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RESEARCHES ON THE BIOPESTICIDES OBTAINED BY EXTRACTION WITH NON-TOXIC SOLVENTS AND THE INSECTICIDE EFFECT ON DEPOSIT PESTS

BY

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Abstract. Ensuring food quality is a requirement of our days, because people have become self-aware with their own bodies, especially ensuring and maintaining the health and the quality of their life. In this context, is increasing tendency towards the consumption of products containing as small quantities of chemicals as possible, especially from the pesticide class. Recent data show that a growing number of essential plant oils have been tested against a wide range of arthropod pests with promising results. Vegetal extracts have shown high efficacy, multiple mechanisms of action and low toxicity on vertebrates. Making an overview of the scientific achievements in the field of biopesticides it were created the premises of the experimental study approach to find alternative solutions for the replacement of chemical pesticides which are used in agriculture and the food industry with biopesticides obtained from plants from the spontaneous flora of Moldavia and Bucovina areas. In this context it was

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being investigated the extraction of some chemical compounds with repellent and/or germicidal effect on pests deposit (insect beans - *Acanthoscelides obsoletus*).

Keywords: biopesticides; extraction; insecticide effect; vegetal extract.

1. Introduction

Ensuring food quality is a requirement of our days, because people have become self-aware with their own bodies, especially ensuring and maintaining the health and the quality of their life (Șuteu *et al*., 2010). For that there is an increasing tendency towards the consumption of products containing a small amount of chemicals as possible, especially from the pesticide class. Recent data show that a growing number of essential plant oils have been tested against a wide range of arthropod pests with promising results (Bett *et al*., 2017; Singh and Kaur, 2018; Zoubiri and Baaliouamer, 2014). Although the essential oils have shown high efficacy, multiple mechanisms of action, low toxicity on vertebrates the practice demonstrated that the application of plant extracts in the synthesis of biopesticides is still an incomplete studied in the field and their practical application is at microfarm level. However, the number of commercial biopesticides based on essential oils remains low.

Analyzing the most important qualities and in the same time the flaws resulting from the use of essential oil biopesticides as follows. The main challenges for the future researches are (Roman *et al*., 2016).

(1) the development of effective stabilization processes (*e.g.* microencapsulation);

(2) simplifying the complex and costly authorization requirements for biopesticides;

(3) optimizing plant growth conditions and extraction processes leading to a homogeneous chemical composition.

Obtaining plant extracts can be achieved through variants of classical or modern liquid - liquid extraction, using conventional, non - toxic solvents or solvent mixtures (Galaction and Caşcaval, 2004; Cobzaru, 2014). Choosing an extraction method was based on establishing a balance between the financial, technological and efficiency aspect.

The paper aims was to realize a general view on the specialized literature in this field, in order to be constituted the starting point for the practical experiments: finding alternative solutions, for the replacement of the chemical pesticides used in the agriculture with biopesticide obtained from plants. Starting from all of these informations it was studied the extraction of some chemical compounds with repellent and/or germicidal effect, predominantly from the spontaneous flora of Moldavia and Bucovina areas. Thus, it was being investigated the effectiveness of some extract from *Achillea*

millefolium, *Hypericum perforatum* and *Origanum vulgare* in the field of pest control, respectively an insect beans - *Acanthoscelides obsoletus*, which is specific for storage of cereals and seeds, on which it acts as a pest.

2. Experimental

Materials

The plants selected in this study come from the spontaneous flora characteristic of Moldavia, the Bucovina area and are presented in Table 1.

Table 1
Characterization of the Selected Plants

Plant	Image	Particular characteristic	Ref.
Milfoil - *Achillea millefolium*		Main components in the oils isolated from different chemotypes are chamazulene, sabinene, β-pinene, 1,8-cineole, linalool, α-thujone, β-thujone, ocimene, camphor, ascaridole, caryo-phyllene oxide, β-eudesmol and α-bisabolol.	(Mockute and Judzentiene, 2003; Verma *et al*., 2017)
Common marjoram - *Origanum vulgare*		It is used in the aqueous or ethanol extracts form containing biologically active compounds with immuno-modulatory, cytotoxic, bactericide and antioxidant properties. They have applications in aquaculture and agriculture.	(Beltrán *et al*., 2018)
Thyme - *Satureja hortensis*		Through the GS technique, a number of more important compounds have been identified, such as: thymol 45.9%, gamma-terpinene 16.71%, carvarol 12.81%, p-cymene 9.61%. These are also characterized by antifungal activity on some microorganisms (Candisa albicans).	(Sharifzadeh *et al*., 2016)

