Pesticide Activity Improvement of Azadirachtin for Pests Control in Zambia

Chibika Jacoba[1], Maseka Kakoma[2]

[1]*School of Mathematics and Natural Science, Copperbelt University, Kitwe, Zambia*
[2]*Chemistry Department, Copperbelt University, Kitwe, Zambia*

***Abstract*: Agriculture has proven to be one of the major economic drivers of any country's economy. Most developing countries have been failing to grow their economies through agriculture due to a number of problems; one of these problems is attributed to the damage pests cause on the crops. Examples of pests being tutaabsoluta and army worms which affect tomato and maize crops, respectively. Botanical pesticides have proved to be an effective method of protecting crops as they are cheaply sourced and their pesticide efficacy can be improved.The efficacy of an improved/ modified pesticide of azadirachtin was determined in comparison with its original form on two different types of pests; viz., on army worms and tutaabsoluta. This was achieved by spraying army worms and tutaabsoluta with four solutions of azadirachtin obtained from the commercial source, extracted one, modified commercial one and modified extracted one. Modification of azadirachtin was done by replacing ester groups with amide groups. Unmodified commercial azadirachtin gave mortalities of 00.0% and 28.6% on army worms and tutaabsoluta respectively while its modified form gave mortalities of 28.6% and 42.9% on army worms and tutaabsoluta, respectively. Unmodified extacted azadirachtin gave mortalities of 28.6% on both army worms and tutaabsoluta while its modified form gave 85.7% and 100 % mortalities on armyworms and tutaabsoluta respectively. Botanical pesticides are a potential remedy to a lot of crop pests in our country and their efficacy can be made better by structural modifications.**

***Keywords:* Azadirachtin, Aniline, TutaAbsoluta, Army worm.**

I. INTRODUCTION

Agriculture has proved to be one of the major drivers of a country's economy. In Zambia for instance the dependence on copper and other minerals has proved to be a failed idea as the exported mineral products at times fetch low prices. This is attributed to a lot of factors which include failure by the London Metal Exchange whose mandate is to determine the price of the country's copper. According to York (2015) the economy for Zambia tumbled in the recent past due to declining prices of copper at the international market.

As a result of such draw backs, the government of the republic has made tremendous strides in moving its economy from a copper dependency economy to an agriculture dependency economy

According to the national budget presentation made by Mr Alexander Chikwanda, minister of Finance Alexander Chikwanda, in his 2016 National Budget speech presented to Parliament last October said 'agricultural diversification also remains top on the agenda as demonstrated by the inclusion of other food crops such as cassava,sorghum, millet, sweet potatoes, beans and groundnuts under the farmer input support programme (FISP)'. He further said, to ensure the sector enhances crop production, income generation and economic diversification., Government was to redouble its efforts to expand and develop the agriculture sector by investing in the development of irrigation that year and beyond, with an investment of over K56 million to irrigation programmes.

However, in the quest to improve the country's economy through agriculture, there exist a lot of draws backs such as unfavourable farming seasons characterised by poor rainfall as it was the case with Zambia in most of her districts in 2018/2019 farming seasons and lack of adequate funding towards agriculture.

Besides that, there exists a lot of pests destroying a lot of the crops. These pests include aphids, red spider mite, cutworms, thrips and many more. The latest pests in Zambia noticed in 2016 that are a harm to food security aretutaabsoluta which destroy tomatoes and army worm which is known to cause mass destruction to the production of maize a staple food for the country.

Tutaabsoluta whose common name is tomato leaf miner originated in South America. This was then reported in Europe in 2006 and was noticed in Africa in 2006. The commonly affected plant by tutaabsoluta is tomato. Other crops affected include potatoes, eggplants, tobacco and Cape gooseberry.

According Jan Hendrik(2016) the peststutaabsoluta and army worm undergo four major stages in its life cycle and these are egg, larvae, pupae and moths (Adult).Both army worm and tutaabsoluta are known to develop resistance to synthetic pesticides such as fubendiamide, emamectin benzoate and chlorofenapyr.It is because of this that this study aims at addressing the problems associated with destruction of crops such as maize and tomatoes by pests through an improvement of a natural pesticide azadirachtin extracted from the neem tree by introducing amide groups.Azadirachtin is used in this study because of its availability as it can be extracted from a local neem tree.

