PLANT EXTRACTS AND SECONDARY METABOLITES: A POTENTIAL TOOL IN ALTERNATIVE PLANT PROTECTION IN INDOORS PRODUCTION

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Abstract: An excessive use of most of the synthetic pesticides has created different types of environmental and toxicological problems. Since the plants, as a way of self-protect, can produce the biologicaly active metabolites that prevent the reproduction of the pathogens, the possibilities of using plant extracts and phytopreparations in plant protection from pests in indoors production were observed in this paper. In order to realize the above mentioned, it is necessary, among all, to adjust the technology of obtaining the active plant preparations with the conditions of agricultural production and to develop an adequate advertising, so the produced biopesticides could find their application with consumers.

Key words: plant protection, plant extracts, phytopreparations, protected spaces

Introduction

Global production of agricultural products is the World's largest industry with revenues of about 5 trillion US dollars a year. However, according to approximate estimations, the losses in the plant industry, resulting from the disease-causing agents attack (fungi, bacteria or viruses), harmful insects, rodents and weeds, are as high as 30%, which represents an enormous damage to the economy. Plant diseases and pests are, therefore, economically very significant. Since almost 40% of all plant destruction can be directly attributed to pests, it is understandable that a pest control is of great importance in agriculture (Cowan, 1999). The importance of plant protection is even greater because the crop production intensifies, while the biological sciences are improved. Therefore the methods of plant protection are implemented with a variety of scientific fields, modern technical and chemical agents, as well as breeding and cultivation of species that are resistant to infection. Although plant protection involves a complex system of agro-technical, biological and chemical measures, production, consumption and use of chemicals (pesticides) to protect agricultural and industrial crops from diseases and pests rank first in the control of plant enemies. When the effects of application of a pesticide are observed, then as inevitably raises the question of their residues in vegetables, fruits, agricultural crops, animal products, as well as soil, water, surface water and air (Vitorović et al., 2000). These effects are particularly pronounced in indoors production, where „the greenhouse effect" of the isolated space and, sometimes, insufficient and inadequate ventilation, causes longer presence of pesticides in the air, its pollution and high toxicity to humans. By realizing health benefits and eco-safety, several

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government and non-governmental organizations support conventional farmers to switch over organic farming practices. An increased demand in organic food and beverages is due to awareness among the consumer and producer about the negative consequences associated with synthetic chemical pesticides.

For the purpose of finding the ways to avoid the toxicity of plant and animal foods, as well as negative effects on the environment of many chemicals, in the past few years greater attention is paid to the implementation of the (still not enough) plant preparations in the control of plant pathogens (Raja et al., 2015).

Crops production in indoors systems

Cultivation of the certain plant species in indoors, especially of the several species rotation during the year, allows to each manufacturer its own choice of production, which should include the object capacity and market needs. Today the indoors production is a „fertile garden" that produces vegetables, flowers and aromatic species at a time when, due to climatic conditions, they can not be grown in the open field.

Indoors production under plastic or glass (greenhouses and glasshouses) is always more intense than other forms of production, because, with the necessary general and specific knowledge, higher yields can be achieved. This kind of closed area should provide the optimal conditions for growing plants in the autumn-winter-spring period, which enable the planned harvest in time of the poor market supply, which ensures a higher price (Lazić et al., 2001).

During the growing season, plant species, which are grown in greenhouses or glasshouses, are exposed to constant attack by pests, diseases and weeds. Faced with the damage that they inflict to plant production, one is forced to constantly control them. Nowadays, the most effective way to control the plant pathogens is commonly seen in the application of chemicals - pesticides. However, with the use of pesticides one should be careful, as many of them are very strong poisons, both for people and for animals. Particular danger of mass poisoning is caused by using the crops products immediately after applying pesticides (Maksimović and Simović, 1992).

Because of the consequences that pesticides can cause to humans, domestic animals and environment, it is necessary to develop and promote the alternative and environmentally friendly technologies in the protection of crops from pests and diseases, both in indoors and outdoors production.

