EXTRACTION AND CHARATERIZATION OF NEEM OIL FOR PRODUCTION OF BIO – INSECTICIDE

BY

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Declaration

I DARAMOLA, SIMIDE JOSEPH declare that this project presented for the Award of Bachelor of Engineering in Chemical Engineering under supervision of has not been presented either wholly or partially for any degree elsewhere.

Simide

DARAMOLA SIMIDE .J.

29/11/2005

DATE

Dedication

This project is dedicated to Almighty God, the most gracious Lord for his love, protection, care and who has spared my life to this end academic greatness. May His name be praised forever. And also to my mother (MRS ALABA DARAMOLA) for her prayers and love, not left out, my loving sister (ARINOLA DARAMOLA) for your prayers and supports throughout the programme.

Certification

This is to certify that this project titled Extraction and characterization of Neem oil for production of bio – insecticide was fully carried out by Daramola, Simide .J under the supervision of Mrs. Aisha Bawa and submitted Chemical Engineering Department of the Federal University of technology, Minna in partial fulfillment of the requirement for the award of Bachelor of Engineering (B. Eng) Degree in Chemical Engineering.

Simide — 29/11/2005

Candidate Name — Date

Project Supervisor — 30/11/2005

Date

Head of Department — Date

External Examiner — Date

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Abstract

Extraction and characterization of Neem oil for production of bio- insecticide, was carried out with two different solvent that is methanol and petroleum –ether (40 – 60 C) extraction using Soxhlet apparatus. Five grammes of grounded Neem seed were used for the extraction of interval of 2 hours. The mean of oil extracted using methanol as solvent was 1.60g and that of petroleum –ether was 1.642g respectively. The highest % oil extracted was 41.6% at 9 hours of extraction for methanol and 33.10% at 7 hours for petroleum- ether extraction respectively. That shows that increasing in time for the extraction led to increasing in oil extracted. Also, shows that methanol is better solvent for the extraction than petroleum-ether. By application of 2^5 full factorial design to the extraction process which gives a straight line graph with regression of lipid yield on time of extraction: - $y = 0.96x + 27.40$ shows that both solvents used are significantly appropriate for the process. The characterization of neem oil i.e. melting temperature ($28^{\circ}C$), boiling point ($260^{\circ}C$), pH (5.6) and specific gravity (0.924) However, on the extract tested on the insects it was confirmed that methanol extract has the efficiency of 66.67% and that of petroleum – ether is having 50.00%. While in effectiveness test, where the methanol is applied to food only is having efficiency of 50.00 % and petroleum – ether with the efficiency of 50.00% and petroleum – ether with the efficiency of 20.10 % and that of synthetic chemical is having 93.06 %.

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CHAPTER ONE

1.0 Introduction

1.1 Background of Study:

Indigenous bio-insecticide production through growth of the Neem tree, Azadirachata indica A, the seed kernel and leaf extracts of which process prodigious pest control properties, can be substitute for energy-intensive pesticide imports. (2).

One of the first active ingredients isolated from Neem, azadirachin has proved to be the tree main agent for battling insects. Azadirachata is an active component of neem seed and the seed composed of a shell and a kernel. It is the kernels that are used mostly in the production of pest/insect control. The leaves also contain pesticidal ingredients but they are much less effective than those of the seed. This compound appears to cause some 90 percent of the effect on most pests.

It does not kill insects at least not immediately. Instead it both repels and disrupts their growth and reproduction. Infact, it is one of the most potent growth regulators and feeding deterrents ever assayed. It will repel or reduce the feeding of many species of pest insects as well as some nematodes. Also, it is so potent that a more trace of its presence prevents some insects from touching plant.

Bio-insecticides are any chemical compounds used to destroy any harmful animals and plants. Pesticides are well known to be in three major classes-: Insecticides, fungicides and herbicides (weed killers). There are some other groups which are: - Rodenticides (for control of vertebrate pests), Nematicides (to kill microscopic worms), Mollusicides (to kill slugs and snails), and Acarides (to kill mites).

Neem tree is well known to be useful as a source of shade and medicinal (especially for malaria) and solution for the control of the farm and household pests.

It is believed that extracts of seed kernel or leaves of neem when applied to plant foliage or incorporated into a diet, have adversely affected the development of a number of insect's species. In the initial development; the peasant farmer in India and South America could control food losses due to insect pests, simply by crushing up and

pondering seeds or leaves of the Neem trees and sprinkling them over their crops as a cheap or even free insect pest control.

The production of Bio-insecticide from Neem seed which is naturally made might provide indigenous insecticide for those developing countries that may not afford to buy expensive modern synthetics. In fact extracts from Neem seeds are of excellent Quality with a bitter taste, which is an advantage in using as insecticides.

With the aid of experimental (2^5 factorial design) can be used to achieve the process yields. A design experiment is a test or series of tests in which purposeful changes are made to the input variable of a process or system so that we may observe and identify the reasons for changes in the output response. Experiment design (factorial design) methods play an important role in process development and process troubleshooting to improve performance. The applications of these experiment designs in the production of bio-insecticides are to improve process yields; reduced variability and clear conformance to nominal or target requirement; reduce overall costs and reduced development time.

The development/production of Bio-insecticide from Neem oil attacked many pestiferous species, they seem to leave people, animal and beneficial insects unharmed. Characteristics of Bio-insecticide in this work are based on their physical properties like melting point (temperature), viscosity, specific gravity, pH and Boiling point.

1.2 Aim and Objective of Study:

1. To develop and produce bio-insecticide from the Neem seed.
2. To determine the characteristics of the extract.
3. To reduce the development time.
4. To test the effectiveness of the Neem extract on the insect e.g. Cockroaches, Houseflies, Mosquito e.t.c
5. To reduce the overall cost.

1.3 Justification of Study

With everyday increasing evolution of pests, insects, and lice in our (society) community, synthetic bio-insecticide could be augmented by making use of bio-insecticide from natural source such as Neem plant. Moreso, being source naturally, it would rather serves as nutrient to the soils and plants than environment hazard while its effect on man would not be pronounced.

CHAPTER TWO

2.0 Literature Review

2.1 A Brief History of Bio-Insecticide Production:

Neem is a member of the Mahogany family, *Maliaceae*. It is today known by the botanic name *Azadirachata indica* A. (Indian neem tree)belong to the most well known of the folk medicinal trees in India and East and West Africa.

Neem tree are attractive broad-leaved evergreens that can grow up to 30m tall and 2.5m in girth. Their spreading branches form rounded crowns as much as 20m across. They remain in leaf except during extreme drought, when the leaves may fall off. It is popularly known as "*Dongoyaro* tree" in Nigeria which is widely used as a remedy against malari8a or chewing sticks for brushing the teeth. *Azadirachtin* is one of the most potent antifeedants against the Africa desert locust *Schistocerca gregeria*

(Chapman, 1986) gave example of insect antifeedants as extracts of seed kernel or leaves of neem which when applied of plant foliage have been adversely affected the development of a number of insect species.

(Saxena *et al.,* 1981) reported that confinement of 5th instar larval of the rice leaf folder on plants treated with at least 12% Neem oil resulted in development of larval monstrosities which retained larval cuticle patches, head capsule, thoracic leg e.t.c.

