Analysis of Pyrethrum Plant: Implication for control of Mosquitoes

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ABSTRACT

The study extracted and analysed Pyrethrins and Pyrethrosin from Pyrethrum Plant. The species used for this study is the Chrysanthenum cinerrafolium. Pyrethrins have been analyzed by a number of analytical techniques e.g.HPLC, GLC, TLC, colorimetric, polarographic, etc. This work reports on the simultaneous quantitative HPLC analysis of pyrethrins and pyrethrosin using HPLC column and aqueous methanol (90:10:v/v) as the eluent. Analysis of a mixture of Pyrethrosin and Pyrethrins standards computer printout gave three peaks relating to pyrethrosin (retention time 2.40 minutes); pyrethrins II (retention time 4.00 minutes) and pyrethrins I (retention time of 6.00 minutes). Statistical analysis of the data obtained revealed that there was no correlation between pyrethrins and pyrethrosin concentration. It also shows that there is no significant difference in Pyrethrins for the ray florets for both flowers A(1.80) and B (2.22) studied. It also revealed that Pyrethrins content is highest in ray florets (3.53) in flower B and lowest(0.08) in petals in flower A. For the 2 types of mosquito coils studied, both have shown to have pyrethrins and pyrethrosin, it also revealed that

Mean Pyrethrosin concentration % (w/w) in Swan off mosquito coil is higher (0.70) than that in Ramboh mosquito coil (0.30). Mean Pyrethrins Concentration % (w/w) in Swan off mosquito coil (1.03 is higher) than that in Ramboh mosquito coil (0.11). from the analysis, Pyrethrum plant contains active ingredient(Pyrethrosin and Pyrethrins) that can repel, knockdown a variety of insects but harmless to animals. This paper recommended that safe levels of pyrethrosin should be established to know whether the levels of pyrethrosin in these two types of mosquito coils are high enough to be harmful or not. Further Studies should be conducted to establish the effects of burning a mosquito coil containing pyrethrosin and assess the lethal dose.

Keywords: *flower, mosquito coil, Pyrethrum, Pyrethrins, Pyrethrosin.*

INTRODUCTION

The use of pyrethrum powder as an insecticide originated in the Transcaucasus province of Asia at about 1800. It was introduced to Asia in an unidentified date and its manufacture began at about 1828 (Robbert L. M.2019). Pyrethrum occurs only in plants belonging to the genus *Chrysanthenum* (Pyrethrum) family compositae. The two species which posses a significantly soaring toxic content to be used for the production of insecticides are *C. Cinerrafolium* and *C. coccinuem*(roseum), but the former is currently the only species of commercial importance (Robbert L. M.2019). The species used for this research is the *Chrysanthenum cinerrafolium*

Commercially, Pyrethrum extract is gotten through solvent extraction of ground dry pyrethrum flower using n – hexane. When n – hexane is used as the extraction solvent, the product is a dark viscous oleoresin containing about 35% by weight of Pyrethrins. This extraction has unique properties such as:

1. Repellent, knockdown, paralytic and toxic effects against a great variety of insects.

2. Ability to be highly synergised.
3. Harmless to man and other warm – blooded animals.
4. Hardly any build-up of resistance in insect population
5. Rapid breakdown and no persistent of residue.

These properties permit the use of Pyrethrins against insect pests in the house, even when a treatment is required just prior to harvest or stored food and livestock products.

Synthetic Pyrethrum compounds have now been grown and are now expected to compete with natural Pyrethrum products to some marked extent in the future. However, in contrast to natural Pyrethrins, synthetic ones are toxic to man and other warm blooded animals and pose residue problems. Because of the increasing awareness of the risk associated with widespread use of many synthetic insecticides such as toxicity to mammals, persistence of residues, and insect resistance, the demand for Pyrethrins has continue to grow. Pyrethrins are distributed in the different parts of the Pyrethrum plant.

Synthetic Pyrethroids

Amongst the various synthetic pyrethroids that have been made in the laboratory, allethrin (figure 1) is the cheapest to produce and has been choosen for commercial development.

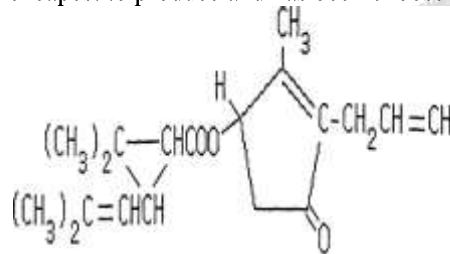


Fig. 1: Allethrin

The product as commercially produced is a clear brownish, viscous liquid containing 75-95% allethrin isomers (Robert L.M. 2019). It is more stable than the natural pyrethrins upon exposure to ultraviolet light and heat. However, it is largely detoxified by hydrogenation of the double bonds of either acid or allyl side chain. It may undergo hydrolysis to form chrysanthemic acid and 2-allyl- 3-methyl-2, 4-cyclopentadienone which readily forms a dimer by the Diels- Alder reaction (La Forge, F et al 1990.)

