REPELLENT AND INSECTICIDE ACTIVITIES OF PLANTS EXTRACTS FROM SPONTANEOUS FLORA USING CONVENTIONAL AND INNOVATIVE ASSISTED EXTRACTION TECHNIQUES

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Abstract. *In this study, we tested 14 plants from the native spontaneous flora to highlight the potential effects of repellents and insecticides on Colorado potato beetle (Leptinotarsa decemlineata Say). Of all the plants tested, only three of them had a good potential to determine an increased mortality (%) of adults and larvae of the species Leptinotarsa decemlineata Say after 168 h, in the order: Primula veris, Origanum vulgare and Achillea millefolium. The highest mortality (%) was observed after treatments with crude alcoholic extracts of Primula veris obtained by the method of extraction based on hot reflux (HAE) in the Soxhlet equipment, 90% in the case of larvae and 80% in adults, respectively. In order to make more efficient the extraction process, respectively to reduce the amount of solvent used as well as the extraction time, we tested another conventional technique, such as maceration (M) and one from the category of "environmentally friendly", ultrasound-assisted extraction (UAE), respectively. We also tried a combined technique: ultrasound-assisted extraction followed by maceration (UAE+M). The highest extraction degree (%) using UAE+M was achieved by the species Origanum vulgare which indicated 70% mortality in the case of adults, after 168h. Our results indicate that spontaneous flora could be successfuly used in combating the adults and larvae of Leptinotarsa decemlineata Say.*

Keywords*: biopesticides, heat assisted extraction, Leptinotarsa decemlineata Say, Primula veris, ultrasound assisted extraction, vegetal extracts*

INTRODUCTION

Plants have been and will be a source of secondary metabolites with a diverse range of uses in many industrial fields (such as pharmaceuticals, cosmetics, food, textiles, etc.), agriculture, etc. Since ancient times, a number of methods of extracting plant compounds have been used mainly for phytotherapeutic purposes. Technological progress has led to the optimization of classical methods, but also to the development of new extraction methods, with increased efficiency, reduced extraction time, low solvent content, conditions that make them "environmentally friendly". In recent years, multiple studies have been based on the replacement of synthetic pesticides with alternative variants of pest and crop pests using biopesticides, which have various mechanisms of action (NABOULSI et al., 2018; SUTEU et al., 2020).

For over two centuries farmers and agricultural researchers were struggling to find a viable solution to control Colorado beetle *Leptinotarsa decemlineata* Say. This pest is native to the state of Colorado, and over time has crossed the ocean on commercial vessels, spreading in the entire world. He has the ability to resist against many treatments administered from the pesticides class, developing over time an impressive adaptation mechanism compared to other pests. Among the most important classes of pesticides to which Colorado beetle has developed resistance we can exemplify carbamates, pyrethroids, organochlorides organophosphates, etc. The economic damage caused by *Leptinotarsa decemlineata* Say is significant. As a result this pest that has been the subject of many studies so as to keep it under control (GÖLDEL et al.,

2020; DARABAN et al., 2018A; ROJHT et al., 2012; SUTEU et al., 2020; SCOTT ET al., 2003). This gives us the opportunity to test the native spontaneous flora from Romania to highlight its pesticide or repellent potential, not just its medicinal effects.

According to the scientific literature, approximately 20,000 species of plants known worldwide are used for medical purposes, of which about 300 are widely used. The territory of Romania offers a relatively large number of plant species known worldwide, many of which have in addition to applicability in medical treatments a potential insecticide effect, which opens new opportunities for crops treated with biopesticides obtained from various plant extraction methods. Literature mentions approx. 1,000 plant species used as biopesticides, some of which can be used with promising results to combat crop and storage pests (ASIMINICESEI et al., 2020A,B; ÖZASLAN and OGUZKAN, 2018).

The plant species considered in our manuscript were chosen according to several principles: (i) the first principle was the period of appearance of perennial species which appear before the crops they are going to protect; (ii) second, the availability in abundance and the fact that being native does not cause an imbalance to the ecosystem; (iii) the third criterion was that the chemical composition of the selected plants doesn't affect the crops or agricultural products to be preserved, and (iv) the last criterion, is the development of plant species with insecticidal potential that have not been well-documented so far. From an economic point of view, the use of plants from spontaneous flora is an advantageous solution, because it doesn't require cultivation or maintenance, but only harvesting and processing to obtain sprayable extracts.

These aspects indicate the timeliness and necessity of studies in the context of replacing synthetic pesticides with a modern and environmentally friendly alternative. Plants from the Moldavian geographical area spontaneous flora were investigated usually for their medicinal characteristics, while their insecticidal effects were less considered. Literature shows several references considering the chemical composition of many plant species of the spontaneous flora (AZMIR et al., 2013; JOVANOVIC et al, 2017), however, attention has been focused on their medicinal importance, ignoring their potential for insecticidal action. These aspects open the way for the investigation of plant species potential in crop pests' control, in the form of biopesticides.