(Saxena *et al.*, 1981) also reported a drastic reduction in feeding by the brown plant-hopper (*Nilaparvate lugens*) or rice plants treated with 'Neem' *Azadirachata indica*; seed oil (Crude oil, extracted from decorticated Neem seed). Neem oil was also reported to have significantly deterred feeding by another rice pest, the rice leaf folder, (*Naphalocrocis medinalis*).

Despite the unique properties of Neem seed oil in their effectiveness against a number of insect pests; little work is in progress in Africa in the use of this compound against crop pest. Chapman (1986) also stated that antifeedants are no less costly to use than an insecticides, especially when repeated applications may be necessary.

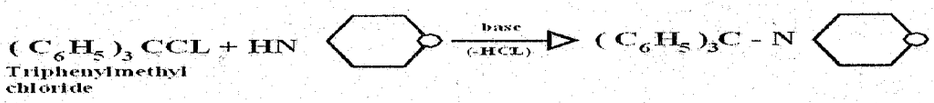
The effectiveness of its oil even in a crude state against pests, safely to environment low cost and ready availability suggests that its potential for pest control be fully

explored. From the information available so far, it seems that antifeedants have an important role to play in the development of effective pest control strategies.

2.2 Some Chemicals Used in the Control of Pests/ Insects:

2.2.1 Mollusicides:

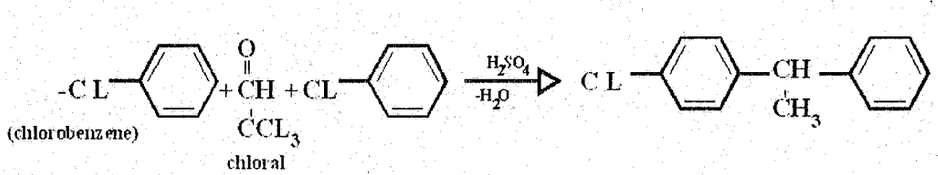
(Shell Research Limited, 1985) developed trifenmorph or frescon and obtained by condensation of triphenyl methyl chloride with morpholine.



Organic Mollusicides, like frescon were not absorbed by clay particles but are adsorbed by organic constituents in the mud but not by clay particles. This was a serious problem in their use to control the aquatic snail vectors of schistosoma as these occurred in relatively stagnant waters containing large amount of mud.

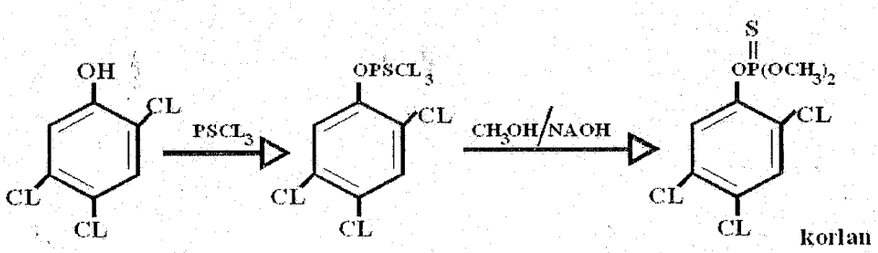
2.2.2 Insecticides:

(Zeidler, 1985), first prepared DDT (dichlorodiphenyl trichloro ethane) as an organochlorine insecticides but its powerful insecticide properties were not discovered until 1939 by Muller of thc Swiss Geigy Company. DDT was manufactured by condensation of chloral and chlorobenzene in the presence of concentrated sulphuric acid.



The pure DDT obtained was a white powder with a melting point 108C by recrystallization from ethanol. It was found to be stable, persistence in its action, also found to kill variety of insects, including domestic insects and mosquitoes but not very cffect against mites.

(Dow Chemical Company 1999) introduced frenchlorphos or korlan, as animal systemic insecticides. It was prepared from 2, 4, 5-trichloro-phenol and thio- phosphoryl chloride as follows.



Frenchlorphos has low mammalian toxicity to 1250-1750mg/Kg and used for treatment of dairt cattle against internal and external pest, example are grubs lice and ticks. It also acted against flies and cockroaches.

2.3 Chemicals Used in the Control of Plants:

(May and Baker Limited, 1998) introduced sulphonyl *carbamata asulam* (Asulox), for control of docks in grass land and against bracken, also believed to act by inhibitors of cell division since the shoots of treated plants do not behave normally.

(Professor Wain of Wye College and May and baker Limited, 1998) were independently discovered several hydroxybenzonitriles where herbicidal; two of the best known example of this group were bromoxynil (24; x-Br) and ioxynil (24; x-1) and could be prepared from p-hydrobenzaldehyde. Ioxynil was chiefly used for general weed control in turf, onion, and leak, while bromoxynil functions as contact herbicides for the pest emergence control of many broad leaved weeds especially chick weed.

2.4 Effects of Chemicals Controller on Life:

Insecticides, as stated earlier, affect the biological processes of many living organisms and may thus act as poisons to a large number of animals, a part from the target species. In some places where chemicals were sprayed against insects, entire populations of birds wiped out or significantly reduced; fish populations drastically lowered and residues of chemical detected in human fat and the milk of cattle.

In West Africa, the use of benzene hexachloride has led to the destruction of natural enemies of pest in the cocoa ecosystem and caused some insignificant insect species to multiply to pest proportions.

Indeed, wherever detailed studies have been made on the influence of insecticides on natural enemies of pest, the evidence is overwhelming that insecticides invariably upset the pest- natural enemy relationship leading not only to the problems of primary pest resurgence and secondary outbreaks but also resulting in direct hazard to applicators and farm workers. This may be not only from mishandling and accidents but from exposure to contaminated surface where insecticides have been sprayed.

2.5 Effect of Neem oil (Bio- Insecticide) on Insects

The growing accumulation of experience demonstrates that neem products work by intervening at several stages of an insect's life. The ingredients from this tree approximate the shape and structure of hormones vital to the lives of insects (not to mention some other invertebrates and even some microbes). The bodies of these insects absorb the neem compound as if they were the real hormones, but this only blocks their endocrine systems.

The resulting deep seated behavioral and physiological aberrations leave the insects so confused in brain and body that they cannot reproduce and their populations plummet.

Increasingly, approaches of this kind are seen as desirable methods of pest control: Pests don't have to be killed instantly if their populations can be incapacitated in ways that are harmless to people and the planet as a whole.

The precise effects of the various neem-tree extracts on a given insect species are often difficult to pinpoint. Neem's complexity of ingredients and its mixed modes of action vastly complicate clarification. But, for all the uncertainty over details various neem extracts are known to act on various insects in the following ways:

- Disrupting or inhibiting the development of eggs, larvae, or pupae;
- Blocking the molting of larvae or Nymphs;
- Disrupting mating and sexual communication;

- Repelling larvae and adults;
- Deterring females from laying eggs;
- Sterilizing adults
- Poisoning larvae and adults
- Deterring feeding
- Blocking the ability to "swallow" (that is reducing the motility of the gut);
- Sending metamorphosis awry at various stages; and
- Inhibiting the formation of chitin.

