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Loss of Pyrethrins Content during Drying of *Chrysanthemum cinerariifolium* Flowers in Direct Sunlight

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ABSTRACT

Pyrethrins are organic compounds derived from the flowers of *Chrysanthemum cinerariifolium* for their insecticidal activities. Pyrethrins I and Pyrethrins II are the two main classes in which Pyrethrins are grouped. The two groups are composed of six organic compounds namely pyrethrin I, jasmolin I, cinerin I, pyrethrin II, jasmolin II and cinerin II. The compounds are degradable on exposure to direct sunlight, moisture and temperatures. Pyrethrins are used as a broad spectrum natural insecticide in agriculture and public health. The aim of this research was to establish the differential total extractable pyrethrins content on drying flowers in direct sunlight and in darkness. Mature pyrethrum flowers from experimental farm, College of Agriculture and Veterinary Sciences, Kabete campus, University of Nairobi, were harvested in brown paper bags, divided into four sets and taken to the laboratory. The first set of flowers were dried in direct sunlight for two weeks and the second set to a constant weight at a temperature of between 16-29^{O}C. The third set were dried in darkness for two weeks and the fourth set to a constant weight at room temperature. Drying was done between 3rd-17th of August 2019. The dried flowers were then ground into fine powder and extracted using Soxhlet extraction method with hexane. The extracts were refined and analyzed by titrimetric method. Pyrethrum flowers were found to achieve maximum moisture loss at varying times depending with the dying method used. The yield of pyrethrins obtained on drying the flowers to constant weight in direct sunlight was 1.02% while in darkness was 1.38%. The percentage of pyrethrins obtained from flowers dried in direct sunlight for two weeks was 0.86 and 1.01 in darkness. Moisture level for the flowers dried to a constant weight was 9%. The pyrethrins I:II ratio was found to vary for the two drying methods used. The best condition to dry the pyrethrum flowers was found to be in darkness to a moisture content of 9%.

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Introduction

Pyrethrum plant, *Chrysanthemum cinerariaefolium*, is from Compositae family and has been widely studied for its commercial importance as a source of insecticidal components known as Pyrethrins (Carcamo *et al,* 2017).

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Chemistry of pyrethrins

Pyrethrins are six closely related esters derived from two acids and three alcohols. Chrysanthemic acid and pyrethric acid are the two acid moieties while jasmolone, cinerolone and pyrethrole are the three alcohol moieties. Chrysanthemates; pyrethrin 1, cinerin 1 and jasmoline 1 make up Pyrethrins I while the esters of pyrethric acid, pyrethrin 2, cinerin 2 and jasmolin 2 makes up Pyrethrins II. The six components together bring the knockdown and kill properties of the pyrethrum extracts. Pyrethrins have been fully isolated, characterized and their chemical structures differentiated as in figure 1. (Sundaramoorthy *et al,* 2016). Pyrethrins are viscous, high boiling gums in pure form. They are insoluble in water but readily dissolve in organic solvents such as alcohols (Njiru, 2006).

