Analele Universităţii din Craiova, seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) Vol. XLIV 2014

CONSIDERATIONS ON PLANTS AND TECHNIQUES FOR OBTAINING BIO INSECTICIDES USED IN ORGANIC FARMING

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Keywords: bio-insecticide, plant, organic, solvent, insects

ABSTRACT

The paper presents a few aspects regarding the development of organic agriculture in our country and abroad in the last years, the plants containing substances that can be used as organic insecticide, respectively the techniques and procedures used for obtaining these insecticides.

INTRODUCTION

Organic agriculture has emerged as an alternative to the intensive, conventional (industrialized) practices in agriculture, which are based on maximizing production by using nitrates, stimulators for production with and energy-intensive character in large quantities, in the purpose of continuously increasing agricultural production, for a population constantly growing, mostly in the urban area.

Emphasizing the intensification factors like: using in high quantities synthesis chemical fertilizers with a rapid intake and accessibility on plants, mobilizing nutritional and biotic reserves in the soil, by drastic interventions on the soil, introducing in the culture plants genome some genes resistant to diseases, pests and weeds by the so called genetic modified organisms (GMO), with an impact on biodiversity and the biotic balance in the soil, water, atmosphere and agricultural products have had extremely severe consequences through the progressive diminishing of the organic matter in the soil, by deteriorating the soil structure, by increasing the danger of erosion, by reducing the number of representatives of mezzo-fauna (earthworms, springtails, ground beetles), by increasing the compaction and setting degree of the soil and, finally, by reducing significantly its natural fertility [7]. Severe damage was caused to the environment by polluting it with nitrites and nitrates in the surface and phreatic water, by accumulations of toxic substances in the soil, in animal feed and in agricultural products, having severe consequences on human and animal health. As a result of toxin penetration in the soil-plant-animal-human circuit irreversible mutations were produced on the micro, mezzo and macro biotic fauna with consequences on the millenary environment balance and especially on human health.

The structure of the new curricula and analytic programs in the higher agronomic education must meet the guidelines for agricultural development on ecological principles in our country. Under this reasons, agricultural scientific research in our country must act on systemic bases, both in creating varieties (hybrids) of plants and animals, and also in the improvement of technologies for plant cultivation and animal husbandry, without polluting, for the protection of flora and fauna, for maintaining ecological balance and environmental protection.

MATERIAL AND METHOD

According to the norms imposed by the legislation in the field, the use of some fertilizers / insecticides free of chemical synthesis substances is absolutely mandatory in organic / bio / ecologic agriculture. Thus, the discovery of new substances and compounds acting as a fertilizer / insecticide is a continuous challenge for field of agricultural scientific research in the XXI century.

The discovery of these new chemical compounds is possible through the study of different vegetal extracts obtained from the variety of available plant on the surface of the earth. These extracts, depending on the biochemical composition can be used as substances acting as biofertilizers / bio-insecticides to be used in organic farming. Their quality is influenced by the fact that they are not dangerous to humans, plants, soil and environment in general. They can be prepared from substances at hand and therefore they do not very high prices. They can be used when the infestation has occurred or may be applied for prevention.

The insects react to odors, vapors, gases, smoke, heat, oil, soap etc., and organic insecticides take all this into account. For example, the strong smell of garlic, tobacco, rhubarb and other plants is repellant for some insects. Chili peppers, alcohol, salt and other substances can burn or destroy pests. Certain oils suffocate insect and soap or detergents added to the recipes are designed to make the substances in the solution stick to the leaves and stems. Organic soaps and detergents can be used in that purpose.

Applying insecticides is not done in sunny weather because in may cause burns to plants and it is recommended before splashing all cultures to sprinkle one plant to see if there are no unwanted effects [5]. Also it should be considered that not only harmful insects can be killed but also beneficial insects. Plants have properties to protect themselves against certain pests and growing companion plants is a good method of pest control. Therefore, the application of insecticides must not be performed every day, but only when necessary.

Pest	Insecticide
Aphids	Rhubarb tea, soap and oil solutions, alcoholic solution, tobacco tea, tobacco smoke, horseradish solution, orange zest spray, mat-grass spray, yarrow tea, coriander tea, sambucus leaves tea, Artemisia absinthium tea.
Whiteflies	alcoholic solution, oil solutions, chili pepper solutions, garlic spray, rhubarb tea, tobacco tea
Thrips	Garlic spray
Acari	Garlic spray, horseradish solution, limestone spray, rhubarb tea, soap spray, coriander tea

