VEGETAL EXTRACT FROM SPONTANEOUS ROMANIAN FLORA WITH BIOINSECTICIDAL ACTION

G. M. DARABAN[1], Marinela BADEANU[2], Lacramioara RUSU[3], Carmen ZAHARIA[1], Daniela SUTEU[1*]

[1] "Cristofor Simionescu" Faculty of Chemical Engineering and Environmental Protection, 73A Prof.Dr.docent D.Mangeron Blvd., 700050, Iasi, Romania

[2] "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine of Iasi, Faculty of Horticulture, 3 Mihail Sadoveanu Street, 700490, Iasi, Romania

[3] "Vasile Alecsandri" University of Bacau, Faculty of Engineering, 157 Calea Mărăşeşti, 600115 Bacau, Romania

Corresponding author: danasuteu67@yahoo.com

Abstract. *Nowadays, the partial or even total replacement of synthetic pesticides with biopesticides in the cultivation and growth of plants on a large scale or only at the level of micro-farms, is in line with the requirements of the concept of "sustainable and sustainable agriculture". In this context, since various active compounds biologically from plant sources present a high efficiency, multiple mechanisms of action, toxicity to mammals, has increased interest in the study (the action of chemical and biological) these compounds with a view to their use as biopesticide in a stabilized form and easy to handle. The paper aim is to present the results of the experimental researches regarding the investigation of the effectiveness as bioinsecticidal effect of alcoholic plant extracts from spontaneous flora of Moldavia and Bucovina (Romania) (i.e. Artemisia absinthium; Primula veris; Origanum vulgare; Achilleia millefolium) in the pests control during the seeds storage (insect bean-Acanthoscelides obsoletus). Obtaining of the plant extracts was achieved by two extractive techniques: maceration (M) and ultrasound assisted extraction (UAE) in association with maceration (M) using as extractant alcohol ethilic 95%. The obtained extracts are considered environmentally friendly since they do not affect the crop plants, but instead they protect it. The quantitative analysis on the experimental data showed that the most efficient method of obtaining plant extracts with a high content of bioactive substances is the combined method ultrasound assisted extraction (UAE) + Maceration (M). Regarding the effectiveness of plants in controlling pests of the species Acanthoscelides obsoletus, the decreasing order is the following: Origanum vulgare, Artemisia absinthium, followed by Achilleia millefolium and Primula veris, whose efficiency is almost identical. Also, the biological assessment highlighted that the extract of Origanum vulgare is much more effective than that of Artemisia absinthium, in combating the studied species, given the mortality recorded among insects (70%).*

Keywords*: biopesticides, maceration, vegetal extract, ultrasound assisted extraction in association with maceration*

INTRODUCTION

Considered as a true natural laboratory, plants have always been a source of biological active substances of organic or mineral nature, really necessary for humanity existence and safe survival (SUTEU ET AL., 2010; DEKEBO, 2019). The applications of these metabolites are various, ranging from obtaining different foods, cosmetics, pharmaceuticals to plant protection products (SAXENA ET AL., 2014; BARBULOVA ET AL, 2015; RIBEIRO ET AL., 2015; ANTOLAK AND KREGIEL, 2017).

The use of biopesticides in the cultivation and growth of plants is part of the concept of "sustainable agriculture" (BRZOZOWSKI AND MAZOUREK, 2018). More and more

research shows that an increasing number of essential oils and plant extracts which have been tested against a wide range of pests with promising results (VILLAVERDE ET AL., 2016; TEMBO ET AL., 2018; SINGH AND KAUR, 2018; ZULHUSSNAIN ET AL, 2020). Thus, it has been shown that various biologically active compounds from plant sources have high efficacy, multiple mechanisms of action, low toxicity to mammals, which has led to the accelerated increase of interest in using them as biopesticides in a stabilized and easy to handle form.

The region of Moldova (NE of Romania) is characterized by an extremely diverse spontaneous flora, specific to the location of plants' growth. It should be noted that environmental factors have a strong impact on it, which translates into different growth particularities (size, blooming, density) but also through a number of chemical compositional characteristics of the plants (leaves, flowers, stems or roots). There is a spontaneous target flora (about 138 species) for obtaining vegetal bioproducts made up of plant species considered as aromatic, melliferous or of medicinal interest. This flora is beginning to be exploited for its potential to provide bioactive compounds with bioinsecticidal action (BRUDEA ET AL, 2012; COISIN ET AL, 2012; PURCARU ET AL, 2018; DARABAN ET AL, 2018A,B; PRUTEANU ET AL, 2018).

The paper aim is to present the results of our experimental researches regarding the investigation of the effectiveness as bioinsecticidal effect of alcoholic plant extracts from spontaneous flora of Moldavia and Bucovina (Romania) (i.e. *Artemisia absinthium*; *Primula veris*; *Origanum vulgare*; *Achilleia millefolium*) in the pests control during the seeds storage (bean insect - *Acanthoscelides obsoletus*). Obtaining of the plant extracts was achieved by two extractive techniques: maceration (M) and ultrasound assisted extraction (UAE) in association with maceration (M). The efficiency of the processes (expressed as the degree of extraction) was investigated considering several physical operating parameters, such as solid / liquid ratio: 1/10, 1/15 and respectively, 1/20; extraction time: 10 minutes and 15 minutes; temperature: 30°C and 40°C. The obtained extracts are considered environmentally friendly since they do not affect the crop plants, but instead they protect it.

