Application of full 4^2 Factorial Design for the Development and Characterization of Insecticidal Soap from Neem Oil

A. S. Kovo

Department of Chemical Engineering, Federal University of Technology, Minna, Nigeria,
kovoabdulsalami@yahoo.com

Abstract

The aim of this paper was to investigate the extraction, characterization and production of insecticidal soap from Neem oil using full 4^2 factorial design. Soxhlet extractor was used for the extraction purpose and two solvent was chosen to determine which is better. N-hexane gives a Neem oil yield of 45.43% while ethanol gives a yield of 46.38%, confirming the earlier literature result giving ethanol as better solvent for Neem oil extraction. The basic properties of the oil were determined as follows, saponification value 215.95ml/g, acid value 1.122g/mol, unsaponifiable matter 19.66 etc. The Neem oil was found to have a colour of golden yellow due to the presence of Nimbidin.

Full 4^2 factorial design and mathematical model was applied to the extraction process and a first order regression equation of the form:

$$Y = 9.548 + 0.144X_1 + 0.1931X_2 + 0.1892\ X_{12}$$

was obtained growing the individual effect of time and solvent type as parameter and their interaction in the entire extraction process the Neem insecticidal soap was found to be effective in insect and pest control.

Keywords

Neem oil; Extraction; Factorial Design; Insecticidal soap; Solvent type

Introduction

Extraction of oil has been of great interest worldwide and this has been as a result of constraint increase the world population. The oil produced cannot cater for all need of the population which includes (soap making, industrial use and edible) [1]. Neem oils extracted from the seed kernel of Neem plant. It has found its use widely in different region of the globe for medicinal and agricultural purpose. It is found to be of great health use and it has an effective anti-germ property which include is use as an insect repellent.

Insecticidal soap are specially formulated biodegradable soap that can kill insect while few adverse effect on people, plant and the environment. Resistance within the insect tend to develop more quickly with material that have specific mode of action (traditional pesticide) but with insecticidal soap, it is less likely that resistance will develop [2]. In this paper, the use of Neem oil as horticultural oil for insect repellent will be emphasized. Therefore this paper intend to investigate the development and production of insecticidal soap by comparing the use of two different solvents for the purpose of extraction, finally the basic characteristic of the soap will determine while also testing the effectiveness of the soap on selected insect.

Experimental Procedure

The process of the extraction of the oil from the seed can be broadly divided into two stages; these are preparatory stage (pre-treatment) and extraction stage. Decorticating the fruit was the first step and it involves the removal of Hull to release the seed embedded inside. Then the shafts and other impurities were separated. The next operation is drying, the clean seed was dried in an oven at temperature of 100°C for a period of 2 hours to remove the moisture content the kernel was weighted before it was placed in the oven (W_1) and thereafter the weight was re-taken at an interval of 1 hour, the procedure was repeated until constant weight (W_2) was obtained.

The percentage moisture of the seed sample was calculated from Moisture content = $100\cdot(W_1-W_2)/W_1$ %, where W_1 weight of wet sample, and W_2 weight of dry sample. The drying was done so that the seed could be stored without deterioration and to enhanced high yield of oil.

Extraction of Oil from Neem Seed with a Soxhlet Extractor

The dried Neem seed was crushed in a mortar. The sample was expressed on a sieve screen to obtain a particular size of 0-0.25mm. This is because from past project work it was determined that the sample with the smallest particle size range gives yields the highest quality of oil.

The weight of the flask and the thimble of the extractor were noted as F_1 and T_1 respectively. 10g of the sample was weighted and placed on the filter paper. The filter paper was folded carefully; the filter paper containing the samples was then inserted into the extractor. The weight of the filter paper and samples weight was taken & noted.

250 ml of the solvent (n-hexane and purified ethanol) was then the assured out suing a assuring asunder and poured into the extractor flash which gave 4/5 of the volume of the flask.

The soxhlet apparatus was then heated up using a heating mantle. The golden yellow colour of a solution was observed in the extractor. The colour solution observed to be the oil remains in the flask.

The oil evaporate along side the solvent and then condensed into the extractor, which is poured back into the flask, this process continued for 2 hours and the same process was repeated for 4 hours, 6 hours and 8 hours.