Regarding the fact that plants, as a form of self-protection, can generate biotic active metabolites, which prevent reproduction of pathogens, for the past few years there is an increasing interest in the application of preparations based on plants to protect cultivated crops. One of the main advantage of the plant pesticides lies in their rapid degradation and lack of persistence and bioaccumulation in the environment, which have been major problems in synthetic use. Unfortunately, nowdays the plant preparations are not numerous, thus there is a consideration that more intensive production and the introduction of biotic active plant products (biopesticides) in the entire system of plant protection should be stimulated (Arnason et al., 2012).

Biotic active plant metabolites

It is generally known that the plants are endowed with the ability to create in tissue the chemical compunds that can prevent the reproduction of harmful and harmless microbes and insects. Several thousand of species of higher plants are tested laboratory and found that the number of analyzed plants that did not have at least some minimal antibiotic properties is very low. It was found that these properties do not only have the volatiles (e.g., essential oils), but many non-volatile matter, especially tannins and other polyphenol compounds. These compounds (phytoncides) can be found in various parts of the plants and their microbicidal and insecticidal properties depend on many internal and external factors (Tucakov, 1997).

Sergeeva (2016) listed the plants, used for pest control experimentally and traditionally in different parts of world, as follows: *Allium sativum*, *Anacardium occidentalis*, *Annona cuneata*, *Azadirachta indica*, *Capsicum frutescens*, *Cassia* spp. *leguminosae*, *Eucalyptus* spp., *Euphorbia tirucalli*, *Melia azedarach*, *Ocimum* spp., *Solanum nigrum*, *Tagetes* spp., *Citrus sinensis*, *Citrus medica limonum*, *Brassica juncea*, *Anacardium occidentale*, *Pimenta dioica*, *Alpinia galangal*, *Anisomeles indica*, *Curcuma longa*, *Carica papaya*, *Annona squamosa*, *Vitex negundo*, *Calotropis gigantea*, *Allium cepa*, *Aloe barbedensis*. She also specified the most popular herbs that repel insects, as follows: Sweet basil (*Ocimum basilicum*), Bay laurel (*Laurus nobilis*), Lavender (*Lavandula augustifoli*), Common tansy (*Tanacetum vulgare*), Wormwood (*Artemisia absinthium*), Citronella grass (*Cymbopogon nardus*).

Studies of the effects of plant metabolites in human, veterinary and herbal medicine gave the positive results, and thus initiated and stimulated further investigations of this type in agriculture and animal husbandry. For example it was found that phytoncides of garlic and mint prevent the occurrence of blight on potato and bacterial diseases of eggplant. At the same time, these phytoncides and phytoncides of corn, cucumber and wild mustard, can prevent disease-causing agents in animals and human (Đokić et al., 1998). Essential oils, such as volatile phytoncides, have a stronger or weaker microbicidal and insecticidal properties, depending on the environmental conditions and varieties of plants. An essential oil *helenin*, isolated from the roots of elecampane (*Inula helenium*), is used as an antihelmintic, antimycotic and bacteriostatic (Sarić, 1989). Among non-volatile phytoncide in agricultural production the commonly used are alkaloids. Nicotine and anabazin are used in control of various crops parasites, and colchicine to increase crop yields by creating polyploids.

Plants have limitless ability to synthesize aromatic secondary metabolites, most of which are phenols or their oxygen-substituted derivatives. According to previous studies (Gurjar et al., 2012), the important sub-classes in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These groups of compounds show antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Table 1).

Although there is not much knowledge on phytoncides and, in particular, on their chemical constitution, it was found that there is not so big difference between classic antibiotics in human and veterinary medicine and phytoncides, because the mechanism of their biological actions is very similar (Tucakov, 1997). Nevertheless, by applying these plant products in indoors crop production the use of pesticides could be avoided, including air pollution and human poisoning.

