oil concentration was prepared by diluting one litre of neem oil in 1000cm^3 of distilled water. Onion samples were dipped into the prepared neem oil concentration and air dried at room temperature. The samples were packed using fibre net-cloth.

3.5.4 Storage study of treated onions: These samples were packaged inside sterile polythene bags and stored under room temperature (29±2^{o}C) for a period of forty-five day. During storage, the onion samples were checked for a possible development and changes in microbial level of the sample and records were made at the interval of seven days. Microbiological analysis was carried out at the end of each weekwhile gross results of the analysis of microorganism were obtained in colony forming units (cfu/g x 10^3) after storage. The analyses were carried out in triplicate.

Plate 3.1: Onion samples with Prepared neem oil concentration.

3.5.5 Collection of locust bean samples: Freshly prepared locally fermented locust beans were obtained from reliable production centre in Ibadan.

3.5.6 Preservation of locust beans with neem oil: The preservation of locust beans took place within the period of forty-five days. About 45g of locust beans samples made in groups of five were treated with neem oil in different concentrations of 1, 2, 3,4, and 0µL/200g locust beans of 0µL/200g which represented the control sample.

3.5.7 Storage study of preserved locust beans samples: The samples were packaged in a sterile plastic containersand stored for a period of 45-days at room temperature. Microbiological analyses (coliform count, total fungi count, *Staphylocoous aureus* count were carried out at the interval of 15-days storage periodwhile gross results of the analysis were obtainedafter 45-days storage period. The analyses were carried out and results were recorded in triplicate. During the storage periods, the locust beans samples were being checked for any possible development and changes in microbial level of the sample and records were made at the interval of seven days.

3.6 Microbial Analyses of Locust Beans and Onions

Microbiological analysis was completed to examine changes in microbial qualities of the sample before and after application of neem oil concentration and also during storage of the samples at one week interval till the end of 45-days storage period.

3.6.1 Determination of total fungal count (TFC): About 2ml of the sample was measured into 9ml of sterile distilled water and serial dilution up to 10^{-6} was carried out in ten folds. About 10 ml of cooled (about 45^{o}C) potato Dextrose Agar was poured aseptically into sterile petri dishes, each containing 1ml of distilled sample (pour plate method). Dilution $10^{-1}$was platedout in triplicates. These plates were swirled gently and allowed to set. All the inoculated plates were incubated at 37^{o}C for 24 hours before they were observed.The samples were inverted after solidification and incubated again at temperature of 25^{o}C for 5 days (Fasoyiro *et al*., 2005).

3.6.2. Determination of total coliform count (TCC): The pour plate method was used. 1ml of the samples were measured into 9ml of distilled water to carry out a ten fold serial dilution up to 10^{-6}. Already prepared Macconkey Agar was allowed to cool before they

were poured aseptically into sterile petri dishes, each containing 1ml of introduced inoculum. Dilution 10^0 and 10^{-6} were plated in triplicates. These plates were gently swirled and allowed to set. All the inoculated plates were incubated at temperature of 37^{o}C for 24 to 48 hours.

3.6.3 Determination of total bacteria count (TBC): Colony count method as described by Jasson *et al.* (2010) was applied for the enumeration of bacteria in the samples. About 2.00 ml/g of locust bean samples were taken in triplicates. Decimal dilutions were performed for all the samples and 0.1ml of 10^{-1} dilutions were inoculated on nutrient agar plates and incubated at 37 ^{o}C for 24h. After incubation, the colonies were counted by using colony counter (Subra Scientific Co., India). The final colonies counts in petri dishes were obtaineddepending on the seeded volume and dilution factor. This provided the initial number of the cells. Analysis was replicated thrice and the arithmetic mean was reported as final result.(Raji *et al.*, 2016).

3.6.4 Determination of other microbial count: Pour plate method with nutrient agar became hired the use of the right media for willpower of the pseudomonas, Staphylococcus aureus and E coli as reported by Singh *et al*., (2015) . About 2g of the pattern changed into taken aseptically by way of using a sterile spatula and transferred into a sterile stomacher, mix very well the use of a Stomacher blender (Huber & Co. AG) that contains 9 ml of peptone water. Incubation of the plates become finished at 30 °C for 24 – 48 h after which the colonies had been counted according to David *et al*., (2018). The test was repeated ultimately for intervals of 15, 39 and 45 days storage intervals.

3.7 Chemical Analysis of Treated Locust Beans samples

3.7.1 Analysis of pH of the samples: pH of the samples wasobtained by using the EDT pH meter, model BA 350. Buffer solutions of pH 7.0 and 4.0 were used for standardization of the meter. Locust bean sample solution of 50 ml was poured in a beaker and the pH of the samples was determined by dipping the electrode into the sample and the reading was read directly from the meter (Akpan and Kovo,2005). This experiment was repeated in triplicate.

3.7.2 Analysis of total titratable acidity (TTA): Total titratable acidity of the sample was determined by titration of the sample solution against standard 0.1M NaOH to an end point of pH 8.1using electronic pH meter. About 10 mL of the sample was diluted to 100ml in a 250ml beaker and standard 0.1M NaOH was titrated against it, using phenolphthalein indicator as described by Olokodana (2005). The total titratable acidity was obtained in form of percentage lactic acid contained in the product (AOAC, 2005). The percentage (%) TTA is calculated as:

$$\text{TTA (\% Lactic acid)} = \frac{\textit{Titre value x Molarity of NaOH x 9}}{\textit{1000xVolume of Sample}} \quad \ldots\ldots\ldots\ldots\ldots\ldots 3.10$$

3.8 Determination of Toxicity level of neem Preserved Samples using Rats

Twelve albino rats of weight 100g were oral fed with trated locust beans samples (2, 3 and 0 µL/200g - untreated) obtained from storage study. After feedig, these rats were observed in animal study house in the department of Veterinary and Biochemistry laboratory, for a period of 14days. After the first day of oral fed with the locust beans samples, these rats were subsequently monitored and fedwith their normal feeds for 14 days. By the end of the animal study period all the rats were sacrificed humanely by using anaesthesia in order to examine the internal structures. The internal tissues like liver, kidney, ovaries, intestine, heart and testis were removed frm the rats and preserved in 10% formal saline immediately for purpse of histopathology examination.Blood samples from the rats were collected in specimen bottles for heamatological examination. The organs were cleaned, dried, embedded and prepared for paraffin block making. Sections of 5 µm thickness wasmade on each organ by using microtome and stained with haematoxylin and eosin stain (Robinson and Gray, 1996). These organs were placed on slides in sectional parts for histological examination under light microscopy (Nikon, Japan).

3.8.1 Haematological Test (Blood Analysis)

Blood obtained from the rat was analysedto determine the effect of neem oil,from preserved samples, onblood parameters such as Red blood cell, white blood cell, hemoglobin, MCV, MCH, MCHC, Platelet, Lymphocytee, Monocytes, neutrophils and Eosiophils with unit XN-100 (Oyeagu *et al.,* 2019).

3.8.2 Calculations of MCV, MCHC, MCH and WBC Differential Absolute:

MCV $= \frac{PCV \times 10 femto\ litre}{RBC}$

MCHC $= \frac{HB \times 100}{PCV}$ %

MCH $= \frac{HB \times 10\ picogram}{RB}$ …….…. 3.12

/Wbc Diff. absolute (white blood cell count)

Lymp $= \frac{Xx\ 5350}{100}$

Neut $= \frac{X\ x\ 5350}{100}$

E o $= \frac{Xx\ 5350}{100}$ …..……..…. 3.13

Where: MCV -mean cell volume; PCV – Packed cell Volume; Rbc – total Red blood Cell count; MCHC – mean cell Hemoglobin concentration; MCH – mean cell hemoglobin; WBc Diffr. Abslt – is the total white blood cell Differential absolute.

3.9 Histopathological Examination of Organs

Hispathological tests were performed toknow the effect of neem oil on various organs such as Liver, Kidney, Testes, and Ovaries in the body system of the animals (Vivek, 2011). The organs (kidney, Liver, Testes/Ovaries were fixed with 10% concentrated formalin underwent several liquor bath of increasing degree (800, 900 and 1000) before passing into toluene mixed with liquid paraffin.Paraffin blocks were mounted on a microtome to make the cuts when sacrificing the animals. The organs were soaked in aqueos dyes (Hematoxylin and Eosin) and observed under microscope.

3.10 Statistical Analysis

Results obtained were subjected to statistical analysis using mean standard deviation and analysis of variance. Duncan multiple range test was used to determine the level of significance between different samples and, significance was set at $p \leq 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Proximate Composition of Treated Neem Seed

Nutritional components in raw and treated neem seed from table 4.1 indicated that **Crude protein** content was retained best in roasted neem seed (19.08%) while the lowest value was observed in water soaked neem seed (16.99%). Equal level of protein contents was obsereved raw seed (18.57%) and alkaline treated seed (18.57%). This was in agreement with the observation of Prakash, (2004) and Wang, (2005) as reported from their cooking effects on nutrient composition of eight legumes.

Crude fat content was significantly increased in roasted neem seed (41.84%) from 41.18% in raw seed. Lowest value of fat content was observed in water soaked neem seed (40.35%) and alkaline treated seed. Crude fibre content was retained best in roasted neem seed (3.64%). The increase was due to protein fibre complexes (Bressani, 1993) formed after possible chemical m odification induced by roasting of seed. The lowest value of 3.53% was obtained from water soaked neem seed. This may occurred as a result of diffusion into soaking water. Equal percentage of crude fibre contents occurred between raw neem seed (3.57%) and alkaline treated seed (3.57%).

Ash content of neem seed was lowest in water soaked neem seed (2.42%). A value of 2.51% was observed in roasted neem seed. No changes occured in ash content between raw seed and alkaline treated seed (2.58%). This value was closer to 3.30% reported for raw melon seed by (Omafuvbe *at al.,* 2004). **Moisture contents** of raw neem seed and alkaline treated seed were (6.03%). Increased moisture content observed in water soaked seed (12.13%) was due to water used in soaking the seed. The moisture content in roasted seed was in the lowest value of (5.89%). This could be due to dehydrating

impacts of roasting treatment. Carbohydrate contents (28.11%) and (28.07%) were obtained in raw and alkaline treated seeds respectively. Lowest value was observed in water soaked neem seed (24.41%), while a value of 27.04% carbohydrate content was obtained in roasted neem seed.

Table 4.1: Proximate Composition of Raw and Pre-treated neem Seeds

Chemical Parameters (%)	Raw Neem Seeds	Water Soaked Neem Seeds	Alkaline Soaked Neem Seeds	Roasted Neem Seeds
Crude protein	18.57±0.09^{b}	16.99±0.27^{a}	18.57±0.09^{b}	19.08±0.11^{b}
Crude fat	41.18±0.03^{a}	40.35±0.03^{b}	41.18±0.03^{a}	41.84±0.05^{a}
Crude fibre	3.57±0.04^{a}	3.53±0.04^{a}	3.57±0.04^{a}	3.64±0.03^{b}
Ash	2.58±0.05^{b}	2.42±0.04^{a}	2.58±0.04^{b}	2.51±0.03^{b}
Moisture	6.03±0.02^{a}	12.31±0.03^{b}	6.03±0.02^{a}	5.89±0.02^{a}
Carbohydrate	28.11±0.02^{b}	24.41±0.43^{a}	28.07±0.02^{b}	27.04±0.02^{b}

Values with a different superscript in the row are significantly different ($p<0.05$).

4.2 Antinutritional Composition of Raw and Treated neem seed

Table 4.2 showed the anti nutritional factors of raw and treated neem seeds. Cyanide content reduced from 2.68 in raw seed to 2.15mg/100g in roasted seed. Highest reduction was obtained water soaked seed (1.46mg/100g) followed by alkaline treated seed (1.98mg/100g). This observation was similar to the report of Nagalaleshmi *et al.,* (1999) on alkaline treatment of neem seed cake.

Nitrate content of the seed was reduced in raw neem seed from 0.16% to 0.13 and 0.14% in alkaline treated and water soaked neem seed. Equal level of nitrate content occurred between raw and roasted neem seeds (0.16%). This was related to the report of (Udedibie *et al.,* 1996) when eliminating terpenoids from raw jackbean by boiling. Azadirachtin content of neem seed was reduced from 0.12% in raw seed to 0.09 % in alkaline treated. This was also reduced by roasting and water soaking method to 0.11% and 0.10% respectively. Phytate content of neem seed was reduced significantly from 0.14% in raw neem seed to 0.11% in alkaline treated seed. A phytate content of 0.13% was obtained from roasted neem seed while 0.12% was obtained from water soaked seed. A similar report of drastic reduction was observed by (Wang *et al.,* 2009).

Level of tannin in neem seed was reduced from 0.004% in raw seed to 0.003% in roasted seed. Equal level of tannin contents (0.002%) were recorded in water soaked, raw neem seed as well as alkaline treated seed (0.002%). The report was similar to the report of (Vijayakumari *et al.,* 1998). Percentage phytate content (14.20- 0.53%) and tannin content (0.42-0.02%) in neem seed was reduced by alkaline soaking. Roasting of neem seed also reduce phytate (14.20-4.73%) and percentage tannin content (0.42-0.02%). Similar result was obtained by Wang *et al.,* (2009).

Percentage nitrate was significantly ($p<0.05$) reduced during soaking with water (16.30-1.81%) as well as during roasting (16.30 – 5.43%) with a significant difference (16.30 – 0.60) in alkaline soaking.

Table 4.2: Contents of Anti-nutrients in Raw and Pre-treated Neem Seed

Antinutritional Factors(%)	**Raw seeds**	**Roasted seeds**	**Water Soaked seeds**	**Alkaline treated seeds**
Cyanide (mg/100g)	26.75±0.04[a]	21.53±0.035[b]	14.75±0.03[c]	19.84±0.04[b]
Nitrate	0.16±0.004[a]	0.16±0.003[a]	0.14±0.005[b]	0.13±0.005[b]
Azadirachtin	0.12±0.00[b]	0.11±0.003[b]	0.10±0.003[b]	0.09±0.03[a]
Phytate	0.14±0.00[a]	0.13±0.003[a]	0.12±0.003[a]	0.11±0.00[b]
Tannin	0.004±0.00[a]	0.003±0.00[b]	0.002±0.00[b]	0.002±0.00[b]
Oxalate	0.10±0.003[a]	0.09±0.004[a]	0.08±0.002[a]	0.08±0.003[a]

Value with a different superscript in the row are significantly different ($p<0.05$).

4.3 Minerals Composition of Raw and Treated neem seeds

Minerals contained in raw and treated neem seed were showed in table 4.3. Content of calcium was significantly reduced from (56.93mg/100g) in raw seed to (52.93mg/100g) in water soaked neem seed. Calcium contents were reduced to 54.20and 54.70mg/100g in roasted and alkaline treated seeds respectively. However, sodium (Na) content of neem seed was increased from 38.80mg/100g in raw seed to 40.20 mg/100g by alkaline treatment. This might due to the sodium content of the alkaline solution used in soaking the seed. Sodium content was reduced in roasted seed (38.19mg/100g) and water soaked seed (37.50mg/100g).

Magnesium (Mg) content of neem seed increased during treatment from (48.50mg/100g) in raw seed to (49.10mg/100g) in alkaline treated seed. Roasting and water soaking brought about a decrease in magnesium content with (47.80 mg/100g) and (45.90mg/100g) respectively. The report was similar to the report of Longe (1983) who recorded decrease of 23% magnesium from autoclaving of mature cowpea seed. There were decrease in phosphorus (P) content of neem seed from (38.40mg/100g) in raw seed to (38.07mg/100g) and (37.30mg/100g) in roasted and water soaked seed respectively. Potassium (K) content of neem seed was decreased from (112.50mg/100g) in raw seed to (112.30 mg/100g), (111.73mg/100g), and (111.20mg/100g) in alkaline treated, roasted and water soaked seed respectively. This was related to the report of Heylowitz and Mattew (1983), who reported 30% loss in potassium during cooking of matured cowpea seed in boiling water.

Copper (Cu) content of neem seed is reduced frm 382mg/100g in raw seed to 381, 379 and 368mg/100g in alkaline, roasted and water soaked neem seeds respectively. Zinc content of neem seed was also reduced drastically from 2463 mg/100g in raw seed to 2429, 2418 and 2398mg/100g in alkaline treated, roasted and water soaked neem seed respectively. This report was similar to the result of Hefnawy (2011) who recorded drastic decrease in zinc content of lentil seed during autolaving and microwave processing methods.

Iron (Fe) contents of neem seed were reduced during treatment from 16482mg/100g in raw seed to 16425, 16418 and 16364mg/100g in alkaline soaked, roasted, and water

soaked neem seed respectively. Manganese (Mn) content of neem seed also reduced from (1182mg/100g) in raw seed to (1179 – 1164 mg/100g) in treated seeds. However, Selenium (Se) content of neem seed increased significantlyfrom 1.50mg/100g in raw seed to 5.10mg/100g in roasted neem seed and also increased to 2.27mg/100g in alkaline treated seed while a reduction in the content was recorded in water soaked seed with 1.10mg/100g. This result was related to the report of Habtamu in nutritional properties of lema bean (Habtamu *et al.*, 2020)

Thus, among the treatment methods, alkaline treatment gave best retention of mineral content of neem.