As noted earlier, neem extracts have proved as potent as many commercially available synthetic pesticides.

They are effective against dozens of species of insects at concentrations in the part – per-million ranges. At present, it can be said that repellency is probably the weakest effect, except in some locust and grasshopper species. Antifeedants activity (although interesting potentially extremely valuable) is probably of limited significance; its effect are short- lived, and highly variable. Blocking the larvae from molting is likely to be neem's most important quality. Eventually, this larviadal activity will be used to kill many pest species.

2.6 Production Process of Chemical Controller from Natural Source:

Neem seed is one of the natural sources of chemical controller. Basically, neem seed extraction involves dissolving out the active insecticidal ingredients, the Azadirachtus, from the dried seed powder with a solvent and the evaporating the solution to recover the solvent and to obtain concentrated crude extract.

For extraction process, the kernels from seeds must first be ground. Mortar and Pestle were used to break down into powder. This operation has to be carefully controlled because if the grounded seeds become too coarse, the achene containing the *Azadirachtins*, are not broken open, and if too fine, a dense mass is produced which the solvent cannot penetrate. After being crushed, the grounded seed kernels of Neem are fed into the extraction vessels where extraction is affected in a counter- current operation.

Simplified picture of the extraction process can be obtained by imaging a vertical extractor where powder is fed in at the top and passes slowly down the extractor while fresh solvent is pumped in at the bottom and rise to the top. As the solvent rises in counter-current flow to the powder (Grist), it first washes the exhausted material at the bottom continuous upwards dissolving what soluble matter remains. In this way, completely exhausted material known as "Mare" leaves the bottom of the extractor and a solvent rich in *Azadirachtins* called "Miscella" leave the top.

The solvent used (petroleum-ether or methanol), i.e. volatile and flammable, so that great care has to be taken in its use. The solvent used can also be recovered for re-use if necessary.

The final stage of the process is distillation. The "miscella" rich in Azadirachtin is pumped into still where it is concentrated by applying moderate heat and high vacuum. When all the solvent has been driven off in this way, remaining is "concentrate" which normally contains between 30 and 35% of *Azadirachtins*, the remainder consisting of a "deo-resins".

Finally, the production of dewaxed and decolorized pale extract is usually done by the manufacturing companies who buy the crude. *Azadirachtins* extract from the producing countries (companies). However, the producing countries are now moving towards doing their own refining. In keeping with this trend, Kenya has already established their own refinery while Rwanda is in the process of installing their own too. Nigeria has to work toward this goal (Plan) too.

2.7 Method of Extraction from Neem Seed:

To obtain Neem oil, the seeds are first broken open and the kernels separated. Therefore neem oil can be extracted from ground up oil bearing seed as kernels using petroleum ether and methanol as solvents. This is also known as leaching that is the solid by means of a solvent. The solvent then moves to penetrate into the solute layer to leach out the oil.

The extraction process is dependent on several parameters such as particles size, type of solvent, temperature, extraction time e.t.c. this experiment is designed to

demonstrate the importance of these parameters by introducing factorial design (experimental design).

The choice of solvent is another factor to be considered and it must exhibit all properties for effective, complete and economical extraction of desirable solute. Therefore, the choices of solvent for extraction are as follows:

2.7.1 Choice of Solvent:

In any separation process where the separating agents is materials as in leaching rather than energy as in centrifugation process, the choice of separation agents in the most importance. In this case selection of the solvent should be considered in the following-:

1.) The solvent should be readily available.

2.) The solvent should be non- corrosive and non-toxic.

3.) The boiling point of the solvent should not be too low otherwise evaporative losses may be high.

4.) There should be wide density difference between the solvent and solute for ease of separation.

5.) The solvent should be cheap so that the cost of solvent replacement.

6.) The solvent should be stable chemically and inert towards the components of the system and materials of construction.

2.7.2 Advantage of Solvent Extraction:

1.) When dealing with seeds of very minute size, the process results in maximum oil yield.

2.) Due to the selective and inert of the solvent used, solvent extraction is free from any undesirable impurities which may lower the quality of the oil.

3.) The extraction rates used in the process are usually higher than that from mechanical methods and residues normally contain less than 2% oil.

4.) It constitutes the most effective method for the recovery of oil from any oil bearing materials. It has advantage over steam distillation since it is not carried at high temperature.

2.7.3 Disadvantage of Solvent Extraction.

1.) Solvent extraction equipment is very difficult to come by, very expensive and costly to maintain.

2.) The process presents danger of fire and explosion especially when flammable solvents are used during leaching.

3.) Separation of liquid from solid provides efficient contacting and extracting time.

4.) The list of solvent to be used is not much since most solvent do not posses the required characteristics for good extraction.

2.7.4 Some Factors that Influence Rate of Extraction.

Some of the factors which may affect the rate of extraction are briefly discussed below:-

A.) Solvent: The solvent used affect the rate of extraction since all solvents used do not posses the same chemical properties. The most common solvents used are benzene, purified petroleum ether, methanol and ethanol e.t.c. In this project petroleum ether and methanol are used. The solvent should posses all the factors discussed in section 2.7.1.

B.) Temperature: This affects the rate of extraction because the solubility of most material generally increases with rise in temperature. Since the process of extraction depends solely on differential solubility, temperature will definitely have effect on the rate of extraction. As rise in temperature will definitely increase the rate of extraction.

C.) Particle Size: This also affect the rate of extraction because the smaller the size, the greater is the interfacial area available between the solid and the solvent and this entails higher rate of diffusion which will lead to increase rate of extraction. If the particle size is big, the smaller will be the rate of diffusion due to small interfacial area between the solid and solvent and hence low rate of extraction.

D.) Time: This is another important factor that should be considered, in this particular project the minimum time for extraction process is below one hour and the

maximum time about ten hours. But it is well desirable to reduce time development.

2.8 Properties of the Neem Oil (*Azadirachtins*)

Azadirachtins have several desirable properties-

Firstly, azadirachtins are rapidly degraded by the combination of sunlight and air and therefore represent little of the environment hazards which are usually associated with certain other classes of persistent insecticides.

Secondly, they have a very low toxicity on mammals in facts, *Azadirachtin* have a very remarkable record of safety towards humans, other mammals and plants.

Thirdly, they are extremely toxic to insects and have a remarkably wide scope of effectiveness. They can be used against most common household pests such as mosquitoes, houseflies, ants, cockroaches, lice and silver fish e.t.c.

They can also be used with success against many pests which affect domestic and farm animals, and also many stored product insects such as grain beetles and the ware hose moth. They are also effective against a number of forest and agricultural pests, but their potential in this field could not be exploited because of their light sensitivity. Their toxicity is characterized by the unusually rapid paralytic "knock down" on flying insects.

All these properties have enabled *Azadirachtins* to compete with successfully in the insecticide market. They are the active ingredients in many house-hold insecticidal preparations and are used as dusts or sprays to get rid of insect pests in food processing plants, dairies and restaurants. Recently, however, it has been shown that very small quantities of *Azadirachtins* are required for the control of some field pests. At present in spite of the progress made in the manufacture of synthetic analogies, the market for natural *Azadirachtins* is believed to be strong. The safety of the products and its non-resistance make it a strong contender as an environmentally save insecticide.