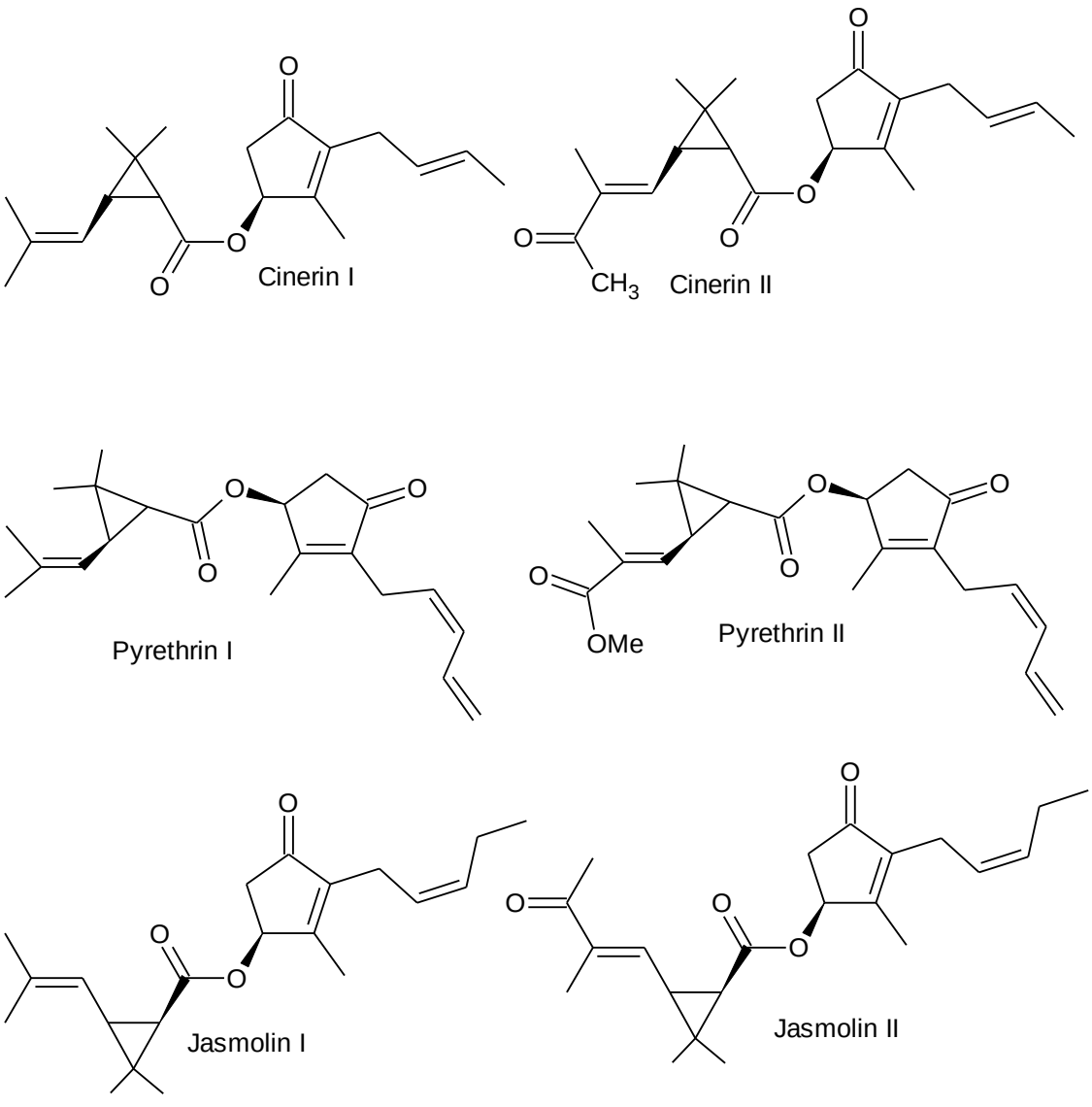

Figure 1 Structures of pyrethrins

Properties of natural pyrethrins

Pyrethrins have a strong “knockdown” and “kill” effects on a wide range of insects. The least dose required to 'knockdown' a mosquito and a housefly are 1.5 x 10 $^{-12}$g and 3.33x I0 $^{-9}$g per kilogram body weight respectively. They also have a repellency and flushing power that make insects to quickly get out of their hiding places (Kotila and Yon, 2015). Pyrethrins are degradable in the environment and in mammalian tissues hence they do not last long in the environment and are also fairly safe to mammals (Romero *et al,* 2017). Pyrethrins being esters, are swiftly hydrolyzed into harmless products in the gut of animals which are subsequently excreted (Fedeli *et al*, 2013). Pyrethrins are fairly free from potentiality to cause the development of resistance or immunity to many insects as opposed to the synthetic insecticides. This sensation is also explained by its non-persistence in the environment. The commercial survival of the pyrethrum industry is attributed to the natural Pyrethrins' low mammalian toxicity, environmental safety and rapid paralysis of a wide range of insects. The pyrethrins extract also displays a repellency effect (Casida, 1973). The competition from the pyrethroids and biotechnological substitutes may be inferior due to these advantages. All the above and other desirable aspects of Pyrethrins have led to the extensively documented use of these compounds in various formulations for household pest control and storage of farm grains. Researchers have also documented on the use of Pyrethrins in formulations for public health and related fields without any reported serious health hazards (Anonymous, 1992). The advancement of agricultural production of pyrethrum is attributed to the successful application of pyrethrum extract in field and horticultural crops insect pest control (Chi, 2014).