Table 4.3: Minerals Composition of Neem Seed during Treatment Methods

Minerals(mg/ 100g)	Roasted Neem Seeds	Raw Neem Seeds	Alkaline Soaked Neem Seeds	Water Soaked Neem Seeds
Ca	54.20[b]	56.93[c]	54.70[b]	52.93[a]
Na	38.10[b]	38.80[b]	40.20[c]	37.50[a]
Mg	47.80[b]	48.50[b]	49.10[b]	45.90[a]
P	38.07[b]	38.40[b]	39.30[b]	37.30[a]
K	111.73[a]	112.50[a]	112.30[a]	111.20[a]
Cu	379.00[b]	382.00[b]	381.00[b]	368.00[a]
Zn	2418.33[b]	2463.00[b]	2429.00[b]	2398.00[a]
Fe	16418.00[b]	16482.00[b]	16425.33[b]	16364.00[a]
Mn	1175.00[b]	1182.00[b]	1179.00[b]	1164.33[a]
Se	5.10[c]	1.50[a]	2.27[b]	1.10[a]

Values with the same superscript are not significantly different ($p \leq 0.05$).

4.4 Chemical properties of Expressed neem kernel oil

The chemical properties of oil obtained from dried *Azadirachta indica* seeds were showed in Table 4.4. A golden yellow oil was obtained with Free Fatty Acid content of 4.94% and a high Iodine value of 81.50. Free Fatty Acid (FFA) value indicated the extent to which the glycerides in the oil have been decomposed by lipase action (Akinoso, 2006). A very high flash point of 226.65ºC indicated the the oil is not volatile. The toxicity level of the oil is revealed from its total content of Azadirachtin level of 11.69% which act as the major active component (Hossain *et al*., 2011) the acts against pest growth during storage.

Peroxide value indicated the degree of spoilage of the oil and also act as index for determining rancidity. Codex Alimentarius Commissiom has set a maximum limit of 10mEq/Kg for nuts and seed oil (SON, 2000). Peroxide value of neem oil was 9.85 mEq/100g which showed that the oil is not liable to rancidity. The value obtained ishigher than 0.92 mEq/Kg reported for piper guineense oil by Ogbonna *et al*., (2015) but lower than 5,05 mEq/Kg obtained by Tsado *et al*., (2018) for Blighia sopida pod oil.

Specific gravityof neem oil was 0.92g/cm^3 from the result obtained. This is comparable to 0.956g/cm^3 reported by Oyeleke *et al*., (2013) for ackee aril oil. This showed that the oilis lss dense than water. It also indicated the molecular weight of an oil in relation to its unsaturation.**Saponification Value:** Saponification value showed the average molecular weight of all the fatty acids present. The higher the saponification value the higher the unsaturated level of the oil (Klaus and Kurt, 2005). The value of 164.7 mg/100 g was obtained for nem oil saponification value. This vlue indicated that neem oil possesses more unsaturated level of the oil. **Iodine value** of neem oil was 84.5mg/100g. Iodine number wasdetermined the amount of unsaturation in fatty acids. The higher the iodine number, the more the double bonds present in the fat (Firestone, 1994). **Unsaponiofiable matters** low level of Unsaponifiable matters provides beneficial properties such as moisturization, conditioning, vitamins, and texture. The proportion of unsaponifiables in oil determine whether the oil is defective or not (Gunstone and Frank, 2016). A very low unsaponifiable matter (0.37) was observed in neem oil, i.e below 1 %.

Table 4.4: Physicochemical properties of Neem oil

Parameter	value
Acid value (%)	2.65±0.03
Free Fatty Acid(mg/KOH/g)	4.95±0.02
Density (g/ml)	0.92±0.00006
Peroxide Value (mEq)	9.89±0.03
Iodine Value (mg/gI)	80.44±0.06
Saponification Value(mg/gKOH)	167.95±0.06
Refractive index	1.47±0.002
Flash point (^{o}C)	226.8±0.3
Smoke point (^{o}C)	126.8±0.3
Unsaponifiable Matter	0.37±0.035
Kinematic Viscousity(kPa)	8.7±0.1
Fire point (^{o}C)	87.8±0.2
Pour point (^{o}C)	11.5±0.3
Cloud point (^{o}C)	9.5±0.2
Azadirachtin content (%)	11.73±0.06

4.5 Azadirachtin Content of Neem Seeds at different Soaking Hours

Azadirachtin, as the most toxic component of neem seed (Hossain et al., 2011) was treated by using methods of alkaline treatment, water soaking and roasting treatments.

Among these methods, water soaking treatment was observed to be the most effective method in removing the azadirachtin content. During process of soaking, it was discovered that the longer the hour of soaking the higher the level of Azadirachtin content removed i.e when soaked for 4 hours to 24 hours the level reduced from 0.72% to 0.09% while the content greatly reduced to 0.048 when soaked for 32 hours (Table 4.6). This was in relation to the discovered by Orwa *et al*., (2009) that azadirachtin is very soluble in water and the level of its effectiveness is reduced when comes into contact with water. This makes it possible to use the soaked water as spray to protect plant against foliage eating insect.

4.6 Content of Fatty Acid in Expressed neem Oil

Oil expressed from neem seed kernel is very rich in long chain fatty acid (Kamalu, 2007). It contained unsaturated Fatty acids (Oleic acid, Erucic acid, Linolenic, Palmitoleic and Linoleic), and saturated Fatty acids (Palmitic acid, Arachidnic, Behenic, Lauric, Lignoceric, Marsgaric, Myristic and stearic acid) but lack in Caproic, Caprylic and Capric acid. The oil expressed from neem seed kernel contained significant amount of oleic acid – 39.07%; linoleic acid – 18.60% (Table 4.5). This showed the oil contained larger amount of un-saturated fatty acid as also reported by Sandanasamy *et al*., 2013.

Palmitoleic acid and Palmitic acid are significantly low (0.11% and 14.5% respectively) which constitute the major saturated fatty acid component. Stearic acid (17.65%) in neem oil is significantly higher than that of palm oil (5.10%) as reported by Uko *et al*, (2008). The unsaturated is richer than the saturated fatty acid.

Table 4.5: Composition of Fatty-Acid in Neem seed kernel Oil

Fatty Acids	Value (%)

Arachidonic acid	1.29±0.025
Behenic	0.75±0.04
Erucic	0.022±0.008
Lauric	4.83±0.005
Linoleic	18.59±0.03
Linolenic	0.38±0.04
Margaric	0.017 ± 0.01
Myristic acid	0.05 ± 0.01
Oleic acid	39.07 ± 0.03
Palmitic acid	14.45 ± 0.03
Palmitoleic acid	0.11± 0.02
Stearic acid	17.65± 0.02

Lauric acid (4.83%) Arachidonic acid (1.30%), Behenic acid (0.75%); Erucic acid(0.02%); Liolenic acid (0.38%); Margaric acid (0.017%); Myristic acid (0.05%) are

significantly low in neem oil while Caproic acid; caprylic acid; capric acid and Lignoceric acid are negligible.

Table 4.6: Effect of Different soaking Hours on Antinutritional content of neem seeds

Time (h)	%Nitrate	%Total Oxalate	%Azadirachtin	%Phytate	%Tannin	%Oxalate	HCN (mg/kg)
32	0.10±0.005[a]	0.08±0.004[a]	0.5±0.035[a]	0.08±0.004[a]	0.0003±0.0002 [a]	0.041±0.006 [a]	13.12±0.035[a]
24	0.14±0.004[b]	0.11±0.005[a]	0.10±0.007[a]	0.12±0.003[a]	0.0023±0.0004[b]	0.083±0.002[b]	14.75±0.03[a]
16	0.11±0.003[a]	0.10±0.004[a]	0.06±0.005[a]	0.087±0.004[a]	0.001±0.0002[b]	0.05±0.004 [a]	15.14±0.035 [a]
12	0.12±0.002[a]	0.100±0.03[a]	0.91±0.01[b]	0.10±0.002[a]	0.002±0.0003[b]	0.054±0.00 [a]	14.65±0.025[a]
8	0.114±0.003[a]	0.10±0.004[a]	0.72±0.05[b]	0.10±0.004[a]	0.001±0.0004[b]	0.058±0.01[a]	17.44±0.04[b]
4	0.13±0.003 [b]	0.11±0.003 [a]	0.77±0.05[b]	0.10±0.0025[a]	0.002±0.0004[b]	0.064±0.004[b]	18.59±0.03[b]

Values are means of three experuiments. Means with the different superscript are significantly different $p<0.05$.

4.6.1 Mass spectrum results of GC-MS analysis

The results of GC-MS evaluation confirmed the presence of fourteen phytocomponents in oil extracted from uncooked and roasted neem seed in Tables 4.7 and 4.8.

In uncooked seed oil, the compounds exist at distinct height percent of; 1.48% of 2,4-heptadienal, (E,E) ; 2.09% of cyclohexane, 1- methyl 1-4 (1- methylethylidene); 4.06% of dodecane; 2.76% of 2(4H) – Benzofuranone, 5,6,7, 7a – tetrahydro – 4,4, 7a – trimethyl-, (R); 8.21% of Hexadecanoic acid, methyl ester; 10.91% of Neophytadiene; 23.93% of n-Hexadecanoic acid; 5.74% of 9,12, 15-Octadecatrien-1-ol, (Z,Z,Z); 5.76% of 9,12- Octadecadienoic acid (Z,Z); 4.10% of Phytol; 3.49% of Oleic acid; 2.67% of 9-Octadecanoic acid, (E); 15.04% of 9, 12, 15-Octadecatrienoic acid, methyl ester, (z,z,z); and four.01%. Of Squalene.

In roasted seed oil, the compounds exist at distinctive top percentage of; 1.62% of 2,4-heptadienal, (E,E); 2.09% of cyclohexane, 1- methyl 1- 4 (1- methylethylidene); 3.83% of Dodecane; 7.81% of Hexadecanoic acid, methyl ester; 17.51% of Neophytadiene; 25.72% of n-Hexadecanoic acid; 12.04% of 9,12,15-Octadecatrien-1-ol, (Z,Z,Z); 5.76% of nine,12- Octadecadienoic acid (Z,Z); 8.21% of Phytol; 2.78% of Oleic acid; 2.19% of 9-Octadecanoic acid, (E); 17.08% of 9, 12, 15-Octadecatrienoic acid, methyl ester, (z,z,z); 4.47%. of squalene and 1.09% of vitamin E. It turned into located that 2(H) Benzofuranone of molecular components $C_{11}H_{16}O_2$ was absent in roasted sample 02 while Vitamin E with molecular formulation $C_{29}H_{50}O$ became absent in un-roasted seed.

These compounds are important within the additives of various pills. Hexadecanoic acid-methyl ester is useful as antioxidant, anti inflammatory, owning hypolipidemic houses and is also beneficial as an antimicrobial agent (Akpuaka *et al.,* 2013). Organic compound 9-Octadecenoic acid (Z)-methyl ester act as antioxidant, anticarcinogenic and additionally beneficial as dermatitigenic flavor. It exists in human blood and urine in which it serves as endogenous peroxisome proliferator activated receptor ligand. Its isomeric compound, 9-Octadecenoic acid, methyl ester (E), additionally posses antioxidant homes and anti cancerous activities (Syeda *et al.,* 2011).

Table: 4 .7 Bioactive Components Identified in Un- Roasted neem seed (sample 01)

S/N	Retention Time	Compound detected	Molecular formula	Molecular weight	Peak area (%)	Compound weight
1	4.50	2,4-Heptadienal, (E,E)-	$C_7H_{10}O$	110	2.74	1.48
2	14.50	Cyclohexane, 1-methyl-4- (1-methylethylidene)	$C_{10}H_{18}$	138	5.13	2.09
3	16.21	Dodecane	$C_{12}H_{26}$	170	5.47	4.06
4	18.98	2 (4H)-Benzofuranone, 5,6,7, 7a-tetrahydro-4,4,7a-trimehyl-, (R)	$C_{11}H_{16}O_2$	180	2.76	1.54
5	25.00	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	8.21	10.03
6	26.00	Neophytadiene	$C_{20}H_{38}$	278	10.91	13.71
7	28.00	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		23.93	28.62
8	32.61	9,12,15-Octadecatrien-1-ol, (z,z,z)	$C_{18}H_{32}O$	264	5.74	7.95
9	39.10	9,12-Ocatdecadienoic acid (z,z)	$C_{18}H_{32}O_2$	280	5.76	8.27
10	42.00	Phytol	$C_{20}H_{40}O$	296	4.10	5.73
11	55.00	Oleic acid	$C_{18}H_{34}O_2$	280	3.49	2.08
12	67.00	9-Octadecenoic acid, (E)	$C_{20}H_{38}O_2$	282	2.67	1.05
13	74.12	9,12,15-Octadecatrieonic acid, methyl ester, (z,z,z)	$C_{19}H_{32}O$	292	15.04	17.10
14	78.00	Squalene	$C_{30}H_{50}$	410	4.01	5.08

Table 4.8 Phytocomponents Identified in roasted sample (02) of neem seeds

S/N	Retention Time	Compound detected	Molecular formula	Molecular weight	Peak area (%)	Compound weight
1	5.50	2,4-Heptadienal, (E,E)-	$C_7H_{10}O$	110	1.62	1.48
2	25.50	Dodecane	$C_{12}H_{26}$	170	3.83	2.29
3	28.00	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	25.72	30.21
4	29.00	Phytol	$C_{20}H_{40}O$	296	8.21	10.13
5	33.21	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.57	7.81
6	35.22	Neophytadiene	$C_{20}H_{38}$	278	17.51	20.34
7	36.00	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)	$C_{19}H_{32}O$	292	12.04	17.08
8	44.00	2 (H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)	$C_{11}H_{16}O_2$	180	3.28	1.53
9	50.64	16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_2$	272	2.79	1.19
10	56.50	9-Octadecenamide (z)-	$C_{18}H_{35}NO$	281	1.64	1.06
11	60.10	Oleic acid	$C_{18}H_{34}O_2$	282	2.78	3.11
12	78.92	9-Octadecenoic acid, (E)-	$C_{19}H_{34}O_2$	282	2.19	1.86
13	70.00	9,12-Octadecadienoic acid, (,z,z)-	$C_{18}H_{32}O_2$	280	5.24	6.83
14	76.27	Squalene	$C_{30}H_{50}$	410	4.47	5.18
15	78.92	Vitamin E	$C_{29}H_{50}O$	430	1.09	2.78

Methyl stearate is useful as solvents, cosolvents and oil carrier in agricultural industry. Octadecenoic acid methyl ester is also used as fatty acidsthatcan selectively inhibit eukaryotic DNA polymerase activities in vitro. The chemical bioactive compounds in roasted samples were of higher values than those of unroasted samples. Chromatograms of these chemical bioactive compounds from the mass spectrum were shown in Figures 4.1 and 4.2.

In the compounds observed, some components were biological active in terms of being antiinflammatory, antifungal, antioxidant and anticancer. These compounds are useful in the formulation of different medicines. For instance, Hexadecanoic acid, methyl ester is used as antioxidant, anti-inflammatory as well as an antimicrobial agentdue to its hypolipidemic properties (Akpuaka *et al*., 2013). 9-Octadecenoic acid (Z)- methyl ester has antioxidant activity, is anticarcinogenic; used as dermatitigenic flavour and exists in human blood and urine where it serves as endogenous peroxisome proliferatoractivated receptor ligand (Syeda *et al.,* 2011). Methyl ester (E) 9-Octadecenoic acid, possesses antioxidant properties and anti cancerous activities (Hema, 2011). Also Methyl stearate is useful as solvent, co-solvent and oil carrier in agricultural industry. 1,3-Octadecenoic acid methyl ester is used as fatty acids as a result of its ability to selectively inhibit eukaryotic DNA polymerase activities in vitro.

Fourteen bioactive components were identified on GC-MS chromatogram from un-roasted neem seed (raw) sample showed in Figure 4.1 while chromatograms of fifteen bioactive components were observed from roastedsample as showed in Figure 4.2 below.