2.9 Economic Benefits from Insecticide/Pesticide

The potential benefits of a local pesticide formulation may be very substantial for a developing country. The major benefits can be as follows-:

A.) Saving substantial amount of foreign exchange otherwise use both for the purchase of diluents and its transportation;

B.) Reduction in cost of transportation by locating the formulation plant at a strategically well selected site;

C.) Potential reduction in cost to the farmers;

D.) Reducing dependence on foreign countries;

E.) Increase in national labour input and purchasing power of workers.

F.) Increase the number of industries and provide job opportunity for the people

2.10 Other Benefits from Neem Oil

A.) **Cosmetics**: Neem is perceived in India as a beauty aid. Powdered leaves, for example are a major component of at least one widely used facial cream. Purified neem oil in nail polish and other cosmetics.

B.) **Lubricants**: Neem oil is none drying, and it resists degradation better than most vegetable oils. In rural India it is commonly used to grease cart wheels. It could find many similar lubrication applications in other locations, especially in village settings in the warmer parts of the world where neem can be grown.

C.) **Soap**: India's supply of neem oil is now used mostly by soap manufacturers. Although much of it goes to small scale specialty soaps, large- scale producers also use it, mainly because it is cheap. Generally, the crude oil is used to produce coarse laundry soaps. However, more expensive soaps are made by saponifying the crude oil and distilling the resulting fatty acids before adding the lye. The resulting almost colorless and odourless product is suitable for top quality toilet and laundry soap.

D.) **Fuel**: Cold pressed oil is mainly used as lamps and source of fuel.

E.) **Fertilizer**: The neem demonstrated considerable potential as a fertilizer for this purpose; neem cake and neem leaves are especially promising.

CHAPTER THREE

3.0 Materials and Methods:

In the course of the practical work carried out in this project, the equipment/ instrument and methods used in the work are outlined in this chapter.

3.1 List of Equipment and Instrument

1.) Soxhlet Extractor.

2.) Weighing balance

3.) Heating mantle

4.) Tray dryer

5.) Condenser

6.) Round bottom flask

7.) Spatula

8.) Sample bottles

9.) Thermometer

10).pH meter

11).Retort stand

12). Measuring cylinder

13).African mortal and pestle

14). Beaker / conical flask /test –tube.

3.2 List of Chemicals and Materials

Table 3.2: List of Chemicals and Materials

CHEMICALS/ MATERIALS	*MANUFACTURERS/ SOURCE*
Neem fruits	Bosso Local Government Area
Distilled Water	Chemistry Department Lab. F.U.T, Minna
Methanol	May and Baker Limited, Degenham England
Petroleum-Ether	May and Baker Limited, Degenham England
Filter paper	Water resource, agriculture and fisheries Department Lab, F.U.T. Minna.

3.3 Procedures for the Extraction Process

The process of oil extraction from neem seed can be broadly divided into two stages:-

(1) The preparation stage (pretreatment)

(2) Extraction stage.

3.3.1 Pretreatment Operation

These are the operations carried out on the neem seeds before the extraction of the oil in order to obtain high yield of oil.

3.3.2 Decortications and Seed Cleaning

Sample of Neem seeds were collected from Bosso, in Bosso local Government Area, Niger State, Nigeria.

Decorticating the fruit (i.e. removal of hulls) to release the seed embedded inside, then the chafts and other impurities were separated. Then the sample were cracked and crushed.

3.3.3 Determination of Moisture Content of the Neem Seed

Moisture content of the seed was determined so that the seed could be stored without deterioration and to enhance high yield of oil. After the decorticated of the Neem seeds and removal of the hulls, the clean seed was dried in an oven at temperature of 80^0C for a period of 3 hours to remove the moisture contents. The weight of the seeds before and after drying was denoted as W_1 and W_2 respectively.

The % moisture contents was calculated as $\frac{W_1 - W_2}{W_1}$ x 100%

3.4 Procedure for the Extraction of Neem Seed

Five grammes (5g) of the sample was weighed and placed on the filter paper. The filter paper was folded carefully. The filter paper containing the sample was weighed and recorded, then inserted into the thimble; the thimble containing the loaded sample was then put inside the Soxhlet extractor.

200ml of the Petroleum-Ether (40 -60°C) was measured out using a measuring cylinder and about two-third of the solvent was poured into 250ml rounded bottom flask

of the Soxhlet apparatus and the remaining solvent was poured into the thimble containing the loaded sample.

The Soxhlet apparatus was then assembled and heated up for 1hr (one hour) using a heating mantle.

After heating and cooling the sample was brought out, and the solution left in the round bottom flask was distilled using the same Soxhlet apparatus. After recovery a reasonable amount of the solvent in the extractor, the source of heat was switched off and the solvent in the Soxhlet extractor was then poured into a separate bottle meant for the storage of recovered solvent.

The remaining solution in the round bottom flask was removed by pouring it to previously weighed sample bottle which is put in an oven so that it can evaporate. The weight of the sample bottle with oil was measured on hourly basis until a constant weight was obtained.

The constant weight of the oil and sample bottle minus the weight of the empty sample bottle gave the weight of the oil extracted.

An approximate method to get the weight of oil extracted is to take the weight of the thimble before and after extraction. The difference between the weight of the thimble before and after extraction gives the approximate weight of the oil extracted.

The experiment was repeated twice as above on (1hrs, 3hrs, 5hrs, 7hrs and 9hrs) and by using different solvent methanol (CH_3OH).

The whole process is shown in figure (1), the schematic flow diagram for neem oil extracted from the Neem seed and figure (2) shows set up apparatus for the extraction process.

3.5 Characterization/ Analysis of Extracted Oil

This is the test carried out with oil recovered from the solvents i.e. determination of melting temperature, boiling point, specific gravity, pH value and colour.

3.5.1 Determination of Neem Oil Melting Temperature

The oil was first solidified using refrigerator, followed by melting the oil over the boiling water with thermometer incorporated. The temperature at which the oil started to melt and the temperature at which it all melted was noted.

3.5.2 Determination of Neem Oil Boiling Point

The boiling point of Neem seed oil was determined by these procedures:

The oil was poured in a test-tube and a thermometer immersed in it, was heated on a heating/plate.

After about 15 minutes of heating it was discovered that the oil in the test - tube started boiling, the heating was continued until the boiling was continued until the boiling was throughout the oil and the value was noted.

3.5.3 Determination of Specific Gravity

The specific gravity of oil was determined using specific gravity bottle. The empty specific gravity was weighed to get the weight of the bottle (W_1), the bottle was then filled with the oil and stopper inserted, this was weighed (W_2). The bottle was thereafter emptied and rinsed several times with water. It is then filled with water; the stopper inserted then weighed again (W_3).

The specific gravity of a substance is defined as:

$$\text{S.P Gravity} = \frac{\text{mass of substance}}{\text{mass of an equal volume of water}} = \frac{W_2 - W_1}{W_3 - W_1}$$

3.5.4 Determination of pH of Neem Seed Oil

The pH was selected and the electrodes were lowered into a buffer solution. The temperature control was adjusted to the temperature of the buffer solution. The calibrate control was adjusted until the meter indicate exact pH of buffer solution. The electrodes were raised and rinsed with buffer, then with the oil. The temperature control was adjusted to temperature of the Neem seed oil. The electrodes were then lowered into the Neem seed oil, and the meter reading was noted.

