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Crop Protectant From Pest By Neem (*Azadirachta Indica*) Oil Bioinsecticide

R. E. Nnam, O. I. Okoro, A. N. Oko And C. O. Oji

¹Department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Ebonyi State, Nigeria

²Chemistry unit, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic Unwana, Ebonyi State, Nigeria

Corresponding Author: Nnam Raphael Eze raphelnnam@gmail.com +2348063013921

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Abstract- Agriculture plays an important role in the socio economic status of country because it provides a lot of products like food, biofuel, fibre. However, these products most especially, food is highly threatened by pests hence, farmers have resulted to the use of synthetic pesticides in order to control or manage the adverse effect of pest on crops and stored food produce. These chemical pesticides are both environmentally and humanly unfriending. Therefore, this work seeks to evaluate the use of Neem oil as a bio-pesticide by characterizing the extracted oil. To ensure that the seed used for the extraction was of good quality and ecofriendly, the moisture content (%) and the Ash Content (%) were evaluated. From the result obtained, both quantities were 9.67% and 0.143% for moisture content and Ash content respectively. Further analysis showed that the oil Thin Layer Chromatography analysis yielded 0.7065. This showed that the oil has a high level of azadirachtin, the compound responsible for the insecticide ability of Neem. The water solubility of the oil was 99.93 while the specific gravity, flash point, pH, saponification value, peroxide value, refractive index, acid value and the iodine value were 0.934 g/cm³, 248°, 4.133, 196mgKOH/g, 7(meq/Kg), 1.4567, 14.33(mgKOH/g), and 75.08(mg I₂/100 g) respectively. These values were found to be with the range of Neem oil used in the product of bio-pesticides.

Key word Neem oil, Crop production, Azadirachtin, Protectant, Biopesticide,

INTRODUCTION

Agricultural sector is the life wire of any nation most especially, a developing and agrarian nation like Nigeria. This is because of its role in the provision of food, fibres, biofuels and other relevant products for the wellbeing of human life [1]. Agriculture also plays vital role in improving the socio economic status of country [2, 3, 4, 5]. However, the increase in human population made worst with the unavailability of farming space (land), has led to intensified agriculture to meet the ever increasing demands for food and fiber [6]. Again, food security is highly threatened by the losses that occur during

post-harvest handling, processing, storage and distributions. The losses that can vary between 20-60% [7, 8, 9] are caused by the enemies namely fungi, insects and rodents. They not only damage a considerable part of the stored product but also lead to post-harvest deterioration like changes in the odor, taste, appearance and nutritive value [10, 11, 12]. These effects cause a lot of economic losses to both the farmers and the nation at large.

Due to the fact that insects are always present in field crops during cultivation and in storage of the food produce [13], farmers have resulted to the use of chemical fertilizers and pesticides to improve food availability and crop yielding capacity [Liu et al., 2004]. These chemicals and fertilizers are not environmentally friendly as they are the primary causes for the reduction in soil fertility and degradation [14].

Pesticides are substances used to prevent, destroy, repel, attract, sterilize, or mitigate pests [15]. These odoriferous substances which are essential oils are rich sources of biologically active secondary metabolites such as alkaloids, phenolics, and terpenoids [16, 17]. Plants such as catnip, basil, artemisia, borage, dahlia, ginger, hyssop, chrysanthemum, lime, black pepper, clove, neem and garlic contain bioactive metabolites, which show antifeedant, repellent and toxic effects on a wide range of insect pests [18, 19].

[20] noted that interest has surged in the use of natural compounds extracted from various aromatic plants, as an alternative to agrochemicals for pest control in agriculture and food storage. The need to use biopesticides as an agrochemical alternative is because bio-insecticides are easily biodegradable, effective on pests control, minimizes pesticide residues, ensure safety of the consumers of the treated grains and the environment. Further, the production of organic extracts of plant origin for pest control may be easier and less expensive than the synthesis of some complex chemical formations [21, 22, 23].

The Neem tree (*Azadirachta indica*), and popularly called 'Dogoyaro tree' in the Northern part of Nigeria, is a tropical evergreen tree originally from India and now found throughout the tropics, particularly in the dry regions of Africa [24]. Each part of neem tree has some medicinal properties and it is commercially exploitable. Neem oil can be gotten from different parts of the tree such as the leave, flower and the seed. These oils' bio-active compounds are at least 100 and triterpenes constitute one of the major constituent while the other constituents include meliantriol, nimbin, nimbidin, nimbinin, nimbolides, fatty acids and salannin. Triterpenes also known as limonoids contains azadirachtin as the most important biologically active compound and it causes about 90% of the effect neem oil has on pests [25, 26]

The essential oil obtained from the leave and flower of Neem tree only contains 0.08% of volatile oil but mainly caryophyllene (85%) [27]. The main source of volatile oil gotten from neem comes from the Neem seed as it is mainly composed of essential oil and fatty acids [28].