II. MATERIALS AND METHODS

2.1 Collection and preparation of neem fruits

The neem fruits were plucked using hands from a neem tree situated near Ndola's High court on the 15th of April, 2020. The collected neem seeds were depulped, Depulping is a process of removing the seed coat and the pulp from the neem seed. This was done using a mortar and pestle at Northern Technical College. Depulped neem seeds were then washed using tap water to remove the seed coats and the pulps.

2.2 Obtaining and pounding of neem kernel

The obtained dried neem seeds were broken using a hammer and block support so that neem kernels could be obtained. The resulting neem kernels were then dried for a period of two weeks. These were then pounded using a mortar and pestle so that a fine powdered sample could be achieved. The neem kernels were then pounded using a mortar and pestle so that a fine powdered could be achieved.

2.3 Extraction of neem oil from powdered neem kernels

58.4151g of the sample of powdered neem kernel was weighed using a weighing balance, Azadirachtin pesticide was then extracted using Soxhlet extraction with n-hexane and ethanol in a 1:1 ratio as a solvent for one hour thirty minutes. The total volume of the extracting solvent was 450 cm^3 at a temperature of 90 ºC. The excess volume of the solvent was of n-hexane and ethanol was removed using a simple distillation using a temperature of 95 ºC for a period of an hour

2.4 Characterization of commercial and extracted neem oil using Shimadzu 2010 HighPerformanceLiquid. Chromatography (HPLC).

20µL of the sample aliquot of commercial and extracted neem oil were injected into a C-18 column of internal diameter 4.60mm and 250mm length, then a mobile phase composing of aceto nitryl: methanol: triethylamine (60:40:1) was run at a flow rate of 1 mL/min for a period of 20 minutes at room temperature for each sample. The absorption was detected at 210 nm wave length using a UV-Vis detector.

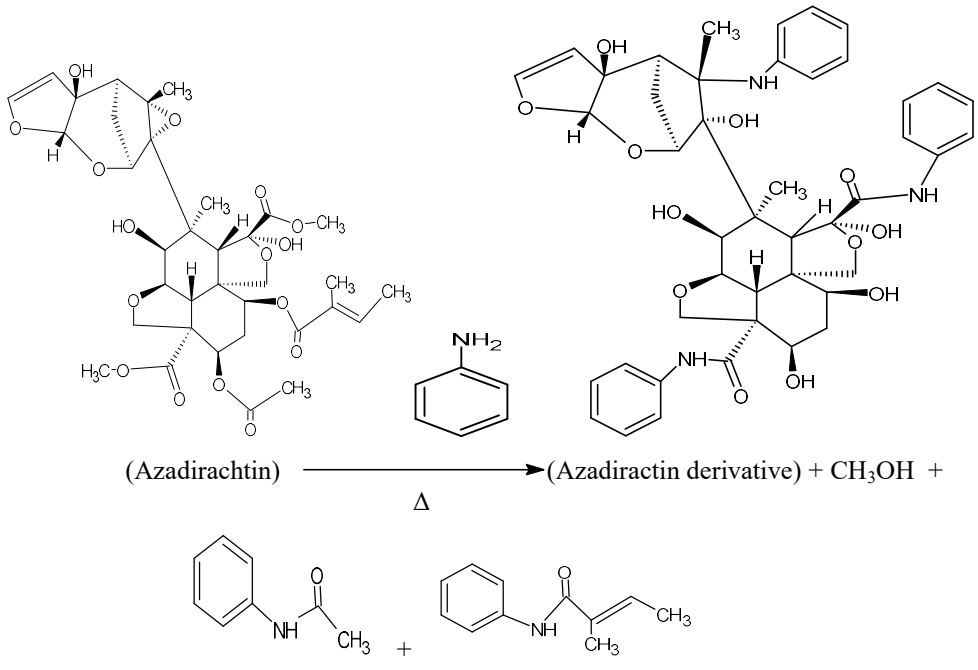
2.5 Determination of azadirachtin from in the need oil extract using Hach DR 2800 UV-Vis Spectrophotometer.