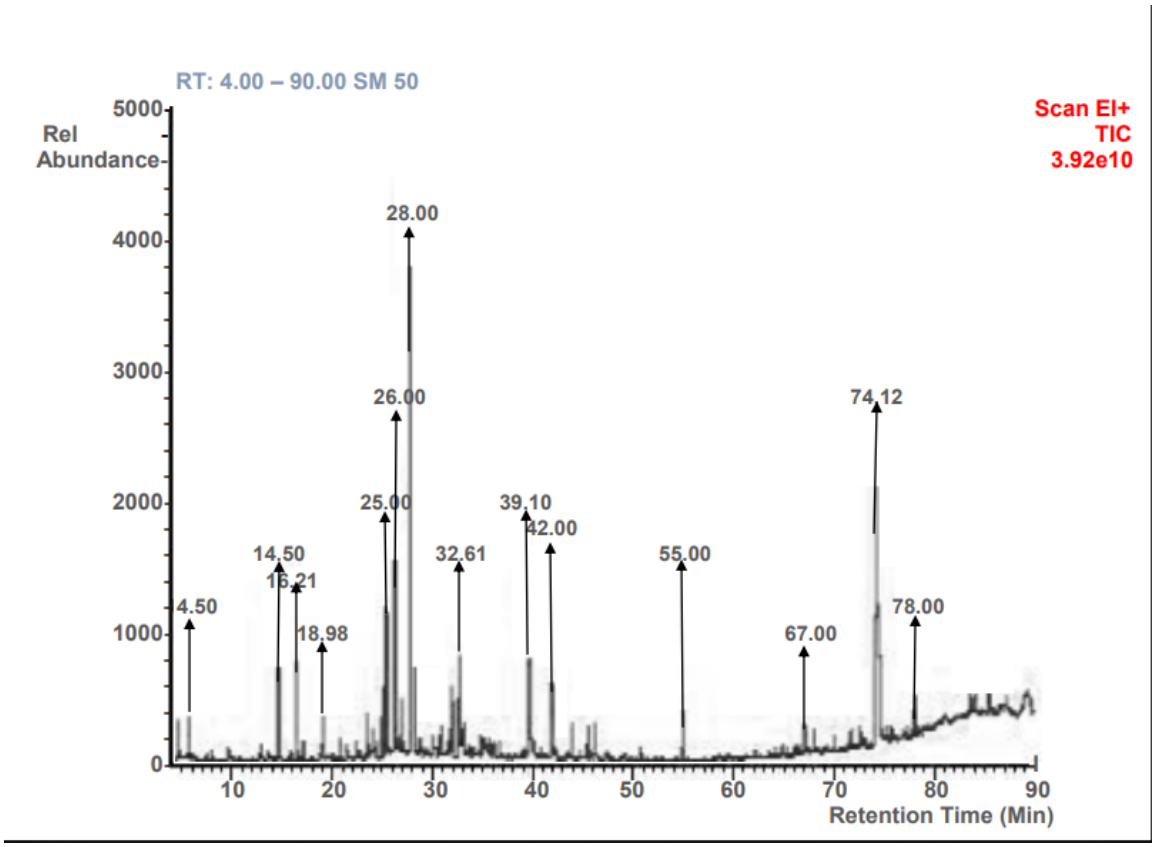

Figure 4.1: Chromatogram of 14-bioactive additives of un-roasted neem seed (pattern 01)

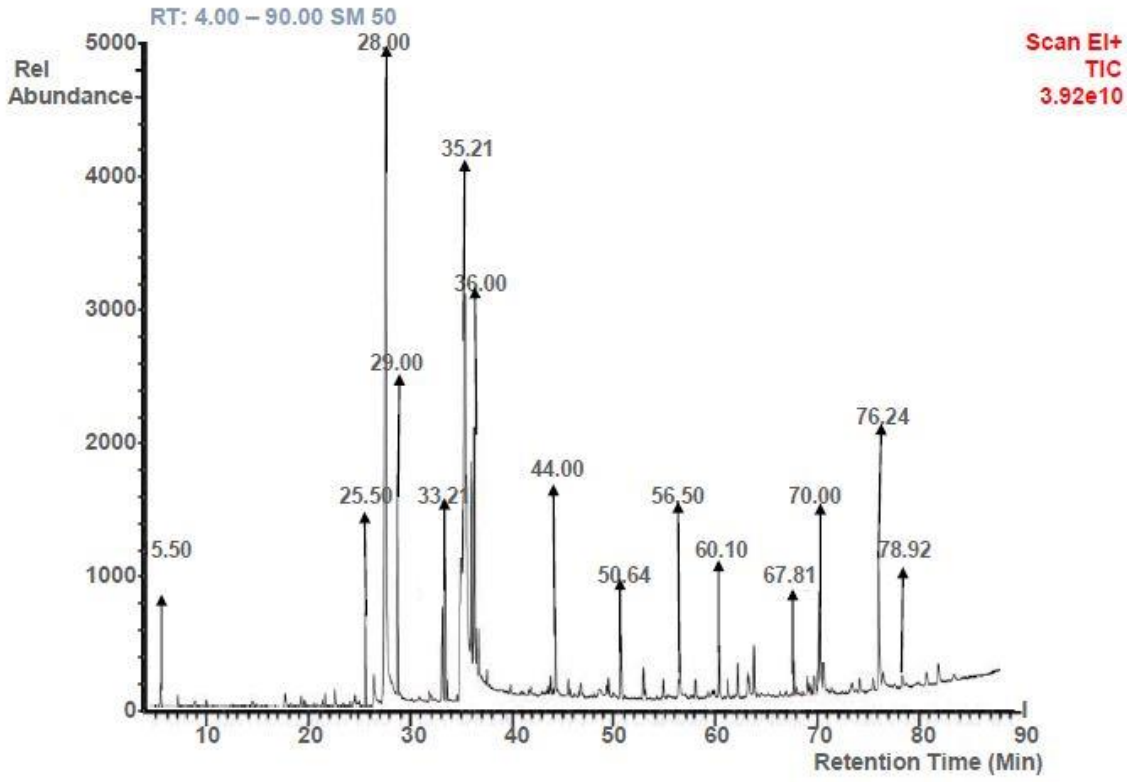

Figure 4.2 Chromatogram of 15- bioactive components of roasted neem seed (pattern 02).

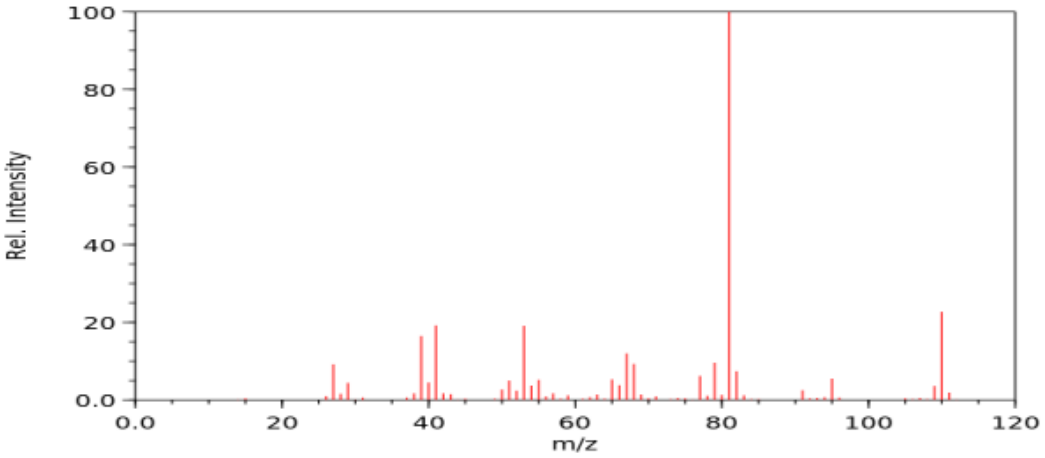

Figure 4.3: mass spectrum of 2, 4 - Heptadienal, (E,E) (2.Forty seven%, RT four.50)

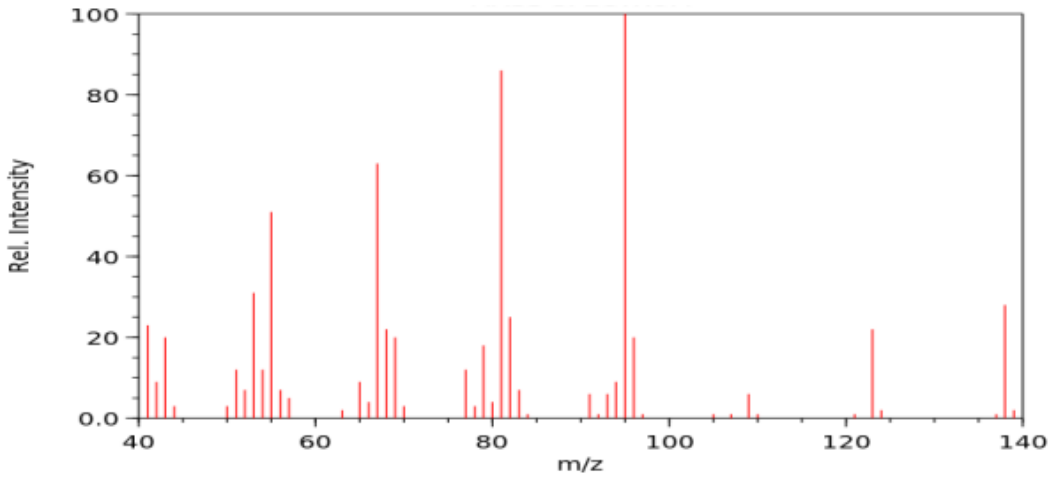

Figure 4.4: Mass spectrum of Cyclohexane (PA 5.13%. RT 14.50)

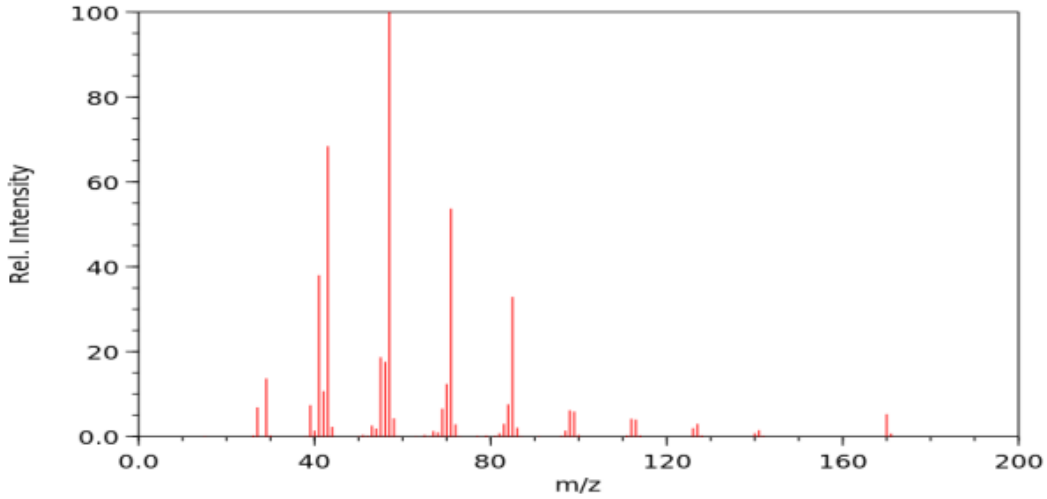

Figure 4.5: Mass spectrum of Dodecane (PA 5.Forty seven%, RT 16.21)

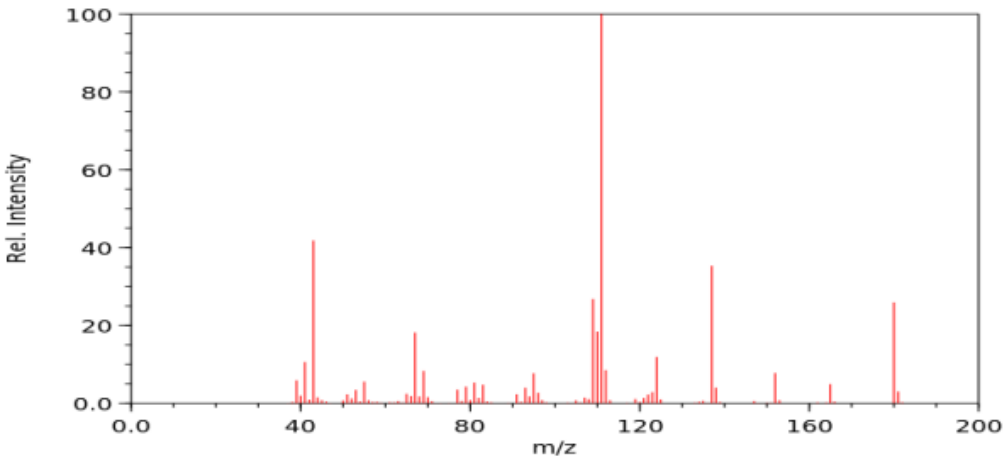

Fig. 4.6: Mass spectrum of 2 (4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)- (PA 2.76% RT 18.98)

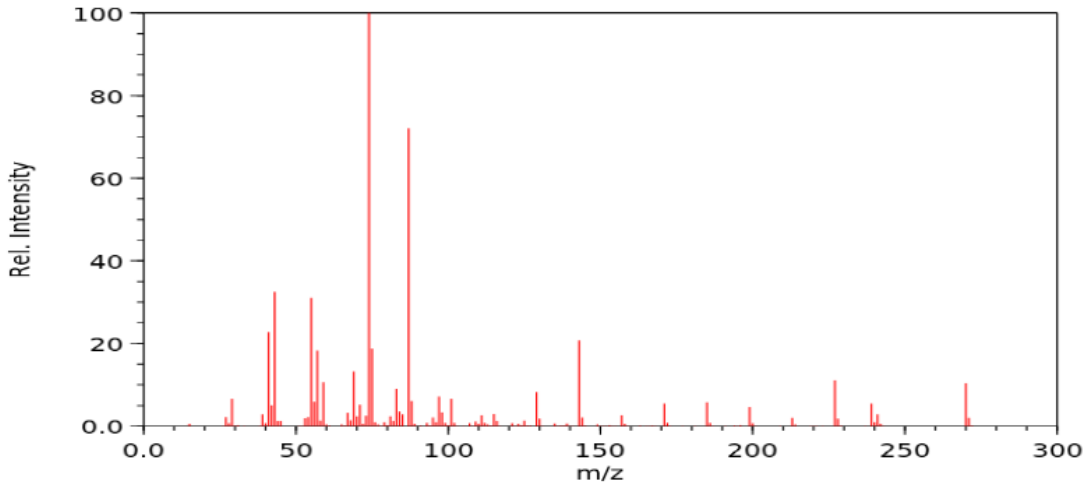

Fig. 4.7: Mass spectrum of Hexadecanoic acid, methyl ester (PA 8.21, RT 25.00)

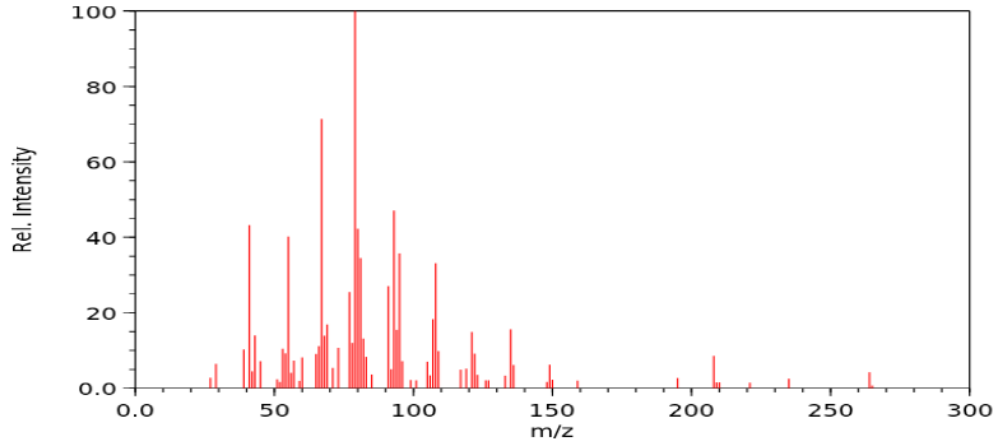

Fig 4.8: Mass spectrum of nine,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (five.74%, RT 32.Sixty one)

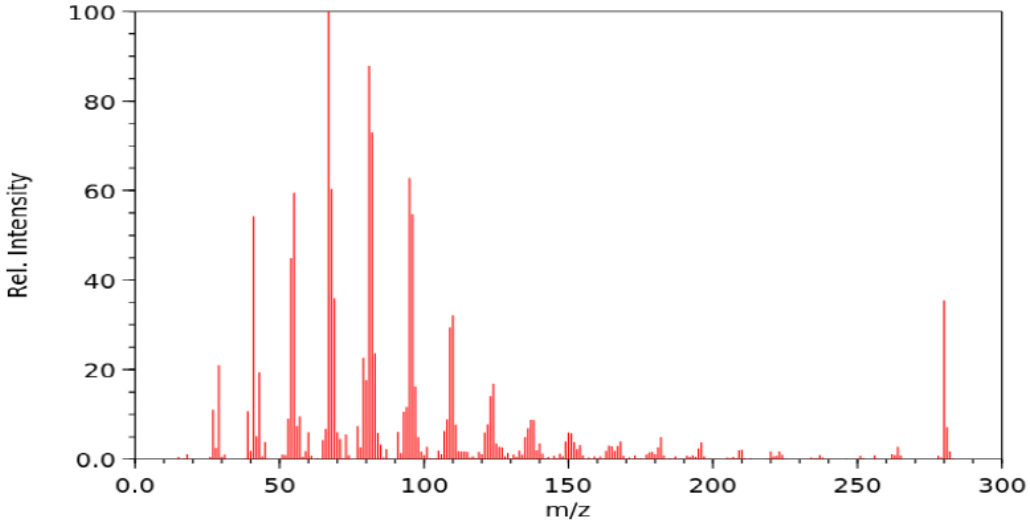

Fig 4.9: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z) (5.76%, RT 39.10)