Table 1. Mode of antimicrobial action of phytochemicals (Cowan, 1999)
Tabela 1. Načini antimikrobne aktivnosti fitohemikalija (Cowan, 1999)

Class *Klasa*	Sub-class *Potklasa*	Mechanism of action *Mehanizam delovanja*
Phenolics *Fenolna*	Simple phenols *Fenoli*	Membrane disruption *Oštećenje membrane*
Phenolic acids *Fenolne kiseline*	Phenolic acids *Fenolne kiseline*	Bind to adhesins, inactivate enzymes *Vezivanje za adhezine, deaktiviranje enzima*
Terpenoids, essential oils *Terpenoidi, esencijalna ulja*	-	Membrane disruption *Oštećenje membrane*
Alkaloids *Alkaloidi*	-	Intercalate into cell wall *Ugradnja u ćelijski zid*
Tannins *Tanini*	-	Bind to proteins, enzyme inhibition *Vezivanje za proteine, inhibicija enzima*
Flavonoids *Flavonoidi*	-	Bind to adhesins, inactivate enzymes *Vezivanje za adhezine, deaktiviranje enzima*
Coumarins *Kumarini*	-	Interaction with eucaryotic DNA *Interakcija sa eukariotskom DNK*
Lectins and polypeptides *Lektini i polipeptidi*	-	Form disulfide bridges *Formiranje disulfidnih mostova*

Extraction of the plant material

Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology (Table 2). Solvents diffuse into the solid plant tissues and solubilize compounds of similar polarity (Gurjar et al., 2012).

Table 2. Solvents used for active component extraction (Cowan, 1999)
Tabela 2. Rastvarači koji se koriste za ekstrakciju aktivnih komponenti (Cowan, 1999)

Water *Voda*	Ethanol *Etanol*	Methanol *Metanol*	Chloroform *Hloroform*	Dichloromethanol *Dihlorometanol*	Ether *Etar*	Acetone *Aceton*
Tannins *Tanini*	Alkaloids *Alkaloidi*	Terpenoids *Terpenoidi*	Terpenoids *Terpenoidi*	Terpenoids *Terpenoidi*	Alkaloids *Alkaloidi*	Flavonols *Flavonoli*
Saponins *Saponini*	Tannins *Tanini*	Saponins *Saponini*	Flavonoids *Flavonoidi*	-	Terpenoids *Terpenoidi*	-
Terpenoides *Terpenoidi*	Terpenoids *Terpenoidi*	Tannins *Tanini*	-	-	Coumarins *Kumarini*	-
-	Flavonols *Flavonoli*	Flavones *Flavoni*	-	-	-	-

In the process of plant extraction numerous essential oils are obtained. These oils usually contain both volatile and liposoluble components, as well as hydrophilic components. By this way it is achieved maximum effect of the active components of a particular plant species. Selection of a suitable solvent is very important to obtain a high degree of selectivity of the extraction and preparation with the standard and high efficiency. Besides the choice of solvents, quality of plant extract also depends on plant material and the extraction methods (Kišgeci, 2002).

In recent years a method of extraction is used the most for obtaining a range of products with a wide application in the pharmaceutical, cosmetic and food industries. Examples of plant extraction for the purpose of crop protection are not so numerous, since most of the methods for evaluation of efficacy of plant extract are based on *in vitro* investigations. *In vitro* antimicrobial susceptibility testing includes diffusion test, such as agar well diffusion, agar disk diffusion, poison food technique, bio autography, and dilution methods - agar dilution, broth micro dilution assay and broth macro dilution assay (Gurjar et al., 2012). Thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* should be subjected to *in vivo* testing to evaluate the efficacy in controlling the incidence of disease in crops, plants and humans.

From the plant immortelle (*Helichrysum arenarium*) it was obtained a resinous fragrant extract *arenarin*, which has a feature to stop the development of many microorganisms on plants, stimulates the germination of tomatoes and protect plants from bacterial attack, which is why it is used in agriculture, particularly in horticulture and vegetable crops (Tucakov, 1997). Also, extracts of *Artemisia absinthium* and *Tanacetum vulgare* are used in the suppression of the spider (*Tetranychus urticae*) because they possess acaricidal properties (Chiasson et al., 2001).

