EXTRACTION OF PYRETHRINS FROM *Chrysanthemum cinerariaefolium* PETALS AND STUDY ITS ACTIVITY AGAINST BEETLE FLOUR *Tribolium castanum*

Muayad S. Shawkat, Ali Q. Khazaal, Majid R. Majeed
Department of Biotechnology, College of Science, University of Baghdad. Baghdad- Iraq

Abstract

The production of Pyrethrins as an insecticide from *Chrysanthemum cinerariaefolium* flowers was studied. The crude active compound was extracted using organic solvents. Detection by HPLC proved that the extract contained Pyrethrin I and Pyrethrin II compared to the standard compounds. The flowers extract showed active biological effect against beetle flour *Tribolium castanum* reached 100% at the concentration 40% while the mortality percent reached 60, 77% when the concentration decreased to 20 or 30% respectively.

استخلاص مركبات البايرثرين من أزهار نبات الداوودي *Chrysanthemum cinerariaefolium* ودراسة فعاليتها ضد حشرة خنفساء الطحين *Tribolium castanum*

مؤيد صبري شوكت،علي قاسم خزعل ،ماجد رشيد مجيد
قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد– العراق.

الخلاصة

درس انتاج مركبات البايرثرين ذو التأثير القاتل للحشرات من مستخلص ازهار نبات الداوودي *Chrysanthemum cinerariaefolium* المزروع في الحدائق المحلية. استخلص المركب الخام بالمذيبات العضوية واثبت الفحص النوعي بتقنية HPLC احتواء المستخلص على مركبي Pyrethrin I and PyrethrinII مقارنة بالمركبات القياسية. ابدى مستخلص الازهار تأثيراً حيوياً فعالاً ضد حشرة خنفساء الطحين*Tribolium castanum* بنسبة قتل بلغت ١٠٠% عند تركيز ٤٠% فيما بلغت نسبة القتل ٦٠ و ٧٠% عندتقليل التراكيز الى ٢٠ و ٣٠% على التوالي .

Introduction

There are many insects cause damage to crop. The losses caused by insect estimated 6 billion $ annually in the U.S.A. To solve this problem many pesticides were investigated. These chemicals have the ability to kill the target insect and non target organism such as beneficial insect. Also they accumulate in food and many human systems and cause cancer. In addition,the insect will have the ability to resist some types of pesticides.Therefore, there is a need to evaluate the materials from natural sources as a biopesticides since it is safe to human and environment. (1, 2) The human made form of Pyrethrins is called Pyrethroid which is toxic, adheres to substances and not solved in water. The exposure to Pyrthroids may cause many symptoms like dizziness, headache, nausea, vomiting, eye irritation, fatigue, muscular twitching, unconsciousness, brain and nervous disorders and immune systems

failures.(3) Although folklore is not considered a valid scientific method to solve this problem, it can serve as an excellent way for fast screening many species of plants before chemical analysis is done. The most important of natural materials is Pyrethrin which extracted from *Chrysanthemum cinerariaefolium* . Pyrethrin has many advantages like rapid degradation, wide range of effect, less toxic to beneficial insects than other insecticides. Also it kills the target insects at low concentration and doesn't accumulate in the food or water.Recently, Pyrethrin is used in many ways like using in organic farms, anti-lice shampoo and ass sprays to inhabit the insects' house. (4, 5, 6)

C. cinerariaefolium Is cultivated in Iraq under the director-general project of agricultural research and Project Abu Ghraib since the soil and weather is suitable for its culture. (7)

The purpose of this research is to extract Pyrethrin from *C. cinerariaefolium* in order to produce insecticide from natural product as a substitution to synthetic products.

Figure 1: *Chrysanthemum cinerariaefolium* flowers

Plant material

C. cinerariaefolium Plants were collected from local nurseries in Baghdad city between October and November 2008. The plant was identified by prof.Dr.Ali Al-Maosawii, Biology Department, college of Science-Baghdad University. The plants were placed in college of science plant field for watching their growth. Flowers with yellow petals were picked by hands. After full blooming and spread under sun light for 3 days then they were moved to shadow until they are completely dry and it can be known through smashing of their petals. The process of drying under sun light helps in increasing Pyrethrene production (3).

