# *Pyrethrins* In Soil and Water From Selected Pyrethrum Growing Areas In Nakuru County, Kenya

*Elizabeth V. Mumbi Kigondu[1], Willie Njuguna[2], Phillip Ndemwa[3], Simon Mwangi[1], Peter G. Kirira[2], Lucia Keter[1], Peter G. Mwitari[1], Festus Tolo[1]

1. *Centre for Traditional Medicine & Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI), P. O. Box 54840-00200 Nairobi, Kenya.*
2 *Research & Development Directorate, Mount Kenya University (MKU), P. O. Box 342-01000 Thika, Kenya.*
3 *Centre for Public Health Research (CPHR), Kenya Medical Research Institute (KEMRI),*

***Corresponding Author:*** Elizabeth V. Mumbi Kigondu,
Centre for Traditional Medicine and Drugs Research *(CTMDR)*,
Kenya Medical Research Institute, (KEMRI). P.O. Box 54840-00200, Nairobi, Kenya.
Tel: +254-20-2722541; Email: ekigondu@kemri.org

## Summary

### INTRODUCTION

**Pyrethrum also known as *Chrysanthemum* is a plant from which compounds known as *pyrethrins* are derived. The *pyrethrins* have been used for many years as insecticides. Incidentally due to their high instability they have slowly been replaced by synthetic *pyrethroids*. *Pyrethrins* are generally regarded as safe compared to the *pyrethroids*.**

**However, the amounts released into the environment have not been well documented especially in pyrethrum growing regions.**

### OBJECTIVE

**The aim of the study was to determine the concentration of *pyrethrins* that come from pyrethrum plants and released into the environment through their use as insecticides, thus, into drinking water and soil, in pyrethrum growing regions in Kenya.**

### METHODOLOGY

**Quantification to amounts of *pyrethrins* from pyrethrum plants, in soil and water bodies in and around pyrethrum farms in Kiambogo and Naivasha (Nakuru County). The study was carried out using High Performance Liquid Chromatography (HPLC). Water samples (0.5L) were collected from the following water bodies: rivers, streams, dams, wells and boreholes near or within pyrethrum farms.**

### CONCLUSION

**It was established that, the quantity of *pyrethrins* present in water and soil samples were below detectable levels within the WHO recommended range. Hence safe for the environment, more so for the farmers and the people living around pyrethrum farms.**

***Key words:*** Pyrethrum, *pyrethrins*, chromatography, water, soil.

[***Afr. J.*** Health Sci. 2019 32(2) : 64 - 71]

## Introduction

Pyrethrum [*Chrysanthemum Cinerariaefolium (Trev.)*] belongs to *Asteraceae* family whose aromatic flower heads are powdered to produce pyrethrum insecticide (*pyrethrins*).

Kenya has been the leading producer of pyrethrum extract producing approximately 50% of the world's consumption, followed by Tanzania, Rwanda, Tasmania, China, Ecuador among others [1]

Pyrethrum cultivation in Kenya dates back to 1928. The plant is mainly found in the Lake Victoria region, Northern Rift Valley, Southern Rift Valley and Mount Kenya regions.

Co-operative Societies have been the major producers of pyrethrum. However, self - help groups and individuals have also been registered as producers and suppliers of pyrethrum flowers to Pyrethrum Board of Kenya (PBK).

