STUDY REGARDING BIOCHEMICAL CHARACTERIZATION AND SOME PREPARATIONS FROM NETTLE AND WORMWOOD IN ORDER TO CAPITALIZE THEM AS BIOINSECTICIDE / BIOFERTILIZERS IN ORGANIC AGRICULTURE

POPESCU C.[1);3)], PRUTEANU A.[2)], VOICEA I.[2)], IVANCU B.[2)], GĂGEANU G.[2)], POPA L.[4)], VLĂDUT V.[2)]

[1)] S.C. HOFIGAL Export Import S.A.; [2)] INMA Bucharest; [3)] Faculty of Medicine, Pharmacy and Medical Dentistry, "Vasile Goldiş" Western University, Arad; Elias Hospital Bucharest

Keywords: organic agriculture; bioinsecticide / biofertilizers, plant extracts

ABSTRACT

Plant extracts contain substances that may induce, in treated plant, stimulation of defense mechanism, which will result in increased crop resistance especially against pathogenic microorganisms and pests that are causing the biggest economic losses and propagate the infection risk in time. Plant extracts are topics approached for a long time in support of organic agriculture, but still represents a generous particularly concerning on this essential aspect of a real organic agriculture. This paper is aimed at biochemical characterization of plant extracts of nettle and wormwood in order to capitalize them further as organic bioinsecticide / biofertilizers in organic agriculture. Extracts of nettle and wormwood are repellents and usually used preventative against pests, but also with economic advantages.

INTRODUCTION

Organic agriculture promotes the sustainable production systems, diversified and balanced, in order to prevent environmental and harvest pollution and with direct involvement on human health and safety. Organic production in the crop without using harmful traditional products, possess a special concern for several decades in economically developed countries. The interest in products and organic production is steadily increasing in our country, being supported by an increasing trend of Romanian population culture regarding the benefits of organic agriculture [8].

The therapeutically "miracles" of the plants were born in protohistory of mankind, but they also exist today. As the evolution of human thought, miracles become reality when researchers succeed to find them scientific explanation [2].

As is known, at the base of therapeutic efficacy of medicinal plants, essential is the quality and safety of raw materials used to obtain various pharmaceutical preparations, food supplements, cosmetics, food and active ingredients of plant origin.

The first element is to know the part of the plant with the highest content of active substances according to the intended purpose (roots, rhizomes, aerial parts, bark, leaves, flowers, fruits, seeds).

The second important *element,* related to the content of the active substances, is the optimal harvest time as it is subject to the growth stage of the plant (before flowering, in buds, blooming), season, the time of day and the weather conditions.

The third element is to respect the correct method of harvesting. In general, the plants are harvested during dry in the morning. In contrast, plants containing volatile oils are harvested, especially on cloudy or morning before sunrise.

All these vegetal raw materials after harvest, they dry immediately in dryers specially arranged in well-ventilated areas, to ensure keeping quality of active substances during drying. Before drying, the foreign bodies, parts of plants altered, yellowed or browned and plants attacked by insects are removed. This preprocessing is necessary because the medicinal plants used as such or in the form of various preparations must have purity as high as possible.

Therefore it is preferable to use plants grown in organic conditions for obtaining a *BIO plant material* with superior biological qualities. On the basis of plant protection, in organic agriculture exist two principles [1]:

- Stimulating natural factors on adjusting pest populations;
- Quitting of synthetic products as well as organisms and products obtained using genetic engineering.

They are required in order to limit the introduction into the nature of organisms and artificial substances. The bioinsecticide are effective natural substances derived from plants, used to combat pests or parasites, and the biofertilizers are natural substances able to enrich the substrate plant nutrients for a proper growth and functioning of the plant.

Plant extracts are substances which are likely to stimulate plant defense mechanism, which will cause increased crop resistance, especially against pathogens and pests that cause the greatest losses. Many of these products act primarily on increasing plant resistance by thickening the cuticle, which prevents the penetration of hyphae mycelia or tube of insects, stimulates plant vigor and does not act directly through toxicity. Plant extracts come from a long time in support of organic agriculture [8].