3.6.0 Testing the Effectiveness of the Neem Oil (Extract)

The aim of test and analysis of any chemical composition is for an assessment of quality, purity as well as for their identification

However, in this research work the method used to determine the effectiveness of the Neem extract is on the insects, e.g. house-flies, mosquitoes, cockroaches and Grasshopper.

3.6.1 Test Carried Out on Extract Obtained from Petroleum-Ether

The first test was carried out using the Petroleum-Ether extract. Four covered perforated cans (cages) were placed and labeled sample A, B, C and D respectively.

Sample "A" was labeled the control where 5 cockroaches were placed with sufficient food that will last for three days (72 hours) or more, only distilled water was sprayed on both the insects and the food.

In sample "B" 5 cockroaches were also placed with sufficient food, but here the Petroleum-Ether was sprayed on the insects, leaving the food (unsprayed).

However, in sample "C" the 5 sets of cockroaches were placed with sufficient food. In this case the extract was only sprayed on the food leaving the insects.

Similarly, in sample "D" the 5 cockroaches and foods were treated with the synthetic insecticides (Mobil insecticide), the chemical was sprayed on the insects and food.

Finally, the same procedure was applied to extract obtained from methanol.

CHAPTER FOUR

4.0 Experimental Results and Discussion

4.1 Experimental Results.

In this chapter, all the results obtained from the experimental work carried out are outlined. They include the extraction process, physical properties of the extracted oil, and application of 2^5 full factorial designs to the extraction process.

Table 4.1: Percentage (%) Yield of Extract Obtained Using Petroleum- Ether Solvent

Time taken (hrs)	*Before exp. (gramme) (g)* w_1	*After exp.(gramme) (g)* w_A	*After exp.(grammes) (g)* w_B	*Amount of oil extracted* w_A(g)	w_B(g)	*% Yield* w_A	*% Yield* w_B
1	5	3.39	3.37	1.61	1.63	32.30	32.60
3	5	3.37	3.37	1.62	1.66	32.60	32.60
5	5	3.37	3.36	1.63	1.64	32.60	32.80
7	5	3.34	3.35	1.66	1.65	33.20	33.00
9	5	3.33	3.55	1.67	1.45	33.40	29.00

Table 4.2: Percentage (%) Yield of Extract Obtained Using Methanol as Solvent

Time Taken (Hrs)	*Before Exp. (gramme) (g)* w_1	*After Exp. (gramme) (g)* w_A	*After Exp. (gramme) (g)* w_B	*Amount of oil Extracted* W_A(g)	W_B(g)	*% Yield* W_A	*% Yield* W_B
1	5	3.88	3.92	1.12	1.08	22.40	21.60
3	5	3.45	3.44	1.55	1.56	31.00	31.20
5	5	3.36	3.38	1.64	1.62	32.80	32.40
7	5	3.35	3.32	1.65	1.68	33.00	33.60
9	5	2.92	2.97	2.08	2.03	41.60	40.60

Table 4.3: Properties of Oil Extracted

Properties	*Standard Values*	*Experimental Values*
Boiling Point	$(25 - 30)^0$ C	28^0 C
Melting Point	(250 - 265)° C	260°C
pH Value of the Oil	5	5.6
Specific Gravity	(0.920 – 0.930)	0.924
Colours		
Petroleum- Ether	____	Yellowish
Methanol	____	Brown

Table 4.4: Observation of Various Tests Carried Out on the Neem Oil (Extracts)

Chemical applied	*Sample "A" (five cockroaches) with control time(72hours)*	*Sample "B" (five cockroaches) with control time (72 hours)*	*Sample "C" (five cockroaches) with control time(72hours)*	*Sample "D"(5 cockroaches) with control time(72 hours)*
Methanol	*No insects died within the first three days *1 insect died on the 5th day. *2 insects died on the 9th day.	*After 12 hours 4 out of the 5 insects died. *At 24 hours none of the insects is alive.	*After 12 hours it was discovered only 2 insects died. *At 24 hours it was 3 insects that died. *But at 36 hours it was confirmed that no insects remained alive.	*All the 5 insects died after 5 hours.
Petroleum-Ether	*No insects died within the first 72 hours. * None of the insects died on the 5th day. *1 insect died on the 9th day.	*Only one insect died after 24 hours. * After 24 hours 3 insects out of 5 died. *At 36 hours none of the insects was alive.	*After 12 hours it was discovered only one insect died out of 5 insects. *After 24 hours it was 3 insects that died. *After 42 hours it was confirmed that no insects remained alive.	*No insects remained after 5 hours.

Table 4.5: Percentage Concentration of Solution and Efficiency of the Extracts

	Extract using methanol as solvent	*Extract using petroleum-ether as solvent*
% concentration of solution	2.5% weight/ volume	2.5% weight/ volume
efficiency of extract at		
Sample "A"	Negligible	Negligible
Sample "B"	66.67%	50.00%
Sample "C"	50.00%	41.67%
Sample "D"	93.06%	93.06%

Table 4.6: General Linear Model: % Lipid versus Solvent, Time

Factor	*Types*	*Levels*	*Values*
Solvent	Fixed	2	1,2
Time	Fixed	5	1,3,5,7,9.

Table 4.7: Shows Factorial Design Representation

Level	*N*	*Mean*	*St.dev*	*Individual 95% cis for mean based on pooled st. deviation.*
1	2	32.400	0.283	(- - x - -) b
2	2	32.600	0.000	(- x - -)b
3	2	32.700	0.141	(- - x -)b
4	2	33.100	0.141	(- - x -) b
5	2	31.200	0.111	(- - x - -)b
6	2	22.000	0.566	(- - x -)a
7	2	31.100	0.141	(- - x - -)b
8	2	32.600	0.283	(- x - -)b
9	2	33.300	0.424	(- x - -)b
10	2	41.100	0.727	(- -x -)c

4.2 Discussion of Results

The results of the percentage of oil extracted for both petroleum ether (40 – 60°C) and methanol were presented in the table 3 and 4 respectively. The percentage of oil yield extracted (lipid) were plotted against time in which straight line graph were obtained. The calculated values of the percentage amount of oil extracted of petroleum ether and methanol were confirmed to be higher at amount of mean of the value to be 33.10% at 7hrs and 41.60% at 9hrs respectively. The extract obtained from methanol was about 20times more concentrated than extract obtained from petroleum- ether.

For both extraction using petroleum- ether and methanol as solvents, the amount of oil extracted increases as time increases.

Reference to the table 4.3, the colour of the extracts, using methanol as solvent is brown and this is due to the presence of oil in conjunction with the chemical composition in the solvent which corresponded with standard value. Similarly, the petroleum-ether extract has a yellowish colour. Extracted oil obtained from using methanol as solvent is stronger in odour of *Azadirachtin* than extracted obtained using petroleum – ether as solvent, and the volume of extract obtained from methanol is twice the volume obtained from petroleum-ether. Therefore, methanol is a better solvent for the extraction of the Neem oil than petroleum – ether.