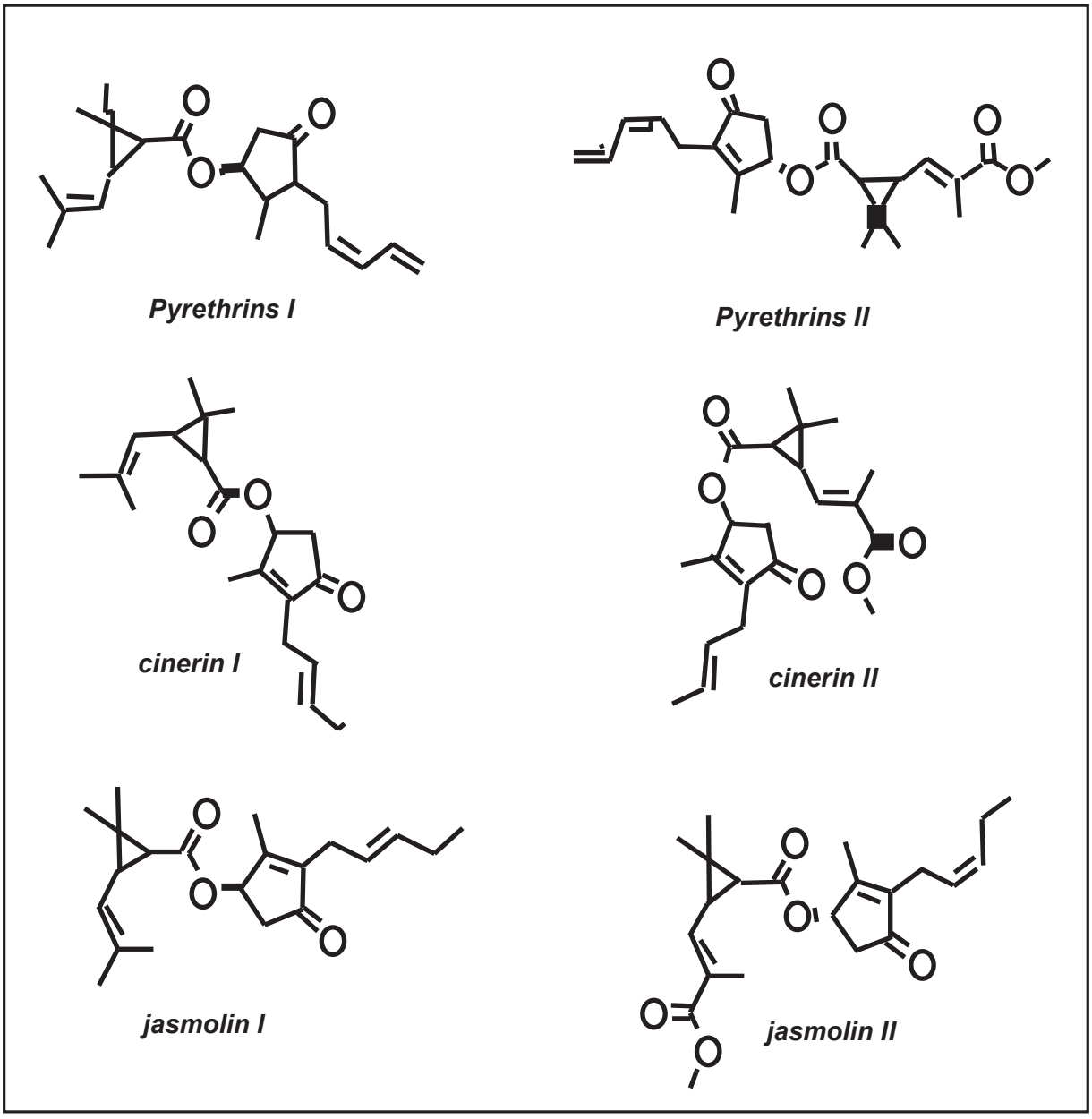


***Figure 1:*** *Pyrethrins*

*Pyrethrins* refer to the six insecticide components occurring naturally in pyrethrum. The six individual *pyrethrins* are *pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I,* and *jasmolin II* (***Figure 1***) [2,3].

The *pyrethroids* are synthetic analogs and derivatives of the original *pyrethrins* and represent a diverse group of over 1,000 powerful insecticides.

Although they are based on the chemical structure and biological activity of the *pyrethrins,* the development of synthetic *pyrethroids* has involved extensive chemical modifications that make these compounds more toxic and less degradable in the environment [1,2].

*Pyrethrins* and *pyrethroids* are released into the environment through their use as insecticides. They can be applied to crops from aerial and ground-based sprayers or applied indoors from commercially available sprays or aerosol bombs [1].

On the other hand, *pyrethrins* may also percolate into water and soil through extraction from the pyrethrum plants especially when rains occur.

The compounds are readily degraded in the atmosphere by natural sunlight and usually do not persist for more than several days to a few weeks.

*Pyrethrins* and *pyrethroids* have always been considered to be the safest insecticides

However, some studies have indicated that the latter exhibit high toxicity to fish, aquatic *arthropods,* and honey bees.

*4 Pyrethroids* have also been reported to exhibit neurotoxicity and increased oxidative stress in mice and rats while in humans an allergic dermatitis and hypersensitivity pneumonitis have been reported [3,5–7]

My team noted that the amounts of *pyrethrins* released from the pyrethrum plant into the environment have not been well documented especially in regions where the plant grows in Kenya. According to WHO (2000), the lowest lethal oral dose of pyrethrum is 500 - 1,000 mg/kg.