COMPOSITION OF PYRETHRUM EXTRACT

a. Pyrethrins

Pyrethrins are a combination of six components. Although the relative proportions of these pyrethrins in extracts from different plants is variable (Head, S.W. (1967), the insecticidal made up of the commercial pyrethrum extracts is relatively constant. Pyrethrins are esters formed by a combination of two acids and three alcohols. The two acids are chrysanthemic acid and pyrethric acid. The three alcohols are pyrethrolone, cineralone and jasmololone. The esters of chrysanthemic acid are pyrethrin 1, cinerin 1 and jasmolin 1, respectively, and are together known as the pyrethrins 1 fraction .The esters of pyrethric acid, on the other hand, are pyrethrin II, cinerin II and jasmolin II, which represent the pyrethrin II fraction (Rose, W.G. and Haller, H.L., (2000), Barton, D.H.R., and de Mayo, P. (2013).

It has been discovered that the structural requirements of the acid component of a pyrethroid for knockdown are much less specific than for high toxicity (Eliot M 1951). For toxicity, a ring system (preferably cyclopropane), a 6 to the carboxyl group is essential and the activity is further dependent upon an isopropenyl, or, much better, an isopropylidene group attached to the cyclopropane ring. Stereochemical configuration is also an essential factor as the pyrethroids are among the most complicated of insecticidal molecules and their toxicity is readily wipe out by the action of heat, light and alkaline hydrolysis. Thus, the activity depends upon the intact esters whereas, chrysanthemic and pyrethric acids as well as pyrethrolone, cinerolone and jasmololone are not significantly toxic (Staudinger, H. and Ruzicka, L. 1999). Efficiency is dependent upon optimum geometric and optical isomerism. Toxicity in this case refers to the harm caused to insects which is ordinarily defined in terms of the lethal dose. Lethal doses are customarily expressed in milligrams of substance per kilogram weight of the subject (i.e. parts per million on body weight basis). Lethal doses are gotten by introducing various dosages of the substances to be tested into laboratory animals or insects. That dosage which would be lethal to 50% of a large number of the animals or insects under controlled conditions is called LD_{50}. Thus if a statistical analysis of a large population tested showed that a dosage of Img per kg was lethal to 50% of the population tested, the LD_{50} for this portion would be lmg per kg.

From toxicity data available at present, there is no evidence that pyrethrins are carcinogenic, teratogenic or

mutagenic in test animals and by extrapolation, to man (Ndalut, P.K. 2019). The supposed allergenic properties of pyrethrins and other pyrethrum extracts have been tentatively shown to be caused by impurities in the pyrethrum flower extracts. The main reaction causing agent has now been shown to be pyrethrosin (Tamura, T. and Matsubara, H. (1955).

b. **Pyrethrosin**

Pyrethrosin (Fig. 2) is a sesquiterpene lactone and was first isolated from pyrethrum extract by Rose, W.G. and Haller, H.L., (2000), but its structure was fully made clear by Barton and de Mayo (Barton, D.H.R., and de Mayo, P. (2013). Pyrethrosin was also isolated by Ndalut, P.K. (2019). After soxhlet extraction of marc by n-hexane, the solvent is removed using a climbing film evaporator and the residue dissolved in warm acetone and filtered. The filtrate on cooling, deposits a white crystalline substance which on recrystallisation several times with acetone yields pure pyrethrosin crystals. Pyrethrosin was the first sesquiteipenoid shown to have a ten-membered carbocyclic ring and the ease of cyclisation pointed to its possible importance in the biosynthesis of connected bicyclic sesquiterpenoids (Rickett, F.E. Tyszkiewicz, K. (1973). Pyrethrosin has been reported to have a synergistic action on "pyrethrins" (Gnadinger, C.B. (1936). Widespread literature survey clearly indicates that chromatographic techniques available for the analysis of Pyrethrum extract have dealt at great lengths with the six insecticidally active constituents, i.e. pyrethrins. Neither the implication/action of the analysis of Pyrethrosins and Pyrethrins on mosquitoes has been reported.

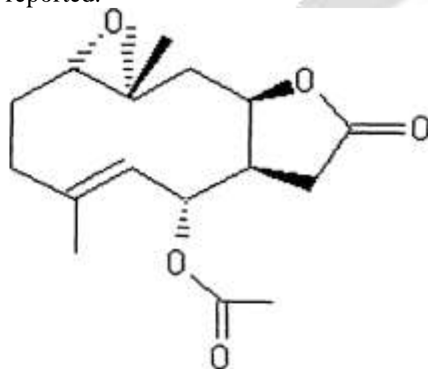


Figure 2. Pyrethrosin

Pyrethrum and its preparations are considered non-toxic to warm blooded animals and harmless to human beings. This opinion has been held up by many researchers who have experienced no ill-effects in the course of years of work on pyrethrins. As further proof of its safe use, it may be mentioned that pyrethrum has been employed in compositions for external use for not only insect repellent properties, but also in the control of scabies, miscellaneous skin affectations and body lice (Mungarulire, J. 1990).