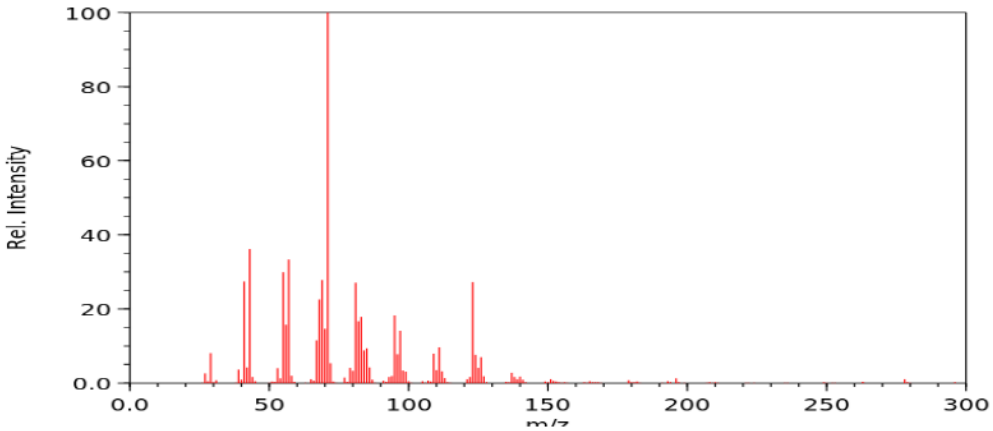

Fig 4.10: Mass spectrum of Phytol (4.10%, RT 42.00)

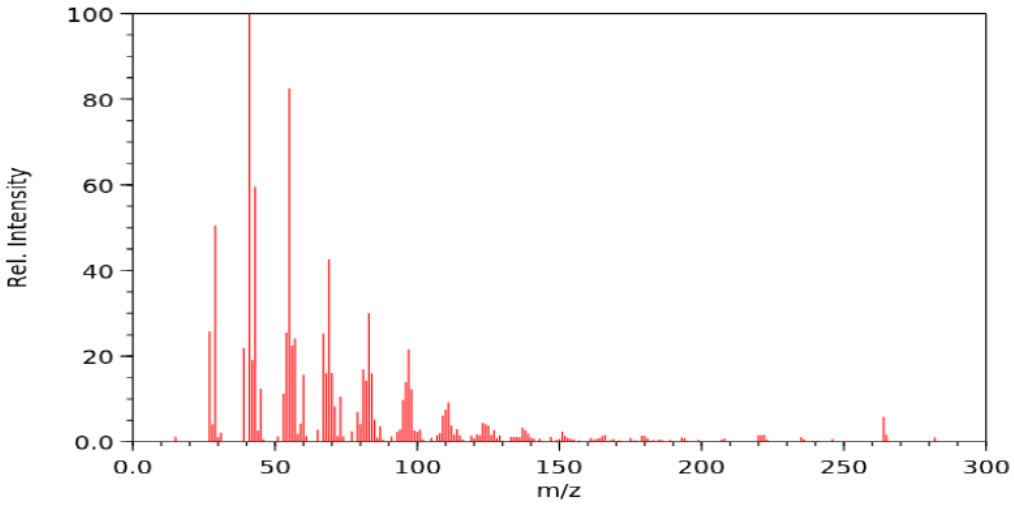

Fig 4.11: Mass spectrum of Oleic acid (3.49% RT 35.00)

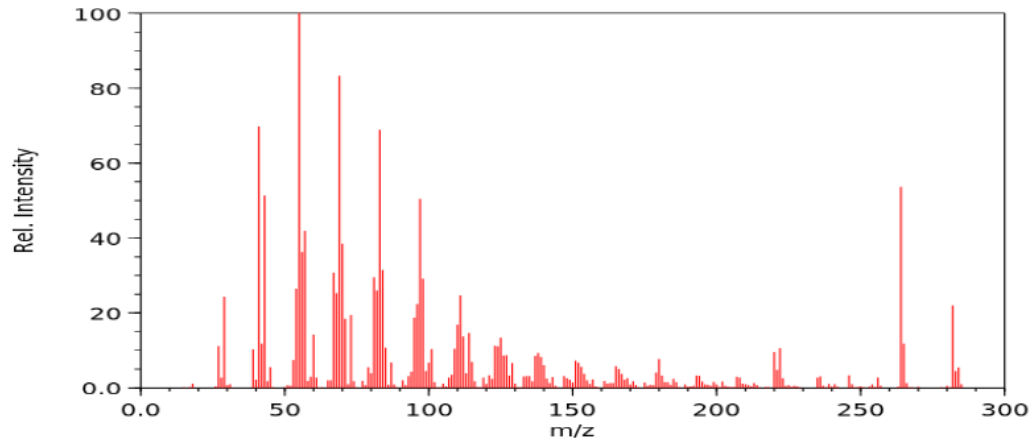

Fig 4.12: Mass spectrum of 9-Octadecenoic acid, (E)- (2.19%, RT 67.81)

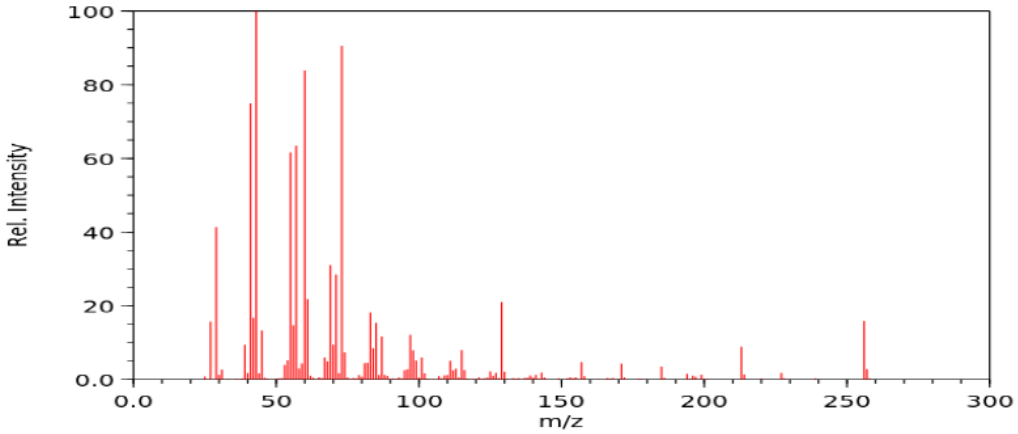

Fig 4.13: Mass spectrum of n-Hexadecanoic acid (23.93% RT 28.00)

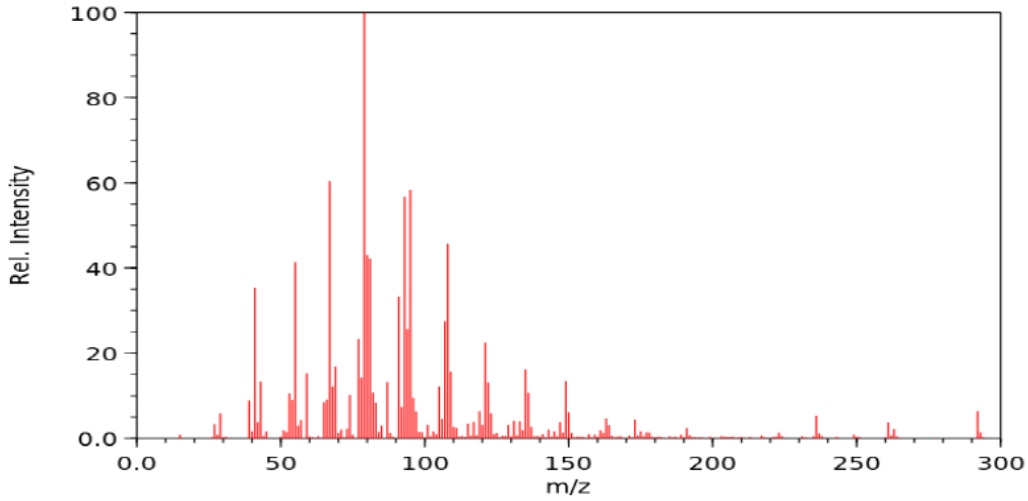

Fig 4.14: Mass spectrum of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-(5.74%; RT 32.61)

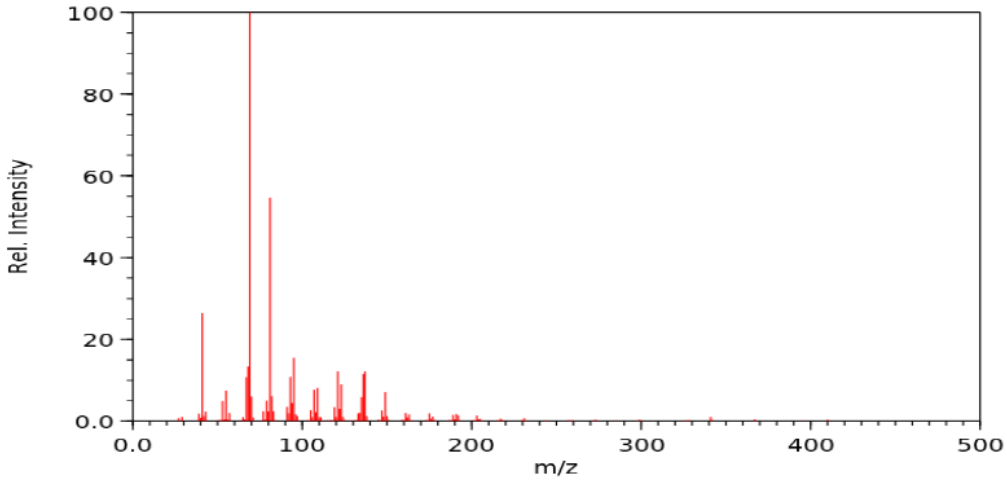

Fig 4.15: Mass spectrum of Squalene (PA 4.47%, RT 76.27)

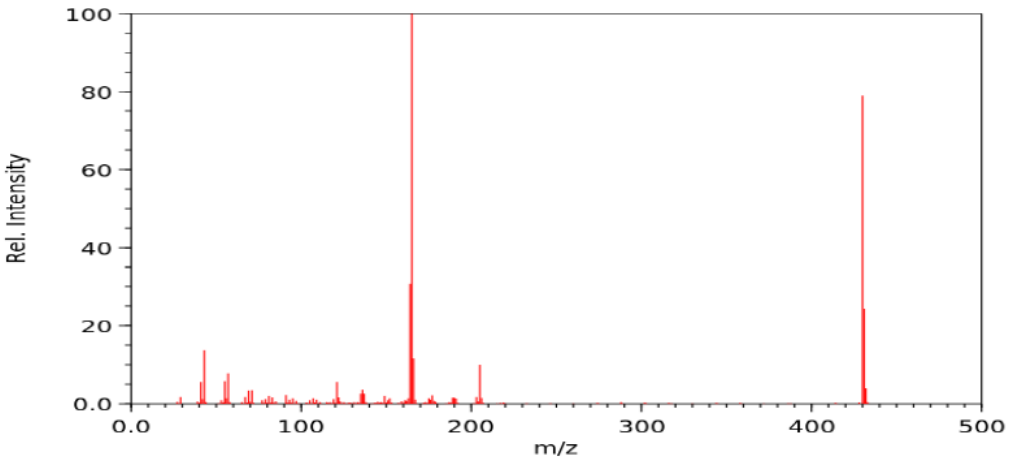

Fig 4.16: Mass spectrum of Vitamin E (1.09%, RT 78.92)

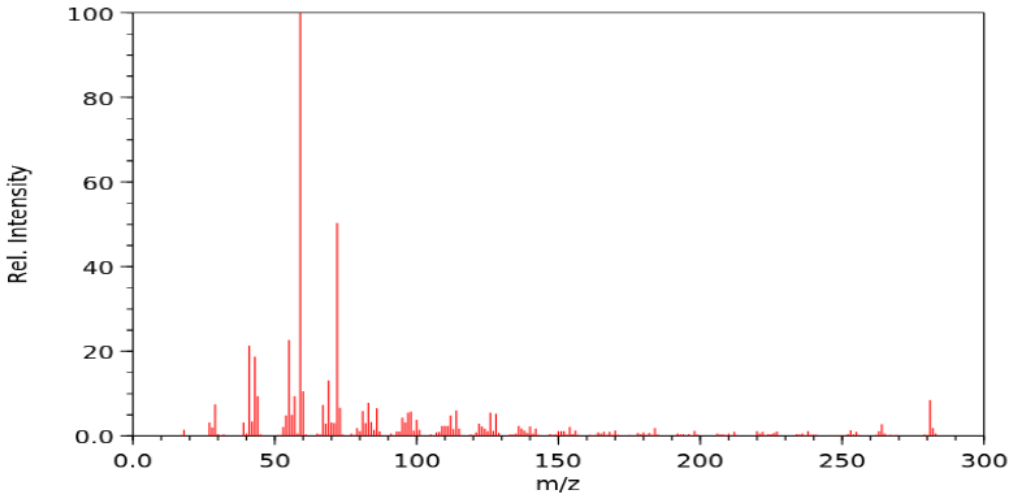

Fig 4.17: 9-Octadecenamide, (Z)- (1.64%, RT 56.50)

4.7 Effects of Neem Oil on infected Grains during Storage

In beans grain treated with neem oil concentration of 1.0mL/200g, the total pest population was reduced from 1200 beetles to 57 pests. Percentage mortality of 41.25; 58.16; 77.65; and 64.91% were recorded by the end 7, 14, 21, and 28 days storage period. During the storage period of 7 to 28 days, the total mortality recorded was 495 deaths at the end of 7days to 37 deaths by the end 28days of storage period with the percentage mortality of 41.25% and 58.16% respectively. However, by the end of 21days storage period, a total mortality of 198 deaths was recorded out of total population of 255 pests, while a total mortality of 410 deaths was recorded out of total population of 705 pests by the end of 14 days storage period. This gives the percentage mortality of 16.5% and 34.17% respectively (Table 4.9).

In beans grains treated withneem oil concentration of 0.7mL/200g, percentage mortality of 63.20; 72.28; 84.31 and 100% were recorded by the end of 7, 14, 21, and 28 days of storage period. This was indicated in table 4.10. Also from bean sample with total pest population of 1200 beetles, percentage mortality of 41.25; 58.16; 77.66 and 64.91% were obtained at the end of 7, 14, 21 and 28 days of storage peiod..This shows that the effect of neem on on the pests are dose and time dependent as discovered by Koul *et al.*, (1987).

In maize grain treated with neemoil concentration of 1.0mL/200g, from total pest population of 100 weevils, percentage mortality of 25.00; 44.00; 54, 76 and 19.00 % respectively were recorded by the end of 7, 14, 21 and 28-day of storage periods. Also in maize grain treated with 0.8mL/200g neem oil concentration, from the total pest population of 65 weevils, percentage pest population of 18.46; 26.41; 46.15 and 81.81% respectively were recorded at the end of 7, 14 21 and 28-day storage period. (Table 4.10).

In Rice grain treated with neem oil concentration of 0.8mL/200g, from the total pest population of 11aphids, percentage mortality of 36.67; 52.63; 0; 77.78% respectively were recorded at the end of 7, 14, 21, and 28-day storage periods (Table 4.11).

Table 4.9: Effect of Neem oil on pest population in beans grain

Grain Mass(g)	Storage Days	NOC (mL/200g)	TPP	TM	% Mortality
	7	1.0	1200	495	41.25
200	14	1.0	705	410	58.16
	21	1.0	255	198	77.65
	28	1.0	57	37	64.91
	7	0.7	500	316	63.20
200	14	0.7	184	133	72.28
	21	0.7	51	43	84.31
	28	0.7	08	08	100.00

Where:

NOC= Neem Oil Concentration

TPP = Total Pest Population

TM =Total Mortality of Pest

Gross Natality: 00; 20

Table 4.10: Effect of Neem oil on pest population in maizegrain

Grain Mass(g)	Storage Days	NOC (mL/200g)	TPP	TM	% Mortality
	7	1.0	100	25	25.00
200	14	1.0	75	33	44.00
	21	1.0	42	23	54.76
	7	0.8	65	12	18.46
200	14	0.8	53	14	26.41
	21	0.8	39	18	46.15
	28	0.8	11	09	81.81

Where:

M = Maize

NOC= Neem Oil Concentration

TPP = Total Pest Population

TM =Total Mortality of Pest

Gross Natality: 00; 02 respectively

Table 4.11: Effect of Neem oil on pest population in Rice grain

Grain Mass(g)	Storage Days	NOC (mL/200g)	TPP	TM	% Mortality
	7	0.8	30	11	36.67
200	14	0.8	19	10	52.63
	21	0.8	09	-	-
	28	0.8	09	7	77.78

Where:

NOC= Neem Oil Concentration

TPP = Total Pest Population

TM =Total Mortality of Pest

In maize grain treated with neem oil concentration of 0.6mL/200g, from the total pest population of 30 weevils, percentage mortality of 28.00; 44.44; 45.00 and 100% respectively were recorded at the end of 7, 14, 21 and 28-day storage periods. a total mortality of 14 deaths were recorded from the total population of 50pests by the end of 7days storage period and a total mortality of 16 deaths at the end of 14 days storage period with the percentage mortality rate of 28% and 32% respectively. However, a total mortality of 9dea.ths and 11 deaths wererecorded at the end of 21days and 28-day storage periods with the percentage mortality rate of 18% and 22% respectively. This showed that the mortality rate was reduced.