The fatty acid composition of Neem seed oil is oleic acid (50%–60%), palmitic acid (13%–15%), stearic acid (14%–19%), linoleic acid (8%–16%), and arachidic acid (1%–3%) [27, 29] also reported the Free fatty acid of Neem flower oil to be palmitic (31.76%), linoleic (18.57%), linolenic (12.64%), oleic (9.74%), arachidonic (7.38%), and docosatrienoic (5.7%).

[30] noted that the oil is brownish yellow, non-drying oil has an acrid taste and unpleasant odor. It is considered a contact insecticide [31] and shows diverse biological effects on insect that help in controlling pests. These biological effects include repellent, feeding and oviposition deterrent, affecting hormone function in juvenile stages, reducing ecdysone, deregulating growth, altering development and reproduction, suppressing fertility, sterilizing, repelling oviposition, and disrupting molting processes [32, 33]. Among the botanical insecticides currently marketed, neem oil is one of the least toxic to humans and shows very low toxicity to beneficial organisms while targeting a wide range of pests [34, 35, 36, 37, 38].

Related Work

In a related work, [39] observed that the physicochemical properties of Neem oil varies with location. According to the research, the variation in the characteristics of neem oil is as a result of difference in soil type, the genotype of the trees and climatic conditions such as temperature and rainfall. The work reported moisture content 9.533 for neem oil gotten from Maroua location and 9.470 for neem oil gotten from Zidim location. However, the reported noted that the standard moisture content for high quality neem oil is between the ranges of 9.04-10. They also reported volatile matters to be 0.07 for neem oil gotten from Maroua and 0.83 for neem oil gotten from Zidim respectively.

[40] in their work reported amongst others the following physicochemical properties pH (5.0 and 4.0), boiling (230 and ND) and melting points (9.0 and ND) for two samples gotten from two different locations Sudan (OSD) and Malaysia (OMY). From their findings, the values were slightly different from one another but fall within the range specified. The colour of the oil extract was brownish-yellow for oil from Sudan (OSD) and greenish brown for oil gotten from Malaysia neem tree (OMY) while the odour was peanut for OSD and combination of peanut and garlic for OMY [41] reported a pH of 4.63, refractive index of 1.463 and a flash point of 260°C. The work further reported that the Chromatographic and spectrophotometric analysis reveals that the Neem Seed Kernel Extract contains four methyl esters close to the structure of azadirachtin. These methyl esters are active, easily distilled and vaporized into the air; hence their effectiveness in causing mosquito repellency action of the extract. 11, 14-Eicodadienoic Acid is the most active because of the presence of two double bonds. The standard thin layer chromatography value of Azadirachtin is 0.70 [42].

3. MATERIALS AND METHODS

50kg of matured neem seeds were sourced from Unwana in Afikpo North Local Government Area of Ebonyi State, Nigeria and processed in the Department of Food Technology Akanu Ibiam Federal Polytechnic Unwana Afikpo North Local Government Area of Ebonyi State, Nigeria. Sourced Neem seeds were cleaned, dried in the sun and sorted to remove any unwanted material. The dried seeds were shelled and winnowing to obtain clean kernels. The dried sample was then milled by an attrition miller to make it ready for oil extraction. Oil was extracted from the crushed neem kernels by Soxhlet extraction method using hexane as the solvent. An organic pesticide was formulated by diluting 0.25ml of neem oil by 20litres of water which can be applied directly on crops by spraying.

A. *Quality characterization*

Moisture content of Neem seed

The percentage moisture in the kernel was calculated using the following:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where: W₁ = Original weight of the sample before drying; W₂ = Weight of the sample after drying.

