

## QUANTITATIVE ANALYSIS OF PYRETHRINS FROM *CHRYSANTHEMUM CINERARIAEFOLIUM* AND *CHRYSANTHEMUM LEUCANTEUM* PETALS

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**Abstract.** Pyrethrins content was determined for two chrysanthemum species namely: *Chrysanthemum cinerariifolium* and *Chrysanthemum leucanthemum*.

In order to determine the most efficient solvent for pyrethrins extraction three solvents were used: petroleum ether (I), a mixture of petroleum ether, ethanol and acetone (1:1:1) (II) and methanol (III).

Spectrophotometric method used for determination of pyrethrins is based on pyrethrins colour reaction with 2,4-dinitrophenylhydrazine followed by measurements of resulting compound at 377 nm wavelength. Pyrethrin content was determined comparing to  $\alpha$ -cypermethrin calibration curve.

According to the obtained results for the analysed chrysanthemum extracts the most efficient solvent for pyrethrins extraction was methanol and the highest pyrethrins content was found in *Chrysanthemum cinerariifolium* petals.

**Keywords:** pyrethrins,  $\alpha$ -cypermethrin, *Chrysanthemum* spp.

### 1. INTRODUCTION

The number of chemical insecticides is significant higher than natural insecticides. Worldwide around 1450 plant species have insecticide properties from which more than 200 can also be found in Romania [1].

One of these natural insecticides is pyrethrum. It is isolated from dried flower heads and is present also in *Chrysanthemum* spp. [2].

Pyrethrum is a valuable bio pesticide, very efficient, biodegradable and nontoxic, with a wide area of action, used as a contact insecticide or as a repellent [3].

Generally the most relevant studies are performed on pyrethrins extracted from *Chrysanthemum cinerariifolium* (also known as *Tanacetum cinerariifolium* or *Pyrethrum cinerariifolium*) [4-8].

Pyrethroids are the synthetic analogues of the naturally occurring pyrethrins, which are derived from the flowers of *Chrysanthemum cinerariifolium*.

$\alpha$ -Cypermethrin is the main representative of pyrethroids class, a nontoxic substance with a wide acaricide and insecticidal effects which activates against parasites through contact [9-10].

Pyrethroids acts as neurotoxins and is targeting central nervous system of insects [11].

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Pyrethroids are considerably less toxic to mammals than organochlorines, organophosphates and carbamates [12].

It is mentioned that pyrethrins extracts in hexane present maximum absorption between 219-229 nm. Regarding this pyrethrins two types are mentioned: pyrethrins I with characteristic absorptions bands between 219-220 nm and also between 222-223 nm range wavelengths and pyrethrins II with maximum adsorptions between 227-229 nm range wavelengths [13].

In literature is mention the reaction between 2,4-dinitrophenylhydrazine with  $\alpha$ -hydroxaldehyde group or  $\alpha$ -hydroxicetone group which results in formation of yellow coloured hydrazine compound that can be determined by spectrophotometric method. Generally pyrethrins and pyretroids presents  $\alpha$ -hydroxaldehyde group or  $\alpha$ -hydroxicetone group [13].

Some data is mentioning that maximum absorption of the pyrethrin's 2,4-dinitriophenylhydrazone were shown at 377 nm [14-15].

It is known that  $\alpha$ -cypermethrin is a pyretroids derivated from pyrethrins II and can be used as reference substance for quantitative analysis of compounds containing aldehydic group or cetonic group [13][16].

Following these ideas we have determined by spectrophotometric method the content of pyrethrins in extracts of *Chrysanthemum cinerariifolium* petals and also in *Chrysanthemum leucanteum* petals.

## 2. MATERIALS AND METHODS

### 2.1. MATERIALS

*C. cinerariifolium* and *C. leucanteum* were picked up in June 2015 from Dobrogea County, Romania (gardens).

Petals were dried in oven for 3 days at 30°C and then crushed and grinded in a mortar till a powder resulted.

The solvents used for pyrethrines extractions were: petroleum ether (I), a mixture of petroleum ether, ethanol and acetone (1:1:1) (II) and methanol (III)

All used reagents were of analytical reagent grade.

Standard of  $\alpha$ -cypermethrin was obtained from Sigma-Aldrich.

2,4-Dinitrophenylhydrazine was obtained from Merck.

### 2.2. METHODS

#### *Extraction of Pyrethrins*

Extractions were achieved by vigorously stirring 50 g of petals powder in 100 mL solvent for 12 h and let to set for 2 days in darkness.