Volumes of 2 mL, 3 mL, 4 mL, 5 mL and 6 mL of the standard azadirachtin were placed

2.6 Pesticide improvement

To a volume of 2 mL of the extracted sample, 20 mL of aniline was added followed by heating on a hot plate for a period of 1 hour 30 minutes in a fume hood and a dark-brown solution was observed in a reaction mixture from an initial colour light-brown. This procedure was also observed using a solution of a commercial pesticide containing azadirachtin. The reaction scheme is given below;

The reaction scheme is given below;



2.7 Characterization of modified commercial and extracted Neem oil using High Performance Liquid Chromatography (HPLC).

20 mL of the sample aliquot of commercial and extracted neem oil were injected into a C-18 column of internal diameter 4.60mm and 250mm length, then a mobile phase composing ofaceto nitryl: methanol: triethylamine (60:40:1) was run at a flow rate of 1 mL/min for a period of 20 minutes at room temperature for each sample. The absorption was detected at 210 nm wave length using a UV-Vis detector.

2.8 Determination of azadirachtin from in the need oil extract using UV-Vis Spectrophotometer

Volumes of 2 mL, 3 mL, 4 mL, 5 mL and 6 mL of the standard azadirachtin were placed in five different 50 mL volumetric flask, followed by an addition of ethanol: n-hexane as a diluent to the mark and the absorbance of each determined using a UV-Vis spectrometer at a wavelength of 542nm. This was done in order to obtain a calibration curve. An aliquot of 3 mL of the unknown sample was measured using a 5 mL pipette and this was then transferred into a 50 mL volumetric flask and addition of ethanol: n-hexane as a diluent to the mark. The absorbance of the unknown was also determined using a UV-Vis spectrophotometer at a wavelength of 542nm wavelength using distilled water as a blank to zero the machine. The concentration of the unknown was determined by the equation of the calibration curve.

2.9 Characterization of modified commercial and extracted neem oil using Agilent 5975 Technologies Gas Chromatography Mass Spectrometry (GC-MS).

5 mL of the modified pesticide was washed using a mixture of ethanol andwater in the ratio 1:1 (50 mL of ethanol and 50 mL of water) followed by separating the organic layer from the aqueous layer, using the organic layer the pesticide was recovered through a simple distillation at a temperature of 80 ºC. Using the particle-free diluted pesticide (10 µL) was then taken in the GC-MS which used an auto sampler

The Model Agilent 5975 Technologies GC- MS was equipped with a fused silica capillary column (30m×0.25i.d.film thickness 0.25m) with an electron ionization system with ionization energy 4.5 eV for the detection of compounds. Inert helium was used as a carrier gas at a constant flow rate of 1ml/min. The oven temperature was programmed started from 40 °C for 2 minutes then this was elevated to 100 °C Celsius at 20°C/min for 3 minutes, then and finally raised to 250°C at 20°C/min for 7.5 minutes.

2.10 Evaluation of pesticide efficacy

To compare the pesticide efficacy of the modified pesticide of azadirachtin to that of a natural one;

2 mL of commercial azadirachtin pesticide was dissolved in 500 mL of tap water and the mixture was shaken. This was then sprayed on two petri dishes, one containing 7 larvae of tutaabsoluta and the other petri dish containing 7 larvae of army worm. The specimens were cultured in a way that the army worms were fed with fresh maize leaves while the tutaabsoluta were fed with fresh tomato leaves and the experiment was allowed to stand for 1 hour 30 minutes at room temperature. Similarly, 30 mL of a modified commercial pesticide of azadirachtin was dissolved in 500 mL of tap water and mixture together through shaking and this was sprayed on two petri dishes one containing army worms and the other one containing tutaabsoluta both prepared as explained above.

An aliquot of 1.5 mL of an extracted pesticide of azadirachtin was dissolved in 500 mL of tap water and the solution was shaken and then sprayed on two different petri dishes; one containing army worms and the other one containing tutaabsoluta prepared as explained earlier, the experiment was also allowed to stay for a period of 1 hour 30 minutes at room temperature. Similarly, 30 ml of a modified extracted pesticide of azadirachtin was dissolved in 667 mL of tap water and the mixture was shaken and this was then sprayed on two petri dishes one containing army worms and the other one containing tutaabsoluta prepared as earlier explained. The experiment was also let to stand for a period of 1 hour 30 minutes.