Extraction

The process of extraction of Pyrethrin was performed by two ways:

1. Method 1:

   A quantify of 50 grams of dry flowers were soaked in 100 ml of Petroleum ether for 3 days in shadow (8).

2. Method 2:

   A quantify of 50 grams of dry flowers were soaked in a mixture consisting of 100 ml of Ethanol, 100 ml of Acetone and 100 ml of Petroleum ether for 3 days in shadow (9).

   In both methods the mixture of solvents and flowers was filtered by using filter paper (Watman no. 1) then the filtrate was mixed with Methanol 80% at ratio 20:80 (extract: alcohol) the mixture were shaked vigorously and left to settle in order to be separated into two layers.

   The layer tend to yellow color which contain the Pyrethrin was isolated. The solution was concentrated by using Rotary evaporator, and different concentrations were prepared; then stored in refrigerator for quality and biological test.

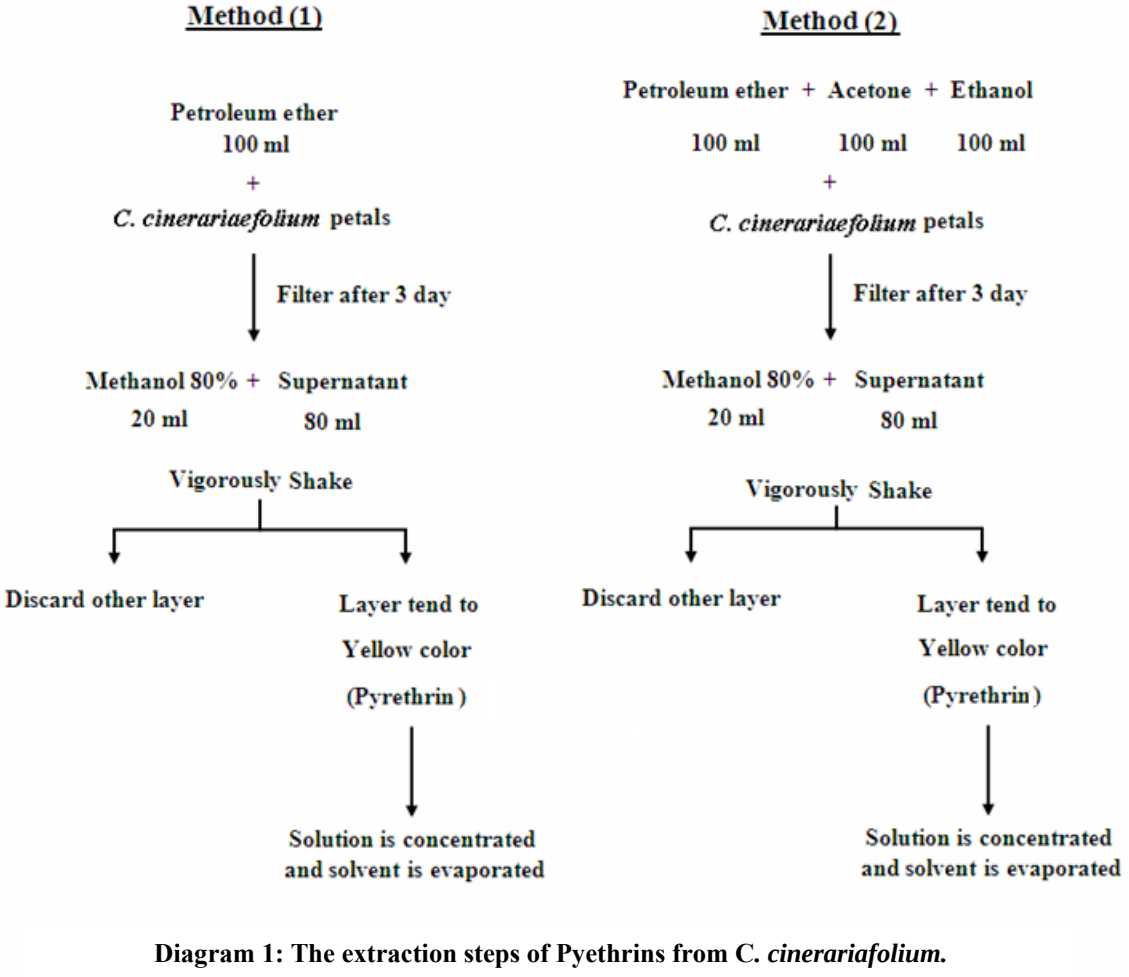

Diagram 1: The extraction steps of Pyethrins from C. *cinerariafolium.*

Quality detection

HPLC technique is used to detect the quality of the Pyrethrins in crude extract. The chromotographic analyses were performed on a 250mm×4.6mm i.d C18 (ODS) column, shimadzu ,Japan with Acetonitril: H2O 80:20(v/v) as the mobile phase at a flow rate of 1 ml/min and a volume injection was 20 µl. Detection and column temperature were set at 30 Cº.

The detection wave length was 350nm.Curves are measured on length wave 280 nanometer and are compared to curves of the standard components (10).

Biological effect test

The biological effect test was assessed by using the test cage described in the American Society for Testing and Materials (ASTM) (11) as follow:

1. Biological test is performed to know the effect of Pyrethrins extract from *C. cinerariaefolium* in the flour beetle insect. Three concentrations 20%, 30% and 40% were prepared from the final extract. Insects were raised in glass container and are fed with wheat flour. Insects were spread with the extract and the percent of killing were recorded.

2. Sample with 40% concentration is sent to the Energy and Environmental research center / Public institution for Industrial Research and Development in the Ministry of Industry and Mineral as a neutral side to perform the test of the compound activity that extracted in killing the insects. A report has been printed explained the test result.

Results and Discussion

According to figure (2) which shows the components of the *C. cinerariaefolium* extract by using method 1. Nine curves with clear peaks appeared six of them are clear which represent the active Pyrethrins and their retention time.
Through comparing between two of the curves peaks which appeared clearly with two of the standard compounds, we found that the value of retention time was 3.577 minute for curve peak No.6 and 5.560 minute for curve peak No. 7.

Figure (2) While the retention time for the standard compound (Pyrethrin I) was 3.620 minute, figure (3) and for the standard compound (Pyrethrin II) was 5.600 minute, figure (4). We couldn't detect the other curves because of unavailability of the standard compounds to make comparison and they are believed to be Jasmolin I, II and CinerinI,II.

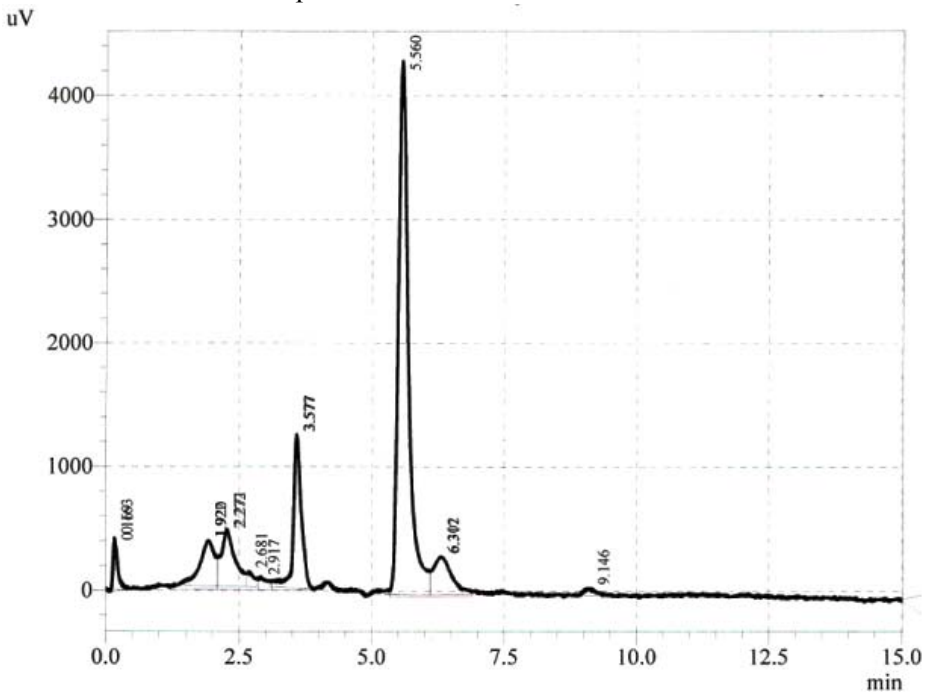

Figure 2: HPLC chromatography of flowers extraction by method 2.