Isolation and Analysis of Pyrethrins and Pyrethrosin from Pyrethrum Plant.

Pyrethrins have been broadly studied and very numerous methods of analysis reported. However, only little information is available on pyrethrum analysis, especially the species *Chrysanthenum cinerrafolium*, no implication analysis of pyrethrins and pyrethrosin on the control of mosquitoes has been reported in the literature. Various analytical techniques of isolating pyrethrins are as follows:

a. Column Chromatography using Adsorptive Charcoal.

This is one of the techniques used for the separation and estimation of the four insecticidal constituents of pyrethrum (cinerins 1 and II and pyrethrins I and II). It is effected by elution from a column of adsorptive charcoal (Head, S.W. (1996). The four pyrethrins separated are analysed by using I.R. spectroscopy. However, many techniques for the separation of pyrethrins by column chromatography have been illustrated. But most of this work was done before the advent of gas liquid chromatography with the result that jasmolins were not detected. Separation by adsorption chromatography either on alumina (Debon, A., Segalen, J.L. (1989) or on a silica gel-plaster of Paris mixture is very helpful in acquiring concentrates of pyrethrins 1 and II.

b.Titrimetric Method

A titrimetric technique has also been used for the analysis of pyrethrins. The method has been referred to as the mercury-reduction technique and was at first put forward by Wilcoxon ((Head, S.W. 1996). The method has been the most widely used for pyrethrins evaluation and consists basically of an alkaline hydrolysis of the mixed pyrethrin esters to give chrysanthemum mono- and dicarboxylic acids. Interfering fatty acids are removed as barium salts and

the monocarboxylic acid is extracted by petroleum ether. This acid reacts with Denige's mercury reagent. Iodometric titration of the mercurous salts formed, determines accurately the quantity of monocarboxylic acid. The dicarboxylic acid, insoluble in petroleum ether, is extracted by diethyl ether and subsequently determined alkalimetrically. The amounts of these two acids are expressed as pyrethrin I and II and jointly give the "pyrethrins" content. The Association of Official Agricultural Chemists (A.O.A.C.) method uses sulphuric acid in a similar titrimetric analysis but gives low down results.

c. Colorimetric Method

Pyrethrins have been estimated using a colorimetric method known as the "sulphur-colour" test. In this method, microquantities of an alcoholic solution of pyrethrins deposited on paper is heated to 70°C with a solution of sodium disulphide. A red brown colour develops whose intensity is proportional to the amount of pyrethrins present. In the "sulphur-colour test according to Head, S.W. (1996) suggested that the method was based on measurements of the red-brown colour produced by heating pyrethrins with solutions of sulphur and lithium hydroxide. The method was useful for a period of about 5 years (Ochieng T, (1992),) in estimation of small quantities of pyrethrins (3-5 mg). The method was discontinued due to accuracy problems.

d. Gas Liquid Chromatography (GLC)

The separation of pyrethrins by gas liquid chromatography was first achieved by Findlay, G.M (2000), and has proved to be the most helpful advance in pyrethrins analysis in recent years. The pyrethrins respond to electron-capture detector and the sensitivity is such as to supply a method suitable for residue determination. The relative response of the six components to the electron- capture detector appears to be proportional to that given by the flame ionization detector. The major benefit of the electron-capture detector is its specificity. However, its use in measurements requires considerable care as it is very sensitive to changes in operating conditions and its linear range is strictly limited. The hydrogen flame ionisation detector does not suffer from these defects; furthermore it allows the use of temperature programming (Gnadinger, C.B. (1996). This detector is non-specific.

e. High Performance (Pressure) Liquid Chromatography H.P.L.C.

It is sure that recent advances in high-speed liquid chromatography has proved to be very helpful in pyrethrins analysis. Pyrethrins have been analysed by high pressure liquid chromatographic method and one such method uses a zorbax ODS column of 25 cm x 4.6 um i.d. using acetonitrile- water (7:3) as the eluent, a flow rate of 1 ml/minute and detection at 240 nm. The following retention times were observed:- rotenone - 7 minutes, pyrethrin II - 11.5 minutes and pyrethrin 1-25.5 minutes.The six pyrethrin esters have also been separated and quantified by high performance liquid chromatography using a double u-porasil column and a 1:1 mixture of anhydrous dichloromethane and water saturated dichloromethane as the mobile phase (Debon, A., Segalen, J.L. 1989). The wavelength used for the detection was 254 nm.