Table 1
Continuation

Plant	Image	Particular characteristic	Ref.
Patience - *Rumex patientia*		The leaf extract and the derived fraction show inhibitory and bacterial and fungal proliferation ability in vitro and in vivo. The ethyl acetate fraction showed the strongest antibacterial and antifungal activity compared to the other extracts that were tested.	(Mhalla *et al.*, 2017)
Marigold - *Calendula officinalis*			(Besil *et al.*, 2017)
Anise- *Pimpinella anisum*		The ultra performance liquid chromatography-tandem mass spectrometric (UPLC/ MS/MS) method revealed the major ingredients of the essential oil: phenyl-propenoids, monoterpenes, trans-anetholes, cis-anethol, estragol, linalool, α-terpineol and methyl eugenol. Essential oil has a high inhibitory effect on *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Micrococcus luteus*.	(Abdel-Reheem *et al.*, 2015)
Nettle- *Urtica dioica*		The presence of hydroxycinnamic acids (chlorogenic acid, caffeic acid, rosmarinic acid) and flavonoids (quercetin) offers high antibacterial potential.	(Zenão *et al.*, 2017)
Rattle - *Hypericum perforatum*		The extract shows antibacterial action against *Escherichia coli*, *Shigella dysenteriae Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*.	(Heydarian *et al.*, 2017)

The ethylic alcohol with 96% concentration and analytical grade purity was the solvent used in extraction experiments.

Extraction methodology

It was selected the maceration, made by suspending the dried plants in 96% alcohol, respecting a 1:10 solid: alcohol ratio. It were used 10 g of plant (inflorescence and/or strains) which was chopped or milled, if was necessary, after which it's were placed in an experimental installation with 100 mL of ethylic alcohol. The mixture was kept under intermittent stirred at room temperature (15-20°C). Finally, was made the extracts phase separation and storage the liquid phases in a tight containers in a cool place. Stirring allows a more uniform and efficient migration of the active principles of plants. In the first step the extracts have been sensitively analyzed.

Preliminary test insect

The solutions that were used in the experiments were prepared with a concentration of 5% from the three types of macerated (*Achillea millefolium*, *Hypericum perforatum* and *Origanum vulgare*) and were sprayed a populations consist of 7-10 insect specific of dry bean stored.

It followed their evolution monitoring during a period of 7 days. The method used is adapted after Asawalam *et al.* (2006). The percentaje of insect mortality was calculated using Eq. (1) (Asawalam *et al.*, 2006).

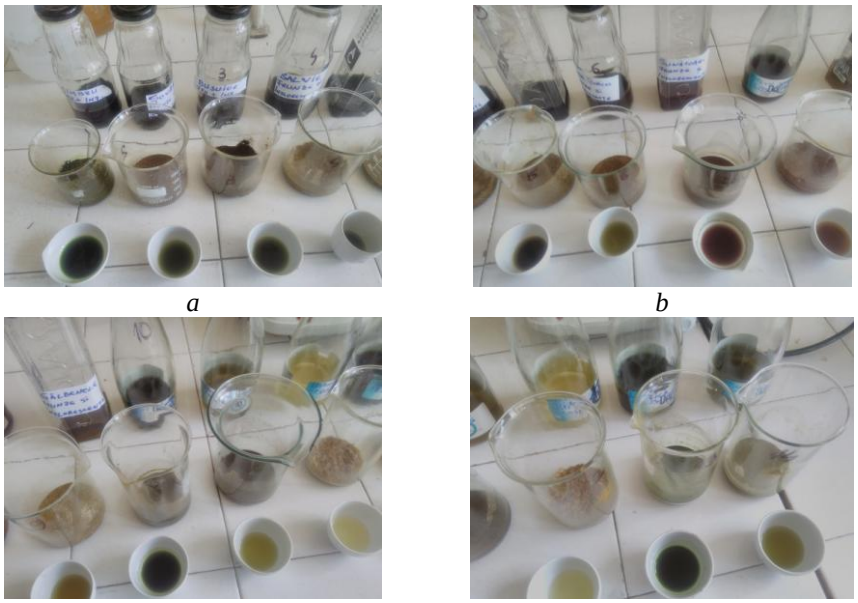
$$\%\text{mortality} = \frac{N_d}{N_0} \cdot 100 \quad (1)$$

where N_d represent the number of dead insect and N_0 represent the number of initial test insects

3. Results and Discussions

Organoleptic characterization of extracts

It were obtained alcoholic extracts having flavors specific to the plant from which they came, and different colours from dark green to yellow very light. The color and consistency of the liquid phases depend on the part of the plant subjected to the extraction, respectively on their chemical composition: inflorescence, strain or seeds (Fig. 1).



a *b*

c *d*

Fig. 1 – Vegetal alcoholic extract: *a – Satureja hortensis, Origanum vulgare, Ocimum basilicum* and *Salvia officinalis*; *b – Matricaria chamomilla, Achillea millefolium, Hypericum perforatum* and *Rumex patientia*; *c – Calendula officinalis, Pimpinella anisum* and *Equisetum arvense*; *d – Allium sativum seeds, Urtica dioica* and *Primula veris*.