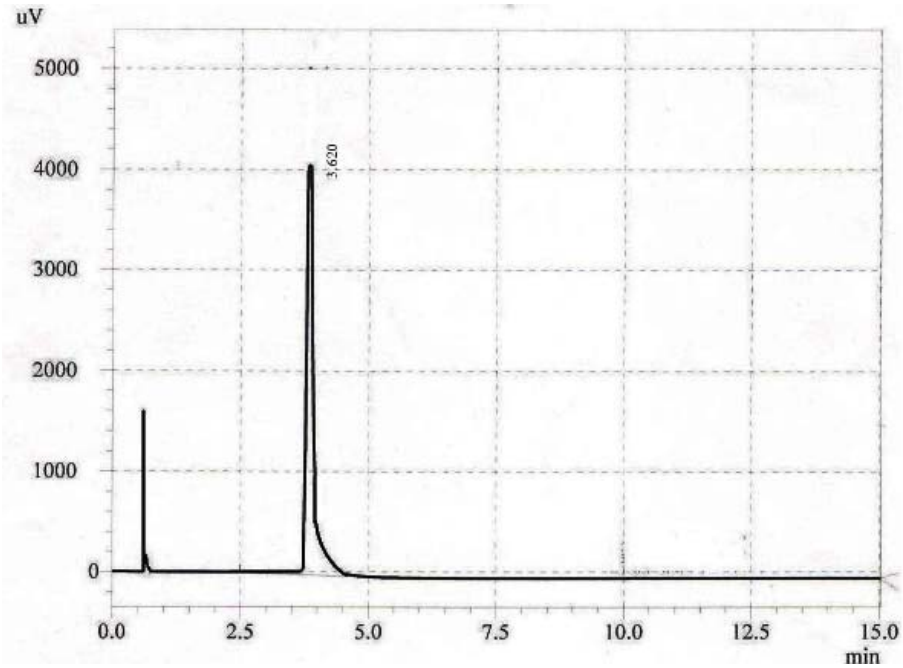

Figure 3: HPLC chromatography of standard pyrethrin I.

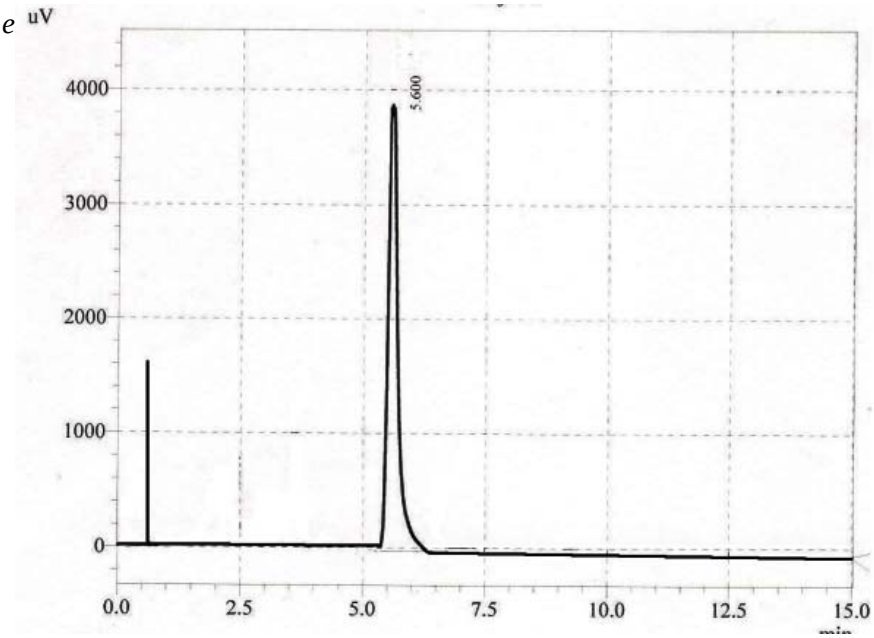

Figure 4: HPLC chromatography of standard pyrethrin II.