Lastly two petri dishes one containing 7 caterpillars of army worms and the other one containing caterpillars of tutaabsoluta were let to also stand for a period of 1 hour 30 minutes but without being sprayed anything. From all the eight petri dishes, the number of dead army worms and the number of dead tutaabsoluta were counted and tabulated data was obtained leading to the determination of percentage mortality in each case. The percent mortality was determined using the formula below;

$$\% \text{ Mortality} = \frac{\text{No. of died larvae}}{\text{Total No. of larvae}} \times 100$$

III. RESULTS

Table I: Mass of prepared sample

Mass of filter paper + neem cake	59.9837g
Mass of an empty dry filter paper	1.56868g
Mass of the Sample	58.41502g

Table II: Table for Calibration curve

S/N	Volume of the known used	Absorbance
1.	2 mL	0.333
2.	3 mL	0.610
3.	4mL	0.858
4.	5 mL	1.748
5.	6 mL	2.525

Table III: Table for absorbance of the unknown

No. of Samples	Vol. used	Absorbance
1	3mL	1.314

Concentration of Azadirachtin from neem oil was determined using the calibration curve below.

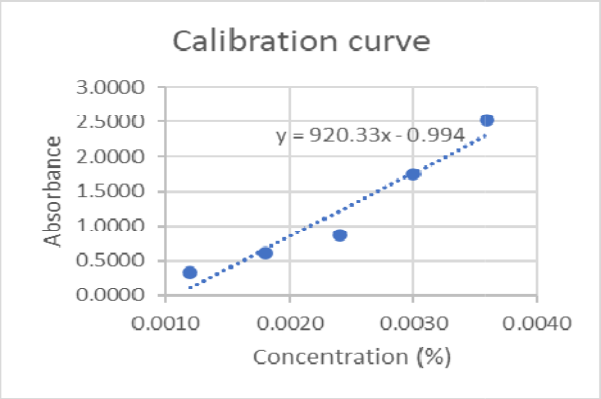


Figure 5: Calibration Curve

Concentration of azadirachtin in a dilute solution was determined from the calibration curve to be 0.00250779612.

To find concentration of azadirachtin in the extract the formula below was used;

$M_1.V_1 = M_2.V_2$ and this was found to be 0.04

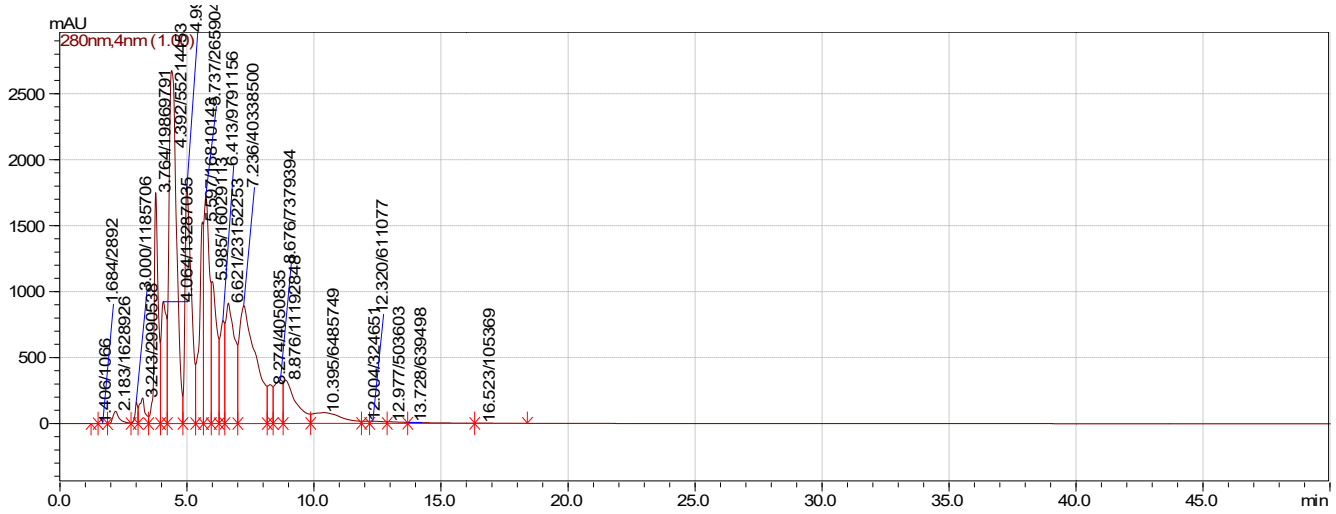


Figure 1: Characterization of commercial neem oil using High Performance Liquid Chromatography