Ash content of Neem seed

Ash content of the organic sample is determined by the method described by [43] and calculated as:

$$\text{Ash Content (\%)} = \frac{W_3}{W_2} \times 100 \quad (2)$$

Where W₂ = Ash(g); W₃ = Sun dried specimen (g)

$$\text{Organic matter (\%)} = 100 - \text{Ash content (\%)} \quad (3)$$

Determination of percentage (%) yield

The percentage (%) yield was calculated using the equation (4) [44]

$$\text{Percentage yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of sample (Neem seed)}} \times 100 \quad (4)$$

Thin Layer Chromatography (TLC) Analysis

The thin layer analysis was carried out according to the method described by [45]. 10g of neem oil was dissolved in ethanol. The solution was spotted on a TLC plate and placed in a beaker consisting Iso-propyl alcohol: hexane (2.5:17.5) ratio as mobile phase. It was run till 3/4th of the plate distance, and then placed in Iodine chamber. Two pale yellow colored spots were observed with the upper spot indicating Azadirachtin while the below spot indicating nimbin [46]. The difference in colour can be attributed to the density variation between nimbin and Azadirachtin. The R_f value which simply indicate the presence of Azadirachtin in the extract is calculated:

$$R_f = \frac{\text{Distance travelled by Component}}{\text{Distance travelled by Solvent}} \quad (5)$$

Determination of Specific gravity

Density of the sample is directly proportional to unsaturation and inversely to molecular weight. The determination of density was done according to the method described by [47] and calculated using the equation stated by [45]:

$$\text{Density of sample} = \frac{W_1}{V_1 - W_2/\rho} \quad (6)$$

W₁ - Mass of sample (g); V₁ - Volume of Pycnometer (cm³); W₂ - Mass of Hexane (g) and ρ - Density of Hexane, (g/cm³)

From equation (6) the specific gravity of the sample can be calculated the density is divided by the density of water.

Determination of refractive index:

According to the method reported by [48].

Determination of Flash point:

The flash point was determined by the method described by [49]

3.5.5. Water solubility

The water solubility of the extracted oil was calculated as follows [45]:

$$\text{Water Solubility} = \frac{W_1 - W_2}{V_w} \times 100 \quad (7)$$

Where: - W_1 – Mass of sample before dissolution (g); W_2 – Mass of sample after dissolution and (g); V_w – Volume of solvent (i.e. water) (mL)

Determination of pH value

The pH of the extracted oil was carried with a pH meter.

Determination of Saponification value:

The saponification value (I_s) is the number of milligrams of KOH (Potassium hydroxide) needed to saponify 1g of fat [39]. It is determined using the colored indicator (phenolphthalein) according to the method described by [50]. The saponification value (I_s) was calculated according to the formula:

$$I_s = 56.1 \times N (V_o - V_1)/m \quad (8)$$

where 56.1 is the molecular weight of KOH, N is the normality of the HCl solution, m is the mass in g of the test sample, V_o is the volume of HCl used for the blank test, V_1 is the volume of HCl used for the test portion.

Determination of acid value:

The acid value (I_A) is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of material [39]. It was determined using the colored indicator (phenolphthalein) according to the method described by [49] as:

$$I_A = 56.1 \times N_{KOH} \times (V_0 - V_1)/m \quad (9)$$

where V_0 is the volume of the standard KOH solution used for the blank test, V_1 is the volume of the standard solution of KOH used for the sample, N_{KOH} is the normality of the KOH solution, m is the mass in grams of the test sample, 56.1 is the molar mass of KOH.

Determination of peroxide value:

The peroxide value (I_p) was determined by the colored indicator method using starch paste as a colored indicator [51]. The peroxide value expressed in milliequivalents of active oxygen per kg of fat is given by:

$$I_p = 1000 \times N (V_0 - V_1) / m \quad (10)$$

In Equation (8), M is the mass in g of the test sample, V_o is the volume of the thiosulfate solution used for the blank test, V_1 is the volume of the thiosulfate solution used for the sample, N is the exact normality of the sodium thiosulfate solution used.

Determination of iodine value:

The iodine value (I_I) which indicates the degree of unsaturation of the fatty acids in the oil was determined by the Wijs reagent method described by [52]. The iodine number was calculated through the relation:

$$I_I = 12.69 \times T (V_0 - V_1)/m \quad (11)$$

In Equation (5), V_0 is the volume of 0.1 N thiosulfate solution used for the blank test, V_1 is the volume of the thiosulfate solution used for the sample, T is the exact title of the thiosulfate solution, m is the mass in g of the test sample.