However, daily acceptable levels in humans is 0-0.04 mg/kg body-weight.8–10 In that regard, the study was designed to quantify pyrethrins from pyrethrum plants, present in soil and water samples collected from regions where pyrethrum is grown in Kenya.

This would help to establish the safety of the given samples, based on International Program on Chemical Safety (IPCS) recommendations [8–10]

## 2. Methodology

### *2.1 Study site*

Water and soil samples were collected from Naivasha, Kiambogo and Bahati area (Nakuru County). Preparation and analysis of the samples was carried out at the Centre for Traditional Medicine & Drug Research (CTMDR), Kenya Medical Research Institute (KEMRI) and Research & Development Directorate, Mount Kenya University (MKU).

### 2.2 Sampling Design

In Kenya, the number of farms in which pyrethrum is grown has reduced significantly since the mid 1990s.

Farmers have been uprooting the plant due to disease infestation of the crop, delay of payments to farmers by Pyrethrum Board of Kenya (PBK), high production costs and a drop in demand for the crop.

Consequently, sampling methods could not be applied to select the farms since all farms where pyrethrum grows were considered for this study.

### 2.3 Sample collection

Water samples (0.5L) were mainly collected from the following water bodies: rivers, streams, dams, wells and boreholes near or within pyrethrum farms.

Soil (500g) samples were collected from the pyrethrum farms within the same locality as the water bodies, at a depth of 20cm within a 10m radius. All samples were collected in duplicates (***Table 1***). *next page*

| Location | Area | Soil samples | Sample source | Water samples | Sample source |
|---|---|---|---|---|---|
| Naivasha | Nyamamithi | 001 | Farm 1 (upper) | 001 | seasonal river |
| | | 002 | Farm 1 (lower) | 002 | *tank-rain |
| | | | | 003 | Bore hole |
| | | | | 004 | Gakarara water project |
| | Kijabe Kenton, Mai Mahiu | 001 | Farm 1 (lower) | 001 | *Tank-dam |
| | | 002 | Farm 1 (upper) | 002 | Dripping water from pipe |
| | | 003 | Farm 2 | | |
| | Munyu, Nyaing'oya | 001 | Farm 1 (upper) | 001 | *Tank-rain |
| | | 002 | Farm 1 (lower) | 002 | *Tank-rain |
| | | 003 | Farm 2 (lower) | 003 | Munyu primary borehole |
| | | 004 | Farm 2 (upper) | 004 | Kwawanini borehole |
| | | | | 005 | Warimui stream |
| | Mwega | | | 001 | Mwega bore hole |
| | Maraigushu (Canaan village) | 001 | Farm 1 (lower) | 001 | *Tank-borehole & Rain |
| | | | | 002 | Rain |
| | | | | 003 | Stream |
| | (Kajogoo village) | 002 | Farm 2 (upper) | 004 | Shallow well |
| | | 003 | Farm 2 (lower) | 005 | *Tank-rain |
| | Kiambogo (Kahuho village) | 001 | Farm 1 | 001 | Rain |
| | | 002 | Farm 2 | 002 | Dam |
| | | 003 | Farm 3 (lower) | 003 | Rain |
| | | 004 | Farm 3 (upper) | 004 | River/stream |
| Nakuru | Bahati area (Kabatini) | 001 | Gacura farm | 001 | Rain |
| | | 002 | Gatitu farm | 002 | *Tap-river |
| | | 003 | Farm 3 | 003 | Muriundu river |
| | | | | 003 | Muriundu river |

***Tank - borehole:** water sample collected from a tank filled with borehole water;
***Tank - rain:** tank with rain water;
***Tank - dam:** tank with water from a dam;
***Tap - river:** water collected from a tap connected to a river source.

## 2.4 Chemicals

The chemicals used in this study were sourced from different Kenya Medical Research Institute's (KEMRI's) prequalified local suppliers. The standard (*pyrethrins* extract) and the analytical grade solvents were purchased from Sigma Aldrich.

## 2.5 *Pyrethrin* Extraction

Water samples (500ml) collected from Naivasha and Nakuru areas, were placed in the water bath at 60°C for 2 hrs. The samples were then filtered and freeze-dried using a freeze drying machine [4,11]

The extracts obtained weighed and were placed in amber vials. Soil extracts were extracted by the method described by [12]

Briefly the samples (500g each) were extracted with petroleum ether at room temperature (3 x 24 hours) and filtered. The filtrate was further partitioned with methanol at a ratio of 4:1 (filtrate: methanol) vigorously shaken and allowed to settle.