In maize grain with total pest population of 500 weevils treated with oil concentration of 0.6mL/200g, percentage mortality of 59.2, 47.06; 69.44 and 62.50 % were recorded at the end of 7, 14, 21 and 28-day storage periods, respectively. A total mortality of 296 deaths were recorded by the end of 7days storage period. This gave the percentage mortalityof 59.2%. By the end of 14 days storage periods, the total mortality of 96 deaths were obtained from total population of 204 pests, given the percentage mortality of 19.2%. After a storage period of 21days, a total mortality of 75 deaths were recorded out of the total population of 108 pests while a total mortality of 18 deaths were recorded out of total population of 24 pests at the end of 28 days of storage period given the percentage mortality of 15% and 3% respectively as showed in Table 4.12 below.

Table 4.12: Effect of Neem oil on pest population in maize grain

Grain Mass(g)	Storage Days	NOC (mL/200g)	TPP	TM	% Mortality
	7	0.6	50	14	28.00
200	14	0.6	36	16	44.44
	21	0.6	20	09	45.00
	28	0.6	11	11	10.00
	7	0.6	500	296	59.20
200	14	0.6	204	96	47.06
	21	0.6	108	75	69.44
	28	0.6	24	15	6250

Where:

NOC= Neem Oil Concentration, TPP = Total Pest Population, TM =Total Mortality of Pest

Gross natality from maize grain of mass 200a= 00

Gross natality from maize grain of mass 200b = 09

4.8 Changes in Microbial level of Onion samples during Storage

The data from table 4.13 shows the total colony forming unit per gram for Total *Bacterial* Count (TBC), Total *Coliform* Count (TCC), *Staphylococcus aureus* Count (S.aureus), Escherichia coli count (E. coli), *Bacillus* count (Lactic Acid Bacteria), *Pseudomonas* count and *Total Fungal* Count TFC for treated onion samples contained neem seed oil concentrations of 0.1% NO; 0.2% NO sample; 0.4% in sample; 0.3% NO sample and 0 % NO (control sample).

4.8.1 Effects of neem oil concentrations on Quality of onions during storage

From the table 4.13 shown below, during the first 15 days of storage period, highest colony count ($0.7cfug^{-1}$) of TBC was observed in control onion sample with the lowest colony count $0.1cfug^{-1}$ from the sample treated with o.4% neem oil (NO) due to short period of application**.** Highest colony $0.53cfug^{-1}$ of TCC was observed in control sample due to absence of neem oil as well with lowest colony (0.1cfug-1) in onion treated with 0.4% NO. *Staphylococcus aureus* count $0.33cfug^{-1}$ was also the highest value in control sample, lowest count in onions with 0.2% and 0.3% NO ($0.1cfug^{-1}$) while no colony count of *Staphylococcus aureus* was recorded for treated sample 0.4% NO. This could be due to higher concentration of neem seed oil added. *E.coli* count of $0.47cfug^{-1}$ was recorded for in control sample with 0% NO. Lowest count ($0.1cfug^{-1}$) of *E.coli* colony was recorded for treated sample 0.4%. *Bacillus spp* count ($0.33cfug^{-1}$), *Pseudomonas spp* count ($0.43cfug^{-1}$) and Total *Fungal Count* ($0.23\ cfug^{-1}$) were also highest in control sample 0% NO with the lowest value of each of these microbial count ($0.1cfug^{-1}$) for all the treated samples 0.1%, 0.2%, 0.4%, 0.3%. The pattern of these colony growth were indicated in fig. 4.10.1, 4.10.2; 4.10.3 and 4.10.4 respectively.

By the end of 30 days storage periods, there was a significant difference between the TBC colony count ($1.07cfug^{-1}$) of the control sample 0% NO compared to the treated samples 0.1 – 0.4% neem oil with count ($0.2\ 0.4cfug^{-1}$). Also there was a significant difference ($p<0.05$) between the control sample 0% with the colony growth of Total *Coliform count* ($0.73cfug^{-1}$), *Staphylococcus aureus* count ($0.5cfug^{-1}$) and *E.coli* count ($0.7cfug^{-1}$) and the treated sample C that showed the lowest colony counts $0.2cfug^{-1}$,

0.2cfug^{-1} and 0.2cfug^{-1} for TCC, *S.aureus* and *E.coli* count respectively. Treated onion sample with 0.1% NO showed the highest TCC count and *S.aureus count* of 0.47cfug^{-1} and 0.37cfug^{-1} respectively while sample with 0.2%NO showed highest *E.coli* count (0.57cfug^{-1}). The highest value of colony count for *Bacillus spp, Pseudomonas spp* and Total *Fungal* count observed in control sample 0% Neem oil were 0.3 cfug^{-1}, 0.5 cfug^{-1} and 0.37cfug^{-1} respectively while lowest colony of these microbial count recorded from treated sample 0.4% NO were 0.1 cfug.$^{-1}$, 0.2 cfug^{-1} and 0.1 cfug^{-1} respectively. The treated sample A showed the highest *Bacillus* count 0.27cfug^{-1}, *Pseudomonas* count 0.37cfug^{-1} and TFC count 0.27cfug^{-1} in compared to control sample 0.1% NO. The highest colony recorded from sample treated with lowest concentration of neem seedoil 0.2% and the control sample that contains no treatment were attributed to environmental condition, and processing , storage of onions and the quality of onion bulbs according to Samuel and Ifeanyi (2015). However the resultant low counts of microbial colony in treated samples (0.4% NO and sample with (0.3%) neem oil showed the preservative impact of neem seed oil during the microbial storage periods. These findings also agree with Shehu and Muhammed (2011) who reported a high frequency of occurrence for Fungi in the onion bulbs they studied.

By the end of 45day of the storage period, no significant difference ($p<0.05$) between the control sample E in the TBC count (1.57cfug^{-1} and the treated sample (0.2% NO) with count (1.13cfug^{-1}). The total *Coliform* Count TCC was highest in control samplewith 0% neem oil (1.0cfug^{-1}). No significant difference between the *Staphylococcus aureus* counts (0.57cfug^{-1}) of control sample and the treated sample. There were highest values of *E.coli* count of 0.73cfug^{-1} from treated sample 0.2%NO and Control sample 0% NO (0.77cfug^{-1}) with lowest count of 0.3cfug^{-1} recorded from treated sample C. *Bacillus* count 0.67cfug^{-1} was recorded from control sample while 0.2cfug^{-1} count was obtained from treated sample 0.4%NO. *Pseudomonas* count 0.73cfug^{-1} was highest in control sample 0% and lowest in treated sample with 2% neem oil as well.

The control sample 0% NO compared with treated samples 0.1% - 0.4% neem oil in total fungal colony count of 0.6 cfug^{-1} with the lowest count of 0.2cfug^{-1} in sample

0.4% NO. This report showed that the higher the concentration of neem oil used the lower the count of colony forming unit by the end of the storage. This agreeg with Ropa *et al.,* (2014) who isolated three different bacteria responsible for causing onion spoilage which include *Staphylococcus spp, Bacillus sp* and *Erwinia sp*.

Table 4.13 Changes in Microbial Quality of Preserved Onion Samples during Storage

N/O (mL/200g)	Total Bacteria Count	Total Coilform Count	(cfug^{-1}) S.aureus	E coli	Bacillus spp	Pseudomonas spp	Total Fungi count
15days							
0	0.70± 0.10bc	0.53± 0.06b	0.33± 0.06a	0.47± 0.06b	0.37± 0.06a	0.43 ±0.06b	0.23±.00a
0.1	0.47 ± 0.06b	0.30 ± 0a	0.20 ± 00a	0.233 ± 0.058a	0.20 ± 00a	0.27 ± 0.06a	0.10 ± 00b
0.2	0.20 ± 00a	0.20±00a	0.10± 00a	0.40 ± 00b	0.167 ± 0.058a	0.20 ± 00a	0.10 ± 00a
0.3	0.23 ± 0.06b	0.20± 00a	0.10 ± 00a	0.20 ± 00a	0.17 ± 0.06a	0.17 ± 0.06a	0.10 ± 00a
0.4	0.10 ± 00a	0.10± 00a	-	0.10 ± 00a	0.10 ± 00a	0.10 ± 00a	-
30 days							
0	1.07 ± 0.12c	0.73 ± 0.06b	0.50 ± 0.10b	0.67 ± 0.06b	0.40± 0.10a	0.53 ± 0.06b	0.37 ± 0.06b
0.1	0.63 ± 0.06bc	0.47 ± 0.06a	0.37 ± 0.06a	0.47 ± 0.06a	0.27 ± 0.06a	0.37 ± 0.06a	0.27 ± 0.06a
0.2	0.37 ± 0.06b	0.27 ± 0.06a	0.20 ± 00a	0.57 ± 0.06b	0.20 ± 00a	0.30 ± 0a	0.20 ± 00a
0.3	0.27 ± 0.06a	0.37 ± 0.06a	0.20 ± 00a	0.27 ± 0.06a	0.20± 00a	0.30± 0a	0.17 ± 0.06a
0.4	0.20 ± 00a	0.2 ± 00a	0.10 ± 00a	0.20±00a	0.10± 00a	0.20 ± 00a	0.10 ± 00a
45 days							
0	1.57 ± 0.06c	1 ± 0c	0.57 ± 0.15c	0.77 ± 0.06c	0.67 ± 0.06c	0.73 ± 0.06c	0.6 ± 0b
0.1	1.13 ± 0.12bc	0.70±0.07bc	0.57 ± 0.06c	0.57 ± 0.06b	0.47 ± 0.06b	0.60 ± 0bc	0.60 ± 0b
0.2	0.50± 0.10b	0.50± 0b	0.40± 00b	0.73 ± 0.06c	0.40 ± 00a	0.47 ± 0.06a	0.43 ± 0.06a
0.3	0.37 ± 0.06a	0.50± 0b	0.30 ± 0a	0.50 ± 0b	0.27 ± 0.06a	0.40± 00a	0.30 ± 0a
0.4	0.27 ± 0.06a	0.30± 0a	0.20± 00a	0.30 ± 0a	0.20 ± 00a	0.20± 00a	0.20 ± 00a

Values with different letters in the column are significantly different ($p<0.05$)

4.9 Changes in Chemical Composition of Locust Bean samples after storage

Table 4.14 below showed the various change in chemical composition of locust beans samples preserved with neem oil concentrations during the storage periods of 15, 30 and 45days.The pH value of treated samples, with neem oil concentration between 1-4µL/200g, ranged from 6.28 – 6.40. Lower pH value 6.22 was recorded in control sample at the end of 15days storage period.

By the end of 30 days storage period, control sample with 0µL/200g neem oil concentration showed a decrease in pH value from 6.22 to 5.01 while the treated samples with 1-4µL/200g neem oil also showed a decrease in pH value (6.40-6.12). Also at the end of 45days of storage period, there was significant difference ($p \leq 0.05$) between pH of treated samples (6.12- 5.91) and the pH of control sample (6.01 – 5.48), with 0µL/200g neem oil concentration. These result indicated a greater reduction in acidity level of control sample while the presence of neem oil showed a more protective action in maintaining the level of fermentation in terms acidity of the treated locust beans samples.

The Total Titratable Acidity (TTA) of treated locust beans samples with 1-4µL/200gneem oil concentration by the end of 15days storage period showed no significant difference ($p < 0.05$) compared to the control sample. However locust beans sample (4µL/200g) and sample (3µL/200g, treated with neem oil concentrations of 4µL/200g and 3µL/200g respectively, gave the lowest values of TTA as 0.05mg/100g and 0.01mg/100g. respectively. By the end of 30 days of storage period, there was a significant difference ($p \leq 0.05$) in TTA (0.32mg/100g) of control sample with 0µL/200gneem oil and the treated sample that contained 4µL/45g neem oil having TTA (0.06mg/100g). During the storage period for the last 45days, treated sample, with 2µL/200gneem oil concentration, showed a lower TTA value (0.28mg/100g) in comparison with the control sample that contained 0.32mg/100g) TTA.

Crude protein content of treated Locust beans (3µL/200g) sample gave (30.72%) while that of control sample 0µL/200g was 32.52% by the end of fisrt storage period of 15-days. Also, the treated sample 4µL/200g with Crude Protein 30.26% compared favourably with the control sample that contains 0µL/200g of the oil havig crude protein

(31.11%) at the end of 30 days storage period. However, by the end of 45 days of storage period, treated sample 1µL/200g showed a percentage of crude protein (18.21%).
The % fat content of treated 4µL/200g sample (27.4%) by the end of 15 days storage period compared with that of control sample 0µL/200g (28.17%), showed in table 4.13. By the end of 30 days of storage period, no significant difference occurred between fat content of treated 4µL/200g sample (26.42%) and control sample 0µL/200g (26.75%) as indicated in table 4.17. During the 45days of storage period, the treated with neem oil 1µL/200g sample gave (23.20%) and the control sample gave (25.14%) while 4µL/200g neem oil treated sample gave percentage fat content of 24.21% at the end of 45days storage period.

Crude Fibre (CF) content of 4.06- 4.3% was recorded for neem oil treated (1-4 µL/200g) samples while a value of of 4.23% was recorded for control sample of locust beans by the end of 15days of storage period. This value was close to the value 3.6% reported for cowpea (Ojimelukwe *et al.,* 1999, but lower than the 8.8% value reported for African locust beans seed by Oyenuga (1968). A massive variations ($p \leq 0.05$) occurred between crude fibre content of manage sample (0µL/200g) sample (4.06%) and that handled neem oil samples 1-4µL/200g (3.22-3.7) by using the end of 30 days of storage duration. By the end of 45 day of storage period, there has been no difference ($p<0.05$) between the percentage Crude Fibre of (3.53%) in control sample with 0µL/200g and the treated samples with (1-4 µL/200g) neem oil attention of crude fibre content material (3.22-3.77%).

Table 4.14: Changes in Chemical composition of Preserved Locust Bean samples during Storage Periodsof 15, 30 and 45-days

Neem oil (µL/200g)	Storage days	pH	TTA (mg/100g)	%crude Protein	% Fat	%Crude Fibre
1		6.29 ± 0.01c	0.23±0.17c	29.13±0.03bc	25.75±0.05b	4.21±0.06c
2	15-days	6.37 ± 0.05c	0.27 ± 00c	29.38 ± 0.39bc	25.89 ± 0.08b	4.33 ± 0.0c3
3		6.28 ± 0.03c	0.09 ± 0.01a	30.72 ± 0.28c	26.62 ± 0.19bc	4.06 ± 0.05c
4		6.40± 0.04c	0.05 ± 0.01a	31.27 ± 0.52c	27.40± 0.02c	4.25 ± 0.05c
0		6.22 ± 0.03b	0.29 ± 0.01b	32.52 ± 0.50c	28.47 ± 0.31c	4.23 ± 0.03c
1	30-days	6.18 ± 0.03b	0.04 ± 00a	28.6 ± 0.06bc	24.23 ± 0.06a	3.22 ± 0.02a
2		6.20 ± 0.02b	0.28 ± 0.01b	28.72 ± 0.07bc	24.62 ± 0.05a	3.36 ± 0.07a
3		6.12 ± 0.02b	0.03 ± 0.01a	29.12 ± 0.15bc	25.81 ± 0.01b	3.57 ± 0.05b
4		6.22 ± 0.03b	0.06 ± 00a	30.26 ± 0.02c	26.42 ± 0.11bc	3.77 ± 0.12ab
0		6.01 ± 0.02b	0.32 ± 0.01b	31.11 ± 0.18c	26.75 ± 0.10bc	4.06 ± 0.06c
1	45-days	5.64 ± 0.05a	0.03 ± 00a	18.2167 ± 1.57a	23.20 ± 0.04a	3.16 ± 0.06a
2		5.77 ± 0.06a	1.12 ± 1.44c	27.27 ± 0.04b	23.34 ± 0.49a	3.23 ± 0.02ab
3		5.82 ± 0.03a	0.02 ± 00a	28.18 ± 0.23b	23.91± 0.04a	3.36 ± 0.02a
4		5.91± 0.01a	0.06 ± 00a	28.70 ± 0.05bc	24.21 ± 0.06a	3.40 ± 0.01a
5		5.48± 0.03a	0.03 ± 0.01a	29.50 ± 0.36bc	25.14 ± 0.19b	3.53± 0.05ab

NO neem oil. The experiment was carried out thrice. Values were obtained as mean ± SD of the values. Mean values with different letters in the column are significantly different ($p<0.05$).

4.10 Effects of Neem oil on Microbial Quality of Locust Beans at storage points.

During storage of locust beans samples for durations of 15; 30 and 45 days, there have been exclusive ranges of microbial improvement within the samples saved with extraordinary awareness of neem seed oil from 0 - 4µL/200g. Four Samples were treated with 1µL/200g of neem oil, sample (2µL/200g); sample (4µL/200g), sample treated with 3µL/200g and control sampletreated with 0 µL/200g of neem oil.

By end of the storage period, sample treated withneem oil concentration of 4µL/200g undergo complete spoilage. This was due to larger amount of neem oil added compared to the remaining samples. Neem oil being extracted from a highly nitrogenous source, this led to development of odour perceived from the spoilt sample.