Photochemical degradation of pyrethrins

Pyrethrins undergo photochemical reactions which are mainly the photochemical isomerization. An indication is made that the stereochemical change of pyrethrins II majorly occurs in the cinerins and jasmolins (Kawano et *al,* 1980). Knowing these properties of Pyrethrins, it is then necessary to evaluate the analytical techniques used to quantify these esters. Figure 2 shows the photochemical degradation of pyrethrins.

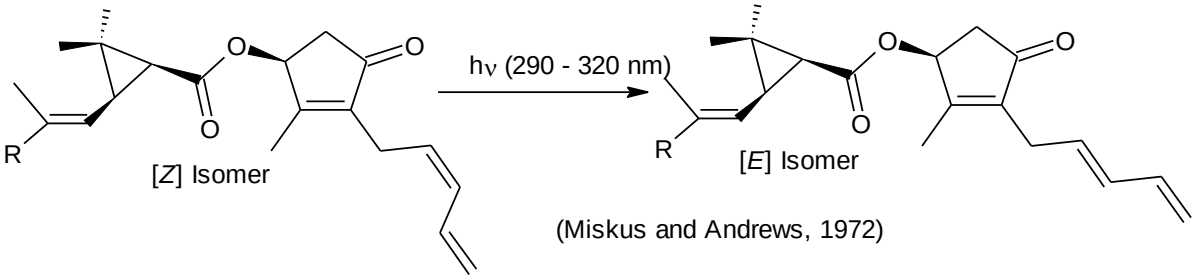

Figure 2 Photochemical degradation of pyrethrins

Exposure of pyrethrins to air and direct sunlight leads to their degradation. Due to the presence of the UV in sunlight, the resonance conjugation of the unsaturated side chain with the cyclopropane ring encompasses the disappearance of the vital activated methylene next resulting to reduced biological activity of iso-pyrethrins (Kawano *et al.,* 1980).

Transformations

Pyrethrins are prone to be to be converted to other chemical compounds when exposed to various conditions.

2.5.2.1 Dark reactions

Xenobiotics are prone to environmental reduction, oxidation, hydrolysis and other chemical transformations. Even in the dark, some reactions with environmental reagents can occur at ambient conditions. Natural pyrethrins; carboxylic esters, are expected to undergo hydrolysis, especially in alkaline waters. The rate will be low; at 25^{o}C and pH 7 as the ester linkage is between alcohols and aliphatic acid (Kumar *et al,* 2011). The hydrolysis $t_{1/2}$ of the analogous isopropyl acetate is 8.5 years and that of allyl acetate 9.7 years; P_{ka} of cyclopropanecarboxylic acid is 4.6 close to that of acetic acid. The P_{ka} of Chrysanthemic acid or the hydrolysis rate of Chrysanthemic esters have not been reported, but the carboxymethoxy group of the Pyrethrins II series is much more stable to alkaline hydrolysis than in the cyclopropane ester, allowing the isolation of pyrethric acid (Sigh *et al,* 2012).

Rapid and extensive reactions of the cyclopropane ring arise when ethyl chrysanthemate is treated with 50 % aqueous sulphuric acid at room temperature. Similar reactions occur with Chrysanthemic acid at a temperature of 210 ^{o}C in the presence of an acid catalysts and the purely thermal degradation of Pyrethrins II probably follows a similar trend at a temperature of 400^{o}C. These are rarely “environmental” transformations (Casida, 1973). However, the thermal elimination of Chrysanthemic acid and its relatives from rethrins, driven by conjugation of the generated rethrolone double bond with the ketone carbonyl would be expected at lower temperatures and maybe responsible for thermal instability of pyrethrins upon gas chromatography. When allethrin is treated with sodium hydroxide in aqueous ethanol at room temperature, this elimination occurs. Simple Chrysanthemic acid derivatives are known to react with gaseous ozone at the olefince double bond to produce the corresponding ozonide (3), aldehyde (4), acid (5) and epoxide (6). Although intact, natural pyrethrins appear not to have been examined except for jasmolin I (WHO, 2015).