Sabadilla is the seed extract of the neotropical lily *Schoenocaulon officinale* which contains veratridine alkaloids which have a neurotoxic mode of action. The extract has low mammalian toxicity and is a useful contact insecticide against a number of agricultural insects such as lepidoptera, leafhoppers, and thrips. Ryania is an extract from the South America shrub *Ryania sp.* containing the diterpene alkaloid ryanodine, which is a contact and ingested insecticide against horticultural and ornamental crop pests. It exerts its toxicity by blocking Ca++ ion channels. However, the market for these phytopreparations is relatively small.

Pyrethrum is now the most important traditional plant insecticide on the market, derived from the African daisy, *Chrysanthemum pyrethrum,* which produces an insecticidal oleoresin that can be extracted with organic solvents and pyrethrum extract. This plant product is valued for its effectiveness against a wide variety of home and garden insects, due to its action on the insect nervous system at the Na+ channels. Nowdays, the potential use of pyrethrum in organic agriculture has created new issues for the pyrethroid industry (Arnason et al., 2012).

Zarins et al. **(2009)** described eight phytofungicides, invented to limit infections caused by phytopathogenic fungi on vegetable cultures, both in greenhouses and in field. Their active components were extracts of various wild and artificially cultivated plants in combination with additives – matrixes. Plant extracts used for production of these phytofungicides were as follows: "Fitoekols-IF" – pine (*Pinus sylvestris*) and spruce (*Picea abies*) green extract; "Fitosativum" – garlic (*Allium sativum*) extract; "Fitocapsicum" – chili pepper (*Capsicum annuum*) extract; "Fitokrisanthemium" – chrysanthemum (*Chrisanthemum* sp.) leaf extract; "Fitoarmoracium" – wild horse radish (*Armoracia rusticana*) root and leaf extract; "Fitotabacum" – tobacco (*Nicotiana tabacum*, *N. rustica*) extracts; "Fitopelargonium" – geranium (*Pelargonium* sp.) leaf extract; "Fitosinepium" – white mustard (*Sinapis alba*) plant and seed extract. Tested fungicidal phytopreparations were produced in different forms – concentrate, liquid, paste and dry form, with pH 8.8-9.0, and their effects compared to those of already approbated fungicides "Bordo", "Mycostop" and "Timorex". The results obtained in the study of these authors showed that an efficiency of these phytofungicides was in the ranges of 65-88 % and 60–80 % under greenhouse vegetable and field conditions, respectively.

Conclusion

In agricultural indoors and outdoors production there are produced biological products used for the control of pests (biopesticides). Essential oil products have recently emerged as the most important biopesticides, although there is a great potential of a number of other experimental plant natural products, particularly as bioinsecticides, including piperamides, acetogenins, thiophenes and limonoids.

The main advantage of phytopreparations use are as follows: sustainable solutions in agriculture; reducing crop losses; ecological safety; no negative effect on treated plants; easily bio-degradable; cheaper integrated diseases management; suitability for prophylactic treatment; organic farming.

The examples of bioprotection should be a role model for more active production of biopesticides and their use in crop production, not only in greenhouses or glasshouses, but also in the open field, if the natural resources, environment and human health want to be protected and preserved.

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EKSTRAKTI I SEKUNDARNI METABOLITI BILJAKA: POTENCIJALNI „ALAT“ U ALTERNATIVNOJ ZAŠTITI BILJAKA GAJENIH U ZATVORENOM PROSTORU

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Izvod

Intenzivna primena većine sintetičkih pesticida može prozrokovati brojne probleme su ekološke i toksikološke prirode. Polazeći od činjenice da biljke, kao jedan vid sopstvene zaštite, mogu da sintetizuju biotički aktivne metabolite, koji sprečavaju razmnožavanje patogena, u radu su razmatrane mogućnosti primene ekstrakata i gotovih preparata na bazi bilja u zaštiti biljaka od bolesti i štetočina u zaštićenim prostorima. Da bi se to postiglo, potrebno je, između ostalog, prilagoditi tehnologiju dobijanja aktivnih biljnih preparata uslovima poljoprivredne proizvodnje i razviti adekvatan marketing kako bi proizvedeni biopesticidi našli svoju primenu kod potrošača.

Key words: zaštita bilja, biljni ekstrakti, fitopreparati, zaštićeni prostori

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