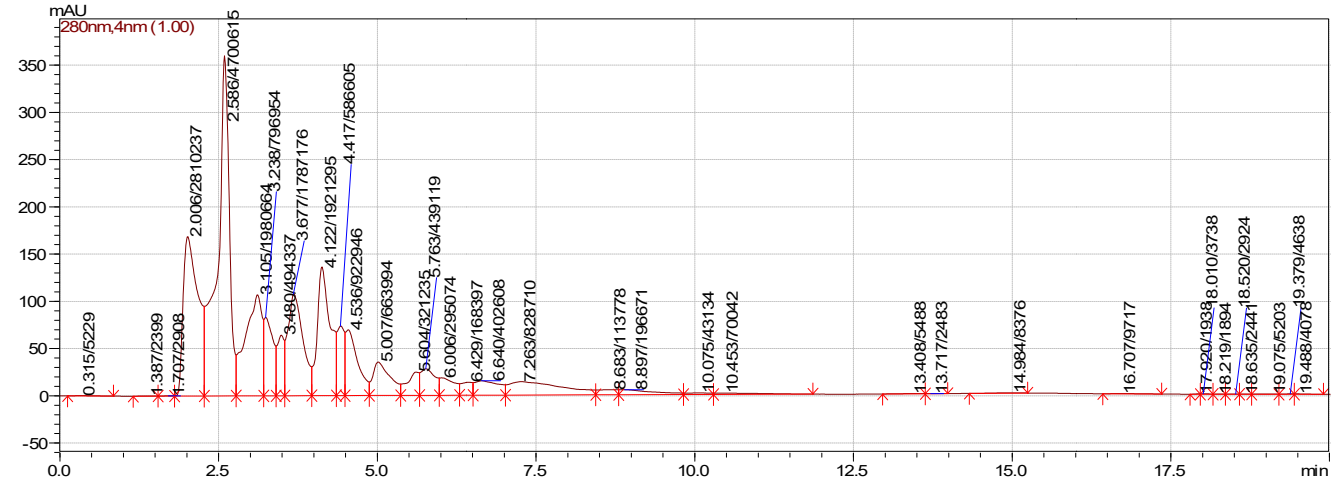


Figure 2: Characterization of extracted neem oil using High Performance Liquid Chromatography

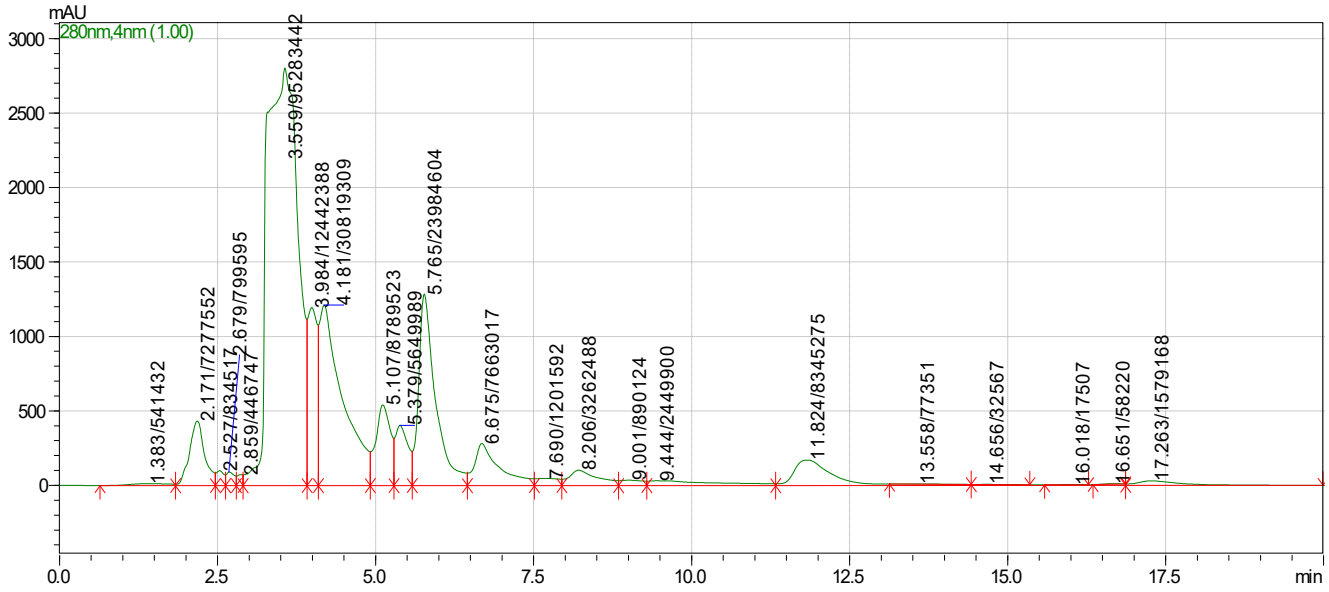


Figure 3: Characterization of modified commercial neem using High Performance Liquid Chromatography.