MATERIAL AND METHODS

Plant material

The used plant material is represented by yarrow (*Achillea millefolium*), wormwood (*Artemisia absinthium*), oregano (*Origanum vulgare*) and primrose (*Primula veris*) plants collected from Tomesti (Iasi, Romania) location, which were dried in a ventilated space protected from direct sunlight. The entire dried plants were crushed using a food mill and after stored in an appropriate container, until later use.

All necessary reagents or reference standards were of analytical quality (p.a.), being purchased from the Chemical S.A. Company, Romania).

Extraction methodologies

In the extraction process 96% ethanol (Chemical Company, Romania) was used as solvent, respecting the quality conditions required by the agriculture and food industry. The established amount of vegetal powder (1 g), weighed with an analytical balance, RADWAG type (AC 230V/400W, 50Hz) was separately dispersed in the established volume of ethanol, in order to respect the considered solid-liquid ratio (1/10, 1/15 and 1/20).

The description of the used extraction techniques is achieved in Table 1.

Table 1

The presentation of the used extraction techniques

Extraction technique	Description
Ultrasound-assisted extraction (sonoextraction)	✓ It was performed by using an ultrasonic bath, SONOREX RK 100 H type (produced by Bandeline Electronic GmbH &Co.KG, Berlin, Germany, bath frequency 35 kHz, power 320 W). The set-up allowed the control of time and temperature. ✓ The variables of the extraction process were: two constant temperatures (35 °C and 45 °C), two different extraction times (10 and 15 min), with or without initial agitation of the phases. ✓ After reaching of the extraction time, the phases were separated and the filtrate was used for the quantitative and qualitative characterization as biopesticides.
Maceration	✓ It was performed at temperature of 20-23ºC, for 9 days ✓ It was used the extract resulting from the sono extraction procedure.

Determination of extraction degree

To calculate the extraction degree it was used the equation 1.

$$\eta\,\% = \frac{m_{\text{residue}} \cdot V_{extract}}{n_{extract} \cdot m_{solid\ sample}} \cdot 100 \quad (1)$$

where, m_{residue} represents the mass of the residue obtained after evaporation to dryness of the established volume for each extract, (g); V_{extract} - the volume of the extract sample for evaporation to dryness (5mL) at constant temperature up to 60°C using a thermostatic oven, (mL); n_{extract} - the total volume of extract obtained after the liquid-solid extraction, (mL); $m_{\text{solid sample}}$ - the mass of vegetal powder introduced in liquid-solid extraction process (g).

Biological assessment

For this purpose, the behavior of the bean insect located in ventilated plastic enclosures together with beans was followed under the conditions of spraying with the obtained extracts, applied in different concentrations,. The pest monitoring was carried out at well-established intervals of time (2, 8, 12, 24, 48, 72, 96, 120, 144, 168 h) after the application of the treatments. The administration of the extract solution was done by controlled release, using an adsorbent cellulosic material placed at the top of the cage impregnated with the solution extract. In the experiments, extract solutions of two concentrations were used: 60% and 100%.

The method of work and the calculation of the degree of mortality among adults and larvae was adapted after the method described by ASAWALAM *ET AL.* (2006).

$$Mortality\ \% = \frac{N_d}{N_0} \cdot 100 \quad (2)$$

where N_d represents the number of dead insects and N_0 represents the number of initial test insects.

RESULTS AND DISCUSSIONS

The extraction degree

The vegetal extraction efficiency using the considered extraction techniques (maceration and sonoextraction in association with maceration), in the case of selected solid/liquid ratios, is evaluated considering the selected working methodology, extraction conditions and plant type, being presented in Figure 1.