Table 4.15: Changes in Microbial Quality of Preserved Locust Bean samples during storage Periods of 15, 30 and 45 days.

Neem oil (µL/200g)	Storage days	TBC	TCC	S.aureus	E.coli	Bacillus spp	Pseudomonas spp	TFC
	15-days							
1		0.77 ± 0.06b	0.43 ± 0.06b	0.37 ± 0.06a	0.60 ± 0b	1.2 ± 0.1c	0.47 ± 0.06b	0.20 ±00a
2		0.97 ± 0.06b	0.27 ± 0.06a	0.47 ± 0.06b	0.43 ± 0.06b	0.77 ± 0.06bc	0.27 ± 0.06a	0.20 ±00a
3		0.47 ± 0.06a	0.20±00a	0.30 ± 0a	0.23 ± 0.06a	0.57 ± 0.06b	0.2 ± 00a	0.17 ± 0.06a
4		0.37 ± 0.06a	0.10±00a	0.20±00a	0.30 ± 0a	0.4 ± 00a	0.1 ± 00a	0.10 ±00a
0		0.63 ± 0.06b	0.53 ± 0.06b	0.60 ± 0b	1.50 ± 0.1a	0.57 ± 0.06b	0.57 ± 0.06c	0.27 ± 0.07a
	30-days							
1		1.133 ± 0.058	0.667 ± 0.048	0.467 ± 0.058	0.767 ± 0.058	1.533 ± 0.115	0.667 ± 0.058	0.267 ± 0.05
2		1.3 ± 0.1	0.467 ± 0.058	0.533 ± 0.115	0.6 ± 0	0.933 ± 0.115	0.467 ± 0.058	0.2 ±00
3		0.667 ± 0.058	0.367 ± 0.058	0.433 ± 0.058	0.367 ± 0.058	0.767 ± 0.058	0.267 ± 0.058	0.167 ± 0.058
4		0.467 ± 0.06	0.2 ± 3.4E-17	0.2 ± 3.4E-17	0.267 ± 0.058	0.567 ± 0.058	0.2 ± 3.4E-17	0.1 ± 1.7E-17
0		1.567 ± 0.058	1.233 ± 0.058	0.7 ± 0.1	0.7 ± 0.1	1.733 ± 0.058	1.033 ± 0.058	0.533 ± 0.058
	45-days							
1		1.767 ± 0.058bc	1 ± 0c	1 ± 0c	1.167 ± 0.058bc	1.833 ± 0.058c	0.867 ± 0.058bc	0.367 ± 0.058b
2		2.033 ± 0.058c	0.8 ± 1.36E-16bc	0.833 ± 0.153b	0.633 ± 0.289b	1.133 ± 0.115bc	0.733 ± 0.058b	0.267 ± 0.058ab
3		0.967 ± 0.058b	0.5 ± 0b	0.367 ± 0.153a	0.567 ± 0.058b	0.967 ± 0.058b	0.433 ± 0.058a	0.2 ± 00a
4		0.667 ± 0.058a	0.4 ± 00a	0.4 ± 0.265a	0.467 ± 0.058a	0.467 ± 0.416a	0.3 ± 0a	0.1 ± 00a
0		2.033 ± 0.058c	1.467 ± 0.115c	0.8 ± 0.346b	1.4 ± 0.1c	2.1 ± 0.1c	1.133 ± 0.115c	0.733 ± 0.058c

4.10.1 Changes in Microbial Quality of Locust Beans after of storage.

Colony forming units of microbes developed from the samples at each treatment level were presented in table 4.15 during microbiological study of locust beans, from the results obtained in the table above. At 15day of storage there was a total bacteria count (0.97cfug…$^{-1}$) increase in treated (2µL/200g) sample (with 2µL/200g of neem oil) while the control sample (with 0µL/200g of neem oil) gave a total bacterial count TBC of (0.6cfug^{-1}). Total *coliform* count TCC was highest in control sample (0.5cfug^{-1}) with lowest count of 0.11cfug^{-1} from treated sample with 4µL/200g of neem oil. There was an increase count of *Staphylococcus aureus* (0.6cfug^{-1}) in control sample, with lowest count (0.2cfug^{-1}) in treated sample with 4µL/200gneem oil. *Escherichia coli* count (0.2cfug^{-1}) was high in sample treated with 3µL/200g nem oil compared to 1.5cfug^{-1}count in control sample wih 0µL/200g neem oil. Significant difference ($p<0.005$) occurred between treated sample with 2µL/200g in *Bacillus spp* count (0.77cfug^{-1}) and the control sample with *Bacillus spp* count (0.57cfug^{-1}).

Highest count (0.57cfug^{-1}) of *Pseudomonas spp* was observed in control sample. Also highest Total *Fungal* Count (0.27cfug^{-1}) and *Pseudomonas count* (0.57cfug^{-1}) were observed in control sample with the lowest count of 0.1cfug^{-1} in sample treated with 4µL/200g. These findings were related to the workfrom Kolapo *et al.*, 2007, who observed reduction in microbial count of treated locust bean cake and increase in that of untreated sample.

At 30 days of microbial study during storage of locust bean, highest count of TBC, TCC and *S. aureus* occurred in control sample at 1.57cfug^{-1}; 1.23cfug^{-1} and 0.7cfug^{-1} respectively while lowest count was reported at 0.47cfug^{-1}; 0.2cfug^{-1} and 0.2cfug^{-1} respectively for treated sample with 4µL/200g. *E. coli* count (0.70cfug^{-1}) was recorded for control sample with the lowest count of 0.27cfug^{-1} for sample treated with 4µL/200g neem oil. There was a significant difference ($p<0.05$) between the control sample with *Bacillusspp* count, *Pseudomonas spp*. Count and Total *Fungal* Count TFC of values 1.73cfug^{-1}, 03cfug^{-1} and 0.53cfug^{-1} respectively and the treated sample treated with 4µL/200g that gave lowest count 0.57 cfug^{-1}, 0.2 cfug^{-1} and 0.1 cfug^{-1} for *Bacillus spp* count, *Pseudomonas spp* count and TFC count respectively.

Sample treated with 4µL/200g neem oil had low records of microbial count against the control sample. This can be due to the fact that the treated sample undergone continuous moisture discount which preventsmicrobial boom (Ademola *et al.*, 2011).

At 45 days of storage period, the predominant microbial counts in sample were Total Bacterial Count TBC and Bacillus spp count of 2.0 and 2.1cfug-1 respectively. The lowest of these microbial counts in sample treated with 4 µL/200g neem oil were 0.67 and 0.47cfug^{-1} respectively. This result was relatedto the results of Rabi *et al.*,2013.

4.11 Toxicity Effects of Neem Oil Treated samples on Rat

During the course of animal study, one mortality was recorded among the female rats used. The rat died at the end of twelveth day and it was among those fed with locust beans sample treated with 2µL/100g of neem oil concentration. After sacrificing the animals on the fourteenth day, a swollen stomach was noticed from the internal structure of all the male animals fed with 3µL/100g of neem oil treated sample of locust beans while no physical defect was noticed from the rats fed with the control sample of locust beans in which no concentration of neem oil was added.

4.11.1 Effects of treated samples on Biochemical Parameter of blood in Rat

The result of biochemical parameters are indicators of toxicity raising the effectiveness of or the installation of a toxicity of liver as one of the vital organs.

In ALP (Alkaline Phosphate) parameter, blood from female rat fed with treatd samples 2µL/100g showed ALP level of 73µL while the level in blood of male rat fed with 3µL/100g treated sample was 102µL. A significance difference occured between these level and the control male rat that showed ALP level of 117µL fed with untreated sample.Table 4.16 below showed the neem oil tends to reduce the alkaline level of blood in rats feed with treated sample.

In AST (Aspartate aminotransferase) parameter, significant difference ($p<0.05$) occurred between male and female rats fed with treated samples 2-3 µL/100g and the control rats 0 µL/100g. The AST levelof blood was reduce to 39.8 and 39.00µL in male and female rat fed with 2 µL/100g and 3 µL/100g treated sample from 46.67µL AST level in male rat fed with contol sample that contains untreated locust beans sample. This was related to the result from protective effect of flavonoid on the liver (Lin and Jian, 2018).

Table 4.16: Effects of Neem oil on Serum Biochemical parameter of blood samples in Rat

Parameters	0 µL/100gNO	2 µL/100g NO	3µL/100g NO
AST (µL)	46.67±1.8b	39.80±2.85a	39.00±2.24a
ALP (µL)	117.00±1.63c	73.00±24.82a	102.00±4.00b
SEX	M	F	M

NO – neem Oil, AST – Aminotransferase Stearase, ALP – Alkaline Phosphate level.
values with difference letters in the row are significantly different.

4.11.2 Effect of neem oil on Histopathological Examination of Intestine and Liver

The microscopic examination of the selected organs; intestine, liver and heart, revealed some deleterious effects on the whole histopathological characteristics of the organs in treated rats (Table 4.17). There was severe mucosal erosions of the intestine with atrophy of intsestine villi and several sunted villi in animals fed with treated (2µL/100g) samples (+ +). No symptom was observed in the liver and intestine control animals (- -). Intestine with stunted villi (+) and liver that contained mild portal congestion, and periportal cellular infiltration was observed in animal fed with 3 µL/100g treated sample. Also it showed mild diffuse hydropic degeneration of hepathocytes (+++). Also female animal fed with the same reated sample showed the same effect as observed in their intestine and liver, (+) and (+++) respectively.

Male animal treated with 3 µL/100grevealed affected intestine with severe portal and central venous congestion (++) with no visible lesion on the liver. The animal fed with control sample that contains 0µL/100g neem oil showed no visible lesion in the liver (--) and their intestine (- -). However female animal fed with sample treated with 2µL/100gsample showed very mild diffuse hydropic degeneration of the hepatocytes (+) while male animal fed with 2 µL/100g showed a focus of mild vascular degeneration of the hepathocytes (+) and a visible lesion on the intestine. There were no visible changes observed from the heart of the animals.

Table 4.17: Histopathological Examination of Selected Organs inTreated Rats

Slide	Intestine	Heart	Liver
10mgPN	++	ND	-
100mgPN	-	ND	-
100mgPN (b)	++	ND	-
500mgPN	-	ND	+
1600mgPN	-	ND	-
2900mgPN	-	ND	+
100mgGL (b)	+	ND	++++
100mgGL	+	ND	+++
500mgGL	+	ND	++
1600mgGL	-	ND	-
2900mgGL	-	ND	-

ND- not determined, + one symptom, ++ two symptoms, +++ three symptoms, ++++ four symptoms, - no visible lesions

4.11.3 Haematological Analysis of Blood Samples of Sacrificed Animals

During haematological analysis, some hematological parameters were used to evaluate and determine the anomalies induced by the plant oil extract. (Table 4.18), these parameters are indicated from the table below. The blood analysis of Mean Cell Hemoglobin, neutrophils and monocytes indicated difference between the animal group fed with treated locust beans sample 2µL/100g and the group fed with control locust beans Sample that contains no treatment 0µL/100g. Monocytes and Neutophils are constituece of White Blood Cell (WBC) that make of its Percentage when combined with lymphocyted. These gave the resultant WBC differential Absolute the showed the overall impacts after completed the table.

High blood platelet count (143 x 10^3) occurred in animal fed with treated locust beans (3µL/100g) of Neem Oil. Other haematological parameters such as white blood cell (WBC), Red Blood Cell (RBC), Hemoglobin (Hb), Mean Cell Volume (MCV), Mean Cell Hemoglobin Concentration (MCHC), Lymphocyte count. Animals fed with treated samples 2µL/100g and 3µL/100g showed the percentage lymphocyte counts, 60.50 and 59.60%, respectively while the control animal group showed the percentage lymphocyte of 65.33%. This indicated the toxicity impact of neem oil on the animals fed with treated locust beans sample by their lower percentage lymphoteric difference compared to the animals fed with untreated samples. This was not the casein work of Emmanuel *et al*.,(2018), who reported no significance diffence and no toxicity effect of aqueos extract of Amsrantaceae in their animal study (Emmauel *et al*., 2018).

Eosinophil level of blood in animal fed with 3µL/100g is increased (2.00%) when compared wih Eo blood count in control group (0µL/100g) fed with control sample of locust beans that showed (1.67%) Eo. This gave another toxic effect of the samle treated with 3µL/100g of neem oil while samples with lower concentration 2µL/100g of neem oil was different from the control sample. One mortality was recorded and symptoms of toxicity were discovered after administration of dose limit (3µL/100g). This shows that the lethal dose is above this dose limit.

Thus, this study has a hematological change which showed that, (3µL/100g) of neem oil concentration is not within the normal health for this studied animal species but the animals can tolerate only as low as (2µL/100g) concentration of neem oil (Gikinis and Clifford, 2008).

Table 4.18 Effects of Neem oil on Hematological Parameter of Rat Blood.

Parameters	Control (0 µL/45g)	2 µL/45g	3 µL/45g
PCV (%)	47.33±1.53c	31.00±8.49a	35.00±7.04
Hb (g/dL)	16.20±1.74ab	10.35±2.76b	11.32±2.23b
MCV (fL)	61.42±1.12b	58.50±0.07	61.46±2.13b
MCHC(g/dL)	34.17±2.57a	33.42±0.25a	32.37±0.43b
MCH (Pg)	20.97±1.25a	19.55±0.17a	19.89±0.51a
RBC (10^6/µL)	7.71±0.36a	5.30±1.6b	5.70±1.19b
WBC	6.57±0.99a	6.08±1.52a	6.77±1.12a
Pt (103/dL)	110.00±12.49ab	106.50±4.95a	123.00±12.94c
Lymp (%)	65.33±4.93ab	60.50±6.36a	59.60±6.80a
Neut (%)	28.00±7.00a	36.00±7.07b	36.80±6.06b
Mo (%)	1.67±0.58a	2.00±0.00b	1.60±0.55a
Eo (%)	1.67±0.58ab	1.50±0.71a	2.00±1.41c
WBC Diffr.Abste (%)	6.57±0.99ab	6.08±1.52a	6.77±1.12c

Mean values with different letters in the row were significantly different ($p<0.05$)

NO – neem oil, PCV – packed cell volume; Hb – HemoglobinMCV – mean cell volume, MCHC – mean cell Hemoglobin concentrationMCH – mean cell Hemoglobin, RBC – total red blood cell count, WBC - total white blood cell count, PT – platelet, lymp – lymphocyte, Neut - Neutrophil, MO – monocyte, Eo – Eosinophil.

Table 4.19: Histopathological Examination of Treated Animal Organs

NO (µL/200g)	Kidney	Liver	Heart	Testes	Ovaries
2 (M)	-	+	+		
2(M)	-	++	-	-	
3 (M)	-	+	-	-	
3 (M)		+	-	+	
2 (M)	+	++	-	++	
2 (F)	-	++	+		+
2 (F)	-	++			+
0 (M)	-	+	-	-	
0 (M)	++	+	-	+	
0(M)	+	++	-	-	

ND- Not detected, + one adverse effect, ++ two adverse effects, +++ three adverse effects, ++++ four adverse effects, - no visible lesions F- female rat; M- male rat

4.11.4 Effects of Neem oil on Histopathology Examination of Testes, Ovaries, Kidney, Liver and heart.

The optical microscope examination of the heart, kidney, liver, ovaries and testes showed some deleterious effects on the whole histopathological characteristics of the heart, liver, kidney, ovaries and the testes of treated rats (Table 4.19)

Liver from animals fed with 2µL/100g showed moderate periportal vacuolar with degeneration of the hepatocytes. Also there was necrosis of hepatocytes in rats treated with 3µL/100g samples and mild portal congestion with periportal cellular infiltration in animal fed with 3µL/100g of sample.

Testes of male rats with 3µL/100g treatment showed a mild interstitial congestion with severe congestion of sub capsular blood vessels. This was also noticed in male rat fed with 2µL/100g of the sample. Kidney showed a severe congestion of renal cortex and oedema of the cortex. Few tubules have protein cast in their lumina in rats treated with 2µL/100g of the sample. Ovaries of female rat treated with 2µL/100g of the sample showed a matured Graffian follicles.

A mild to severe congestion of coronary vessels were observed in the heart of animals with 3 µL/100g sample. This was a reverse to studies of Emmanuel *et al., (*2018) on the repeated administration of Amaranthaceae leaves extract (Emmanuel *et al*., 2018).

The following plates showed Photomicrographs structures of the tissues.