Table (1) shows the retention time of standard Pyrethrins and the active compound of flowers extraction. Pyrethrins have been extracted and characterised into six closely related irregular monoterpens esters form by conjugation of two acids (chrysanthemic acid and pyrethric acid) with three monotrerpien-alcohols, jasmolin-3, cinerolone-4 and pyrethrolone-5. The acid moiety of Pyrethrins is formed by deoxy-D-xylulose-5-phosphate (DOXP) pathway while alcohol moiety via linolenic acid. The collective of chrysanthemates ester jasmolin-1, cinerin-1 and Pyrethrin-1 are called Pyrethrin I while Pyrethrin II refers to ester of Pyrethric acid jasmolin-2, cinerin-2 and Pyrethrin-2 (12, 13). Figure (5) shows the result of the quality detection for Pyrethrins extraction according to method 2 in which we have used a mixture of solvents. According to the work method, we found only one curve is appeared clearly at retention time 3.826 and it could not be compared with the curves of the standard compounds. In figure (6) we notice the chemical structure for Pyrthrin I and Pyrethrin II (4, 11).

Table 1: The retention time of standard Pyrethrins and the active compound of flowers extraction.

Peak No.	Retention time (minute)	Pryethrin I	Pyrethrin II
1	0.163		
2	1.920		
3	2.271		
4	2.681		
5	٢.٩١٧		
٦	3.577	3.620	
٧	5.560		5.600
٨	6.302		
٩	9.146		

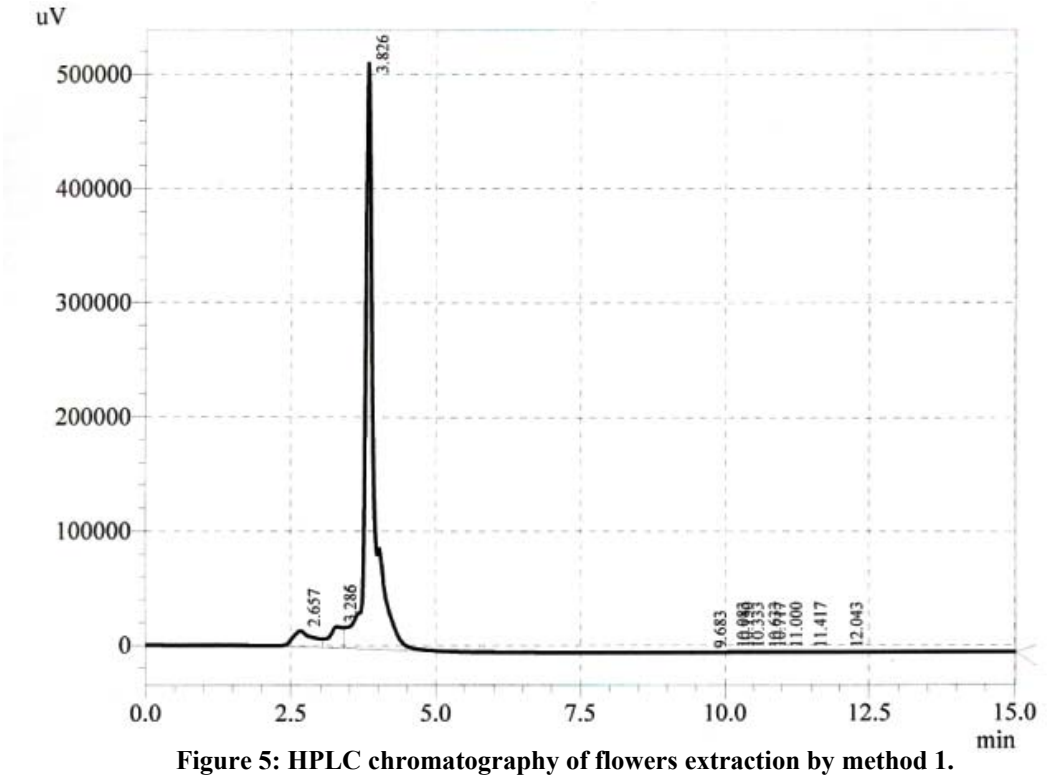

Figure 5: HPLC chromatography of flowers extraction by method 1.