In the case of extracts for the control of insects some of which are repellent to pests, but others contain toxic compounds. Treatments of plants in fields and greenhouses are made using natural products, organic. If the attack of pests and diseases is in its infancy, it can be controlled using natural remedies. In the category of natural remedies makes part the herbal products, which, properly applied have high efficacy especially if they are prepared and properly applied. The advantage of using these products is that most often they have very low cost, does not pollute the environment and, in particular, are not toxic to humans and animals.

These preparations are the decoction, the extract, the infusion and the macerate.

- *The decoct* is prepared in cold water leaving plants for 24 hours after which it is heated to near the boiling temperature.
- *The plant extract* is prepared leaving the vegetal material in water at ambient temperatures for about 3 days.
- *The infusions* are prepared by pouring boiling water over the vegetal material, which allow macerating at least one day.
- *The macerate* is obtained fermenting plant material in rainwater for two three days or more; it can be used when the fermentation process is over and the water is dark.

Plants, with potential as biofertilizers and bioinsecticide, are: wormwood, horsetail, garlic, fern, nettle, tobacco, peppers and others.

Wormwood is effective as a natural antibiotic and insecticide.

Nettle is probably one of the most used natural combat of pests and diseases. Due to the high content of formic acid, keep away insects, but has positive effects in combating fungal diseases and bacterial infections. At growing vegetables in small spaces, these plants are used successfully in the treatment of diseases and pests. Organic agriculture is based, largely, on the knowledge of the properties of these plants and uses them to keep away pests and diseases. Extracts of nettle and wormwood are repellents and usually used preventative against pests [1].

Particularities for organic preparations of vegetal origin from nettle (*Urtica dioica L*) [1]:

- Botanical particularities: perennial plant, herbaceous, spread across the country in plain area and up on the mountain; the leaves and the stem are covered with numerous stiff bristles and urtica;
- Plant organs used: aerial part apply when fresh or dried;
- Chemical composition: protein substances with a large number of amino acids, carbohydrates, fatty substances, organic acids, vitamins, chlorophyll and salts of Ca, Mg, Si;
- Action: trophic (stimulating plant growth) and antiseptic (slows the attack of insects);

- Preparation: macerate (1 kg of fresh plant material in 10 l of water for 12 hours and it's let to macerate, then strain) and purine fermentation (from 1 kg of fresh plants or 200g of dried plant material in 10 l water);
- Usage: It is used to stimulate growth of young plants (especially vegetables) and preventively against soil fungal diseases (blight) and plants (leaf chlorosis of fruit trees) [11].
- Nettles have a beneficial impact on our health condition and on nature health. They are rich in chlorophyll, vitamins, and contain numerous minerals, all of which are needed for every living organism.
- But besides the fact that nettles are a miracle for our health, they have many benefits in the garden, acting both as a fertilizer and as a natural fungicide and insecticides.

The particularities for organic preparations of vegetal origin from wormwood (*Artemisia absinthium L.)* are [1]:

- Botanical particularities: perennial plant with white gray appearance because of densely and silky bristles, very little pretentious to soil conditions and widespread in lowland and hilly areas;
- Plant organs used: the aerial part (stem, leaves and flowers), apply when fresh or dried;
- Chemical composition: wormwood grass contains volatile oil;
- Harvesting the plant material is during the flowering season and the drying, only in the shade, in thin layer and in aerated and dry spaces;
- Action: protects crops from some pests;
- Preparation: purine (300 g of fresh plant or 30 g dried in one liter of water), purine mixed with sodium silicate (1%), infusion and decoct;
- Usage: each preparation has specific action: the purin against ants, caterpillars and aphids; the infusion against blackberry and raspberry dust mites and larvae of Colorado potato beetle; and decoction against cabbage fly (*Chortophila brassicae*) and apple worm (*Carpocapsa - Cydia pomonella*) [11].
- The nettle leaven is not only a natural insecticide but also a highly effective natural fertilizer, strengthen the plants, making them resistant to pest and disease attacks.

MATERIAL AND METHOD

The study was carried out on samples of plant material from Hofigal own organic cultures.

Fig. 1 - *Nettle crop (Urtica dioica L).*
(Organic crop of SC Hofigal Export Import SA Bucharest)

The samples studied were represented by the dried and grinded aerial parts, herba, of nettle (*Urtica dioica L*) and wormwood (*Artemisia absinthium L*).