Pyrethrins have also been analysed using normal-phase liquid chromatography (Heywood, V.H et al 1977). In this technique samples were dissolved in tetrahydrofuran and analysed on a 5µ m amino-column of 25 cm x 4.5 µm i.d. with hexane.tetrahydrofuran (9:1) as the mobile phase, a flow rate of 1.5 ml/minute and detection at 240 run. Total elution time was 7 minutes.

High performance liquid chromatography has also been used for determining trace levels of pyrethrins and piperonylbutoxide down to 0.1 jxg/litre in tap water. In this method, a 5 /xm ODS $C_{]8}$ 25 cm x 4.6 */.µm* i.d. was used. The mobile phase was methanol/water, 90/10, v/v (helium degassed), flow rate 0.8 ml/minute and the detection wavelength was 230 nm (pyrethrins) and 209 nm (piperonylbutoxide) (60). Under these conditions, retention times were as follow:-

Pyrethrins II------------------------------------ 4.5 to 5.2 minutes
Piperonylbutoxide.. 6.0 to 6.8 minutes
Pyrethrin I -------------------------------------- 6.6 to 7.6 minutes

Objectives of the study

The objectives of this study include:-

1) To determine the concentration (amounts) of pyrethrosin and pyrethrins in different pyrethrum plant/flowers.
2. To establish how the pyrethrosin content varies in the three different parts of the pyrethrum flowers viz:- Ray Florets, Petals and Sepals. Pyrethrins content in the three different parts of the flowers has been established.
3) To determine the pyrethrins and pyrethrosin content in two types of mosquito coils.
4) Implication of Analysis for the control of Mosquito

EXPERIMENTAL

Materials and Chemicals

HPLC grade methanol was used, this was gotten from Near East University and C.I.U.TRNC, Mersin 10 Turkey, this

was where one of the researchers studied. Double distilled, deionized water normally meant for X-Ray fluorescence analysis was used as the eluent in the HPLC. The chart papers used were Kipp and Zonnen BD41. The flower samples were picked from Pankshin, Mangu (Plateau Central) and Shendam Local Government Area (Plateau South) of Plateau State

Two types of mosquito coils, Swan off mosquito coil and Ramboh mosquito coil were purchased from a leading supermarket and analysed for their pyrethrins and pyrethrosin content. Both indicated as being pyrethrins/pyrethroids based.

INSTRUMENTATION

A varian micropack ODS C_{18} analytical HPLC column, 5 mm internal diameter by 5 cm was used together with a check pre-column. The U. V.-Vis variable spectrophotometer used was a Hitachi Model 100-40 fitted with an analytical flow cell. For normal data analysis, a Beckmann Kipp and Zonnen BD 41 recorder was used and was equipped with Kipp and Zonnen BD 41 chart papers.

Most of the data was collected and analysed using a BBC model B micro-computer. The output voltages of the detector had been tested for compatibility with the micro-computer input and found to be within the recommended range that is sufficient for detection and not of a magnitude to harm the computer. As an added precaution, the protective circuit was designed to "cut off" at + 2.3 volts and -0.3 volts.

A series of programs were then fed into the computer to do the following:-

1) Collect data from the output of the detector into the memory of the computer. Sampling of the detector output was done at the rate of ten points per second, but only an average of every five were taken; thus, only two points had to be conserved due to its relatively small size. These were discovered to be sufficient data points from which to reconstruct a continuous chromatograph.

2) Data from electrical equipment is contaminated with electrical and electronic random noise which had to be removed before the chromatograph could be analysed. A routine to perform this task smoothing' afterwards was included in the data collection program.

3) After entering the data and subsequent 'smoothing', the spectra was saved on diskette, from where it could be recalled and analysed.

METHODOLOGY

Preparation of Pyrethrosin Standard

A few grams of pyrethrosin dust were dissolved in warm acetone and filtered. The filtrate on cooling gave small brownish white crystals. The crystals formed were redissolved in warm acetone and filtered. This filtrate emerged yellowish in colour. The process was repeated until the filtrate lost the yellowish colour and became colourless. After repeating this procedure four times, the colourless filtrate was allowed to cool and form crystals. Clear white bipyramidal crystals resulted which were stored in the refrigerator awaiting analysis. The melting point of these crystals was 197-198°C which is within the reported range of 193-200°C (Parlevliet, J.E. 1970). The sample was analysed by HPLC under various conditions and only a single peak was observed. This clearly indicated that the sample was pure.