After heating and cooling for the required time, the waded thimble containing the sample was brought out and the oil was distilled off the solvent or evaporation of the solvent was allowed to occur while the oil was collected as extract and the solvent as raffinate.

An approximate method of obtaining is to take the weight of thimble with samples before and after extraction. The diff between the weight of the thimble before and after extraction gives the approximate weight of the oil extracted

The experiment was repeated twice for the various time (2 hrs, 4 hrs, 6 hrs & 8 hrs) using the different solvent in-hexane and ethanol).

The oil extracted can be calculated from weight of oil extracted (W_1-W_2) and % of oil = 100·Oil_extracted/Weight_of_sample, where W_1 weight of filter paper and sample before extraction, W_2 weight of filter paper and sample after extraction.

The Experimental Full Factorial Design Method

The two-variable four-level factorial experiment, which indicated the run-by-run experimental design, is shown in table 1. The runs were replicated two times (r = 2) giving a total number of samples of 8×2 = 16 (n × r) samples. In the 4^2 full factorial experimental design, the low and high levels of the factor code are "- "and "+" respectively. The level of the two factors is listed in standard order in column X_1, X_2 in the table and the interaction in column X_{12}. To obtain the matrix, start x_1 with the minus sign and alternate the sign until you reach row (run) 4^k start column X_2 with two minus sign and alternate sign in block of 2. The sequence of + and – sign in the column tell us how to combine the observation to get the main effect and their interaction so as to determine the yield. Using the generalized regression equation of the factorial model: $Y = b_0X_0 + b_1X_1 + b_{12}X_{12}$

Table 1 design matrix plan for 4^2 full factorial experimental design

Run N	X_0B_0	X_1B_1	X_2B_2	$X_{12}B_{12}$
1	+	-	-	+
2	+	+	-	-
3	+	-	+	-
4	+	+	+	+
5	+	-	-	+
6	+	+	-	-
7	+	-	+	-
8	+	+	+	+

Analysis and Characterization of Extracted Oil

Determination of Specific Gravity and Boiling Point

The specific gravity of oil was determined using specific gravity bottle. The empty specific gravity bottle was weighed to get the weighed 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 types with water. It is then filled with water; the oil stopper inserted, and then weighed again W_3. Some amount of oil is put into beaker and a thermometer was inserted in it for 18minutes on a heating mantle it was observed that the oil in the beaker started circulating leading to boiling of oil. The temperature at the point was 259°C and that was the boiling point.

Melting and Solidification in Temperature of Neem Oil

The process of solidifications of the oil involves the use of ice blocks and a thermometer immersed read in the oil. The oil was observed to solidity at 10°C the oil melted over boiling bam water at a temperature of 29°C.

Determination of Refractive Index

This is a physical attribute of triglycerine measures by the angle through which a beam of light is bent when passing through a thin film of melted fat.

Few drop of oil were placed on one face of a glass prism of a refractometer and it was gently spread, closed and tightened. Ample time is allowed for the prism and oil to attain steady temperature. The refractive index was then read form demarcation line.

Determination of pH and Viscosity of Neem Oil

The pH was selected and the electrodes were lowered into buffer solution. The temperature control was adjusted until the meter indicates exact pH of buffer. The electrodes were raised and rinsed with buffer, and then with the oil. The temperature control was adjusted to temperature of Neem seed oil. The electrodes were then lowered into the Neem seed oil & the meter reading was noted. A little quantity of the oil was poured into a test tube and a viscometer was used to measure the viscosity.

Determination of Acid Value

2g of the test portion was dissolved in some neutral solvent (toluene/ethanol mixture) the solution was thoroughly mixed and then titrated with 0.1 KOH using 1ml of phenolpthalein indicator or solution. The end point was reached when pink colour persisted for 30 seconds. Two determinations was carried out on the same test sample. The acid value is given by the expression: Acid value = v·c·56.1/m, where v is volume of potassium hydroxide (ml), c is concentration of potassium hydroxide, m is mass of the test portion (g), and 56.1 - Molar mass of potassium hydroxide.