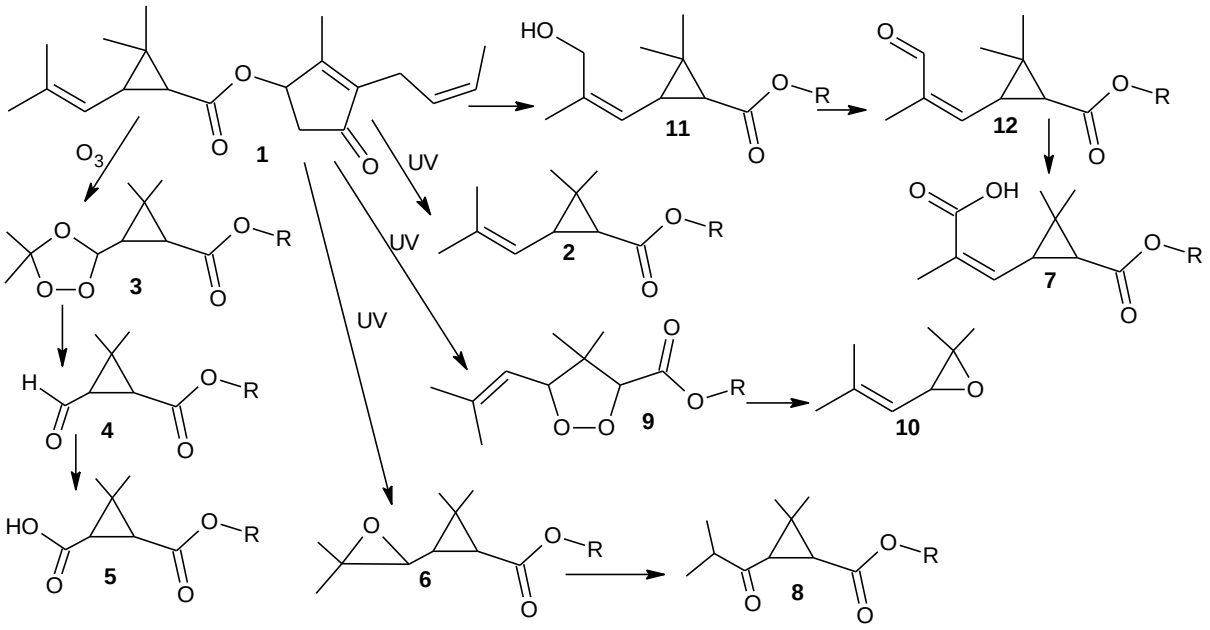

Figure 3 Photodegradation and ozonolysis pathways of pyrethrins

Materials and Methods

Sampling

Mature flowers were identified by their horizontal petals and yellowish center. They were randomly hand-picked from the nurseries in the cultivar plots. All the plots from which samples were taken had been subjected to uniform breeding treatment. Sampling was done at an experimental farm in the College of Agriculture and Veterinary Sciences, University of Nairobi in Kenya.

The samples were picked in the morning into brown paper bags and weighed immediately to get their fresh weights. The samples were then transported on the same day to the laboratory in The University of Nairobi, department of chemistry for drying and analysis. Transportation was done on the same day to ensure that the quality of the flowers were never compromised by rotting. The picked fresh flowers were weighed and then dried.

Drying of the pyrethrum flowers.

The first portion of the flowers were spread on a large polythene sheet and exposed to direct sunlight. The flowers were turned daily for two weeks of the drying period. The flowers were kept away from other factors that would alter the results as rainfall or exposure to water. The second portion were spread on a large polythene sheet and exposed to direct sunlight. The flowers were dried monitoring the change in weight until a constant weight was achieved. The third portion of flowers were spread on a large polythene sheet and dried in darkness for two weeks. The fourth portion were spread on a large polythene sheet and put in darkness. The flowers were dried monitoring the change in weight until a constant weight was achieved. The temperature within Chiromo Campus and its surrounding during the drying period was between 16-29 ^{O}C which was

confirmed with the Meteorology department at The University of Nairobi. The moisture contents of the dry flowers were tested and percentage weight loss was calculated using equation 1

Percentage weight loss = **Weight of wet flowers–Weight of dry flowers/ Weight of wet flowers** ×100% ….Equation 1.

Grinding

The dried flowers were ground using a pestle and mortar. After grinding, the powder was sieved using a BS 410 mesh and put in labeled brown paper bags awaiting extraction.

Reagents

Dinige's reagent, potassium iodate standard solution, iodine mono-chloride all prepared using AOAC method 936.05, n-hexane, sodium hydroxide, barium chloride and methanol.