Fig. 2 *– Wormwood crop (Artemisia absinthium L).*
(Organic crop of SC Hofigal Export Import SA Bucharest)

Analyses were performed in the laboratories of SC HOFIGAL Export Import SA, producer of medicines, food supplements, cosmetics and natural and organic active pharmaceutical ingredients, certified manufacturer GMP (= Good Manufacturing Practice) by the competent national authority (National Medicines Agency) recognized in the European Union. All tests were performed in accordance with the European Pharmacopoeia 8th edition and of specialized literature. All the methods have been validated in accordance with the current regulations.

Fig. 3 - *Wormwood crop (Artemisia absinthium L) - detail.*
(Organic crop of SC Hofigal Export Import SA Bucharest)

The macroscopic identification of dry herba sample of nettle and wormwood was performed in accordance with the referential above and all of the characteristics corresponded with the provisions of Hofigal Technical Specification, developed by the same referential.

Fig. 3 *- Nettle crop (Urtica dioica L).*
(Organic crop of SC Hofigal Export Import SA Bucharest)

The results of microscopic characteristics presented in Table 1, are within the limits of admissibility imposed by the same pharmacopoeia.

Table 1

The results of verification the microscopic characteristics of the dried aerial parts of nettle (*Urtica dioica L*) and wormwood (*Artemisia absinthium L*)

NETTLE characteristics	**Admissibility limits**	**Obtained results**
Foreign bodies, total [% max.]	2.0	1.4
a. Parts of the same plant:		
- Stems with a diameter greater than 5 mm [% max.]	1.5	1.0
- Lignified stems [% max.]	Lack	Lack
b. Foreign bodies:		
- Organic (insect larvae)	Lack	Lack
- Inorganic (soil, sand), [% max.]	0.5	0.4
WORMWOOD characteristics	**Admissibility limits**	**Obtained results**
Foreign bodies, total [% max.]	7.0	3.2
a. Parts of the same plant:		
- Stems with a diameter greater than 4 mm [% max.]	5.0	2.5
- Yellowed plants,[%, max.]	1.5	0.6
b. Foreign bodies:		
- Organic (insect larvae)	Lack	Lack
- Inorganic (soil, sand), [% max.]	0.5	0.1

For ***nettle***, the chlorogenic acid content and the caffeoylmalic acid, expressed in chlorogenic acid, is determined according to the European Pharmacopoeia provisions, current edition, "Folium URTICAE" monograph and chapter 2.2.29 through high performance liquid chromatography (HPLC). [10]

The reagents used are:

- Methanol R, 40% solution (v / v);
- P-coumaric acid R;
- Chlorogenic acid CRS;
- Phosphoric acid R;

- Water R;
- Internal standard solution: dissolve 20,0 mg of p-coumaric acid in methanol R, 40% solution (v / v) and dilute to 200 ml with the same solvent;
- Test solution: to 0,200 g of the sample (355) was added 25 ml of methanol R, 40% solution, (v / v);
- Reference solution: dissolve 10,0 mg chlorogenic acid CRS in 100,0 ml of methanol R, solution 40%, (v / v), and dilute to 25,0 ml with the same solvent;

Working conditions:

Precolumn:
- Length = 4,0 mm, diameter = 4,0 mm;
- Stationary phase: octadecylsilyl silica gel for chromatography R (5 µm).

Column:
- Length = 0,125m, diameter = 4,0 mm;
- Stationary phase: octadecylsilyl silica gel for chromatography R (5 µm);
- Temperature: 250 C.

Mobile phase:
- Mobile phase: a mixture of 15 volumes of methanol R and 85 volumes of water adjusted to pH 2,0 with diluted phosphoric acid R;
- Mobile phase B: methanol R.

Time	The mobile phase A	The mobile phase B
[minutes]	[%, v/v]	[%, v/v]
0-1	100	0
1-25	100→85	0→15
25-35	85	15
35-36	85→0	15→100
36-37	0→100	100→0
37-41	100	0

Flow rate: 1 ml/minute.
Detection: spectrophotometric at 330nm.

Work technique.

It injects about 20 µl test solution and reference solution. The relative retention on chlorogenic acid (retention time = about 13 minutes) and caffeoylmalic acid = about 2,2.