Determination of Specification Value

2g of Neem seed oil was weighed into a conical flask. 25ml of alcoholic KOH was added to the test portion with using a pipette. A reflux condenser was connected to the flask

was placed on the heating mantle and boiled gently at temperature of 35°C shacking from time to time for 60 minutes.

1ml of the phenolpthalein solution was added to the hot solution and titrated with 0.5 HCL and until the purple colour of the indicator disappear to give light yellow.

The blank test was carried out following the above procedure but test portion was omitted. The saponification value is given by the formula: $(V_0\text{-}V_1)\cdot c\cdot 56.1/m$.
where V_0 is volume (ml) of the standard volumetric HCl solution used for the blank test, V_1 is volume (ml) of the standardized HCl solution used for the test with the oil, c is exact concentration (mol/l) of the standard volumetric HCl solution, m - mass in gram of the test portion, 56.1 is molecular mass at KOH solution.

Determination of Iodine Value

2g of sample was weighed into a conical flask. 20ml of carbon tetrachloride and 25ml of DAM's reagent was added to the flask. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 1 hour 30 mins, at the end of the time, 20ml of potassium iodide solution and 150ml of water were added, The content of the flask was titrated with 0.1mol/l sodium thiosulphate solution until the yellow color due to iodine has almost disappeared. Few drops of starch were then added and titration continued until the blue color disappeared after vigorous shaking. The same procedure was used for blank. The iodine value is given by the expression $12.69c(V_1 - V_2)/M$, where c = concentration of sodium thiosulphate used, V_1= volume of sodium thiosulphate solution used for blank, V_2 = volume of sodium thiosulphate solution used for determination, M = mass of test sample.

Determination of Free Fatty Acid Value

25ml of toluene was mixed with 25ml of ethanol in a beaker the solution was poured on to 2.0g of Neem seed oil in a flask and 1ml of phenolphthalein was added. The mixture was then titrated against 0.1m NaOH with constant shaking for which a dark pink color was observed and the volume of 0.1m NaOH used (v_b) was noted. The free fatty acid value: ffav = $v_b\cdot 2.82\cdot 100$ %; where 100ml of 0.1NaoH = 2.82g of oleic acid.

Determination of Unsaponifiable Matter

5g of Neem oil was weighed into a conical flask, 50ml of 1M solution of ethanolic potassium hydroxide was added with a few anti-bumping granules. The flask was attached to a reflux condenser and the content was allowed to boil for one hour. 100ml of water was added through the top of the condenser with continuous swirling of the flask. The flask was allowed to cool and the contents now transferred into 500ml separating funnel. The conical flask was rinsed by using 100ml diethyl ether. Stopper was inserted into the separating funnel and then inverted after vigorous shaking.

The content was allowed to stand until there was complete separation of the two phases. The lower layer was then run off completely as possible into a second separating funnel. The ethanolic soap solution was extracted twice more each time in the same way with 25ml of diethyl ether and the contents of the extract was collecting in separate funnel containing 40ml of distilled water. The separating funnel containing the combine extract and 40ml of water was gently rotated.

The layers were allowed to separate completely and the lower aqueous layer was drowning off. The ethercal solution was washed successfully with 40ml of potassium hydroxide solution and 40ml of water. The washing continues until the solution no longer gives a pink colour on addition of a drop of the phenolphthalein solution. The ethercal solution was transferred through the top of separating funnel into 250ml conical flask previously dried and weighed.

The solvent was evaporated by placing the flask on a boiling water bath. 5ml of acetone was added and the content completely evaporated in a gently current of air. The residue was dried in an oven maintained at 103ºC for 15mins. This was allowed to cool in a desiccator and then weighed to the nearest 0.1mg.

The unsaponifable matter content expressed as a percentage by mass of the sample is: $100\cdot(M_1 - M_2 - M_3)/M_0$, where M_0 = mass of the test sample, M_1 = mass of the residue, M_2 = mass of residue obtained with the blank, M_3 = mass of free fatly acid and equal to 0.28Vc, V = volume of 1 molar ethanol potassium hydroxide solution used for titration, c = concentration in molar of the ethanolic potassium hydroxide solution.