RESULTS AND DISCUSSIONS

Organic treatments are based on herbs or derivatives from herbs, with raw material that costs nothing; everyone can buy plants for *treatments from the nature* which are found in abundance. The main recipes prepared are decoction which is made leaving the herbs in cold water for 24 hours and then heating them for 20-30 minutes or even longer for those which have wooden terminations [1]. The extract is prepared leaving the herbs in

water at a room temperature, for about 3 days, the infusion is prepared pouring boiled water on the herbs that must be allowed to macerate for another day. The macerate is prepared by fermenting the herbs for a longer period of time in rain water, stirring from time to time. The macerate usually send an unpleasant smell, it is ready for use when the fermentation stops, meaning when it doesn't produce foam. In all cases, the liquid obtained must be filtered and applied using a sprayer. The main plants that can be used successfully for the organic treatment of other plant are: garlic and onion, artemisia absinthium, equisetum arvense, fern, stinging nettle and chili pepper.

- ***Garlic and onion*** contain essential oils and substances with antibiotic properties, the treatment based on these plants are efficient for the elimination of insects and bacterial and fungal diseases. An infusion of onion and garlic is prepared using about 100 g grinded bulbs for 10 liters of boiling water. After it was cooled and filtered it is sprayed on the surface of the plants.
- ***Artemisia absinthium*** contains insecticide and antibiotic substances that keep insects at a distance and strengthens the plants immune system. Around 500 grams of fresh branches (30 grams of dried leaves) are put in 10 liters of water to prepare an infusion that is sprayed on the leaves keeping insects at a distance, warding of ants, it is also efficient for treating leaves against de cabbage butterfly.
- ***Equisetum arvense*** has an epidermis that contains large quantities of silica, which fortifies the plants teguments and has positive effects of treating fungal diseases. A decoct is prepared using 100 grams of plant for 10 liters of water, it is used diluted, 1 liter of decoct for 5 liters of water. The plants are sprayed using a sprayer and it is also possible to use the macerate that can be prepared following the same recipe and can be mixed with the macerate made from stinging nettle.
- ***Fern*** contains venom that destroys vitamin B1 essential for life. It can be also used as an insecticide for treating plant rust. Dry fern leaves used for mulching keep snails at a distance. A kilogram of leaves and roots are left to macerate and the product obtained is diluted – one part of product and 10 parts is sprayed on the plants as insecticide and also to fight against plant rust.
- ***Stinging nettle*** is one of the most commonly used plants in the biological fight, it contains formic acid (an irritant substance), the leaves have a high content of iron, nitrogen and other substances that stimulate the plants immune system and positively influence their development.
- ***Chili pepper*** contains a strong alkaloid that keeps insects at a distance.

The efficiency of *organic treatments* depends on the attention with which they are applied and the growth phase of the plant, the maximum effects being obtained when *preventing treatments* are made.

The Common Agricultural Policy (CAP) and Europe's agriculture proposes to support an agriculture that guarantees food safety (in a context of climatic changes) and to ensure a sustainable and balanced development for all European rural territories, including the regions confronting with difficult conditions for production.

This agriculture must be multifunctional. It has to respond to the concerns of the citizens regarding food (availability, price, diversity, quality and safety), to protect the environment and to allow farmers to earn their existence from their activity.

In the production of medicinal and aromatic plants, the quality of the products is given by the content of active principles. The quantity of active principles in the plant is conditioned by ecologic factors, by the zoning of the plant, by the cultivation technology, the biological value of the cultivar (population, variety, hybrid, etc.) and not last, by the processing (manufacturing) ways. Concerning the processing of medicinal plants, it can be divided in two stages: primary processing and advanced processing [2].

Primary processing consists in drying, conditioning and packaging plants, and advanced processing consists in transforming raw material obtained from the primary

processing into products that are commercialized: phyto-therapeutic products (water extractive solutions, hydro-alcoholic extractive solutions, lyophilized powders from extractive solutions), cosmetics, food and diet food supplement, flavor food additives.

Solvent extraction is the most commonly used type of extraction for bioactive compounds from plants. This technique of separation implies the extraction of some components from a solid or semi-solid sample in an adequate solvent. In the extraction operation, the choice of solvent is made depending on the nature of the substance that is going to be extracted and on the nature of the raw material. The actual solubilization of bioactive compound is made by treating the finely grinded plant with water, saline solutions, hydro-alcoholic solutions, etc. [6].