The used solvents were: petroleum ether (solvent 1); mixture of petroleum ether: ethanol: acetone 1:1:1 (v/v/v) (solvent 2) and methanol (solvent 3) (Table 1).

After complete extraction, all samples were filtered by using filter paper (Watman no. 1).

The samples extracted with solvent 1 and solvent 2 were mixed with methanol (extract: alcohol at ratio 20:80), the mixture were shaken vigorously and the methanolic layer isolated. All collected extracts were brought to mark with methanol in 100 mL volumetric flasks.

**Table 1. Pyrethrin extraction from *C. cinerariifolium* and *C. leucanteum* using different solvents.**

No.	Sample (abbreviation)	<i>Chrysanthemum</i> spp.	Extraction solvent
1.	CRYSCIN01	<i>Chrysanthemum cinerariifolium</i>	petroleum ether
2.	CRYSLEU01	<i>Chrysanthemum leucanteum</i>	petroleum ether
3.	CRYSCIN02	<i>Chrysanthemum cinerariifolium</i>	petroleum ether:ethanol:acetone
4.	CRYSLEU02	<i>Chrysanthemum leucanteum</i>	petroleum ether:ethanol:acetone
5.	CRYSCIN03	<i>Chrysanthemum cinerariifolium</i>	methanol
6.	CRYSLEU03	<i>Chrysanthemum leucanteum</i>	methanol

### *Spectrophotometric analysis of $\alpha$ -cypermethrin*

Is a well-known fact that the reaction between  $\alpha$ -cypermethrin and 2,4-dinitrophenylhydrazine results in a yellow compound. In the same way the reaction between pyrethrins and 2,4-dinitrophenylhydrazine also results in a yellow compound [13].

In both cases, resulting compounds absorb at 377 nm [14-15].

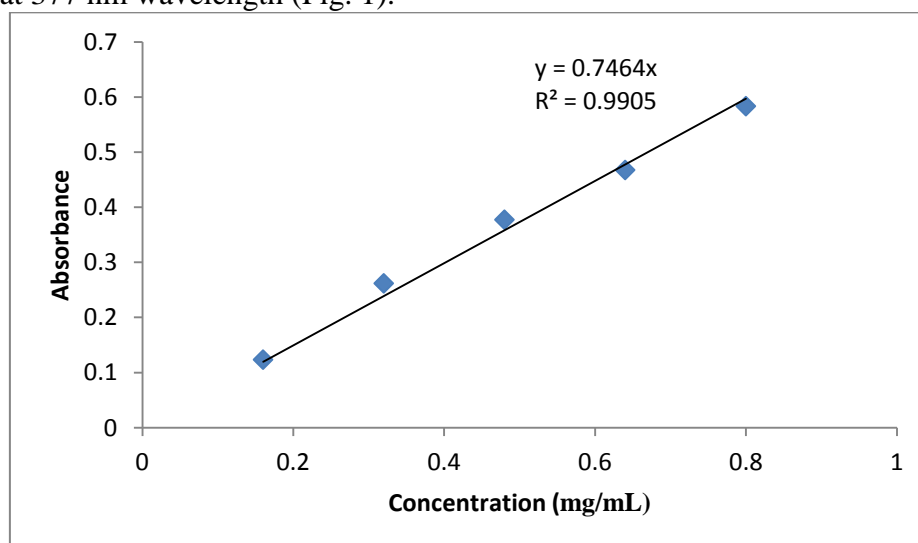
Based on this characteristic the concentration of pyrethrins in the samples were determined using  $\alpha$ -cypermethrin calibration curve.

For determinations a standard solution of methanolic  $\alpha$ -cypermethrin 40 mg/mL and methanolic 2,4-dinitrophenylhydrazine 2 mg/100 mL were prepared [14-15].

The measurements were performed using a Jasco V 550 Spectrophotometer at 377 nm.

### *Calibration curve*

In a series of 25 mL volumetric flasks were introduced volumes of 1, 2, 3, 4 and 5 mL of 40 mg/mL  $\alpha$ -cypermethrin standard solution and also 0.1 mL of 2,4-dinitrophenylhydrazine 2 mg/mL solution each. All samples were brought to the mark with methanol and right away measured at 377 nm wavelength (Fig. 1).