RESULTS AND DISCUSSIONS

Table 1: Physiochemical Properties of Extracted Neem Oil

S/N	Properties	units	Values
1.	Yield	%	44.6
2.	Moisture content (%)	%	9.67
3.	Ash Content (%)	%	0.143
4	Thin Layer Chromatography		0.7065
5	Specific gravity	g/cm ³	0.934
6	Water solubility	-	99.93
7	Flash point	⁰ C	248
8	pH	-	4.133
9	Saponification value	mgKOH/g	196
10	Peroxide Value	meq/Kg	7
11	Refractive index at 40°C	-	1.4567
12	Acid value	mgKOH/g	14.33
13	Iodine value	mg I ₂ /100 g	75.08

Moisture content of Neem seeds

Moisture content of the seed indicates the extraction kinetic of the oil in the seed. This is because the level of diffusivity of the solvent towards the surface is dependent on the moisture content of the seed as moisture blocks the diffusivity of solvent to the seed surface thereby stopping the extraction of the bio-active compounds present in the seed as oil. According to [53], neem oil with high moisture content yield less bio active substance during extraction. [39] further noted that the standard range of moisture content for high quality neem oil is 9.04-9.10. From the result obtained from this research, the moisture content of the oil produced is recorded at 9.67%. This is slightly higher than the standard obtainable value for moisture content. However, it is in agreement with earlier work reported by [39, 54] and lower than that obtained by [45, 53]. From the result, high level of biologically active compounds of the neem seed was extracted and contained in the oil. Therefore it will be a good source of biopesticide for crops protection and stored grains and legumes.

Ash contents of Neem seeds

The non-volatile and non-decomposable compounds which are harmful to the environment are represented by the ash content of the material. Therefore, the ash content of neem seed simply represents the nonorganic matter or compounds of neem seed. The ash content which is mostly metallic oxides cause different kinds of health issues on different floral and faunas. The higher the quantity of nonorganic material the more the substance is not ecofriendly. Therefore, the value of the ash content should be as small as possible. From table 1, the ash content of the neem seed is 0.143% of total weight of the matter which therefore gives the organic content of the neem seed to be 99.857%. This shows that neem seed product (oil) is biodegradable and environmental sustainable and can be used comfortably on the environment with little or no side effect to the environment and its living organization. This finding is lower than the 10% ash content reported by [55], and the 12% reported by [56] but agrees relatively with the 0.6% reported by [57] and the 0.183% of [45].

Percentage Oil Yield

The efficiency of the extraction method is evident in the quantity of oil extracted from the yield. From literature, the quantity of extractable oil from the dried seed mass can be as high as 25-49% of the dried mass [58, 59, 60]. From the result obtained, the extraction of the oil from Neem seed using Soxhlet extraction method was viable as a yield percentage of 44.6% was recorded. This result was similar to the result obtained by [61, 62, 63] that used the same hexane as the solvent for extraction and reported 43.71%, 44% and 43.48% respectively. It was however, higher than the result reported by [55, 64, 65] 18.00%, 34.85% and 24.8% respectively.

Thin Layer Chromatography (TLC) Analysis

The Thin layer Chromatography (TLC) is a qualitative measure of the presence of certain compounds with respect to other compounds found simultaneously by dissolving in a mobile phase. In other words, it is an indication of the qualitative characteristics of the compound i.e. azadirachtin with respect to other compounds or elements extracted together which can possibly dissolve in the same mobile phase [45]. The standard TLC value of Azadirachtin is 0.70 [42]. Table 1 shows that the standard R_f value reported in this research is 0.7065. This near proximate value to the literature obtained can be attributed to errors due to measurement and calibration of instrument. This result reported in this work agrees with works of [45, 66].

4.2.3. Specific gravity

The specific gravity is an important factor in the characterization of oil as it describes the heaviness of the oil compared to that of water, and it is expressed without units [67]. The result found from the analysis as can be seen from table 1, shows that the specific gravity of the oil is 0.934. This value agrees with the standard specific gravity of neem oil which is between 0.905-0.975. It also agrees with earlier report of [40] that obtained 1.06 for OSD and 0.95 for OMY densities, [67] reported 0.887, [39] reported 0.833 and 0.850 for oil extracted from neem seed gotten from Maroua and Zidim respectively while [68] reported 0.8363 to 0.8432 for edible vegetable oil.

Refractive Index

The refractive index for the oil reported in this work was 1.4567. This shows that the oil has high number of carbon atoms [69]. This result agrees with the result reported by [58].

Flash point

The flash point is the minimum temperature at which a lubricant's vapour, when mixed with air, will ignite but will not continue to burn. The flash point can also be used to determine the transportation and storage temperature requirements for lubricants. Lubricant producers can also use the flash point to detect potential product contamination. A lubricant exhibiting a flash point significantly lower than normal 243 °C while a lubricant with a flash point less than 38 °C will usually require special precautions for safe handling [70]. In this work, the flash point was 248 °C. This is in agreement with [70, 71].