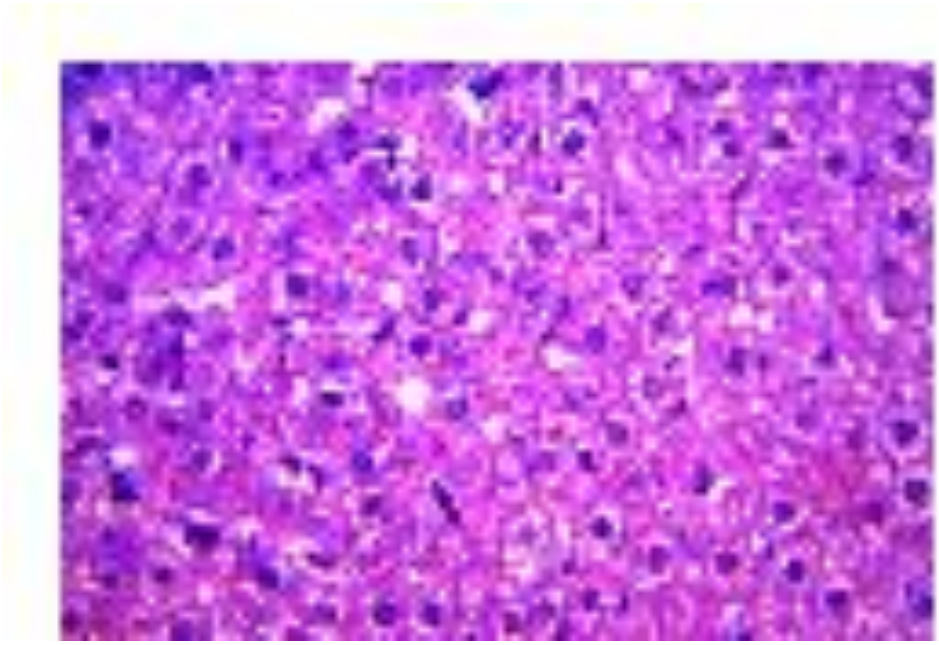

Plate 4.1: E1 Male Rat Liver control fed with 0 µL/100g neem-oil showing diffuse vacuolar

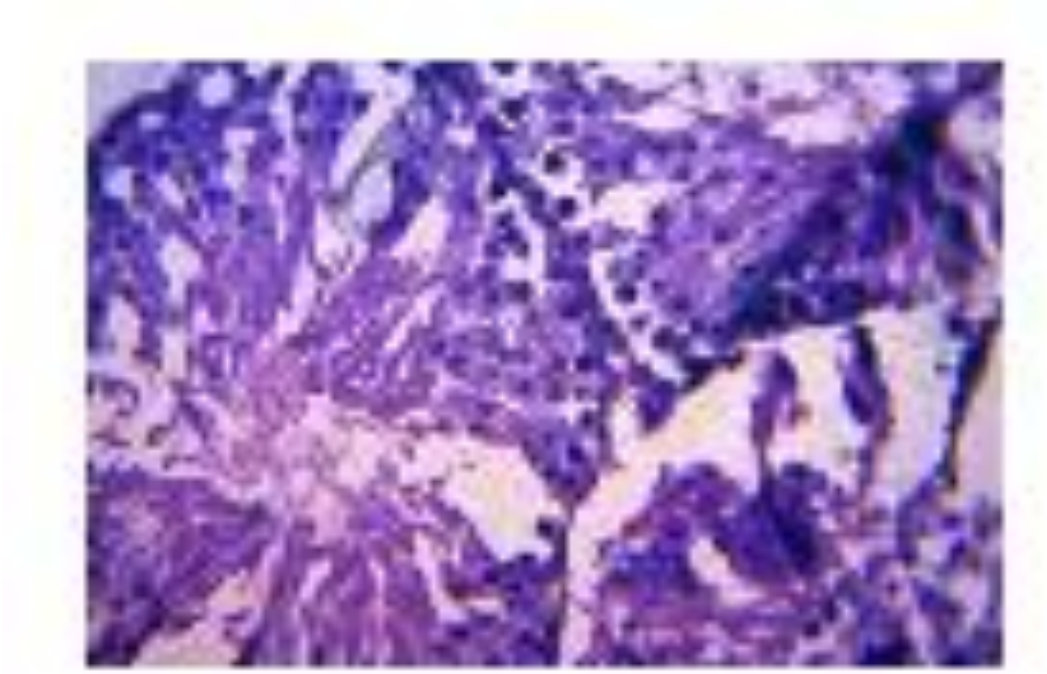

Plate 4.2: E1 Male Rat Testis control fed with 0µL/100g neem-oil

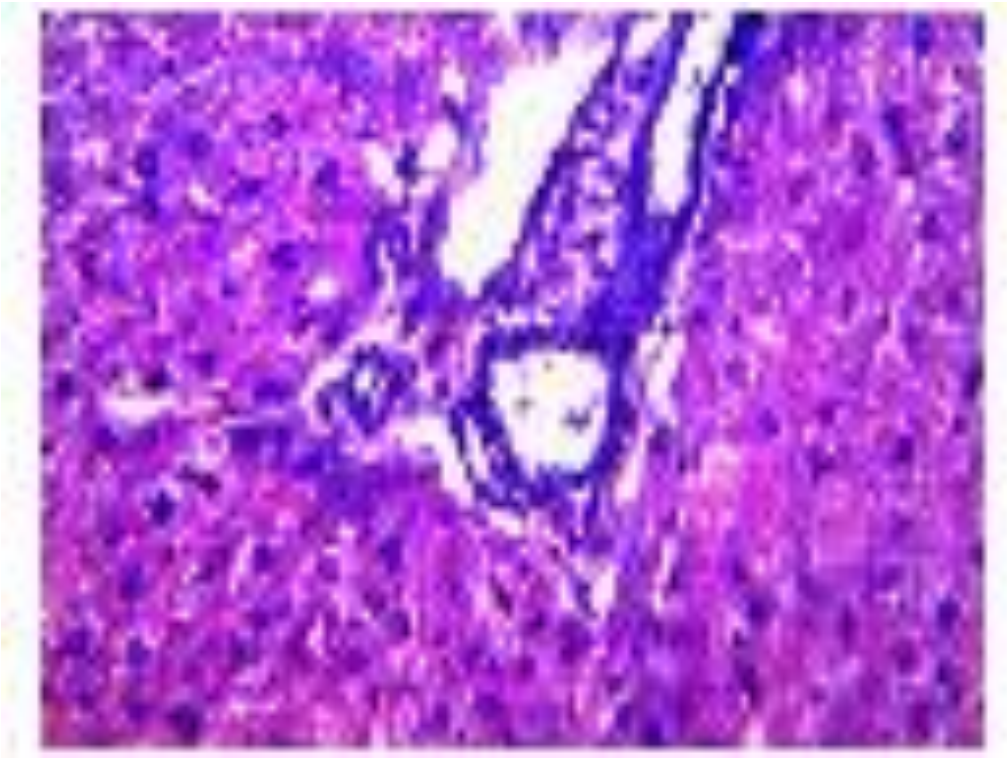

Plate 4.3: E1 Male rat liver fed with 0 µL/100g neemoil showing cellular infiltration

Plate 4.4: D3 Control rat liver fed with 0µL/100g neem oil showing Mild to severe Portal Congestion

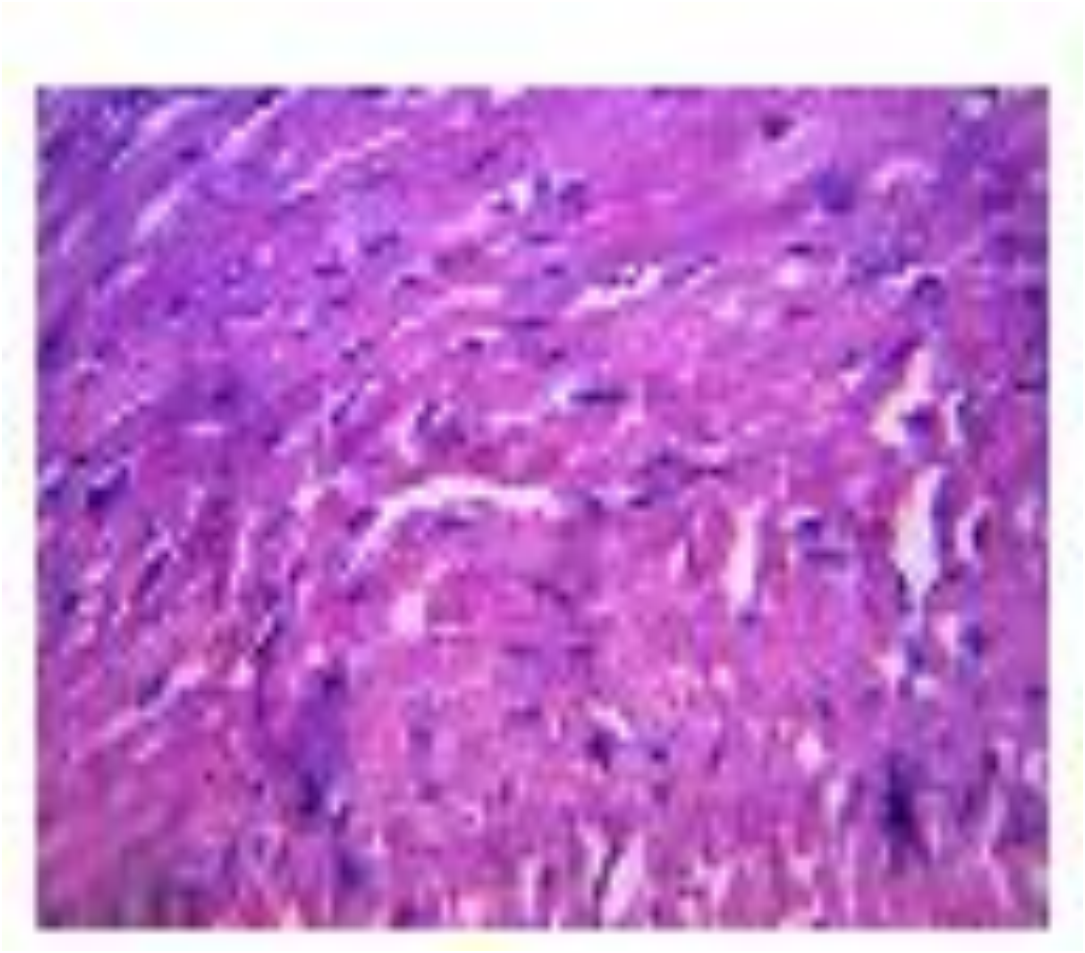

Plate 4.5: E1 Male Rat heart control fed with 0µL/100g neem oil

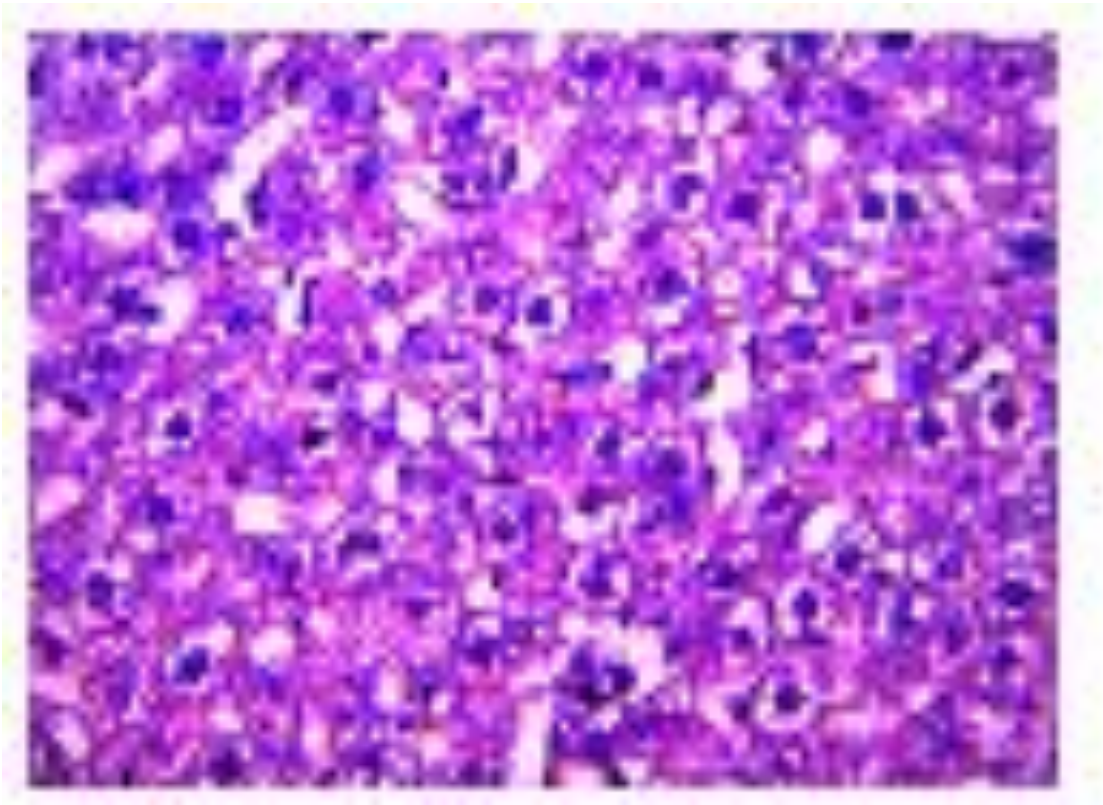

Plate 4.6: B1 Female Rat Liver control fed with 0 µL/100g neem-oil showing Periportal Vacuolar Congestion

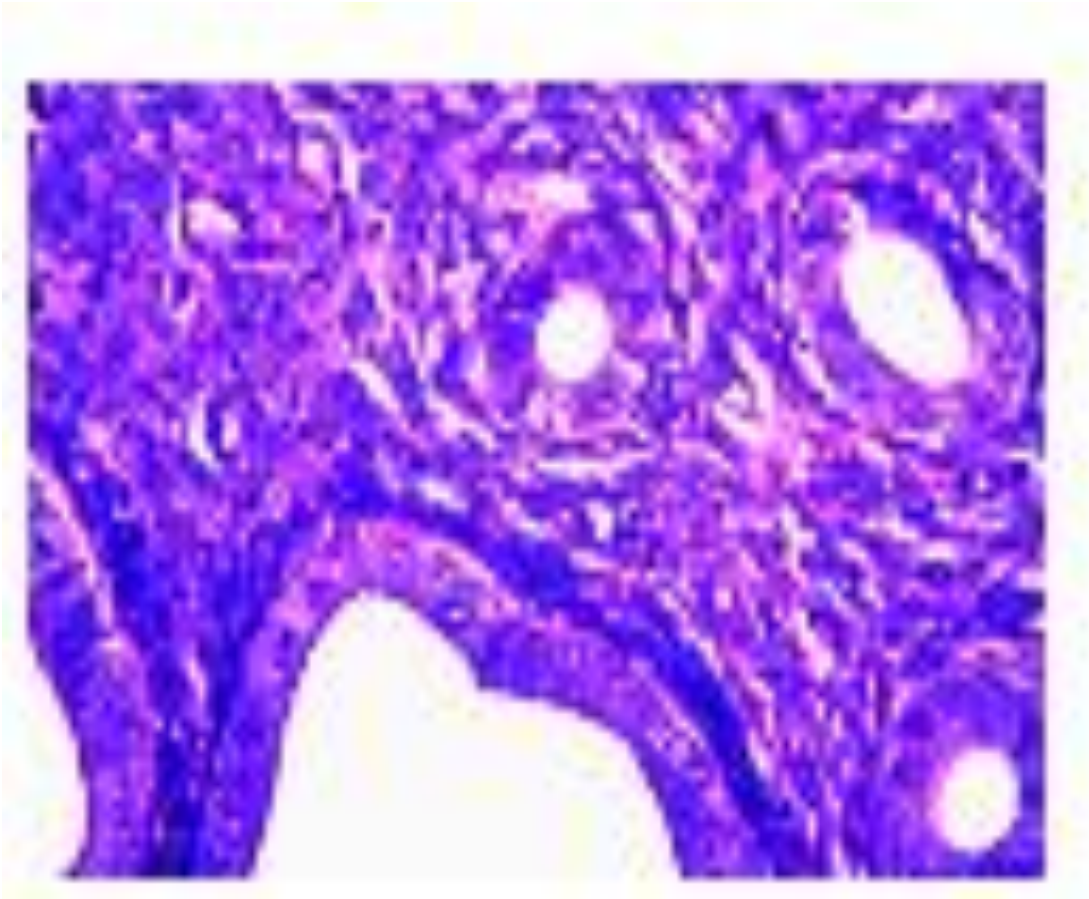

Plate 4.7: B1 Female Rat Uterus fed with 0 µL/100g neem-oil

Plate 4.8: E1- Control male rat heart fed with 0 µL/100g neem oil concentration

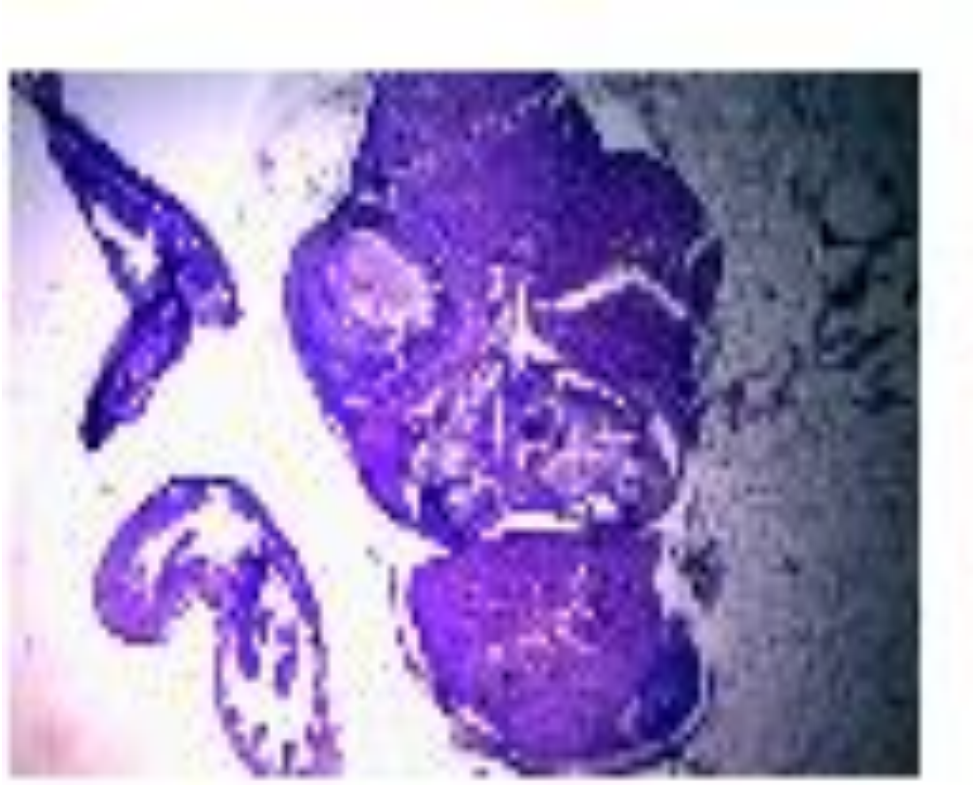

Plate 4.9: A5: Changes in female rat ovaries fed with 2 µL/100g neem oil showing Matured Graffian Follicles