It is calculated the percentage in caffeoylmalic acid and chlorogenic acid, expressed in chlorogenic acid, using the following formula:

$$\text{caffeoylmalic acid and chlorogenic acid, expressed in chlorogenic acid, \%} = \frac{A_1 \times m_2 \times p}{A_2 \times m_1 \times 20},$$

where:

A_1 - the sum of the peak areas determined by caffeoylmalic acid and chlorogenic acid in the chromatogram, obtained with the test solution;

A_2 – the sum of peak areas determined by caffeoylmalic acid and chlorogenic acid in the chromatogram, obtained with reference solution;

m_1 - the mass of the sample used in the preparation of the test solution, in g;

m_2 - the mass of chlorogenic acid CRS used in the preparation of the reference solution, in g;

p = the percentage content of chlorogenic acid in chlorogenic acid CRS.

For ***wormwood***, the chromatographic profile was determined according to the European Pharmacopoeia [10], chapter "Herbal drugs", WORM WOOD monograph, Absinthii herba". The method of analysis was the chromatographic method, the equipment used was the gas chromatograph mass spectrometer equipped with GC-MS.

According to the method of analysis Hofigal CC-MFC 336/2014, the reagents used were: hexane R; absinthin; test solution: the sample is diluted with hexane, if necessary, it's dried on a small amount of anhydrous sodium sulfate R and filtered through 0,2µm filter; anhydrous sodium sulfate R; reference solution: dissolve 5 µl absinthin R in 5 ml hexane R. Working conditions for the column: at 40°C temperature, with the flow rate of 1 µl / minute.

- ***Description of the method of analysis, The chromatographic methods***

Equipment: Gas chromatograph equipped with:

- Detector: mass spectrometer;
- Split injector;
- Automatic system for injection of sample subject for analysis;
- Automatic system for integration of peak areas of the chromatograms obtained;
- The macrogol column 20 000 R (0,25 µm film thickness l = 30 m, Ø = 0,25 mm.

Working conditions:

- Carrier gas - helium for chromatography R with flow rate of 1,5 ml / minute;
- Split ratio of 1/50;
- Temperature:

	Time, (minute)	Temperature [°C]
Column	0 -10	40 40→280
Injector		250
Detector		287

- Injected volume: 1µl.

Reagents:

- Hexane R;
- Absinthin;
- Test solution: the sample is diluted with hexane, if necessary, it's dried on a small amount of anhydrous sodium sulfate R and filtered through 0,2µm filter;
- anhydrous sodium sulfate R;
- reference solution: dissolve 5 µl absinthin R in 5 ml hexane R

Work technique:

- It injects the test solution and the reference solution and it's recorded the retention times.
- The order of elution: the order indicated by the reference solution.

Using the retention times obtained with the chromatogram of reference solution, are located its components in the chromatogram obtained with the test solution. Is determined the percentage content of components of the test solution through the normalization procedure.

RESULTS AND DISCUSSIONS

In Figure 1 are shown the values of quality parameters for dry herba of nettle and wormwood, expressed as a percentage compared to the admissibility limits imposed by the technical specifications.