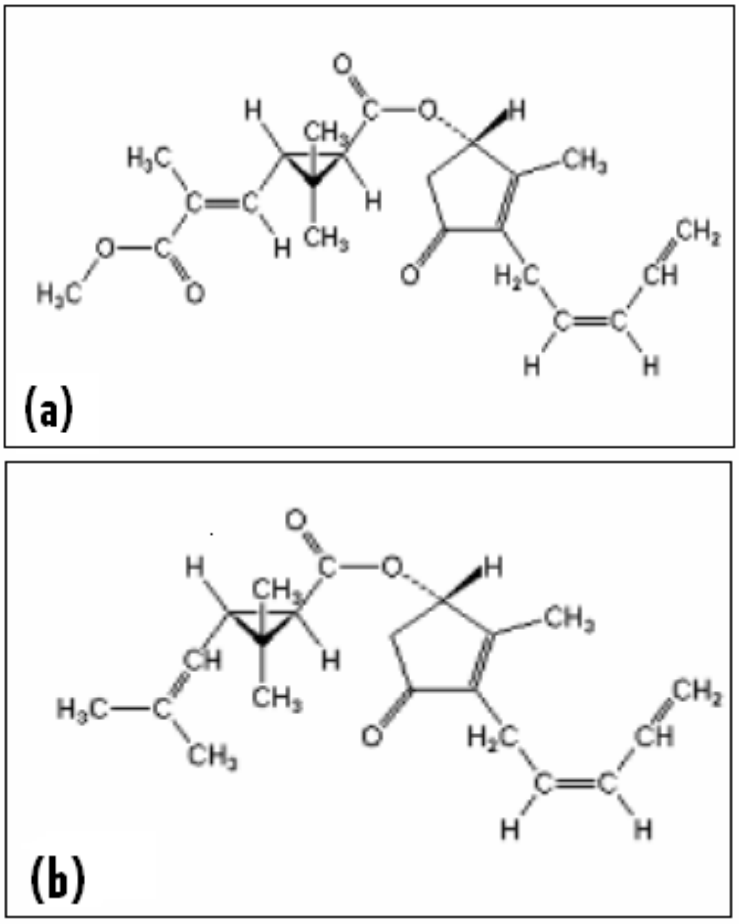

Figure 6: Structure of (a) Pyrethrin I, (b) Pyrethrin II (14).

Biological activity

To prove the activity of the Pyrethrins extract of the flowers in killing insects, an experiment is performed directly on beetle of flour. Three concentrations of the extract were used: 20%, 30%, and 40%.

The results revealed that the percentage of killing was 60%, 77% and 100%, respectively during 24 hours, as it shown in table (2).

Table (3) shows the report results of Pyrethrins extract at concentration 40% on flour beetle certificated by Energy and Environmental Research Center.

To destroy the nervous system of an insect and leads to poison the sodium canals which are cellularcomponents allow for sodium ions to enter the cell and help the transition process of the nervous pulse which leads to the move of the pulses vigorously, repeatedly resulting in paralysis and death is considered as the effects of the pesticide which is from plant origin (14). The normal growth of the insects is paralysed by the Pyrethrins compounds through effecting on the nervous system as well as its role as antifeedents. (15)

Table 2: The effect of different concentration of Pyrethrins extract on flour beetle

Pyrethrins concentration%	Incubation time(hr)	Sample volume (ml)	Killing percent	Beetle number
20	24	50	60*	100
30	24	50	77*	100
40	24	50	100*	100

* The numbers represent average of three replicates.