This research project work coincide with previous work (Ndagana, 2000) carried out on extraction of Neem Oil using ethanol as a solvent, in term of colours (brown) and the efficiency/effectiveness was slightly higher than previous work by 11% table 4.3 and 4.5.

Also, table 4.3 showed the characterization of the Neem oil were pH value 5.6, melting point 260°C, boiling point 28°C and specific gravity 0.924. The pH value gotten was slightly different from standard value of pH 5 which was due to excess heating during extraction process that led to the evaporation of saturated fatty acid present in the Neem seeds. While, the melting point and boiling points were in the range of the standard value of 250 -265°C and 25 -30°C respectively. Sexane *et al* (1981). However, any

in the neem seeds or during extraction process will alter the both melting/boiling point value

According to table 4.4, the various test carried out on the amount of oils produced from (1hr to 9hr) using petroleum- ether and methanol as solvent respectively, were mixed together and used for the test on insects (cockroaches). For experiment number one which used methanol as a solvent. In sample "A" that is, control can, the insects remained alive within the fist three days, until on the fifth day of the control were only one insect died out of five, the dead increased to two (2) insects at the ninth day, but the food still remained and the dung of the insects died not because of the lack of food, but due to their life – cycle of change of their environment.

While in perforated sample "B", where the methanol extracts were sprayed to the insects at the first -12 hours it was observed that four insects out 5 died, and non of the insects remained at 24 hours, in which the food remain untouched and there is on any sign of dung in the can, this indicates that the insects died due to the chemical sprayed on them, and this chemical act as antifeedants to them.

Furthermore, in the sample "C" where the spray was on food only, the food remained untouched, while there are some pieces of dung, after 12 hours it was discovered that only 2 insects died, the death gradually increased until 36 hours where all the insects died, in this sample insects prefer to starve themselves to die than eating the treated food

More so, in the perforated sample "D" the insects died 5 minutes after the application of synthetic chemical and there is no sign of dung and food remained untouched.

Similarly, the sane observation was noticed in second tests, using petroleum – ether test, in this case, it was observed that sample "B" and "C" there are small eaten in the food, and little dung were also present in the can. Reference to table 6

Reference to table 4.5, where the calculated efficiency of the extracts obtained using methanol as solvent, in sample "A" the efficiency of the extract are negligible, where in sample "B" and "C" the efficiency of the extracts were 66.67% and 50.00%

respectively, while in the second experiment were 50.00% and 41.67% respectively, this shows that based on the application of the extract on insects, the methanol extract is better use for Bio- insecticides with 66.67% than petroleum – ether extract 50.00%.

In the case of the application of extracts on food, the methanol extract is better with 50.00 % efficiency, than petroleum-ether with 20.10%. Therefore on comparing the efficiency of extracts with the synthetic chemical applied (i.e. 93.06% efficiency), it was confirmed that methanol extracts (natural insecticides/ pesticides) produced can compete with today's synthetic chemical, since the only target is to eliminate insects/ pests from the environment.

However, table 4.6 showed that data in the same row carrying the same superscript do not differ significantly from each other (probability > 0.05) and data in the same row carrying different superscript differ significantly from each other (probability< 0.05).

CHAPTER FIVE

5.0 Conclusion and Recommendations

5.1 Conclusion

The mass of Azadirachtin extracted from the seeds of Neem using methanol and petroleum – ether were plotted against time where a straight line graph with slope of Y = 0.96X + 27.40 were obtained. This was compliance with fact that the rate of extraction is increasing as the time increases.

For the time 1, 3, 5, 7, and 9, hours, the amount of oil extracted were 1.620, 1.635, 1.655, and 1.566 all in grammes for petroleum – ether and 1.100, 1.555, 1.630, 1.665, and 2.055 for methanol extraction were 32.400, 32.600, 32.700, 33.100, and 41.100 for methanol respectively. The amount of oil extracted in the methanol was found to be greater than that in the petroleum – ether showing that methanol is better solvent to extract more than petroleum – ether. The oil also had a scanted odour.

On the various test carried out, it was confirmed that, in the control can, that is sample "A" the insects did not died to the lack of food, but due to their life cycle and change in their environment while in the other sample "B", it was confirmed that the insects treated with extract are unable to eat food, due to the lack of appetite and the chemical also act as antifeedants to them.

Similarly, on sample "C "where the extracts were sprayed on food, the insect prefer to starve, than eat the treated food.

The calculated efficiency of tested extracts on insects, it was confirmed that in methanol extracts is better with 66.67%, than petroleum-ether extract with 50.00%. On the other hand, the application of extract on food, methanol extract is better with 50.00% efficiency than petroleum-ether with 20.10%.

However, on considering the overall efficiency of the extracts on sample "B" and "C" and comparing with synthetic chemical it shows that methanol with 66.67% while synthetic chemical is having 93.06%. This indicates that natural bio-insecticide can work more efficiency as synthetic insecticide does.

According to table 4.7 shows that the best hour for the maximum oil extraction for both Petroleum – ether and Methanol as solvents was between (3-5 hours).Also the data in the same row carrying the same superscript do not differ significantly from each other indicate that extraction rate were the same during these time used, and data in the same row carrying different superscript differ significantly from each other indicates that extraction rate were different for the time.

5.2 Recommendations

(1.) Further work should also be carried out using different solvent such as Ethanol, Hexane, Pentane, Chloroform e.t.c so that their efficiency can be compared.

(2.) Further work should be carried out using different particular size ranges to find the most economic size to be used. Other factors such as temperature, viscosity, fluidity e.t.c. could then be varied and worked on and the data obtained can be used in the design of pilot plant which can be sealed up.

(3.) Anybody carrying out further research on this particles research work, investigation should be carried out to determine other amount of compound of Salannin, Meliantriol, Nimbrin and Nimbidin in the extract.

(4.) In the present economic situation in Nigeria, due to high price of edible oils, the question of using non-edible oil such as Neem seed oil for making insecticide soap within the country should have to be re-considered, since the soap has beneficial effect on skin.

(5.) Further work should be carried out on other uses of Neem oil such as cake for the production fertilizer and oil for manufacturing of other product such as oil, cosmetics, lubricants, fuel e.t.c.

(6.) Further research work should be carried out on Neem oil to improve the insecticidal properties by the application of the additive (promoters) to boost power. E.g. pipronyl butoxide and pyrethrins, since it has no adverse effect on human being and environment compared to synthetic insecticidal.