The influence of factors for obtaining extracts

When preparing extracts, the influence of the following factors should be especially taken into consideration:

- *the nature of the solvent*: solvents have to dissolve and extract at a high yield the majority of active components and to contains as few as possible inert matter with no therapeutic value; the most commonly used solvents in the industry of vegetal extract are: water (for alkaloid salts, glycosides, saccharides, proteins, enzymes, tannins, etc.) and alcohol;
- 50% or 70% (for essential oils, hydrocarbons, tannins, base alkaloids and their salts, glycosides, resins, chlorophyll, etc.), *diethyl ether* (for base alkaloids, resins, essential oils, etc.), *oil, wine, vinegar*;
- *the grinding degree of the plant:* the more the vegetal product is brought to an advanced grinding degree, the bigger the contact surface is, so the extraction is complete, for the water extractive solutions is recommended the grinding depending on the plant product;
- *the ratio between the quantity of plant and of solvent:* the Romanian Pharmacopoeia provides concentrations of up to 6% for water extracts, 20% for the majority of tinctures and 10% for tinctures prepared from vegetal products containing highly active substances;
- *the contact time between the plant and the solvent:* differs depending on the extraction technique applied, but also on the type of extract; for water extracts it is of 5-6 hours, and for the alcoholic ones of 6-10 days;
- *the effect of stirring:* by stirring, the time for obtaining the extract is shortened;
- *the working temperature:* influences positively the extraction yield, due to increasing the active principles at a warm temperature. The Romanian Pharmacopoeia provides the extraction of some thermo-stable principles, at a temperature of 90-100 °C in the case of infusions and decocts;
- *the separation of the mixture and the manner of recovering the active compound from the solid residue.*

In the case of preparing water or hydro-alcoholic extractive solutions by maceration, the grinding degree plays a very important part. This, correlated with the nature of the solvent used and the intensity of stirring determines the contact time for the extraction of soluble components until reaching the concentration balance between the solid phase and the liquid phase.

Extraction techniques

The extraction can be achieved by *discontinuous processes* (maceration, percolation, infusion, decocting, as well new and advanced methods: accelerated solvent extraction, extraction assisted by microwaves, extraction with supercritical fluid) and *continuous processes* (continuous extraction with organic solvent, continuous percolation, Soxlet extraction).

- **Maceration**: consist in treating the vegetal grinded product with necessary quantity of solvent, maintaining the contact for a determined period of time (water maceration: 8-12 hours), simultaneous with a continuous or intermittent stirring and after that separating the extractive solution from the residue by filtering on decanting it; in the case of macerates in solutions of another nature (alcohol, oil, wine, vinegar), the maceration time increases and could even reach several weeks. Maceration is applied especially in the cases of cold and thermolabile extraction of easily soluble principles. Maceration can be achieved: *in cold conditions* – extracting active principles from the plants into the solvent at room temperature (17-22 °C) or in warm conditions (called digestion) – achieved with the solvent heated at 40-60 °C, generally at a temperature inferior to the one at which the solvent is boiling, so that the active principles can pass through the solution slowly, without being degraded by a temperature too high or by a sudden heating.
- **Percolation**: the process of extracting active principles from plants, cold, using countercurrent solvent. The process that takes place is developing as follows: before the solvent becomes saturated with active principles extracted, it is moved by another layer of solvent in which the vegetal product undergoes a short maceration and gives another part of the active principles. This phenomenon is continuous, each portion of added solvent coming into contact with the vegetal product until its complete exhaustion.
- **Infusion**: consist in humidifying the grinded vegetal product with water, except for vegetal products that contain essential oils, which are humidified with a solution of diluted alcohol; after about 5 minutes the water is added, at a boiling point and is left in contact for 30 minutes. After the infusion time is over, the solution is filtered. Generally, the infusion is used for parts of the plant that have thinner cellular walls (flowers, leaves, herbaceous parts).
- **Decoction**: the preparation technique is similar to the infusion – the grinded product will soak in 5 parts of cold water; it is macerated for 5 minutes and then the rest of boiling water is added to the required proportion (1% or 5%) afterwards it is heated, on a water bath for 30 minutes (at boiling point). Finally it is strained and the residue is washed until it reaches the volume prescribed (medicinal plants with a high content of etheric oils will be humidified using 50° alcohol, then the boiling will be added). In phyto-therapy, the decoct is made for vegetal organs (roots, rhizomes, bark, etc.) from which the principles are harder to be extracted.
- **Alcoholic extraction by fermentation**: the extraction procedure implies soaking the vegetal material either in the form of a decoct, either in a grinded form, for a certain period of time, during which the fermentation and alcohol generation in situ takes place; this facilitates the extraction of active substances contained by the vegetal material. The alcohol thus generated also has a conservation role. At an industrial scale, wooden vats, recipients made of porcelain or stainless steels for the food industry are used.
- **Continuous extraction with organic solvents**: the components present in the raw material are extracted by dissolving in the solvent-liquid. The raw material is placed in a special built extractor, and the solvent must be recycled continuously through the mass of vegetal material, the efficiency of the extraction in the maceration process can be improved by the continuous circulation of solvent through the vegetal product and, moreover, for an extraction as complete as possible of the active principles, a multi-stage process is used.
- **Accelerated solvent extraction (ASE**): is a new extraction method, based on using high temperature and pressure in order to accelerate the kinetics of dissolution and to undo the analyte-matrix ties [7-10]. In addition, by increasing temperature, the viscosity of the solvent decreases, allowing it to penetrate easier into the solid matrix of the sample. This way, the extraction time is reduced form tens of minutes to a maximum of a few minutes, and the extract samples can be in small quantities. The high temperature

(50-200 °C) accelerates the diffusion of solvent molecules in the texture of the sample, and the high pressure obtained maintains the solvent in a liquid state.