A pale yellowish layer containing *pyrethrins* was carefully removed and dried in vacuo using a rotary evaporator[14]. The extracted *pyrethrins* from the water and soil samples were placed in amber color vials and wrapped in foil paper to avoid exposure to light. It should be noted that protection of all samples from light was maintained throughout the experimental analysis process.

## 2.6 Sample Preparation

Approximately 20mg of each sample was weighed and placed in a 100ml volumetric flask and diluted using acetonitrile. The samples were then filtered with 0.45µM polytetrafluoroethylene (PTFE) filters and transferred into HPLC amber colored vials for analysis.

## 2.7 High Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis of the collected samples was carried out at Mount Kenya University, Research Center. A Shimadzu, LC-2010CHT HPLC equipment with an ultra violet (UV) detector was used while a supelcosil LC – 18 5µm, L=250mm, ID=4.6mm column was employed.

Mobile phase consisted of acetonitrile (eluent A) and water (eluent B). 10µl of sample was injected and eluted at a flow rate of 1.4ml/min, using a gradient method ***(Table 2)***. An Ultra Violet (UV) detector was used at 225nm to visualize the *pyrethrins chromatograms*. *Pyrethrin* extract from Sigma Aldrich was used as an external standard.

***Table 2:*** *Gradient Method*

| Time / min. | %A | %B |
|---|---|---|
| *15* | *60* | *40* |
| *25* | *80* | *20* |
| *35* | *Stop* | |

To calculate the amount of *pyrethrins* present in the samples, the following equations were used:

i. $$\% \text{ Ingredient of pyrethrins} = \frac{\text{Sample area x weight of sample x potency of standard}}{\text{Area of standard x weight of standard}}$$

ii. % Pyrethrins /100 x weight extracted from 0.5lit of water samples then extrapolated to 3.2lit (average amount of water consumed by a human being) [13]

## 3. Results and Discussion

Extraction of *pyrethrins* was carried out on 25 water samples however the *pyrethrins* present in 6 samples were below the HPLC detection limit. Seventeen water samples were found to have *pyrethrins* (***Table 3***).

It should be noted that the *pyrethrins* present in 18 soil samples collected were also below the detection limit since the results are not presented here.

Calculation of the amount of *pyrethrins* present in the water samples was carried out using the equations in section 3.5 and the quantities obtained are presented in (***Table 3)***

***Table 3:*** *Pyrethrin Quantities In Water Samples Collected from Naivasha and Nakuru*

| Location | Area | Sample source | Water Samples | Weight (mg) | Pyrethrin quantities (%) | | | | | | | Pyrethrin quantities (mg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | CII | PII | JII | CI | PI | JI | Total | |
| | | | Standard | 8.4 | 1.9 | 15.7 | 1.3 | 2.3 | 26.4 | 1.6 | 49.2 | - |
| Nakuru | *Bahati area (Kabatini)* | Muriundu river | 003 | 1.2 | 0.053 | 0.013 | 0.0073 | 0.00104 | | | 0.07434 | 0.0057 |
| | | Tap-river | 002 | 6.8 | | 0.0077 | 0.075 | | | | 0.0827 | 0.036 |
| | *Kiambogo (Kahuho village)* | Rain | 001 | 1.5 | 0.0568 | 0.019 | 0.015 | | | | 0.0908 | 0.0087 |
| Naivasha | *Maraigushu (Canaan village)* | Tank-borehole & rain | 001 | 0.6 | 0.022 | | 0.0086 | | | 0.012 | 0.0426 | 0.0016 |
| | | Stream | 003 | 9.1 | 0.108 | | | | | | 0.108 | 0.0629 |
| | *Maraigushu (Kajogoo village)* | Tank-rain | 005 | 1 | | 0.0096 | 0.0107 | | | | 0.0203 | 0.0013 |
| | | Rain | 002 | 0.9 | | | 0.011 | | | | 0.011 | 0.0006 |
| | *Kijabe Kenton (Mai Mahiu)* | Tank-dam | 001 | 3.4 | | 0.0056 | 0.0621 | 0.0037 | 0.037 | | 0.1084 | 0.0236 |
| | | Dripping water from pipe | 002 | 10.2 | 0.094 | | | | | | 0.094 | 0.0614 |
| | *Nyamamithi* | Seasonal river | 001 | 10 | 0.133 | 0.0296 | 0.0296 | | | | 0.1922 | 0.1230 |
| | | Tank-rain | 002 | 0.5 | 0.16 | | 0.0047 | | | | 0.1647 | 0.0053 |
| | *Munyu Nyaing'oya* | Wairimu stream | 005 | 0.97 | 0.154 | 0.0104 | 0.012 | | | | 0.1764 | 0.011 |
| | | Tank-rain | 001 | 5.3 | 0.0512 | | | | | | 0.0512 | 0.0174 |
| | | Kwa-wanini borehole | 004 | 8.1 | 0.072 | | | | | | 0.072 | 0.0373 |
| | *Mwega* | Mwega borehole | 001 | 10.5 | 0.293 | | | | | | 0.293 | 0.1969 |