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Appendix I

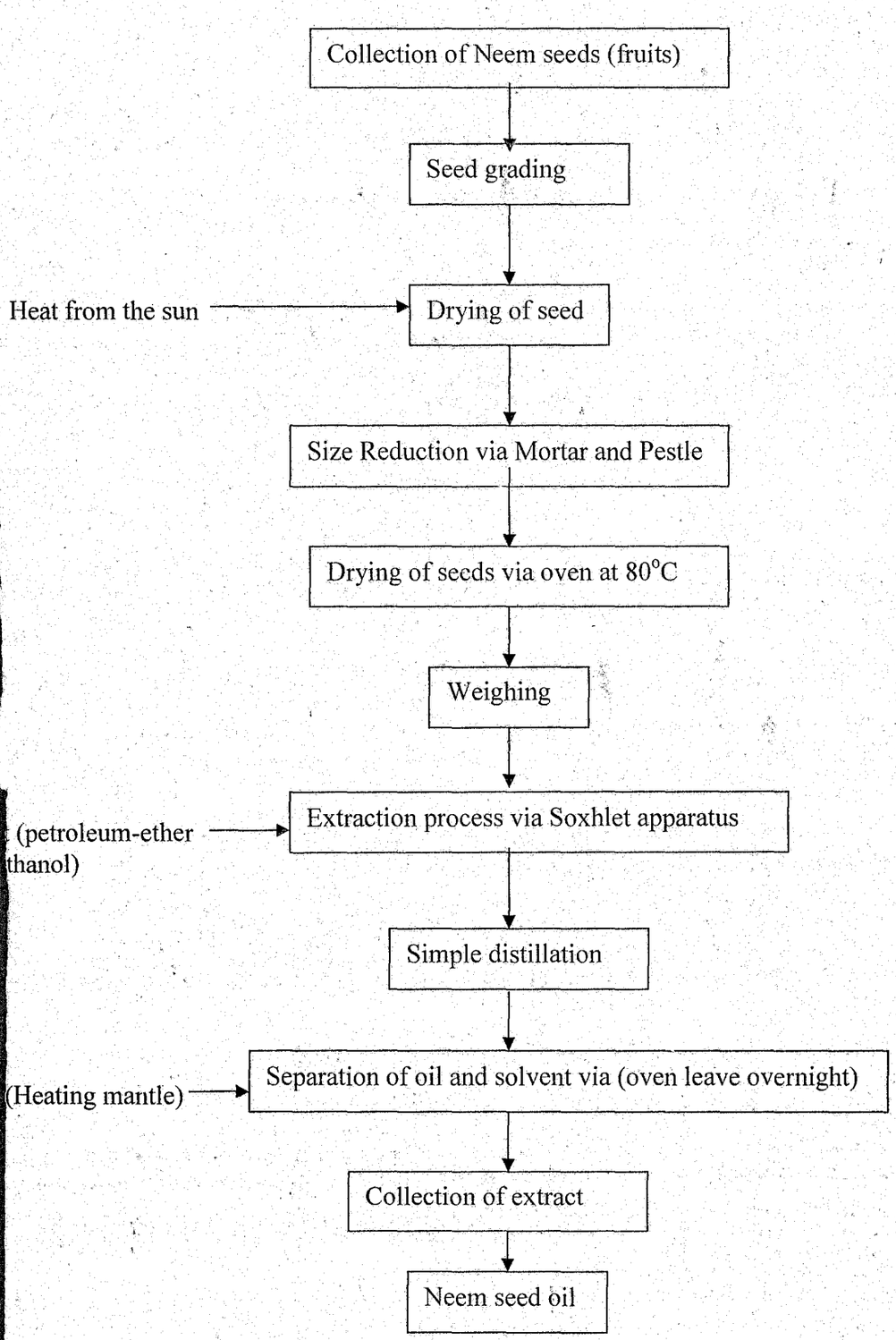

Figure 1:- Schematic Flow Diagram for Neem Oil Extraction from Neem Seeds.