**Figure 1. Calibration curve of  $\alpha$ -cypermethrin.**

The content of  $\alpha$ -cypermethrin expressed as mg/100 g dry weight was calculated using the formula (eq. 1):

$$C = \left[ \frac{V_{s,flask}(mL) \times 10^{-3}}{m_{sample}(g)} \times F \times C_{det.} \left( \frac{mg}{L} \right) \right] \times 100 \quad \frac{\text{mg cypermethrin}}{100g \text{ d.w.}} \quad (\text{eq. 1})$$

The characteristics of the obtained calibration curve are:  $Y = A * X + B$ ;  $A = 0.7464$ ;  $B = 4.1780$ ; Correlation Coefficient = 0.9905; Standard Error = 0.1021;

To determine the pyrethrins in the investigated extracts, in a series of 25 mL volumetric flasks 1 mL of each extract was added, treated with 0.1 mL of 2,4-dinitrophenylhydrazine (2 mg/mL) and brought to the mark. The measurements were performed at 377 nm.

The pyrethrins concentration is determined from the calibration curve of  $\alpha$ -cypermethrin.

### 3. RESULTS AND DISCUSSION

#### 3.1. RESULTS

In Table 2 are presented pyrethrins concentrations in all studied chrysanthemum extracts measured at 377 nm.

**Table 2. Pyrethrins content from *C. cinerariifolium* and *C. leucanteum* extracted with different solvents.**

No.	Sample (abbreviation)	Pyrethrins mg/mL
1	CRIZCIN01	0,23
2	CRIZLEU01	0,17
3	CRIZCIN02	0,32
4	CRIZLEU02	0,21
5	CRIZCIN03	0,41
6	CRIZLEU03	0,30

In Fig. 2. it presented the variation of pyrethrins in the samples of *Chrysanthemum cinerariifolium* and *Chrysanthemum leucanteum* petals expressed as g/100g dry weight.

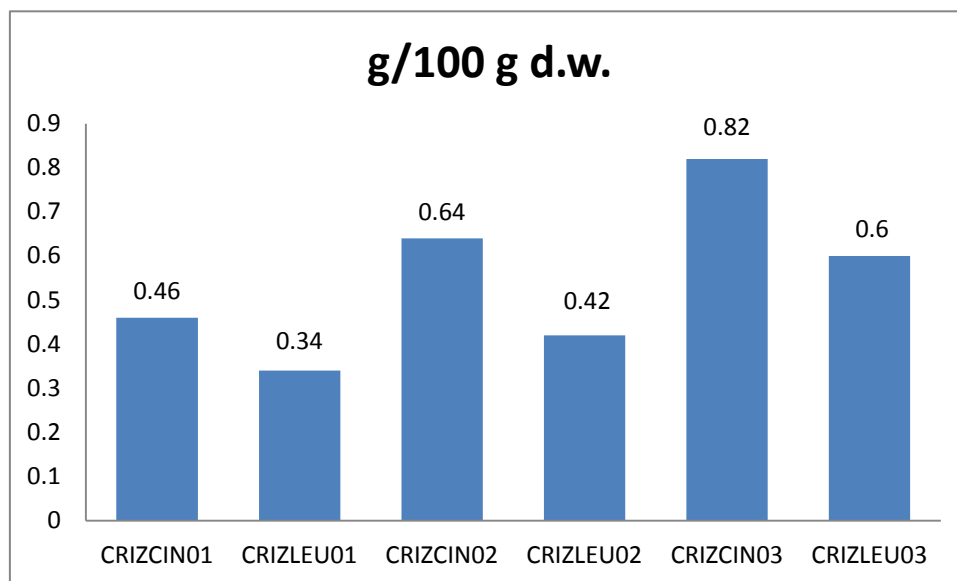


Figure 2. Content of pyrethrins from *C. cinerariifolium* and *C. leucanteum* in g/100g dry weight petals.

### 3.2. DISCUSSION

The obtained data indicate that the content of pyrethrins in all three extracts of *Chrysanthemum cinerariifolium* petals is higher than contents of pyrethrins in all three extracts of *Chrysanthemum leucanteum* petals.

Samples extracted with methanol (CRIZCIN03 and CRIZLEU03) of both chrysanthemum species have the highest content of pyrethrins which indicates methanol as the most efficient solvent.

It was also noticed that the highest content in pyrethrins is found in methanolic extract of *Chrysanthemum cinerariifolium* petals (0.82 g/100g d.w.).

### 4. CONCLUSIONS

The spectrophotometric method described in this study based on the reaction of pyrethrins with 2,4-dinitrophenylhydrazine has given reproducible analytical results.

The content of pyrethrins in *Chrysanthemum cinerariifolium* petals is higher than in *Chrysanthemum leucanteum* petals.

From all solvent used in pyrethrins extractions the most efficient was proved to be methanol.

Both chrysanthemum species have a rich content in pyrethrins which makes them suitable source for obtaining nontoxic and biodegradable insecticides.

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