Results and Discussion

Table 2. Result of Extraction Oil from Neem Seed at Different Time for Different Solvent

Solvent	n-Hexane				Ethanol			
Sample number	1	2	3	4	5	6	7	8
Time (hr)	2	4	6	8	2	4	6	8
W (g)	14.31	13.9	14.23	14.19	14.07	14.19	13.89	14.23
Yield A (g)	10.08	9.30	9.60	9.50	10.12	9.52	9.02	9.30
Yield B (g)	10.08	9.30	9.60	9.50	10.02	9.51	9.02	9.30
Average yield (g)	10.08	9.30	9.60	9.50	10.07	9.52	9.02	9.30
Average yield per time (g/hr)	5.04	2.36	1.60	1.19	5.04	2.38	1.5	1.16

Table 3. Physical Property Determination
Experimental Result of the Characterization of Extracted Oil

Physical property	Value	Units
Refractive index at 28°C	1.3675	-
Boiling point at room temperature	258	°C
Melting point at room temperature	29	°C
Solidification point at room temperature	10	°C
Odour	Garlic-like	-
Colour	Brown	-
pH	5.5	-
Viscosity	0.050	kg/ms
Specific gravity	0.927	-
Percentage yield of oil	46	%

Table 4. Determination of Saponification Value Chemical Properties Determination

Sample	Mass (g)	1st titre (ml)	2nd titre (ml)	Mean value (ml)	S.V (ml/g)
Neem oil	2.00	3.3	3.1	3.2	-
Blank	-	18.6	18.6	18.6	215.98

Table 5. Determination of Iodine Value

Sample	Mass (g)	1st titre (g)	2nd titre (g)	Mean value (g)	Iodine value 1g of beeline 100g of sample
Neem oil	2.00	86.75	86.75	86.75	72.02
Blank	-	199	201	200	-

Table 6. Acid Value Determination

Sample	Volume (ml)	1st titre (ml)	2nd titre (ml)	Mean value (ml)	Acidity value (ml/g)
Neem oil	2.00	0.40	0.40	0.40	1.122

Table 7. Determination of Free Fatty Acid

Sample	Mass (g)	1st titre (ml)	2nd titre (ml)	Mean value (ml)	FFA (ml/g)
Neem oil	2.0	0.40	0.30	0.35	49.35

Table 8. Determination of Unsaponifiable Matter

Sample	M_0 (g)	M_1 (g)	M_2 (g)	M_3(g)	Mean Titre (ml)	U.S.V
Neem oil	5.00	0.43	0.0014	0.392	1.40	19.99

Table 9. Comparison between Standard Literature and Experimental Values

Property	Literature	Experimental	Unit
Iodine value	65-80	71.02	ml/g
Refractive index at 28°C	13610-13705	13675	-
Boiling point	250-265	264	°C
Melting point	25-30	29	ml/g
Saponification value	175-205	215.98	ml/g
Specific gravity	0.908-0.934	0.927	-
pH	5	5.5	-

Test for Effectiveness of Neem Soap

The test effectiveness of Neem was carried out, after collecting insects and mites identified to be leaf hoppers, aphids, hairy caterpillar, grasshoppers, cricket and cockroaches.

Two leaf hoppers and one grasshopper was put into cage A; two crickets was put in cage B; two hairy caterpillars in cage C; four cockroaches in cage D; black ants in cage E.

And their insecticidal soap was sprayed on the plants the insects were on, and the result over the span of 60 hours (2½ days) was noted and shown below.

Table 10. Experimental Results from Test for Effectiveness of Neem Soap

Sample	Cage A	Cage B	Cage C	Cage D	Cage E
6-12 hrs	Stops feeding	Stops feeding	Peels off the hair on the caterpillar	Stops feeding and malformed wings that cant be fold flat	Stops functioning
12-24 hrs	Defected wings	Stops feeding	The caterpillar stopped moving, it stopped functioning		Death of a few
24-36 hrs	Death	Sops feeding	One caterpillar dies	Two out of the four was dead	Death of more
36-48 hrs	Death	Death	Second caterpillar dies	Death of all	Death of all
48-60 hrs	Death	Death	Death of both	-	-

From the above result, the two grasshoppers and the leaf hopper died within 2 days. The case of the two cricked, they died sequentially after they had stopped feeding for more than 24 hours. For case C, the first caterpillar died may be as a result of being smaller in size or maybe in age for case D, after 36 hours, 2 out of 4 cockraches were still left, but due to the defected wings and the inability to move, they didn't survive; for case E where we have ants,

just few of them died in less than 12 hours while others stop functioning but by the 36-th hours, all were dead.