4.2.5. Water solubility

Water solubility of neem oil simply investigates the ability of *Azadirachta indica* to dissolve in water since it will be mixed with water to form a pesticide that can be sprayed on crops. Solubility is defined as the amount of substance that passes into solution to achieve a saturated solution at constant temperature

and pressure. Solubility is expressed in terms of maximum volume or mass of the solute that dissolve in a given volume or mass of a solvent. This property is very crucial for this test. Since, the final extract is going to be used by dissolving in water and spray on field, it should not left a residue over the crop. The more soluble mater in the solvent means the more dispersing material on the environment. Under 20 minutes at a temperature of 25°C, the result showed that the water solubility of the extracted neem oil is 99.93% of the total oil mass (table 1). This result shows that the extracted oil can easily form a homogenous mixture with water to become a pesticide.

4.2.7. pH value

The pH value of a substance is the rate at which hydrogen ion is released when a substance is dissolved in water. In the case of *azadirachtin*, this rate can affect the environment as well as the end user if it goes to much high or low [72] as the liberation of the ions might cause soil infertility depending up on the natural behaviour of the soil. The pH value of azadirachtin has great implication on the target pest depending on the physiological and *Extraction of azadirachtin* enzymatic pattern [73]. The result of the pH value of azadirachtin was observed to be 4.133 in this work.

Saponification Value

Triglycerides' molecular weight present in oil is shown by the saponification value. It also shows the level of oil oxidation when store as it relates the unsaturated free fatty [55, 74, 75]. The saponification value for the analyzed oil was 196 (mgKOH/g). This value was higher than what [47, 55, 76, 77] reported in their works which were 190.84mgKOH/g, 140.00 mg KOH/g, 91 mg KOH/g and 148.8 mg KOH/g respectively. It was however lower than the 200.090 mg KOH/g reported by [39].

Acid Value

The acid value has an adverse effect on the quality of fats present in oil as it measures the breakdown of the triacylglycerol into free fatty acids [78]. According to literature, the acid value (oleic acid) of oil for food applications should not exceed 4% [79, 80]. The acid value reported in this work showed that was determined to be 14.33mgKOH/g above the recommended value for edible oil. This fact was supported by [39, 55] that reported 9.163mgKOH/g and 11.20mgKOH/g respectively. [77] also reported an acid value of 22.37mgKOH/g while [76] reported 30.8 mg KOH/g.

Peroxide Value

The peroxide value indicates the extent of oil oxidation. This oxidation is caused by lipolytic enzymes such as peroxidase and lipoxygenase [81]. It therefore implies that Peroxide value measures the freshness of the lipid matrix and oxidation of oil especially during storage [75, 82]. It was observed that the neem oil sample has a peroxide value of 8.3 (meq/Kg). This value is higher than the 6.9 meq/Kg reported by [39].

Iodine Value

The iodine value is a measure of the unsaturated fatty acids present in the oil. For Neem seed oil the iodine value was found to be than many seed oil [47, 55]. This high iodine value shows neem oil as unstable because it has high level of unsaturated fatty acid content and the higher the iodine value, the less stable the fat becomes [55]. Result in table 1 shows that the analyzed neem oil has an iodine value of 75.08 (mg I₂/100 g). This value is value is similar to result obtained by [39, 47, 77]. It is however lower than the result reported by [55] which was 123.44(mg I₂/100 g).

CONCLUSION AND RECOMMENDATION

The aim of the research which is to extract and characterize Neem oil from the seed so as to evaluate its ability as a bio-pesticide was achieved. From the result obtained, the moisture content of the dried seed was low for a seed (9.67). This resulted in a high oil yield of 44.6%. The extracted oil when analyzed showed that it is a good bio-pesticide even when mixed with water. This is because the Thin Layer Chromatography value implied that the major constituent of Triterpenes (azadirachtin) is very much present. The ash content analysis proved that the neem oil is ecofriendly as it has 99.758% organic content. It was also observed that the extracted oil will form a homogenous solution with water when mixed as it has high water solubility. The physiochemical properties analyzed showed that the oil is of high quality. It can therefore be inferred that bio-pesticide can be produced from neem oil. It is recommended that further research should be carried out on the effect of extraction method on the yield and quality of neem oil. Furthermore, alternative solvents for extraction as well as the effect of drying rate should be investigated.

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