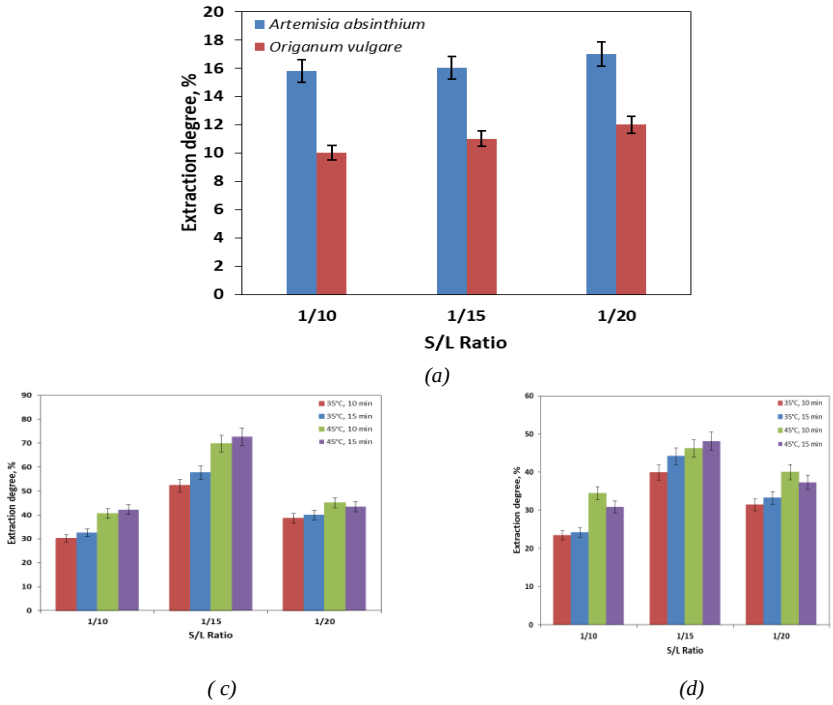
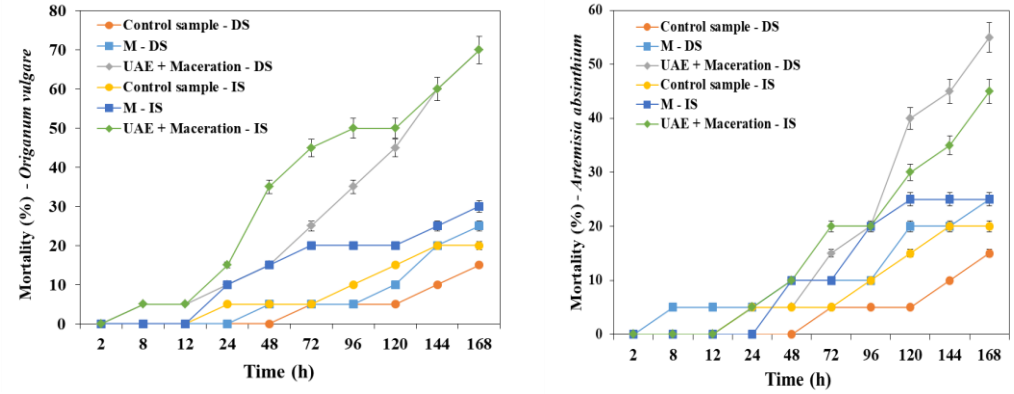


Figure 1. The obtained extraction degree (%) according to the S / L ratio using **M (a)** method and S/L ration, temperature and sonoextraction time using the **UAE+M (c, d)** method for *Artemisia absinthium* (a, c) and *Origanum vulgare* (a,d)

Assessing the bioinsecticidal activity of plant extracts

Using the methodology and study procedure previously applied in our studies on the bioinsecticidal action of these types of plant extracts (DARABAN ET AL, 2018a,b) there were performed some testing on ladybug beans (*Acanthosceledes obtectus*) behaviour, in principal considering the extracts which showed maximum bioinsecticidal action (*Origanum vulgare* and *Artemisia absinthium*) obtained by maceration and ultrasound-assisted extraction in association with maceration.

For the pest control, the used working protocol includes: (i) direct or indirect spraying of the raw form of plant extract, using a cellulose disk that was impregnated with extract, in an enclosure where the ladybug are together with beans as a food source; (ii) pest monitoring carried out at well-established intervals (2, 8, 12, 24, 48, 72, 96, 120, 144, 168 h) after the application of the treatments with obtained extracts. The monitoring consists in the identification of the number of eggs and eggs laid, as well as the establishment of the effectiveness of the treatment applied by studying the neuroleptic manifestations of pests (hyperactivity, inconsistency of the hind limbs, unnatural behaviours) and identification of the mortality degree. The obtained results are presented in Figure 2.



(a) (b)

Figure 2. The bioinsecticidal action of *Origanum vulgare (*a) and *Artemisia absinthium* (b) extracts obtained by maceration related to other used liquid-solid extraction techniques: sonoextraction+maceration (DS – direct spraying; IS – indirect spraying)

Figure 2 shows that the highest mortality rates of insect bean (*Acanthoscelides obsoletus*) are obtained in the case of extracts obtained from the use the sonoextraction in association with maceration method. Also, the most effective method of treatment application was by spraying on an adsorbent surface (indirect spraying), followed by direct spraying on food (beans) at a short distance.

Regarding the effectiveness of plants in controlling pests of the species *Acanthoscelides obsoletus*, it is observed that the extract of *Origanum vulgare* is much more effective than that of *Artemisia absinthium*, given the mortality recorded among insects (70%).

CONCLUSIONS

The experimental results showed that the most efficient method of preparation plant extracts with a high content of bioactive substances is the mixed method of ultrasound assisted extraction (UAE) in association with maceration (M).

For all plant species the highest degree of extraction was obtained at the optimal operating parameters: the S/L ratio of 1/20, temperature of 45°C and extraction time of 15 min.

In the case of the control sample, there are no abnormal manifestations, the individuals feed constantly and naturally.

Regarding the effectiveness of plants in controlling pests of the species *Acanthoscelides obsoletus*, the decreasing order is the following: *Origanum vulgare* and *Artemisia absinthium*.

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