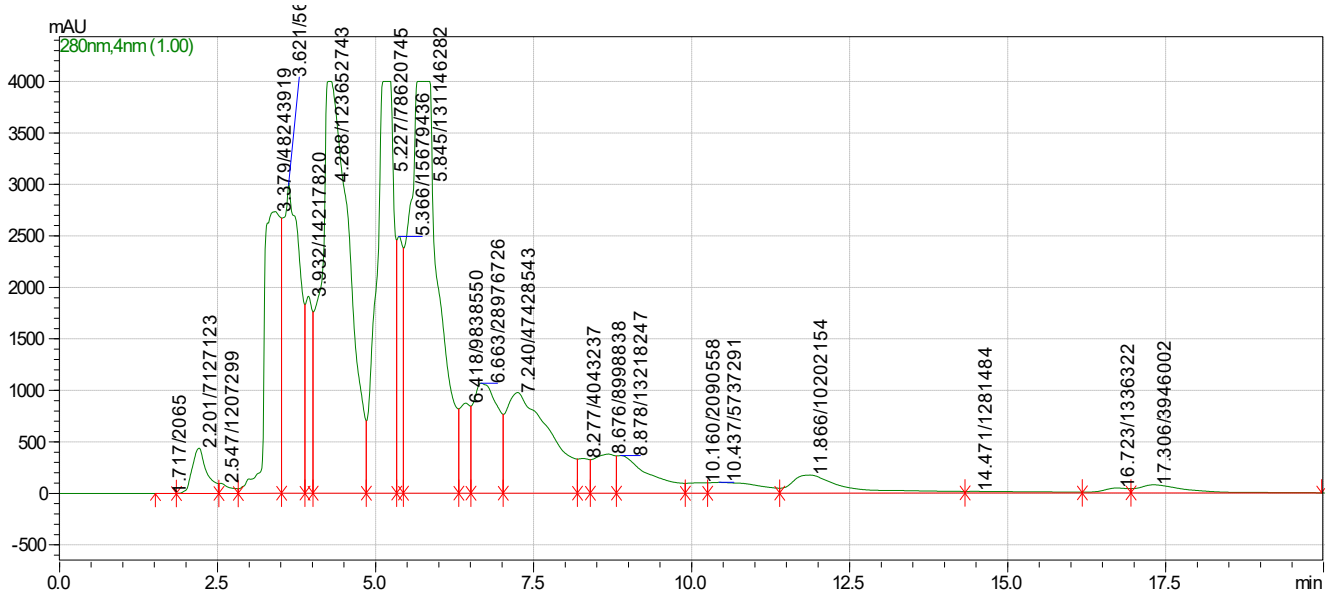
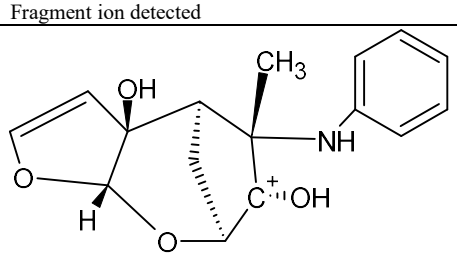
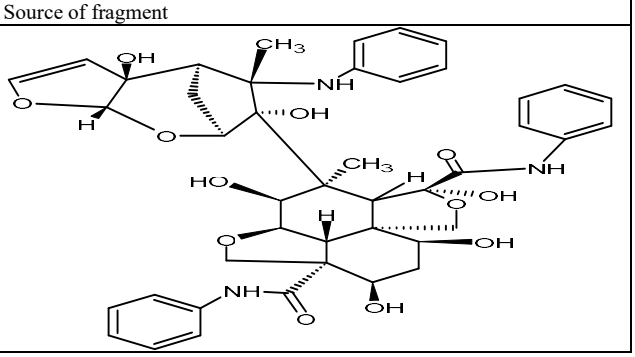