Plate 4.10: A5: Female rat liver fed with 2 µL/100g neem oil showing moderate periportal vacuolar

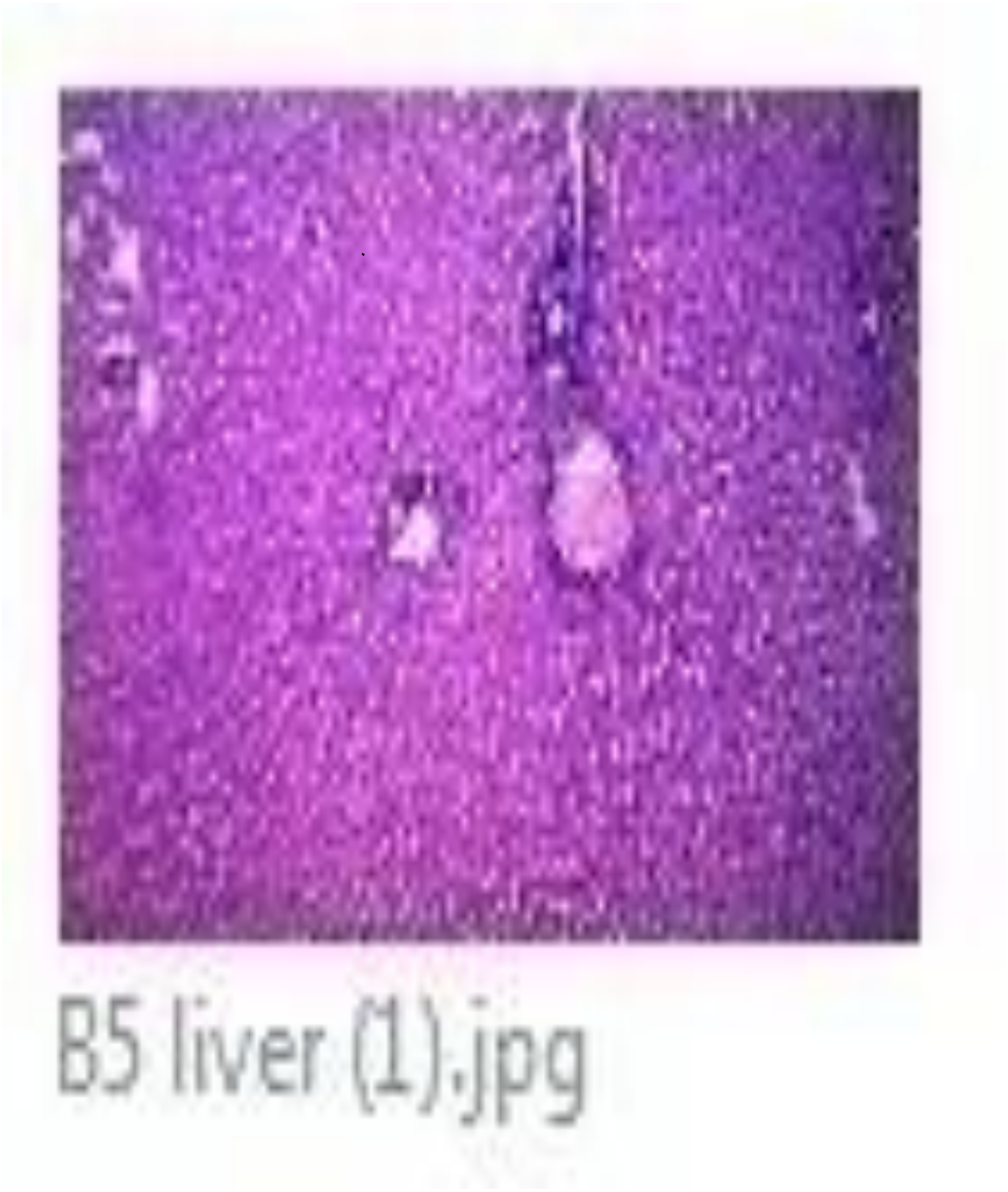

Plate 4.11: B5: Changes in female rat liver fed with 2 µL/100g neem oil showing mild Portal congestion

Plate 4 12: C1 – Male rat liver fed with 3µL/100g neem oil concentration showing Necrosis of Hepatocyte

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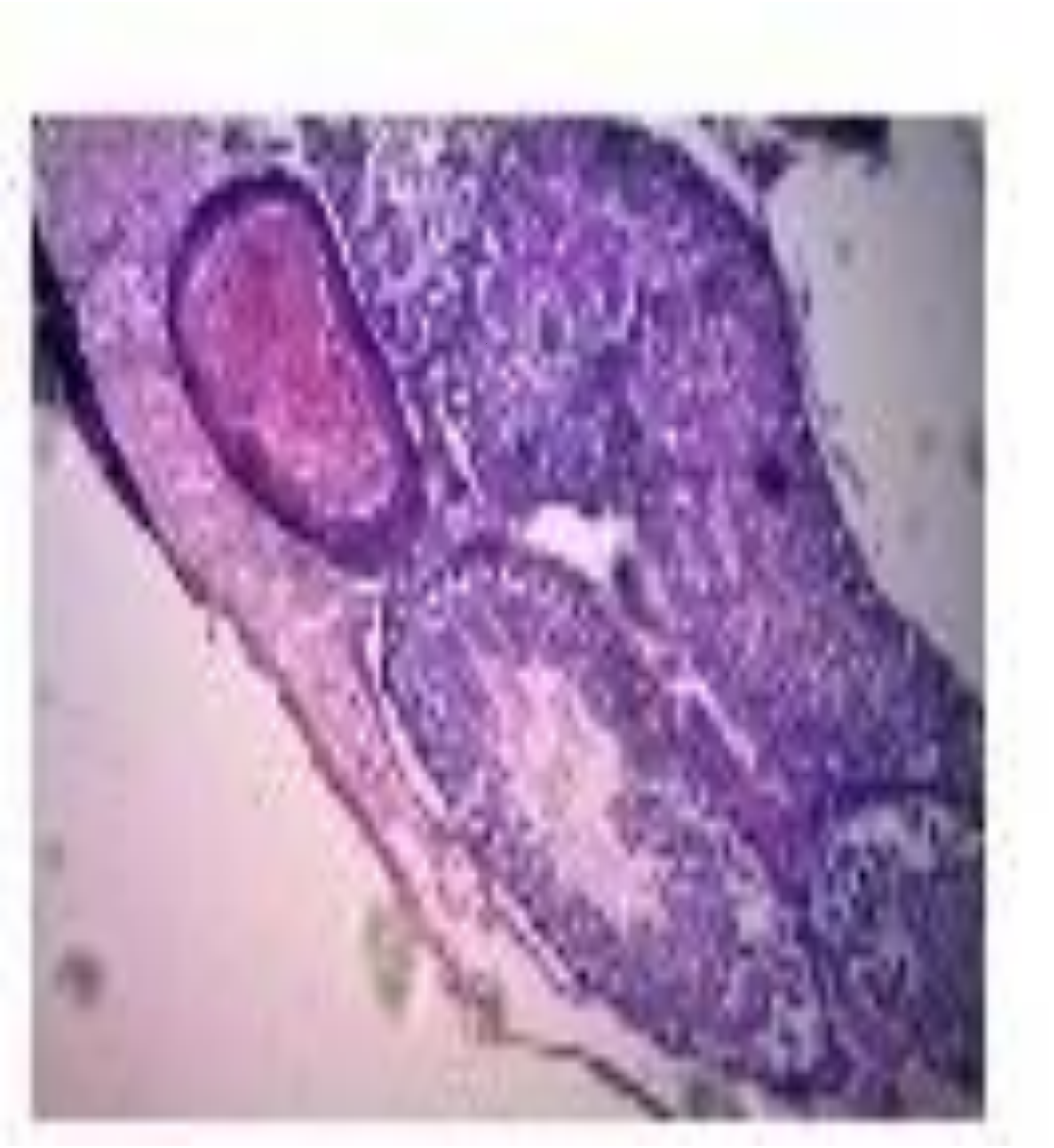

Plate 4.13: C2 – Male rat testis fed with 3 µL/100g neem oil showing severe congestion of interstitial cell

Photomicrograph structures of the tissues sections in Rats

4.12 Photomicrograph Structures of Organs

In the photomicrograph structures of the organs shown above, plate 4.1 with -cotrol male rat liver showed a moderate diffuse vacuolar and degeneration of the hepatocytes, it also indicated a moderate to severe periportal cellular infiltration. Plate 4.2 with - control male rat liver showed a mild to moderate periportal vacuolar with degeneration of hepatocyte. Plate 4.3 with - control male rat testis showed no visible lesion, also plate 4.4; 4.5 with liver and kidney showed no visible lesion. Plate 4.6 with treated male rat heart showed a moderate to severe coronary congestion. Plate 4.7 showed a similarchange as discussed for liver above. This was related to the report of Sunny *et al.*, (2017) Plate 4.8 with treated male rat liver showed mild portal congestion with cellular infiltration.

Plate 4.9 with treated female rat uterus gave no visible lesion. Also no visible lesion was seen on plate 4.10 with control male rat heart as well as in treated male rat heart on plate 4.11 containes treated female liver gave a moderate periportal vacuolar with degeneration of the hepatocyte. Plate 4.13 contained treated male rat testes had a mild interstitrial and sub capsular blood vessel congestion. Plate 4.14 that containes treated female rat uterus with no visible lesion seen and liver gave a moderate periportal vacuolar with degeneration of the hepatocyte. In ovary of treated female rat on plate 4.15, there were matured graffian follicles that predominated. No visible lesion was seen on plate 4.16 that showed treated female rat kidney.

Plate 4.16b with treated female rat liver showed a moderate periportal vacuolar and degeneration of the hepatocytes. Plate 4.17 contain treated female rat liver which also showed changes similar to that of treated female rat liver. Plate 4.18 with treated female rat uterus showed no visible lesion. Plate 4.19a with B- treated female rat uterus also showed no visible lesion while in plate 4 19b with treated male rat heart, there was no visible lesion seen. In plate 4.20 with treated male rat kidney few tubules were seen that contain protein cast in the lumina. Plate 4.21a with treated male rat testis showed effects

as mild interstitial congestion and subcapsular blood vessels were severely congested. Plate 4.22a and 4.22b with treated male rat heart and treated male rat kidney both showed no visible lesion. However plate 4.23 with treated male rat liver showed a mild periportal cellular infiltration. Plate 4.24a and 4.24b with treated male rat heart and treated male rat kidney respectively, both showed no visible lesion. Plate 4.25 with treated male rat liver showed mild portal congestion and mild cellular infiltration with no visible lesion observed on treated male rat testis. Plate 4.26 with treadted male rat kidney and treated male rat testis both showed no visible lesion as well.

Also Plate 4.27 with C2-treated male rat liver and C2-treated male rat heart showed no visible lesion while duplicate of C2-treated male rat liver showed severe diffuse vacuolar and degeneration of hepatocytes, as related to report of Sunny *et al.,* (2017). Plate 4.27b with E3-control male rat heart showed no visible lesion. This was also the case in plate 4.28 with E3-control male rat testis and L3-control male rat heart. However, L3-control male rat kidney showed a mild congestion and oedema of the renal cortex.
Plate 4.29a with B-treated female rat kidney and B-treated female rat heart showed no visible lesion while B-treated female rat liver showed a moderate diffuse vacuolar degeneration and necrosis of the hepatocytes, there was also a moderate to severe periportal cellular infiltration while B-treated female ovary showed several graffian follicles. Plate 4.29b with B2-treated male rat heat showed no visible lesion.

Plate 4.30a contained B2-treated male rat liver that showed a moderate diffuse vacuolar and degeneration of hepatocytes (Sunny *et al.,* 2017) ; B2-treated male rat testes had a mild interstitrial and sub capsular blood vessel congestion. Plate 4.30b with B4-treated male rat heart showed no visible lesion. Plate 4.31a with B5-treated male rat liver showed mild portal congestion and mild periportal cellular infiltration; while in B5-treated male rat testis, no visible lesion was seen. Plate 4.31b with C1-treated male rat liver showed no visible lesions.

From the interpretation of the results above, it can be deduced the control animals has little or no defects on their organs due to the fact that neem oil was absent in their samples. Defects of the liver include diffuse vacuolar, periportal vacuolar, periportal

cellular infiltration portal congestion to degeneration of hepatocytes and necrosis of hepatocytes. The major defect of the heart is the severe coronary congestion. Kidney defects include mild to severe congestion and oedema of renal cortex (Sunny *et al.*, 2017). The defects in testis include severe congestion at the interstitium and congested sub capsular blood vessels. The ovaries showed a defect of predomination of more matured and several graffian follicles. The impact of the oil was not revealed in Uterus.

This result was able to observe that the Neem seed oil has greatest impact on the liver that showed larger number of defects, followed by the kidney and the testes and lastly the heart. The ovaries showed no less than a positive while the uterus has no side effects from the oil as no visible lesion was seen. Therefore among all the organs (i.e liver, kidney, Heart, Uterus, testes and Ovaries) the most affected organ was the liver.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The treatments reduced antinutritional factors in neem seed. Oil from roasted neem seed reduced weevils in grains and retarded microbial multiplication on fermented locust beans and onion. Low oil dosage is recommended.

5.2 Conclusion

Under listed conclusions were drawn from this work;

1. Roasting, soaking in water and alkaline (10% sodium hydroxide) solution affected neem seed anti-nutritional contents including cyanide, nitrate, total oxalate, azadirachtin, phytate, tannin, and oxalate. Levels of reduction depended on roasting temperature and soaking duration. Roasting and soaking in water reduced both cyanide and azadirachtin contents of neem seeds, with water soaking being more effective. Soaking in alkaline solution reduced only cyanide but increase azadirachtin contents. None of the treatments reduced azadirachtin contents to safe level for human consumption.
2. Roasting and soaking neem seed in alkaline solution did not affect percentage distribution of nutritive composition. Conversely, percentage distribution of proximate composition was influenced by water soaking. Predominant minerals in neem seed were iron, zinc, manganese, copper, potassium and calcium. They were not significantly influenced by the treatments.
3. Neem seed has high oil content of 41.18%, and more of saturated than unsaturated fatty acids. Major ones that were present include oleic, linoleic,

stearic and palmitic acids. Azadirachtin, an active anti-nutrient was more dominant in oil than cake. Neem oil effectively controlled weevils in stored beans, maize and rice, and remained potent throughout storage period of 28 days. Also, microorganism including bacteria, coliform, staphulococcus aureus, E-coli, bacillus, pseudomonas and fungi activities in onion and fermented wet locust beans were inhibited.

4. Concentration of the oil in animal fed with preserved loust beans samples affected white blood cell differential, alkaline phosphate and aspartate aminotransferase of the experimental animal. Liver, ovaries of female and testes of male rats fed with high concentration of neem oil were greatly affected.
5. The oil has potential of being used as food preservative but at very negligible quantity.

5.3 Recommendations

1. Neem seeds should be used to control weevils' invasion in grains.
2. It is recommended that appropriate dosage of neem oil concentration in preservation of food should be further investigated.
3. Potentials of other products of neem tree such as leaves, as food preservative should be studied.

5.4 Contributions to Knowledge

1. This study provided information on effects of roasting temperature and duration, soaking in 40 % sodium hydroxide and water duration on chemical properties of neem seed.
2. This research revealed azadirachtinas the most active component of neem seed with its higher concentration in neem oil.
3. Preservative potentials of neem seed oil for rice, maize and beans weevil control, and locust bean as well as onion microbial activities inhibition was established.
4. Information on toxicity level of neem oil was provided.
5. The study showed mass spectra and chromatograms of bioactive compounds that are useful in drug synthesis.
6. The report encouraged traditional application of neem plant that contains various bioactive compounds.

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