Table 3: The biological activity results(Energy and Environmental Research Center)

Beetle number	100
Incubation time	24 hr
Sample volume	50 ml
Sample concentration	40%
Killing percent	100% for 24 hr
Experiment place	Glass container with size 0.016 m^3
Experiment result	The compound is active to killing floor beetle

It is found that there are more than 200 kinds following 17 plants family which have this feature. But a small number of them are used to extract the pesticide commercially maybe complex Ester likes Pyrethrins or alkaloid as Nicotine and Anabusine or non heterocyclic Volatile compound such as Eotenoid. There are many types of insects that can't be killed successfully for having the ability to resist against the chemical pesticide when using them repeatedly. So it is better to use normal pesticides from plant origin which have wide range against many types of insects. In addition to the expensive of the chemical pesticide comparing with natural pesticide (16).

References

1. Joshi, S. R. **2006**. *Biopesticides*. A Biotechnological Approach. New age international (P) limited, New Delhi., India. P: 90.
2. Wells, C.; Bertsch, W. and Perich, M. **1993**. Insecticidal volatiles from the marigold plant (Genus *tagetes).* Effect of species and sample manipulation. *Chrom*. **35**: 209-217.
3. Cox, C. **2002**. Insecticide fact sheet: Pyrethrins/ Pyrethrum. *J. pes*. **22**:14-20.
4. Jovetic, C. and C. de Gooijer **1995**. The production of Pyrethrins by *in vitro* systems. *Crit. Rev. Biochem. Mol. Biol.,* 15, 125-138. (Cited) Dolinsek,A.J.; Kovac,M.; Zel,J. and Camloh,M. **2007**. Pyrethrum (*Tanacetum cineraiifolium*) from the northern Adriatic as a potential source of natural insecticide. *Ann. Ser.hist.nat.***17**:39-46.
5. Schleier, J. J.; Peterson, R.; Macedo, P. and Brown, D. **2008**. Environmental concentrations, fate, and risk assessment of Pyrethrins and Piperonyl Butoxide after aerial ultralow-volume application for adult mosquito management. Environ. *Toxicol. Chem*. **27**: 1063-1068.
6. United States Environmental Protection Agency (US EPA) **2006**. Office of Prevention, Pesticides and Toxic Substances. Reregistration Eligibility Decision for Pyrethrins. June 7, 2006. Washington, DC. P: 13-16.
7. Chakravarty, H.L. **1976**. Plant Wealth of Iraq (A Dictionary of Economic Plants). Volume one. Ministry of Agriculture and Agrarian Reform. Baghdad\Iraq: 121-122.

٨. الشحات، نصر ابو زيد. ٢٠٠٥. المنتجات الطبيعية للوصفات العلاجية من النباتات الطبية والعطرية، الطبعة الاولى، الدار العربية للنشر والتوزيع.

9. Harborne, J. B. **1973**. *Phytochemical Methods*. Science paper backs, Chapman Hall, London.
10. Essig, K. and Zhao, Z. **2001**. Preparation and characterization of a Pyrethrin extract standard.LCGC.**19**(7).www.chromotagraphy online.com
11. Jantan, I. and Zaki, Z. M. **1998**. Development of environmental friendly insect repellents from the leaf oils selected Malaysian plants. *As. Rev. Biodiv. Environ. Cons*. 5:1-7.
12. Odinga, W. A. and Angedu, C. A **2003**. The relationship between Pyrethrin and the yellow pigmentation in Pyrethrum flower. *Afr. J. Sci. Tech*. **4:** 116-123.
13. Haque,S.; Farooqi, A.; Gupta, M.; Sangwan, R. and Khan, A. **2007**. Effect of ether, chlormequant chloride and paclobutrazol on growth and Pyrethrins accumulation in *Chrysanthemum cinerariaefolium*. *Plan. Gro. Reg.* **51**:263-269.
14. Ray, D. E. and Pharshaw, P. J. **2000**. Pyrethroid insecticides: poisoning syndr-omes. Synergies and therapies. Clin. Toxicol. **38**:95-101.
15. Casida, J. F. and Quistad, G.B.**1995**. Metabolism and Synergism of Pyrethrins. In Casida, J. E. and Quistad, G. B (Eds.). Pyrethrum Flowers: Production, Chemistry, Toxicology and Uses.2nd edition. New York NY: Oxford University press pp. 65-66.
16. Ramawat, K. H. **2008**. *Plant Biotechnology*. Chand, S. and company LTD. Ram Nagar, New Delhi.India.