Figure 4: Characterization of modified extracted neem oil using High Performance Liquid Chromatography

Table 1: Characterization of azadirachtin in commercial and extracted neem oil using Gas Chromatography Mass Spectrometry (GC-MS)

m/z	Fragment ion detected	Source of fragment
286		

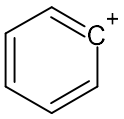
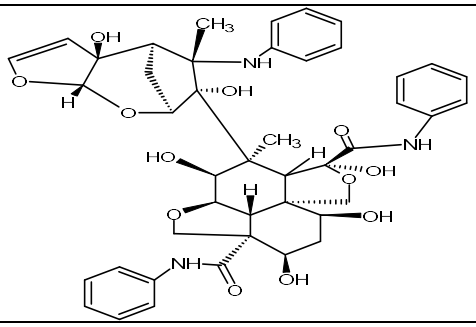
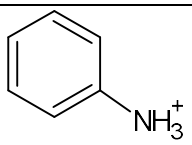
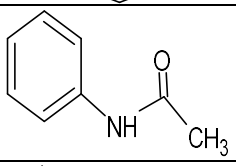
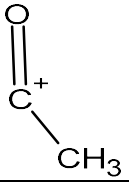
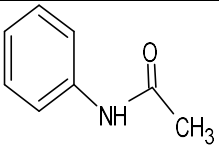
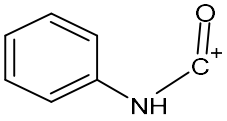
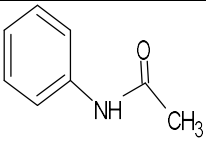
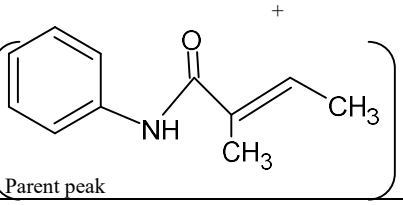
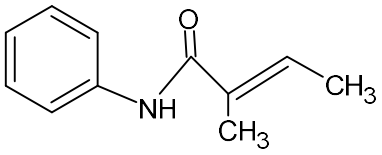
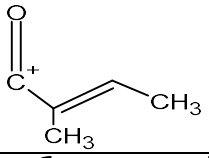
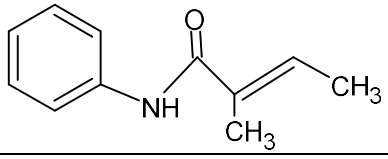
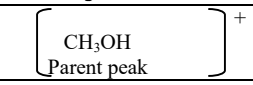
77		
93		
43		
121		
135	 Parent peak	
83		
32	 CH_3OH Parent peak	CH_3OH

Table IV: Pesticide efficacy for commercial, extracted modified and unmodified azadirachtinonarmy worms

	No. of larvae in commercial pesticide	No. of larvae in modified commercial pesticide	No. of larvae in extracted pesticide	No. of larvae in modified extracted pesticide
Live ones	07	05	05	01
Dead ones	00	02	02	06
% Mortality:	00.0%	28.6%	28.6%	85.7%

Table V: Pesticide efficacy for commercial, extracted modified and unmodified azadirachtin on tutaabsoluta

	No. of larvae in commercial pesticide	No. of larvae in modified commercial pesticide	No. of larvae in extracted pesticide	No. of larvae in modified extracted pesticide
Live ones	05	04	05	00
Dead ones	02	03	02	07
% Mortality:	28.6%	42.9%	28.6%	100%

IV. DISCUSSION

The application of plant-based pesticides has been in use as an alternative protection for plant because of their minimal negative effects on human, wildlife and aquatic life. Botanical (natural) insecticides have long been a subject of research for some time in an effort to develop alternatives to conventional insecticides or synthetic insecticides. For now, several insecticides from various plant extracts are used around the world.

Azadirachtin is one of the insecticidal ingredients found in the neem oil, an extract from neem leaves or neem seed kernels and it is a naturally occurring substance that belongs to a class of organic compounds called terpenoids. Azadirachtin is used to control tutaabsoluta, army worms, whiteflies, aphids, thrips, beetles, mushroom flies, leaf miners and other insects in food, greenhouse crops, and ornamental plants. The mode of action of azadirachtin on its target pests is that it acts as an inhibitor which blocks the enzyme ecdysome from binding on its active site hence stopping the insect from feeding, growing, reproducing and moving. In as much as azadirachtin together with other terpenoids such as nembin and salannin found in neem oil are applied as pesticides, these terpenoids undergo photolytic degradation and this tendency tends to lower the efficacy of such compounds to be used as pesticides. Besides this, there exists a competition for the binding site between the ecdysome hormone and azadirachtin; because of this the efficacy of azadirachtin and other neem pesticides has been low.