Standardization of Extraction Procedure

Because chloroform easily dissolve both pyrothrosin and pyrythresin, it was seen as the extraction solvent. The samples were therefore not extracted with chloroform at **68°**C. In order to determine the total number of hours required to extract all the prethrins and pyrethrosin, 10 gm of pyrethrum grist were extracted with 250 ml of chloroform at 3 hour intervals. After every 3 hours interval, the chloroform was evaporated by a rotatory evaporator and the extract dissolved in the HPLC solvent system i.e. methanol: water (90:10; v/v). Aliquots of 20 microlitres were then injected into the HPLC machine and the machine run for 7 minutes. If peaks appeared where either pyrethrosin or pyrethrins were expected to appear, it meant that extraction was incomplete. Another 3 hour extraction interval was then carried out. This process was repeated until no peak appeared where pyrethrins and pyrethrosin were expected.

After repeating this procedure twenty seven times, i.e. the 26th to 27th extraction hours, it was observed that no peaks appeared. This meant that, if all the samples to be analysed were extracted for the same length of time, all other conditions being equal, one would safely assume that any pyrethrins and pyrethrosin that might have remained must have been below the detection limit of the HPLC machine. It was then decided to extract all the test samples for a minimum of 27 hours.

Collection and Preparation of Pyrethrum Flowers

The flowers were picked in Pankshin and Mangu LGAs (Plateau Central), and Shendam LGA (Plateau South) by the researchers and their assistants. The flowers were then dried at ambient temperatures, spread on raised sieves to avoid fermentation which would otherwise lower the pyrethrins content. Separation of the three different parts of the flower i.e. sepals, petals and ray florets either before or after drying does not have any effect on the pyrethrins or pyrethrosin content. However, it was found easier to separate them after drying instead of when they were fresh. Therefore the flowers were grinded using a Mortar and pistil and dust obtained stored in a cotton paper bags awaiting extraction.

Extraction

In all the cases, 10gm of the dust sample was weighed into a cellulose extraction thimble which was then put into a soxhlet extractor. The soxhlet was then attached to a half litre flat bottomed flask containing 300mls of chloroform and some boiling chips added. A condenser already connected to a source of tap water was attached on the upper side of the soxhlet. All the glassware used had quick fit joints. The extractions were done at 68°C for a minimum of 27 hours each. Most extractions were undertaken at the laboratories of National Veterinary Reseach Institute Vom, Plateau State where one of the researchers studied, Near East University TRNC, Mersin 10 Turkey. The resultant solution (extract) was then filtered through cotton wool and chloroform evaporated by a rotatory evaporator. The extract was then allowed to cool after which it was weighed to determine the yield. These weights were important in calculating the percentage concentrations, the extracts were then put in small sample vials, labelled and stored in the refrigerator to await analysis. For each sample, including mosquito coil samples, two extractions were performed.

Sample Preparation

In the case of extracted samples, 10 mg of the extract was weighed and then dissolved in ethyl acetate in a 50 ml beaker. A small glass column, 5 inches long and blocked at the tapering end with cotton wool was packed with approximately 1 gm of celite. A small amount of ethyl acetate was first passed through the column to completely wet the celite. The extract (10 mg) in approximately 5 ml of ethyl acetate was then passed through the column and collected into a 50 ml pear shaped flask. Precaution was taken to avoid the celite drying up. After all the solution in the beaker passed through the column, the beaker was rinsed twice with about 20 ml of ethyl acetate and this also passed through the column. The column was also rinsed twice with 2 ml of ethyl acetate to remove any pyrethrins or pyrethrosin which may have remained on the celite. Ethyl acetate was then completely evaporated by a rotatory evaporator with the temperature thermostatically set at 78°C. The HPLC solvent system, i.e. Methanol: Water; 90:10 v/v was then used to dissolve pyrethrins and pyrethrosin from the pear shaped flask. This solution was carefully transferred to a 50 ml volumetric flask and the volume made up to the 50 ml mark with Methanol: Water; 90:10. After thorough shaking the solution was filtered through cotton wool and a little amount of the filtrate transferred to a 5 ml sample vial. The sample vial was labelled and then stored in the refrigerator awaiting HPLC analysis. For each extract, the above steps were repeated two times resulting in two cleaned samples.

HPLC Analysis and Operating Conditions

The HPLC column was always kept in methanol. Before starting the analysis, methanol was first pumped through the system for about an hour with the detector on till stability was achieved. Methanol and water was then introduced on a linear gradient in two minutes until the ratio of methanol.

RESULTS AND DISCUSSION

Obtaining Retention Times and Peak Areas

Analysis in chromatography requires the determination of the size of the peak either in units of area or weight, both of which are related to concentration and in some instances, peak heights are used. These determinations are sometimes complicated by the inability of the chromatographic system to folly resolve all the components in a sample, thus resulting in poorly defined merged peaks and further, by the instability of the baseline. Since the introduction of computers in chromatography, various methods have been developed for peak area determination. These range from the simple ones such as the height, times, width (at half height) and the trapezium methods, to elaborate gaussian curve - fitting methods (Head, S.W. (1996).