Given this context, our main objective is to identify the potential of spontaneous flora to act as biopesticides considering two main aspects: (i) to investigate different extraction methods with high extraction degree, and (ii) to test the potential of spontaneous flora species on *Leptinotarsa decemlineata* Say pest.

MATERIAL AND METHODS

Plant material

All plant material used was collected from Tomești area (Iași, România) (Figure 1). Prior to analysis, plants from the spontaneous flora were dried in a ventilated space protected from direct sunlight. The initial plant screening was focused on the following species: *Primula veris, Urtica dioica, Allium sativum, Equisetum arvense, Pimpinella anisum, Salvia officinalis, Matricaria chamomilla, Calendula officinalis, Achillea millefolium, Rumex patientia, Hypericum perforatum, Origanum vulgare, Ocimum basilicum* and *Satureja hortensis.* The entire dried plants were grinded using a mill processor. The storage time was maximum 1h from the moment of grinding until the extraction process.

Extraction methodologies

All necessary reagents or reference standards were of analytical quality, being purchased from the Chemical S.A. Company, Romania. For the extraction process we used as solvent 96% ethanol, considering the necessary quality conditions as required by the agriculture and food industry. A previously established amount of vegetal powder (g) was weighed using RADWAG type analytical balance. The vegetal powder was separately dispersed in the necessary volume of ethanol.

Figure 2 shows the schematic representation of the main conventional and modern extraction techniques with a highlight on the ones in our own experimentations.

Fig. 1. Tomești area (Iași, România) considered for plant collection

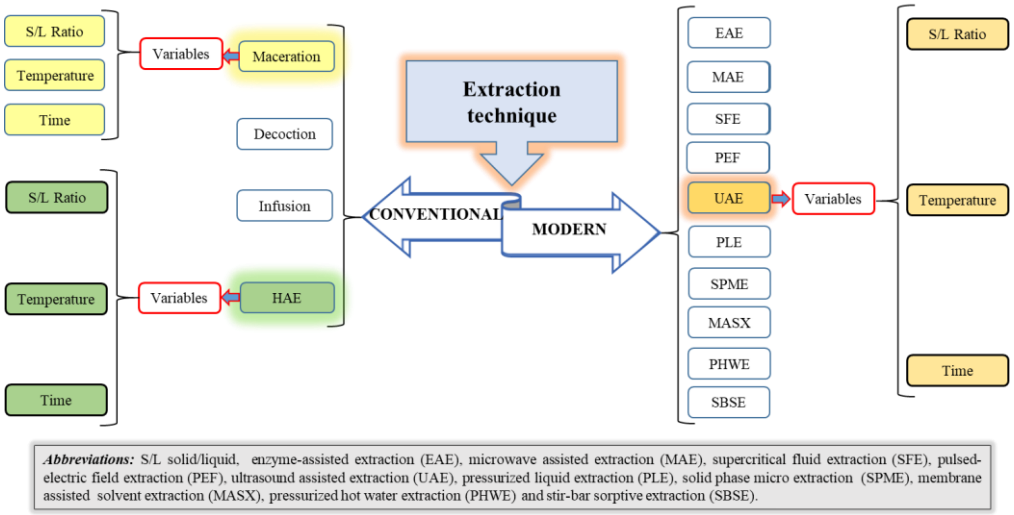

Fig. 2. Schematic representation of the main conventional and modern extraction techniques (highlighted parameters are the ones used in the present experiments)

The determination of extraction degree was performed using the following equation (Eq. 1) (DARABAN et al., 2020, 2021):

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

where: m $_{residue}$ - is the mass of the residue obtained after evaporation to dryness of the established volume for each extract, (g);

$V_{extract}$ – is the volume of the extract sample for evaporation to dryness (mL) at constant temperature up to 60°C using a thermostatic oven, (mL);

$n_{extract}$ – is the total volume of extract obtained after the liquid-solid extraction, (mL);

$m_{solid\ sample}$ - the mass of vegetal powder introduced in liquid-solid extraction process (g).

Biological assessment

In this paper we took into consideration the observation of the effects of different plant extracts on the crop pest of the species *Leptinotarsa decemlineata* Say. A number of 10 larvae and 10 adults were placed throughout the experiment in ventilated plastic enclosures with a capacity of 10 L each. The food represented by young and fresh shoots of *Solanum tuberosum* was constantly administered once or twice per day according to the rhythm of eating of the pests. The scheme of administration of the treatments was at precise time intervals (between 2 h and 168 h), monitoring at the same time the evolution of the pests (DARABAN et al., 2020).

The administration of plant-based treatments was performed by direct spraying on fresh shoots of *Solanum tuberosum*. After the administration of the treatment, we performed also a careful monitoring of the mortality (%) at different time intervals (DARABAN et al., 2018 A, B).