******Tank - borehole:*** water sample collected from a tank filled with borehole water
***Tap - river :*** water collected from a tap connected to a river source.
***Tan k- dam*** : tank with water from a dam. ***Tank-rain :*** tank with rain water.

All the samples analyzed had low amounts of *pyrethrins* (***Table 3 & Figure 2***). Compared to the standard *(pyrethrin* extract) which had 49.2% *pyrethrins,* the computed amount of *pyrethrins* in all water and soil samples was <0.3%. This may have been due to exposure of the soil and water to sunlight in the farms. Hence leading to degradation of the compounds[1, 14].

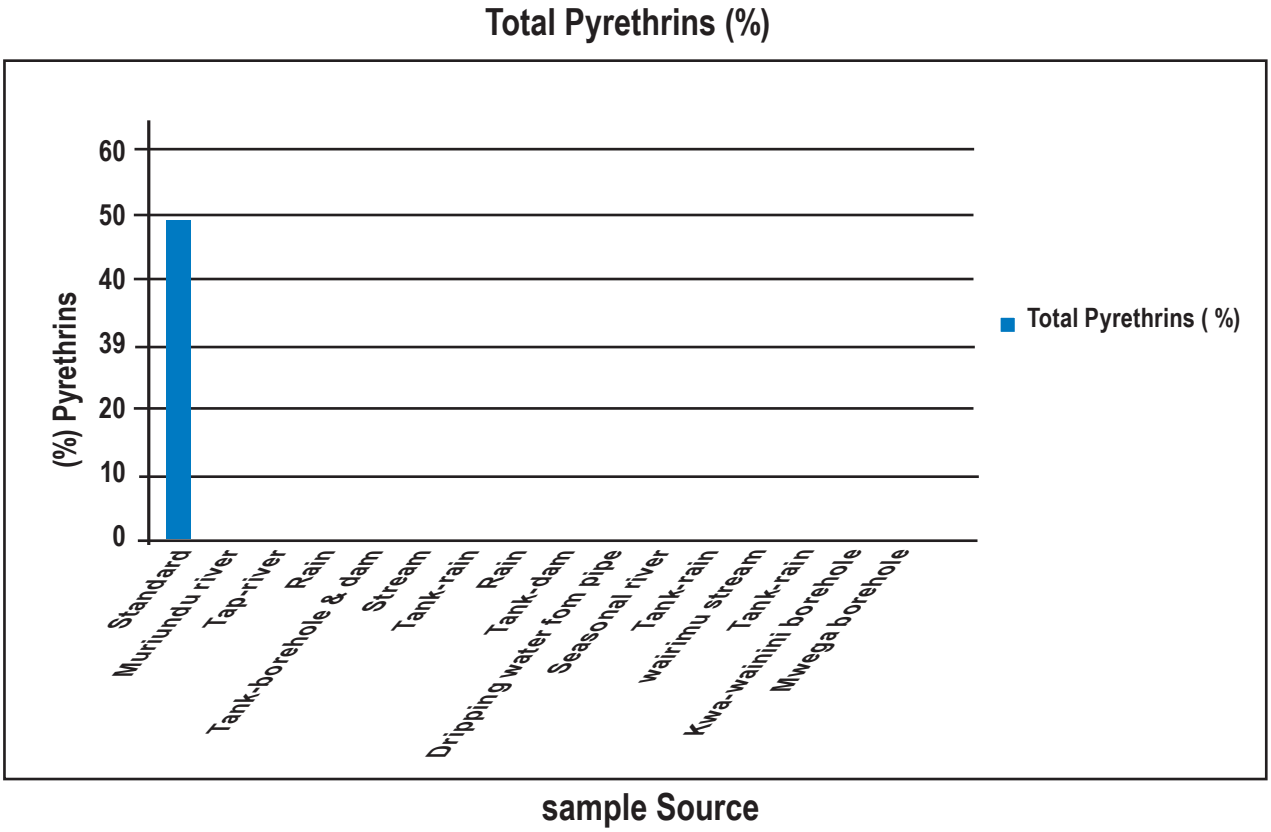


***Figure 2:*** *Total Extracted Pyrethrins In The Water and Soil Samples Compared To The Pyrethrins In The Standard Extract*