Extraction and refining procedures

Extraction was done at varying temperature using soxhlet extraction for 14 hours using 1200 ml n-hexane in a 2000 ml Erlenmeyer conical flask containing a few anti-bumping chips. Ground powder of the dry pyrethrum flowers were weighed and placed in an extraction thimble. The solvent, n-hexane, was removed after extraction using a rotar-vapour to reduce the extracts volume to about 50 ml.

Hexane was then added to the concentrated sample in a flask at a ratio of 3:1 v/v solvent to sample. The solution was then kept in a refrigerator at a temperature of $2^{O}C$ for 24 hours to remove wax from the mixture. The extracts were then filtered after 24 hours through a cotton plug into a conical flask. The extracts were then evaporated to dryness in a water bath.

A volume of 25 ml alcoholic NaOH was added into the extracts and refluxed for 1.5 hours for saponification of the fatty acids. After saponification, the extracts were then transferred into 250 ml beakers. The volume of the extracts were topped up to 200 ml using distilled water and concentrated on a hot plate up to a volume of 150 ml to remove the alcohol. Cooling of the concentrated solution was then done at room temperature using tap water.

The solutions were transferred into 250 ml volumetric flasks after cooling and 1.0 g of filter-celite was added to each solution. A volume of 15 ml of 10% $BaCl_2$was added to the extracts and topped up to the mark using distilled water. Vigorous shaking of the mixtures was done to ensure that removal of the fatty acids by the $BaCl_2$ was successful. A yellowish-orange barium fatty acid salt precipitate was formed. A volume of 200 ml of the extracts were then filtered into 250 ml beakers and three drops of phenolphthalein indicator was added. Neutralization of the filtrates was then done with excess 20% sulphuric acid to precipitate the remaining $BaCl_2$. A white precipitate of $BaSO_4$ formed was filtered off through Whatman paper no. 1 coated lightly with a suspension of filter-celite on a Buchner funnel aspirator. Washing of the precipitates was then done severally using distilled water.

Separation of Chrysanthemic and Pyrethric acid

The filtrates were then transferred into a 500 ml separating funnel and extracted twice with two 50 ml portions of petroleum ether. The petroleum ether layers were transferred into a 250 ml separating funnel and washed twice with 5 ml of distilled water to remove any traces of aqueous layer. The aqueous washings were not discarded. The resultant aqueous portions were emptied into 250 ml beakers. The Chrysanthemic acid is less polar acid than the pyrethric acid and therefore in principle the pyrethrins I are contained in the petroleum ether layer while the aqueous layer contain pyrethrins II. The main goal achieved in this step was the separation of the two acids subsequently used in quantifying the pyrethrins I and pyrethrins II. The aqueous layer was then set to evaporate down to 50 ml at this point for 1 hour. The mixture was then cooled to room temperature using tap water.

Determination of Pyrethrins I

The petroleum ether layers were extracted twice using 5 ml 0.1 NaOH solution. Careful washings and transfers were undertaken during these partitioning to avoid losses of the analyte. Chrysanthemic acid has a high solubility in dilute basic aqueous solutions and so 0.1 NaOH solution was suitably used here. The basic extracts were then placed in 100 ml beakers and the petroleum ether layers were discarded.

Mercury Reduction

A volume of 10 ml of Dinige's reagent was added to the basic extract. The 100ml beaker and its contents were then kept for one hour in a dark water-bath at 25^{o}C. The extract on adding Denige's reagent changed colour to pink and then gradually to purple - light blue - deep blue-green colour. These colour changes are characteristic of Chrysanthemic acid. The intensity of these colours depend on the concentration of the acid. The mixture was then removed from the dark water bath and immediately added 3mls of saturated NaCl solution followed by 20ml amyl alcohol. The saturated NaCl precipitates Mercurous Chloride (HgCl). The precipitate was briefly boiled and filtered through a small filter paper carefully transferring all the precipitate to the filter paper. A further 10ml amyl alcohol was added to the empty beaker and boiled briefly again to precipitate HgCl. The white precipitate of HgCl was filtered through the same filter paper. The filter paper was then washed twice with 10ml of distilled chloroform using the same 100ml beaker above. This helps remove all traces of alcohol from the filter paper. The filter paper was then placed in a 200ml conical flask. The beaker was washed with 50ml of 60% HC1 aqueous solution and the sides of the beaker wiped thoroughly with cotton wool on a small glass rod before emptying both the HCl solution and the piece of cotton wool into the 200ml conical flask-containing the filter paper and HgCl(s). 20ml of $CHC1_3$ was then put into the same 100ml beaker above and added to the 200ml conical flask contents. 1ml of iodine mono chloride (IC1) indicator was added and titration with 0.01MKI03 solution carried out. The titration was done with constant shaking until the pink colour in the chloroform phase just disappeared.