The working method and the calculation of the mortality rate among adults and larvae was adapted according to the method described by ASAWALAM et al. (2006) (Eq. 2).

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

where: N_d - is the number of dead insects, and

N_0 - is the number of initial test insects.

RESULTS AND DISCUSSIONS

Assessing the bioinsecticidal activity of plant extracts

In this initial screening, we evaluated the efficiency of the crude extracts obtained by heat assisted extraction (HAE) using a number of 14 species from the spontaneous flora. We managed to achieve a separation of species with insecticidal and / or repellent potential in controlling the pest *Leptinotarsa decemlineata* Say. The bioinsecticidal activity of the selected plant extracts considering the mortality (%) of adults and larvae of the species *Leptinotarsa decemlineata* Say after different treatments is indicated in Figure 3.

Figure 3 shows that the highest mortality rates of potato beetle (*Leptinotarsa decemlineata* Say) using pulverization of the raw extract obtained by HAE in Soxhlet apparatus was achieved by *Primula veris* which indicated 90% mortality in case of larvae, after 168h, and 80% in case of adults, after 168h.

The other studied species that reached a degree of mortality in the case of larvae of the species *Leptinotarsa decemlineata Say* of 50% and follow the order: *Origanum vulgare* and *Allium sativum*, followed by *Achillea millefolium* with 45% and *Pimpinella anisum* and

Hypericum perforatum with 40%. In the case of adults, with a mortality degree of 40% there is the specie *Salvia officinalis*, followed in a percentage of 30% by the species: *Ocimum basilicum, Origanum vulgare, Cahillea millefolium* and *Pimpinella anisum*. The other species studied did not influence the mortality of pests enough to be taken into consideration.

Given these results, *Primula veris, Origanum vulgare* and *Achillea milefolium* also have a potential use for *Leptinotarsa decemlineata* Say control, if the treatment would be included in the Integrated Pest Management (IPM), which takes into consideration the following principles: crop rotation, selection of those pest-resistant varieties of the species that are least susceptible to pests (*Leptinotarsa decemlineata* Say), predatory animals, mechanical damage to pests, planting curtains of plants or shrubs with repellent effect at optimal intervals, in which the volatile compounds of these plantations keep pests at bay. Therefore, by combining the methods of IPM listed above with plant extracts, a symbiosis can be achieved between them so that pests do not affect the crop or do so in a reduced or insignificant way. These alternatives present a low risk or not at all for people or the environment and are cost effective (CHOWAŃSKI et al., 2016).

Similar results were obtained by GÖKÇE et al. (2007) who showed a toxicity for the larvae of *Leptinotarsa decemlineata* Say ranging from 0% to 91% after only 24 h of incubation for 30 plant extracts. Plant extracts obtained from *H. lupulus, L. temulentum, Reseda lutea* and *Solanum nigrum* showed significant results compared with control samples and exhibit high toxicity for the larvae of the specie *Leptinotarsa decemlineata* Say.

Considering this initial plant screening in the next section we focused on plants having the highest bioinsecticidal performance, e.g. *Primula veris, Achillea millefolium* and *Origanum vulgare.*

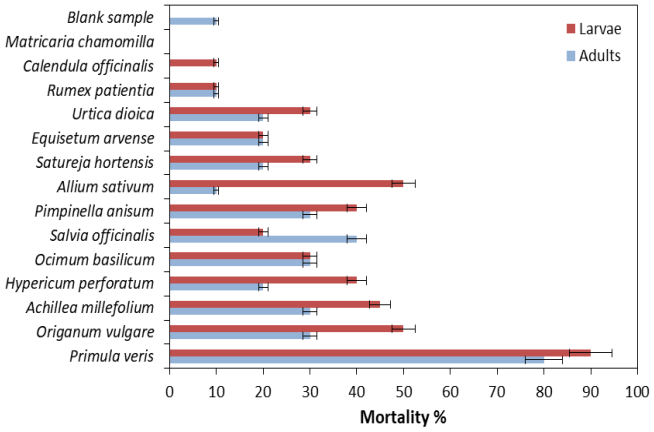

Fig. 3. Mortality (%) of adults and larvae of the species *Leptinotarsa decemlineata* Say after different treatments (using HAE extraction method) with plants extracts after 168h

The extraction degree

Further with our investigation, considering the plant species *Primula veris, Oreganum vulgare* and *Achillea milefolium*, we focused on identifying a method of extraction among those studied with a high degree of extraction and its use in pest control. In this regard, three extraction techniques and a combination of them, maceration (M), heat assisted extraction in

Soxhlet apparatus (HAE), ultrasound assisted extraction (UAE) and combined method between UAE and M (UAE+M) were compared.