WHO (2000) recommends the lowest lethal oral dose of pyrethrum as 500 - 1,000 mg/kg with daily acceptable levels in humans as 0-0.04 mg/kg body-weight [8–10]

It hence follows that in an average adult who weighs 70kg consuming about 3.2 liters of water daily, the acceptable levels would be 0 - 2.8mg [13].

From the calculated *pyrethrin* quantities (0.00004 - 0.0308mg) in 0.5 liters of water samples collected from Naivasha and Nakuru (***Table 3***), it can be deduced that in 3.2 liters of water, the *pyrethrin* levels range between 0.0002 - 0.2mg and are within the acceptable range. Credibly, that water is safe for consumption.

It should be noted that contamination of water and soil by *pyrethrins* from pyrethrum plants in areas where it is grown in Kenya, has not been reported before. Hence, these findings will be valuable to the pyrethrum farmers.

## 5. Conclusion

*Pyrethrin* levels in water and soil samples analyzed in this study are within the acceptable WHO recommended range and can be said to be safe for the environment and human beings around pyrethrum farms in Nakuru County.

## 6. Conflict of Interest

None.

## Acknowledgement

The authors would like to thank the Government of Kenya for funding this Project and the Heads of the Kenya Medical Research Institute and Mount Kenya University where the project was carried out. We can not forget all the participants who took an extra mile to make this findings a success.

## References

1. **Casida JE.** Pyrethrum flowers and *pyrethroid* insecticides. Environ Health Perspect 1980 ; **34** : 189 – 202.

2. **Silvestroni L**. Topics in Current Chemistry, Biochemistry. Bioelectrochemistry Bioenergy 1980; **7**: 401–2.

3. **Mitchell JC, Dupuis G, Towers GHN.** Allergic contact dermatitis from pyrethrum *(Chrysanthemum Spp.). Br. J. Dermatol* 1972; **86**: 568 – 73.

4. **World Health Organization,** Safety IP on C. *Cyhalothrin* / published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. 1990.

5. **Kale M, Rathore N, John S, Bhatnagar D.** Lipid peroxidative damage on *pyrethroid* exposure and alterations in antioxidant status in rat *erythrocytes*: a possible involvement of reactive oxygen species. Toxicol Lett. 199 9; 105 : 197 – 205.

6. **Dorman DC, Beasley VR**. Neurotoxicology of *pyrethrin* and the *pyrethroid* insecticides. *Vet Hum Toxicol* 1991 ; 33 : 238 – 43.

7. **Carlson JE.** Hypersensitivity *Pneumonitis* Due to Pyrethrum. *JAMA* 1977; 237: 1718.

8. **IPCS. WHO** Recommended Classification of Pesticides by *Hazards and Guidelines to Classification* 2000-2002. 2000.

9. ADD / 71.42 W. 1970 Evaluations of some pesticide residues in food. The Monographs. Food Agric Organ United Nations World Health Organization 1971 : 198. *Pyrethrins* (AGP:1970/M/12/1). Available at: http : // www. inchem.org / documents / jmpr / jmpmono/ v070pr21.htm. Accessed February 19, 2016.

10. **Daniel T, Wohlers D, Citra M.** Toxicological Profile for Pyrethrins and Pyrethroids. US Dep Heal Hum Serv Agency Toxic Subst Dis Regist 2003: 328.

11. **Harborne JB**. Phytochemical Methods. Dordrecht: Springer Netherlands; 1984.

12. **Shawkat MS, Khazaal AQ, Majeed MR.** Extraction of pyrethrins from Chrysanthemum cinerariaefolium petals and study its activity against beetle flour *Tribolium castanum*. *Iraqi J Sci* 2011; 52: 456–63.

13. **World Health Organization.** Guidelines for Drinking-Water Quality: *Fourth Edition. Geneva*; 2017.

14. Regulation **P, Gunasekara A.** Environmental Fate of Simazine Amrith S . Gunasekara Environmental Monitoring Branch Department of Pesticide Regulation 1001 I Street Sacramento , CA 95812 April 2004. Pyrethrum flowers; *Prod Chem Toxicol* uses 2004: 194–214.