Calculations of Pyrethrins I concentration

The % (w/w) Pyrethrins I was calculated using equation 2,

Percentage of Pyrethrins I = 0.7125 × $\frac{V_1}{W_1}$ **Equation 2**

Where V1- Titre volume (volume of 0.01M KIO_3 used) (cm3)

W1- Weight of sample (g)

0.7125 - Stoichiometric factor for pyrethrins I

Determination of Pyrethrins II

After evaporating the aqueous layer containing Pyrethrins II acid moiety (step 6) down to about 50ml and cooling to room temp (20^oC) the solution was transferred into a 50 ml separating funnel containing about 20 g NaCl and 10ml concentrated HC1 such that a saturated solution of NaCl was formed. The sodium Chloride solution supersaturates the aqueous layer with NaCl and therefore reduces the solubility of pyrethric acid in the aqueous layer. The aqueous layer was then extracted three times with diethyl ether washing twice with 10ml portions of saturated NaCl solution to remove traces of HC1. This step is very critical such that any traces of acid left over would lead to inaccuracies in the titre volume determination. The total diethyl ether layer was then filtered (to remove undissolved excess NaCl) through a cotton plug and residue washed with additional 10ml of diethyl ether. The total diethyl ether extract in a 500ml conical flask was then evaporated to dryness in a water-bath designed to recover the solvent. The sample was then put in an oven at 100^OC for ten minutes. On removing the sample from the oven, a current of compressed air was blown into the flask to remove HCl fumes. The sample was then dissolved in 2ml of neutral alcohol and added 20 ml of acid free water. Both the neutral alcohol and acid free water were prepared by neutralization of absolute alcohol and deionized water using dilute sodium hydroxide to make PH value to 7. Two drops of phenolphthalein indicator were then added to the conical flask containing the sample and titrated with 0.02N NaOH.

Calculation of Pyrethrins II concentration.

The percentage (w/w) Pyrethrins II = 0.4675 x $\frac{V_2}{W_2}$ **x TF ……. Equation 3**

V2= Volume of 0.02N NaOH (cm3)

W2 = Weight of Sample extracted (g)

TF = Titration factor due to the hygroscopic alkali base NaOH

0.4675 = Stoichiometric factor for Pyrethrins II

Results and discussion

Table 1: Percentage Moisture Loss from Flowers after Drying in Direct Sunlight and in Darkness

Drying condition	Dried for two weeks	Dried to a constant weight
Drying in direct sunlight	70.93%	71.23%
Drying in darkness	56.34%	69.45%

Percentage moisture loss for flowers dried in direct sunlight for two weeks was 70.93% while that of flowers dried to a constant weight was 71.23%. Percentage moisture loss for flowers dried in the dark for two weeks was 56.34% while that of flowers dried to a constant weight was 69.45% as presented in Table 1. The difference in percentage weight loss from flowers dried in darkness for 2 weeks was 14.59 while the difference in percentage moisture loss in drying to a constant weight was 1.78. The difference in percentage weight loss maybe due to temperature variations.

Table 2 Percentage of Pyrethrins on Drying the Flowers for Two Weeks

Condition	**Pyrethrins I**	**Pyrethrins II**	**Total pyrethrins**	**Ratio PI:PII**
Dried in direct sunlight	0.50	0.36	0.86±0.01	1:0.72
Dried in darkness	0.59	0.42	1.01±0.02	1:0.71

Total pyrethrins obtained from the flowers dried in darkness was 1.01 ± 0.02% while in direct sunlight was 0.86 ± 0.01% as presented in Table 2. Pyrethrins content obtained from flowers dried in the dark exceeded that from the flowers dried in direct sunlight by 0.15. The percentage of pyrethrins from flowers dried for two weeks were lower than those from flowers dried to a constant weight. This was due to the presence of moisture in the flowers dried for two weeks which may have hydrolyzed pyrethrins giving lower yields.