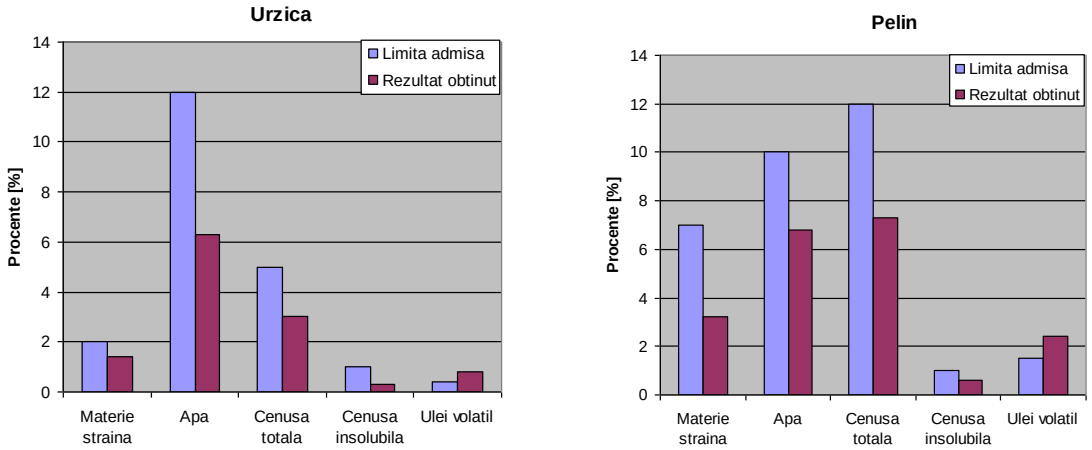
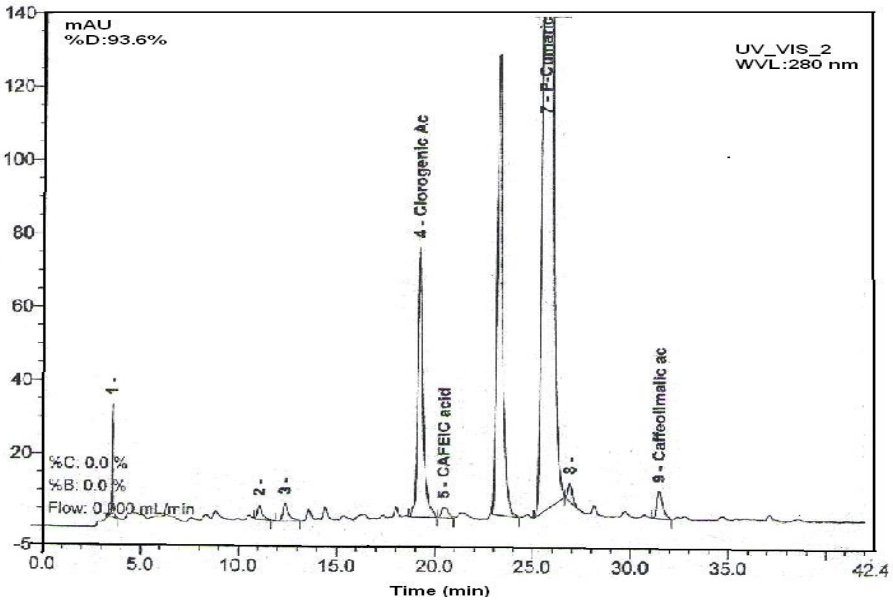


Fig. 1 - Foreign matter content [%]; Water; Total ash; Insoluble ash; Volatile oil - for *dry herba nettle* (left) and *wormwood* (right)

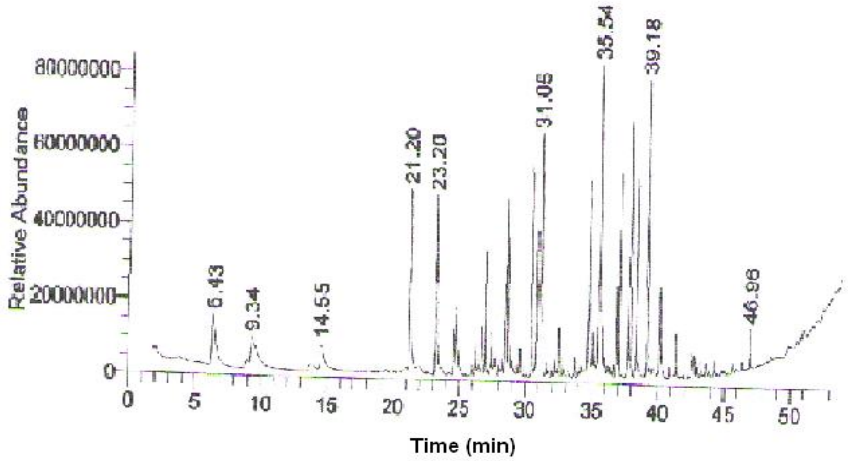
Following the results it is observed that were not exceeded the limits of admissibility quality parameters analyzed for the two types of herbs, in addition, the two plant species used for the experiment are rich in volatile oil, ingredients with antimicrobial properties of choice. Further, the study sought to highlight the chemical composition of the extract obtained from the grinded dried herb of nettle and for volatile oil of wormwood.