As a result of the factors lowering the efficacy of azadirachtin and other neem compounds such as salannin and nembin, this research aimed at altering the structure of azadirachtin by introducing amide groups through a reaction with aniline in the presence of heat. The introduction of amide groups improves the pesticide efficacy of azadirachtin. Amide groups in a pesticide induce uncontrolled release of calcium ions hence paralyzing its muscles which eventually leads to death of the insect. This is the principle on which flubendiamide, an active ingredient found in BELT SC 480 a synthetic pesticide operates. Belt SC 480 is a suspension concentrate containing 480 flubendiamide whose mode of action causes constant opening of the ryanodine receptor allowing uncontrolled movement of calcium ions that lead to generalized muscle contraction (rapid paralysis) and causes death of the larvae.

After administering the unmodified and the modified forms of the pesticide from both commercial and extracted sources, results indicated that modified forms of azadirachtin had better pesticide efficacies when compared to the unmodified forms. That is, the commercial unmodified form of azadirachtin showed a mortality of 0% while its modified form recorded a mortality of 28.6% and the extracted unmodified form of azadirachtin recorded a mortality of 28.6 % while its modified form recorded a mortality of 85.7% on army worms (Table IV). On tutaabsoluta, unmodified commercial pesticide recorded a mortality of 28.6% while its modified form recorded a mortality of 42.9% and the unmodified extracted pesticide had a mortality of 28.6% and its modified form recorded a mortality of 100%. (Table V).Both experiments were conducted for a period of 1 hour 30 minutes at room temperature. With regard to these findings, amide containing forms of azadirachtin in both cases gave good pesticides efficacies compared to their respective unmodified forms because apart from the inhibitory properties of the pesticide, the pesticide could help to kill the pests through the presence of the amide groups which are known to paralyze the pests.

In order characterize the anticipated products from the reaction; a High-Performance Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GC-MS) were used. The HPLC spectra in both modified and unmodified products gave different peaks at different retention times (Figure 1, 2, 3 and 4). Because there were a lot of compounds present in commercial modified pesticide and extracted modified pesticides a Gas Chromatography Mass Spectrometry (GC-MS) was used, a technique which is able to separate and identify the compounds of interest in the reaction mixture. From the GC-MS spectra, compounds of interest were produced as this could be evidenced by the peaks whose mass to charge ratio (m/z) were recorded. However not all the expected fragment ions were recorded since only those that are stable could give some peaks. The stability of the fragment ions depends on a lot of factors some of which include the inductive effect, resonance effect, hyperconjugation and hybridization. It is known that fragment ions with more resonance forms are more stable than those with less resonance structures while a methyl group is less stable than primary carbocation, secondary carbocation and tertiary carbocation.

However, it is not obvious that the molecular peaks of the compounds present in the mixture should show, in some cases such do not show because of less stable it is. Because of this, most molecular ion peaks in the spectra produced could not show.

Another fascinating thing observed from the mixture of the products produced was the presence of adamantane derivatives. Adamantane is an organic compound consisting three cyclohexanemolecules fused together and its derivatives are known to be of medicinal properties. Some of its derivatives have been used in the treatment of viral diseases such as influenza virus, because of this; this can be another area of research interest in the field of medicinal chemistry.

V. CONCLUSION AND RECOMMENDATION

Conclusion

Botanical pesticide azadirachtin was extracted from neem seed kernels, modified through the introduction of amide groups and the modified form of it offered better pesticide efficacy compared to its unmodified form.

Recommendation

Based on the findings in this study, this research makes the following recommendations to policy makers and chemistry research professionals.

1. Government should create a conducive environment for research in chemistry by increasing funding and procurement of research equipment.
2. There is need by science researchers to engage government on the use of locally available botanical pesticides and their derivatives for control of different pests affecting food security in the country.
3. Based on adamantane derivatives produced as part of the products in the research, additional research must be done so as to understand their sources as they are known to have some medicinal importance.

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