Table 3 Percentage of Pyrethrins on drying the Flowers to a constant weight

Condition	**Pyrethrins I**	**Pyrethrins II**	**Total Pyrethrins**	**Ratio of PI :PII**
Dried in Direct sunlight	0.63	0.39	1.02±0.01	1:0.70
Dried in darkness	0.81	0.57	1.38±0.04	1:0.62

Total extractable pyrethrins content was found to vary with drying conditions. The concentration of the pyrethrins obtained from the flowers dried in darkness being 1.38% exceeded the concentration of samples obtained from flowers dried in direct sunlight giving 1.02% as presented in Table 3.

Comparison of the Concentration of Pyrethrins Obtained from the two Methods of Drying.

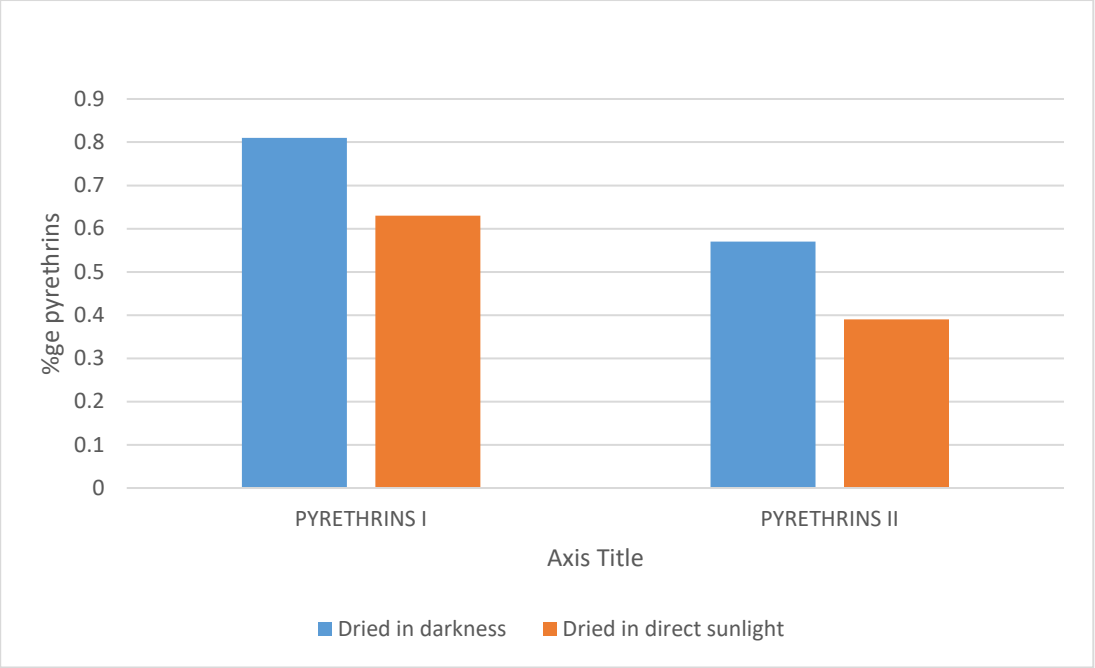

Figure 4 Comparison of the concentration of pyrethrins obtained from the two methods of drying

The concentration of Pyrethrins I was higher than that of Pyrethrins II in all the cases. The average ratio of the concentration of PI:PII was found to be 1:0.68. Pyrethrins I and II obtained from the pyrethrum flowers dried in darkness exceeded the concentration obtained from those dried in direct sunlight. This was due to photolytic degradation of the pyrethrins by the ultraviolet rays in sunlight. Heat causes rearrangements of the pyrethrins structure to form iso-pyrethrins which are thought to be insecticidally inactive. Exposure to air and direct sunlight also leads to degradation of the pyrethrins. This is due to the presence of the UV in the sunlight. There are high chances that the resonance conjugation of the unsaturated side chain with the cyclopropane ring encompasses the disappearance of the vital activated methylene next to the ring and so reduced biological activity in iso-pyrethrins results.

Conclusion

Total extractable pyrethrins content were found to be 1.38% when the flowers were dried to constant weight of 9% moisture content in darkness. The content obtained from the flowers dried in direct sunlight was 1.02%. Drying in direct sunlight lead to a percentage pyrethrins loss of 26.09%. The percentage of Pyrethrins I lost during drying in direct sunlight is 22.22% and 31.58% for Pyrethrins II. Pyrethrins II were found to degrade more than Pyrethrins I. Pyrethrum flowers should therefore be dried in darkness to a moisture content of 9%.

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