Figure 4 shows the results obtained by comparing the obtained extraction degree (%) considering the chosen methods. In Figure 4 it can be seen that the highest degree of extraction was reached in the case of the combined method between UAE+M for all species, the highest extraction degree (%) being in the case of *Origanum vulgare* (75%) followed by *Primula veris* (48%) and *Achillea millefolium*, (45%) respectively. The most spotted efficiency after the combined UAE+M method was recorded by the UAE method following the order *Origanum vulgare*>*Achillea millefolium*> *Primula veris*. In the case of the HAE method in Soxhlet apparatus, the specie that recorded the highest value of the degree of extraction was *Achillea millefolium* (18%) followed by the species *Origanum vulgare* and *Primula veris*, which recorded almost similar values (15%). By the M method, the species with the highest degree of extraction were *Primula veris* and *Origanum vulgare* having approximately the same extraction degree (11%) followed by a small difference for the specie *Achillea millefolium* (10%). A high extraction degree doesn't necesarly indicate a high mortality rate in the case of selected plant species and this is in acordance with the results indicated in Figures 3 and 5.

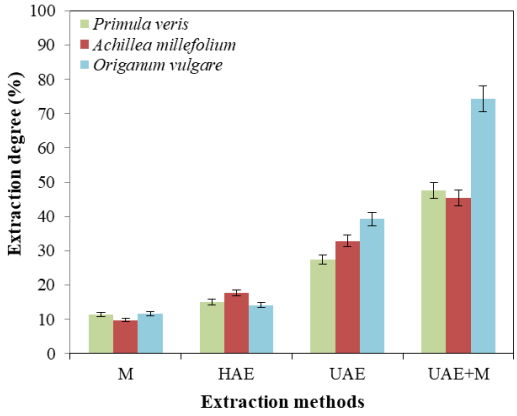

Fig. 4. Comparison between the obtained extraction degree (%) considering chosen methods: maceration (M), heat assisted extraction in Soxhlet apparatus (HAE), ultrasound assisted extraction (UAE) and combined method between UAE+M

Figure 5 shows the mortality rates of potato beetle (*Leptinotarsa decemlineata* Say) using pulverization of the raw extract obtained by UAE+M.

The highest rate (%) was achieved by *Origanum vulgare* which indicated 70% mortality in the case of adults, after 168h, followed by *Primula veris* (60%) and *Achillea millefolium* (40%). It is interesting to notice that there is a difference between control sample and the highest mortality rate of *Origanum vulgare* of 50%.

This is clear evidence that biopesticides of *Origanum vulgare* extracts combined with IPM methods could provide a successful alternative and eco-friendly product, which could be used in a sustainable way for the species *Leptinotarsa decemlineata* Say.

RUSIN and GOSPODAREK (2018) showed that fresh and dried extracts of *Origanum vulgare* at concentrations of 10% and 30% conducted to reducing the amount of food eaten by females and males of the species *Leptinotarsa decemlineata* Say.

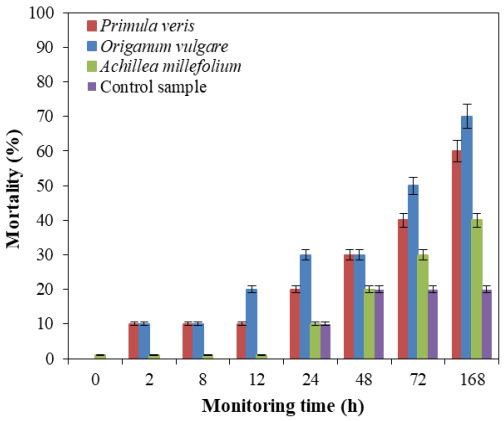

Fig. 5. Mortality (%) of adults of the species *Leptinotarsa decemlineata* Say after different treatments using UAE+M extraction method with plants extracts after 168h

CONCLUSIONS

In conclusion, regarding the effectiveness of the extracts in controlling the targeted pests, after the use of the 14 extracts it was observed that only 3 species of the spontaneous flora had potential effects to be used as biopesticides.

The species with the highest potential studied using HAE extraction in Soxhlet equipment is *Primula veris* with a mortality of 90% for larvae and 80% for adults followed by *Origanum vulgare* and *Achillea milefolium*, which showed similar effects against the adults and larvae of the species *Leptinotarsa decemlineata* Say. On the other side, when we investigated as extraction technique UAE+M, the results showed a high mortality of the adults of the species *Leptinotarsa decemlineata* Say in the case of *Origanum vulgare* (70%).

Among the extraction methods selected in this paper, the method that showed the highest efficiency in terms of the degree of extraction expressed (%) was the combined method between ultrasound assisted extraction (UAE) and maceration (M) followed by ultrasound assisted extraction (UAE), heat assisted extraction (HAE) in Soxhlet apparatus and maceration (M).

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