APPENDIX II

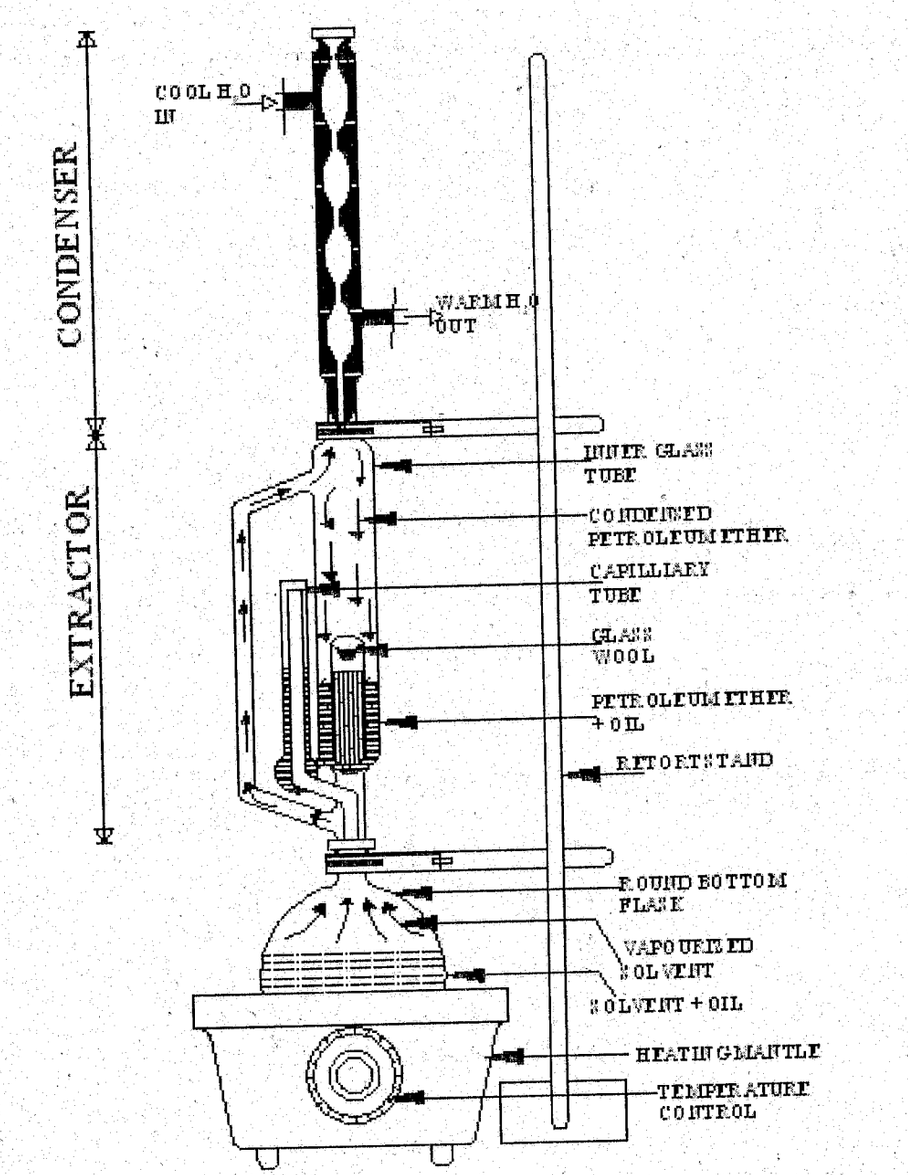

Figure 2: Set up Apparatus for the Extraction Process

Appendix III

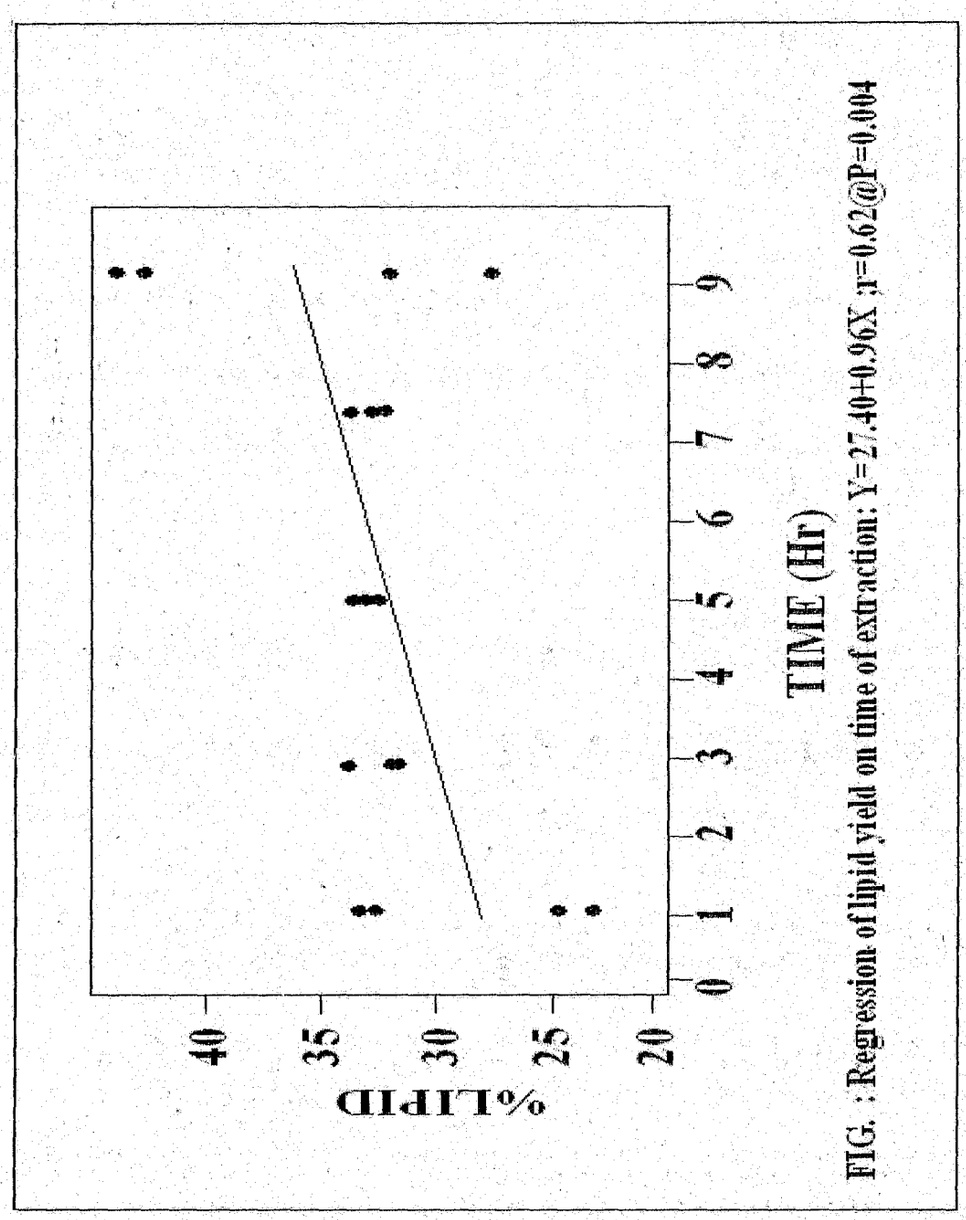

Figure 3: **Graph of Lipid (extract) vs. Time**

Appendix IV

Determination of Percentage concentration of the solution

% Concentration of the solution could be determined by the following way:

Weight of the seed used = 5g

Volume of the solvent used =200ml

$$\% \text{ Concentration} = \frac{\text{Weight of seed used}}{\text{Volume of solvent used}} \times 100\%.$$

$$= \frac{5}{200} \times \frac{100}{1} = 2.5\% \text{ weight/volume (w/v)}$$

1) Determination of amount oil extracted using Petroleum –Ether as solvent

To determine the amount of oil extracted in 1hr:

Weight of sample before extraction = w_1 = 5g

Weight of sample after extraction = w_A =3.39g

Amount of oil extracted =$W_A = w_1 - w_A$

W_A = (5-3.39g) = 1.61g.

2). Determination of Percentage yield of extract using Petroleum –Ether as solvent

Percentage of oil extracted can be calculated as:

Amount of oil extracted = W_A= 1.61g

Weight of sample before extraction = W_1= 5

(Percentage of oil extracted) = W_A/w_1 x 100%.

Percentage of oil extracted for 1hr =1.61/5 x 100/1

=32.20%

Note: others can be calculated as above

3). Determination of extract efficiency using Petroleum - Ether as solvent

To determine efficiency of the petroleum-ether extracted compared to the synthetic insecticides.

Basis: 72 hours (control time).

$$\text{Percentage efficiency} = \frac{\text{(Time taken for the control) - Time taken for the insecticide to die by the application of chemical}}{\text{(Time taken for the control}} \times 100\%$$

Percentage of efficiency of extract in sample "B"

Time taken for insects to die = 24hours

Control time =72 hours.

(% efficiency = $\frac{72-24}{72}$ x 100% = 66.67%

% efficiency of extract in sample "C"

Time taken for insects to die = 36 hours

Control time = 72 hours

%efficiency = $\frac{72-36}{72}$ x 100% = 50.00%

% efficiency of the synthetic chemical in sample "D"

Time taken for insects to die = 5 hours

Control time = 72 hours

% efficiency = $\frac{72-5}{7}$ x 100% = 93.06%.

1). Determination of amount of oil extract using Methanol as solvent

To determine the amount of oil extracted;

Weight of sample before extracted w_1 = 5g

Weight of sample after extracted w_A = 3.88g

Amount of oil extracted =WA = $w_1 - w_A$

(5 – 3.88) g = 1.12g

2). Determination of Percentage yield of extract using Methanol as solvents

% of oil extracted can be calculated as;

Amount of oil extracted = W_A =1.12g

Weight of sample before extracted = w_1 = 5g

Percentage of oil extracted = $\frac{W_A}{W_1}$ x 100

For 1 hr extraction,

$\frac{1.12}{5}$ x 100 = 22.40%

NOTE: Other can be calculated as above.

3). Determination of extract efficiency using Methanol as solvent

To determine efficiency of methanol extracted compared with the synthetic insecticide.

Basis: 72 hours (control time)

% efficiency of extract in sample "B"

Time taken for insects to die = 36 hours

Control time = 72 hours

$$\% \text{ efficiency} = \frac{72 - 36}{72} \times 100 = 50.00\%$$

% efficiency of extract in sample "C"

Time taken for insects to die = 42 hours

Control time =72 hour

$$\% \text{ efficiency} = \frac{72 - 42}{72} \times 100 = 41.67\%$$

% efficiency of extract in sample "D"

Time taken for insects to die = 5 hours

Control time = 72 hours

$$\% \text{ efficiency} = \frac{72 - 5}{72} \times 100 = 93.06\%$$