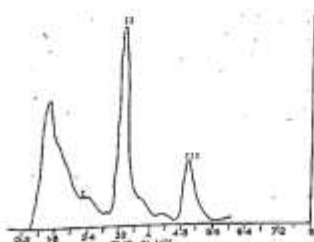
In this study, a fairly simple method was employed to obtain peak areas and to resolve merged peaks. This involved the summation of consecutive heights along a curve and after substraction of the baseline, the values obtained were directly proportional to the area of the peak. As this method follows the actual curve, all points along it are taken into consideration and is hence more accurate than either the trapezium method or the height, times. width (at half height.) methods. It was also feasible to obtain areas of peaks that on an ordinary chromatogram chart would be considered out of range and hence not quantifiable. With the computer system, peaks exceeding the maximum peak height of the chart could be stored in memory and later reconstructed for the

purpose of analysis or display.

Identification in chromatography relies on the location of the peak in the chromatogram, i.e. the retention time which is a factor of the polarity of the substance. Retention times were simply determined even for broad peaks as the program could pick out slight changes in the gradient. Where uncertainty arose in the identification of a peak, the sample was spiked with the suspected compound and an increase in height confirmed the inference. This is illustrated in figures 3 and 4. Figures 3 and 4 represent chromatograms of the same sample with the only difference being that in figure 4 the sample was spiked with pyrethrosin.

The retention times for pyrethrosin ranged from 1.72 minutes to 2.52 minutes, that for pyrethrins II from 3.04 minutes to 4.00 minutes, while that for pyrethrins I ranged from 4.27 minutes to 6.00 minutes for the standards and samples analysed. In all samples analysed, the shortest retention times observed were those for sepal samples whereas some of the longest retentions observed were for the standards. It should however, be noted that in the case of similar samples or extracts, the variations in retention times were very slight.

Chromatogram of the Extract Flower A



Time

Figure 3

<u>Conditions</u>

Column ODS C13, 5mm 1.d. × 5cm

Solvent System: 90:10;Methanol:Water

Flow Rate 0.8 ml/minute

Detection: 230 mm

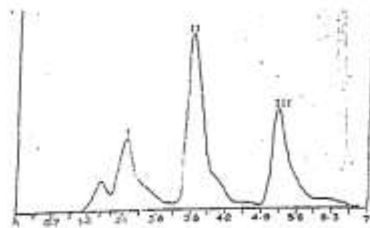
<u>Identification of Peak</u>

I Pyrethrosin

II Pyrethrins II

III Pyrethrein I

Chromatogram of Flower A, spiked with Pyrythrosin standard



Time

Figure 4

Conditions

Column ODS C13

Solvent System: 90:10;Methanol:Water

Flow Rate 0.8 ml/minute

Detection: 230 mm

Identification of Peak

I Pyrethrosin

II Pyrethrins II

III Pyrethrein I

Variations in retention times have also been observed in the analysis of pyrethrins and piperonyl butoxide (Head, S.W. (1996). They have also been observed in the analysis of polycyclic aromatic hydrocarbons in roast meat and smoked fish (Debon, A., Segalen, J.L. (1989). These variations in retention times observed while analysing for the same compounds in different samples is due to matrix effect (Eliot M (2000). This means that retention times for the same compound can vary depending on the chemical environment and concentrations in which the compound is found.
Hence, though sepals and ray florets contain pyrethrosin and pyrethrins, the chemical environment in which these exist is definitely different since both contain other compounds some of which are different.These other compounds interact with the compounds of interest, the stationary phase and the mobile phase in different ways hence causing the phenomena of matrix effect.

Resolution of Peaks

In some of the sample chromatograms, e.g. figure (5), the peak for pyrethrosin has not been well resolved. This is a general problem associated with isocratic development where the resultant chromatogram of a multicomponent combination shows uneven degree of separation between the components of the mixture (Eliot M 2000). That is, the front of the chromatogram shows a series of bunched and poorly resolved peaks whereas the center portion shows good separation and the final portion shows good separation but sometimes long diffuse peaks which if continued would be difficult to detect (Eliot M (2000). This is a general elution problem and is common in all forms of chromatography.