After this, it was noted that other insects refused to perch on the plants when they were return out side amongst others. This is as a result of the repelling property.

From the result, I come to a sure conclusion that Neem is a slow but sure killer of insects and pests.

Table 12. Result of Factorial Analysis

Time	% Lipid for n-Hexane	% Lipid for Ethanol
2 Hours	42.30 ± 0.00	39.75 ± 0.35
4 Hours	46.00 ± 0.00	46.73 ± 0.04
6 Hours	46.30 ± 0.00	48.70 ± 0.00
8 Hours	46.90 ± 0.00	49.30 ± 0.00

Analysis of oil carried out to determine the interaction between the two factor table above shows that data carrying the same subscript do not differ significantly from each other ($p > 0.05$). Data in the same row carrying different subscript differ significantly from each other ($p < 0.05$).

Using the Duncan multiple variation the subscript were assigned to the data. The data with the subscript a has the least yield but the data with the subscript e which is the extraction with ethanol at 6 and 8 hours produce the highest yield of lipid.

A total of 16 runs where conducted for n-hexane and ethanol solvent. The extract in each case can be compared. The average extract obtained was 45.38% for n-Hexane and that of ethanol was 46.2%. The highest yield of oil comes from extraction using ethanol at 6 hours and 8 hours followed closely by extraction using n-hexane for 8 hours, then the extraction using ethanol for 4 hours, the least yield is by ethanol for 2 hours closely followed by n hexane for 2 hours. However it can be deduced from the result obtained that there is a positive effect of the solvent on the yield and as the time increased, the yield increased. From the entire extraction process a 4^2 that is 4 level 2 factors factorial mathematical model of the order of the form:

$$Y = 9.548 + 0.144X_1 + 0.1931X_2 + 0.1894X_{12}$$

From the model equation, it was observed that solvent type X_2 has the highest positive effect followed by time X_1. The interaction X_{12} shows that time increased with both solvent increases oil yield.

Graph clearly confirmed the observation given from result. From the regression analysis, the correlation coefficient (r) is +0.83 growing that there is a strong and positive relationship between yield time of extraction that is to say as time increases, yield increases.

From the entire extraction process, a 4 level 2 factors factorial mathematical model of the fist order form:

$$y = 9.548 + 0.144X_1 + 0.1931X_2 + 0.1892X_2$$

From the model equation, it was observed that time of extraction X_1 has an positive effect 0.144, type of solvent has an higher positive effect 0.1931 and the interaction X_{12} has a positive effect in the extraction process.

From table 9 there is a slight difference between the experimental result and the literature values this might be as a result of specie of the Neem, impurities in the seed kernel, and the solvent for extraction or the condition at which the experiment were performed.

Conclusions

In conclusion, the method of solvent extraction was used in this project. The solvent used for the extraction were n-hexane and ethanol.

Based on the analysis of the experimental result obtained the quantity of oil extracted from Neem seed was found to be directly proportional to the time of the extraction and the solvent type.

The extract has an acid value of 1.122, refractive index of 1.3675 compared to the standard values of 1.3610-1.3705, boiling point of 258°C, and iodine value of 71.02, saponification value of 215.98 compared to the standard value of 175-205, the specific gravity 0.927 and pH of 5.5.

The difference in the physical and chemical properties of the oil extracted compared to their standard value was as a result of slight impurities present in the seed and some of the solvent.

From the regression and correlation analysis, it can be deduced that the yield of oil increases as the time increases due to the model equation.

References

[1] Bringi, N.V., *Non-traditional Oil seeds and Oil in India*, Oxford & IBH, 1991.

[2] Coulson F. M., Crafts A. S., *Mode of Action of Pesticides*, Academic Press, 1974.

[3] ***, *Neem: A tree for solving Global problems*, A report of the beard on science and technology for international development research council, National Academy Press, 1992.