No.	Ret.Time [min]	Peak Name	Height [mAU]	Area [mAU*min]	Rel. Area [%]	Amount [mg/ml]	Type
1	19.17	Clorogenic acid	73.671	21.984	8.62	0.0406	BMB
2	20.44	Cafeic acid	2.700	1.120	0.44	n.a.	BMB
3	25.55	P-Cumaric	505.641	186.845	73.27	0.0954	BMb
4	31.46	caffeoylmalic acid	7.320	2.648	1.04	n.a.	BMB

Fig. 2 *- Chromatographic profile of the nettle extract from the grinded dried herb*

HPLC determinations of the extract, obtained from grinded nettle plant, allowed the identification of quantitatively in plant material of four acidic components (chlorogenic acid, caffeic acid, p-coumaric acid, caffeoylmalic acid). The content of these components is shown in the chromatographic profile from Figure 2. In Figure 3 is shown the chromatographic profile of the volatile oil of wormwood obtained from the grinded dried plant.



No.	Ret. Time [min]	RT	Area [%]	Peak Area	Peak height	S/N
1	Pinene α	6.43	4.46	208436192	12603500	3971.25
2	Allyl cyclohexene	21.20	10.25	479681434	47085736	14836.30
3	Linalool δ	23.20	7.71	360652004	45581001	14362.17
4	Cariophyllene β	26.95	3.38	158250485	30427562	9587.46
5	Himachalene	28.53	4.59	214942084	44031359	13873.89
6	Curcumene β	28.71	3.90	182331979	33091769	10426.93
7	Absinthin cis trans	30.43	5.04	235948936	51658565	16277.16
8	Absinthin trans cis	30.87	3.60	168643764	34671345	10924.64
9	Absinthin trans trans	31.06	9.72	454778312	60799835	19157.49
10	Absinthin cis cis	34.75	5.10	238414801	46745137	14728.98
11	Cedrenol	38.37	4.37	204323806	47065838	14830.03
12	Curcumene α	40.14	3.42	159924828	22319239	7032.60

Fig.3 *- The chromatographic profile of volatile oil of wormwood*

The content of volatile oil and its chemical composition depends on:

- Species, subspecies and cultivar processed;
- Time to harvest during the growing season and the plant part sampled, instead of harvesting;
- Soil pH;
- Climatic conditions of the year when the harvesting is made;
- The type of material (fresh / dried) extracted;
- The type of solvent used for extraction.

The pharmaceutical quality is met by both studied products (phase I of the study) and orientation of subsequent stages of the study, that depends essential of the obtained results at this phase. Pharmacological action of the two products studied is reflected in their organic bioinsecticid / biofertilizers effect, studied in numerous papers, some of which are cited below, but the possibilities of obtaining new products with this activity is very high.

Studies regarding the effectiveness of combating aphids on plum, red currant and bride flower with different nettle extracts are given in [3].

An example of a study conducted to determine the insecticidal and physiological activity of volatile oil of wormwood showed that it reduced the emergence, development and reproduction of moth *Plodia interpunctella* (Lepidoptera: Pyralidae) [9].

Another example of chemical composition of the volatile oil of wormwood: it was studied by gas chromatography coupled with mass spectrometry (GC-MS), which has been identified about 60 bioinsecticid compounds with potential for insect control. It was found that *artimisin-a* extracted from Artemisia vulgaris can repels mosquitoes and other insects [7].

Acaricidal activity of ethanolic extract from *Artemisia absinthium,* against ticks, is detailed in the paper [6]. Another study describes the application of plant extracts of Artemisia vulgaris that can act with prophylactic effect on bean leaves. They have a positive impact on reducing rust disease severity of bean in organic agriculture system [4].

Evaluation of phytotoxic effect respectively allelopathy of aqueous extracts obtained from different plant organs of white wormwood (*Artemisia absinthium L.*), caryopsis germination of wheat (*Triticum aestivum L.*), ryegrass (*Lolium perenne L.*) and tarsac (*Bromus inermis L .*).

Allelopathy effect, respectively of inhibition on the seedlings of grain, on ryegrass and tarsac intensifies with increasing concentration of the aqueous extract used in the germination and the growth substrate [5].