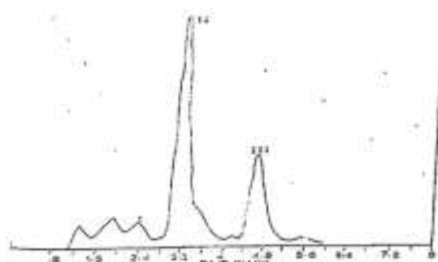
Variation in retention times may be minimized by instituting the following: programming; (i) coupled columns (ii) Flow programming and (iii) temperature. Temperature programming and flow programming are not efficient in solving this problem and have little effect in capacity factor. Temperature programming is also inappropriate for habitual analysis since too much time is required to equilibrate (Ndalut, P.K. (2019). Coupled column technique (same or different columns) has many advantages especially in routine analysis (Eliot M 2000) . To resolve this problem, the computer used for the analysis was equipped with a program for resolving merged peaks, i.e. the perpendicular drop method (Ndalut, P.K. (2019)

Determination of Concentrations

The wavelength of this analysis was chosen as 230 nm since a u.v. scan of pyrethrosin confirmed that pyrethrosin absorbs between 225 nm and 237 nm. The conditions used for the analysis were such that the detector was

more sensitive to pyrethrins than to pyrethrosin with the result that very small peak areas due to pyrethrosin represented higher percentage concentration than higher peak areas due to pyrethrins. This can be resolved by the use of photodiode-array detector which is not available in Nigeria. This can be seen in the case of the standards chromotagram (figure 5) which is a tracing of the chromatogram from the chart paper.

Chromatogram of the Extract of the Ray of Florets



Time

Figure 5

Conditions

Column ODS C13, 5mm 1.d. × 5cm

Solvent System: 90:10;Methanol:Water

Flow Rate 0.8 ml/minute

Detection: 230 mm

Identification of Peak

I Pyrethrosin

II Pyrethrins II

III Pyrethrins

The chromatogram was obtained when 10 mg of pyrethrosin and 10 mg of standard pyrethrins were both dissolved in 50 ml of the solvent system (methanol: water; 90; 10, v/v) and analysed. The pyrethrosin concentration in the prepared standard was 100 percent, that for pyrethrins II in the standard was given as 9.44 percent whereas that for pyrethrins I was given as 13.60 percent (PBK method).

In figure 6, peak I is due to pyrethrosin, peak II due to pyrethrins II and peak III due to pyrethrins I. This is a tracing of the chromatogram from the chart paper.

STDS 1 on figure 7 is another chromatogram of the same standards but in this case printed directly from the computer program used for most of the work. All the chromatograms appearing in the chapter with the exception of figure 6 were obtained directly from the computer. Hence, in order to have standard chromatograms for comparison purposes, the same scale has been used throughout though the computer program had the ability to compress or enlarge the peaks.

Statistical Treatment of the Data Obtained

Turkey's test (usually referred to as HSD (Honestly Significant Difference test) etc. This is also known as the p-test and makes use of a single value against which all differences are compared. In this work, Turkey's test was chosen as it was found to be the most appropriate for the analysis of data for the tables.

HSD is given by:-

HSD - $g_{aiKiN\cdot K}$^MSB

Where

a	Chosen level of significance
K	Number of mean in the experiment
ISF	Total number of observation in the experiment
n	Number of observation in a treatment
MSE	Error Mean square from the ANOVA table
q	Is obtained by entering appendix table H with a, k and N- K

Chromatogram of mixture of Pyrythrosin and Pyrethrins (Chart Paper)

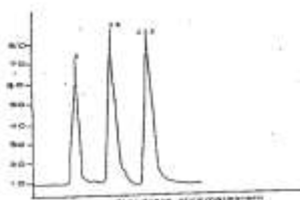


Figure 6

<u>Conditions</u>

Column ODS C13, 5mm 1.d. × 5cm

Solvent System: 90:10;Methanol:Water

Flow Rate 0.8 ml/minute

Detection: 230 mm

<u>Identification of Peak</u>

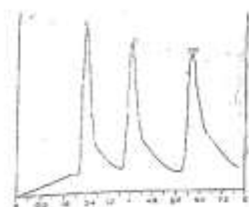
I Pyrethrosin

II Pyrethrins II

III Pyrethrein I

IJARIIE

Chromatogram of Mixture of Pyrothrosin and Pyrethrins standard – Computer Printout



TIME IN MINUTE

Figure 7

Conditions
Column ODS C13, 5mm 1.d. × 5cm
Solvent System: 90:10;Methanol:Water
Flow Rate 0.8 ml/minute
Detection: 230 mm

Identification of Peak
I Pyrethrosin
IIPyrethrins II
III Pyrethrein I

All possible differences between pairs of means are computed and any difference which yields an absolute value that exceeds HSD is declared to be significant. In our case, the level of significance *a* was taken as *oc* = 0.05 (meaning 95% confidence level).
In tables 1 and 2, the statistical method of analysing the data chosen was the student's t-test.

Inference

Since there is no correlation between the pyrethrins and pyrethrosin content, in a breeding program, neither of these compounds can be used to assess the other for selection purposes. Thus, in breeding new strains of pyrethrum that are high in pyrethrins content and low in pyrethrosin content, specimens for propagation would have to be selected for these two characteristics independently of each other. In such a program, one can select flower/plant which show the desired qualities then propagate them or else two lines may be established: one for increased pyrethrins content and the other for reduced pyrethrosin content. These two lines are then crossed after a number of generations when significant improvements in the desired traits are observed. Lack of correlation in the two compounds indicates that the two do not influence each other's mixture in any way. Hence their production could be autoregulatory or else, influenced by other factors other than each others' presence.