- **Microwave assisted extraction (MAE):** is a relatively recent technique that uses the energy from the microwaves to heat the solvent and the sample in order to increase the transfer rate of mass between the substances dissolved from the solvent matrix and the solvent, contributing to their easier passing in the solvent [3]. The advantage of this technique compared to the conventional methods consists in a reduced extraction time, in the conditions of using a low energy consumption and a solvent with a high extraction efficiency [3, 8]. Thus, MAE has gained an increasing interest in extracting active compound as terpenes [8], alkaloids [1, 2], flavonoids [1, 2], glycoside [2] and essential oils [8] of vegetal oils. Polar solvents, as acetone, methanol or dichloromethane are rapidly heated under the influence of microwaves and because they do not a dipole moment, they do not absorb microwave radiation.
- **Ultrasound extraction (UAE):** is one of the most important techniques used for the extraction of valuable compounds from vegetal materials [1] and it is adaptable enough at a reduced or larger scale (for example, in the laboratory or at an industrial scale) [8]. The method implies the use of ultrasounds, with frequencies varying from 20 kHz to 2000 kHz, thus increasing the permeability of the cellular walls and producing cellular lysis (disintegration and dissolution of some cells), favoring the extraction of biologically active compounds.
- **Extraction with supercritical fluid:** was developed in the last years to be used at an analytic scale, building an alternative to the classic solvent extraction. In practices, more than 90% of the extractions with supercritical fluids are made with CO_2 for many practical reasons. Besides having the critical pressure (74 bar) and the temperature (32 °C) relatively low, CO_2 is relatively non-toxic, non-flammable, available at a high purity and low prices and it is easy to remove from the extract [4]. Above these critical values, CO_2 reaches a “supercritical level”. In this form, the viscosity is similar to that of a gas, facilitating it’s penetration in the solid matrix and its capacity of dissolution is similar to that o a liquid. After extraction, the solvent is separated from the extract by decreasing the pressure, converting the carbon dioxide to a gaseous phase and loosing the high dissolution capacity. The product can be separated completely from the solvent, which is again compressed and recycled in the process.

In the case of extraction with supercritical fluids and not only, the characteristic properties of the extract obtained from vegetal materials can differ, in a large extent, from the extraction and separation conditions chosen in operating the experimental installation; thus, along with the temperature increase, the density of the supercritical CO_2 increases, which leads to the increase of solubility for the less volatile compounds from the material prepared. Therefore, by choosing appropriate temperatures and pressures for extraction, as well as appropriate separation conditions, it is possible to optimize both the selectivity and the speed of extraction [4].

Due to its high efficiency, this type of extraction became more and more interesting for the food, aromatics and perfume industries. The advantages of this extraction method are:

- supercritical fluids have a power of solvation similar to that of organic solvents, but with a higher power of diffusion, a lower viscosity and superficial tension;
- the extraction of bioactive compounds from plants at low temperatures, thus avoiding their distortion due to heating or other toxic organic solvents;
- there are no solvent residues;
- supercritical fluids are, generally, inexpensive, simple and safe;
- The cost of eliminating the solvent and lower, and the fluids are easy to recycle;
- Is an “environment friendly” procedure.

CONCLUSIONS

Plant extracts can also be used as an insecticide to prevent the insect attacks on some crops, following the studies made in the last years observing that they can represent a solution for the future in order to support organic agriculture.

In conclusion, for the extraction of active principles from medicinal plants, one can select any of the extraction methods presented, taking into account, besides the advantages and disadvantages presented above, the following factors:

- If the plant contains thermolabile active principles, the cold extraction methods will be chosen: maceration, percolation and counter-current extraction; for thermo-stable active principles usually are chosen the Soxlet extraction (when ethanol or other organic solvents are used as solvents) or decoction (when distilled water is used as a solvent);
- The standardizing of the extraction time: an insufficient extraction time leads to an incomplete extraction, respectively an extraction time that is too long leads to the extraction of unwanted compounds, and the process becomes economically unprofitable;
- The number of extractions necessary for a complete extraction is as important as the duration of each extraction
- At an industrial level, a significant importance is that of the type of extractor used that will ensure a high efficiency, and the equipment costs.

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