CONCLUSIONS

In recent years there has been a shift towards the medicinal product, food supplements, and cosmetics with natural active ingredients of plant origin, search and identification of new biologically active compounds by investigating plant species, capitalization of traditional medicine remedies of various nations after scientific validation of their therapeutic effects.

An insecticide is environmentally friendly as long as any synthetic product is not included in the composite, but may contain essences, extracts, oils, macerated and other substances resulting from natural physical or chemical process, which is also the products case studied by us.

The two plants studied, by their composition, derived from organic agriculture may be sources of substances with properties of organic insecticides that are produced easily without high costs, are very efficient, produce no resistance from pests and treatments can be repeated when needed without to affect crops subject to natural treatment.

Many species of medicinal plants are frequent in our country's flora and can be cheap alternative easily accessible and free of adverse effects in combating diseases and pests, but also use as fertilizers able to enrich the substrate and plant operation.

Phytochemical studies on species from Romanian flora are rather limited, the existing data is referring in particular to the amount and composition of the volatile oil of medicinal species and not to the beneficial effects they can have as insecticides and biofertilizers to promote organic agriculture.

The two studied herbal plant products are rich sources of beneficial compounds, namely both antioxidant, anti-inflammatory but also antiseptic and antimicrobial (bioinsecticide, biofertilizers), which can be used in organic agriculture.

BIBLIOGRAPHY

1. **Atudosiei N.**, 2008 - *Clean technologies used in plant protection* (course for student use), Bioterra University, Cermaprint Publishing, Bucharest, ISBN 978-973-1887-57-9, pp. 139-141;
2. **Bojor O., Popescu O**., 2001 - *Traditional and modern phytotherapy*, Fiat Lux Publishing, ISBN: 973-9250-61-0;
3. **Bozsik A.**, 1996 - *Studies on aphicidal efficiency of different stinging nettle extracts;* Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, Volume 69, Issue 1, pp 21-22;
4. **Chhetry G.K.N., Mangang H.C.**, 2012 - *Evaluation of ecofriendly management practices of french bean rust (Uromyces appendiculatus) in organic farming system*; International Journal of Advancements in Research & Technology, Volume 1, Issue 4, ISSN 2278-7763;

5. **Corbu S., Cachiţă-Cosma D.,** 2009 - *Study on the alelopatic action of the watery extract of Artemisia absinthium l. upon the germination of the caryopses and of the growth of the plantlets of Triticum aestivum l., Lolium perenne l. and of Bromus inermis l.,* Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii Vol. 19, Issue 2, pp.295-301;
6. **Godara R., Parveen S., Katoch R., Yadav A., Katoch M., Khajuria J.K., Kaur D., Ganai A., Verma P.K., Khajuria V., Singh N.K**., 2014 - *Acaricidal activity of ethanolic extract of Artemisia absinthium against Hyalomma anatolicum ticks*, Journal Experimental and Applied Acarology, Springer International Publishing, ISSN 1572-9702;
7. **Haghighian F., Jalali S.J., Aliakbar A., Mohammad J.M.**, 2008 - *The growth regulatory, deterrency and ovicidal activity of worm wood (Artemisia annua L.) on Tribolium confusum Duv. and identification of its chemical constituents by GC-MS*, Pestycydy, (1-2), 51-59; ISSN 0208-8703;
8. **Jitareanu G., Samuil C.,** 2003 – *Technologies for organic agriculture*, Pim Iasi Publishing, ISBN 973-7967-32-1;
9. **Zamani S., Sendi J.J., Ghadamyari M.,** 2010 - *Effect of Artemisia Annua L. (Asterales: Asteraceae) Essential Oil on Mortality, Development, Reproduction and Energy Reserves of Plodia Interpunctella (Hübner). (Lepidoptera: Pyralidae)*; Journal of Biofertilizers & Biopesticide 2:1, http://dx.doi.org/10.4172/2155-6202.1000105;
10. ****European Pharmacopoeia*, the 8th Edition, 2014, European Directorate for de Quality of Medicines and Healthcare;
11. **Felecan A.S., Voevod M. -** *Principles and regulations of fertilizer in organic agriculture,* http://cultagribio.blogspot.ro/**.**