It should be noted that if other positive uses of pyrethrosin (besides molluscicidal activity) are discovered since nearly all sesquiterpene lactones have diverse biological activity or if pyrethrosin's molluscicidal activity could be commercially exploited, then, a high concentration of pyrethrosin in the pyrethrum flower would be more advantageous than it would be disadvantageous. However, if the advantages of pyrethrosin are disregarded, then pyrethrum with high concentration of pyrethrosin would be considered as being disadvantageous since pyrethrosin cause dermatitis (Robbert L. M.2019). The standard deviation for pyrethrosin in the flower A, studied is quite low 0.0153 – 0.075 also pyrethrosin in flower A studied have coefficients of variation ranging from 0.8% to 1.71% showing that concentration of pyrethrosin does not vary much with the seasons.

Pyrethrins and Pyrethrosin Concentration in the Different Parts of the Flowers Studied.

A pyrethrum flower has three parts. These are the ray florets, sepals and petal.
Table 1 shows the mean pyrethrosin and pyrethrins concentration for the different parts of the flowers.

Table 1: Mean Pyrethrins and Pyrethrosin concentration in flowers A and B studied

A Flower	Part of Flower	Mean Pyrethrosin conc in % (w/w)	S.D	C.V	Mean Pyrethrins conc in % (w/w)	S.D.	C.V.
	Ray Florets	2.81	± 2.81	0.8	1.82	± 0.0153	0.8
	Petals	2.68	± 0.1591	5.9	0.08	± 0.0132	2.0
	Sepals	1.07	± 0.0200	1.8	0.43	± 0.075	1.7
B Flower							
	Ray florets	3.53	± 0.1109	3.1	2.20	±0.0430	1.0
	Petals	3.38	±0.1051	0.4	0.11	±0.0120	10.9
	Sepals	2.04	±0.0100	0.5	0.72	±0.0146	2.0

n= 3

Pyrethrosin concentration in Flower A of the three different parts is not significantly different statistically except in the case of sepal which is slightly lower (1.7). Pyrethrosin concentration in Flower B of the different parts studied is not significantly different statistically except in the case of sepal which is slightly lower (2.04).

As for the Pyrethrins content statistical analysis shows that there is no significant difference in Pyrethrins for the ray florets for both flowers A(1.80) and B (2.20) studied. It also shows that Pyrethrins content is highest in ray florets and lowest in petals 0.08 in flower A and 0.11in flower B.

Analysis of Mosquito Coils

The aim of this analysis was not to compare the amount of pyrethrins/pyrethrosin in the mosquito coils but to establish whether pyrethrosin was present in the mosquito coils. The mosquito coils chosen for the study were Swan off mosquito coils and Ramboh mosquito coils which were purchased from a leading supermarket in Pankshin LGA.

Table 2 represents the results obtained after analysis of the two samples.

Table 2:Percentage Concentration of Pyrethrins and Pyrethrosin in two types of Mosquito Coil Studied

Sample	**Mean Pyrethrosin conc in %**	S.D	C.V	**Mean Pyrethrosin conc in %**	S.D	CV

	(w/w)			**(w/w)**		
Ramboh mosquito coil	**0.30**	± 0.019	7.3	**0.11**	± 0.015	15.3
Swan off mosquito coil	**0.70**	± 0.019	2.7	**1.73**	± 0.168	16.7

Mean Pyrethrosin concentration % (w/w) in Swan off mosquito coil is higher (0.70) than that in Ramboh mosquito coil (0.30).
Mean Pyrethrins Concentration % (w/w) in Swan off mosquito coil (1.03) than that in Ramboh mosquito coil (0.11) Swan off mosquito coil has higher mean concentrations for both Pyrethrosin (0.70) and Pyrethrins (1.03) than the Ramboh Mosquito coil as shown in Table 2 above.

INFERENCE/IMPLICATION /RECOMMENDATION

Both types of mosquito coils have been shown to contain both pyrethrins and pyrethrosin.
The implication of this is that since Pyrethrums plant contain active ingredients (abiliy to repel, knockdown, paralytic and toxic effects against a great variety of insects, harmless to man and other warm – blooded animalst, it should be grown by farmers and scientist alike or in the alternative taken from the wild/bush and brought into homes. This will serve as a practical way of controlling mosquitoes at home.
It is recommended that safe levels of pyrethrosin should be established to know whether the levels of pyrethrosin in these two types of mosquito coils are high enough to be harmful or not. It is also recommended that Studies should, therefore, be conducted to establish the effects of burning a mosquito coil containing pyrethrosin and assess the lethal dose.

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