Test insect

The results of the monitoring of insect evolution (*Acanthoscelides obsoletus*) from the beans are systematized in Table 2 and Fig. 2.

Table 2

Time Evolution of Insects on Dried Beans After Spraying with Extract with 5% Concentration

Contact time, [h]	% mortality depending on plant extract with 5% initial concentration			
	Origanum vulgare	*Achillea millefolium*	*Hypericum perforatum*	Blank sample (alcohol 96%)
2	0%	0%	0%	0%
5	28.57%	28.57%	14.28%	10%
8	28.57%	14.28%	0%	0%
24	14.28%	0%	42.85%	0%
48	0%	14.28%	14.28%	10%
72	0%	0%	14.28%	10%
96	0%	42.85%	0%	0%
168	14.28%	0%	14.28%	0%

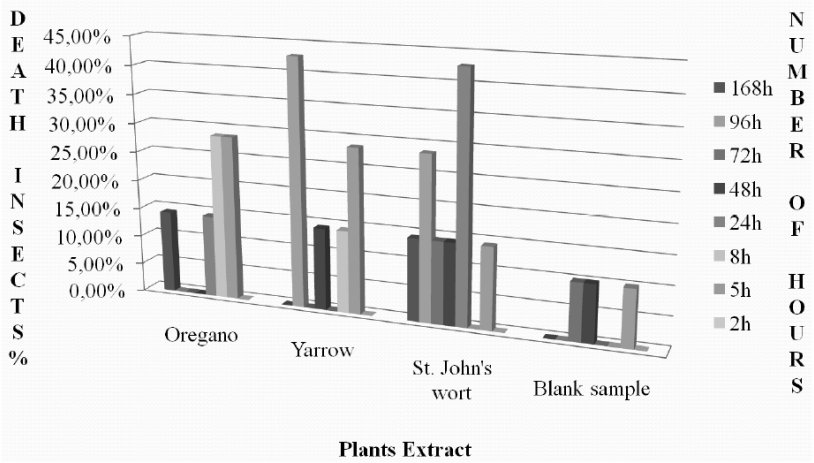


Fig. 2 – Graphical presentation of bean insect mortality over time and depending on the nature of the extract; C_0 = 5%, room temperature (15-20°).

The treatments applied to individuals of the species *Acanthoscelides obsoletus* (*Coleoptera-Bruchidae*) generated different manifestations of adults in different stages of the experiment.

Some of the extracts generated high mortality immediately after application, others induced late mortality after more than 5 days after application of the extracts.

Regardless of the type of extracts applied, the monitored insects had neuroleptic manifestations of different intensity and duration. Therefore, to confirm the results and to establish the plant extracts with the most effective results, it is necessary to repeat the experiments and to test several variants of concentrations.

4. Conclusions

The preliminary experimental results allow a few comments and underline some directions for further studies that should be performed:

- The use of biopesticides is in line with the concept of sustainable agriculture.
- The preparation of vegetable extracts by the maceration technique (cold extraction) in solvents accepted by the agriculture and food industry, is a simple and relatively cheap option, allowing the use of non-hazardous and environmentally friendly solvents. It also ensures good results with potential for application in practice.
- The use of plants in pest control is a useful method especially for small farmers who apply the principles of bio-dynamics agriculture.

➢ Careful monitoring of harmful insect behavior is required since the moment of contacting them with the studied plant extracts, in order to differentiate extracts that cause a higher mortality index than extracts at which the mortality index is low but other effects may occur, such as sterility.

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CERCETĂRI PRIVIND BIOPESTICIDELE OBȚINUTE PRIN EXTRACȚIE CU SOLVENȚI NETOXICI ȘI EFECTUL INSECTICID ASUPRA DĂUNĂTORILOR DE DEPOZITARE

(Rezumat)

Asigurarea calității produselor alimentare reprezintă un deziderat al zilelor noastre, deoarece oamenii au devenit foarte atenți cu propria persoană, respectiv cu asigurarea și menținerea sănătății și a calității vieții. În acest context, se tinde tot mai mult spre consumul produselor care conțin cantități cât mai mici de substanțe chimice, îndeosebi din clasa pesticidelor. Datele recente arată că un număr tot mai mare de uleiuri esențiale din plante au fost testate împotriva unei game largi de dăunători artropozi cu rezultate promițătoare. Extractele vegetale au demonstrat eficacitate ridicată, mecanisme multiple de acțiune, toxicitate scăzută asupra vertebratelor și potențial de utilizare a produselor secundare ca agenți de reducere și găsirea unui mecanism/metodă de stabilizare pentru sinteza biopesticidelor.