Essential oils as bioinsecticides and their application in organic agriculture

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Uncontrolled pesticide use in agriculture pollutes the environment and has harmful consequences on food chain participants. Health-safe plant production, without residues of carcinogenic and toxic pesticides, is a priority in food production. In addition, plant pests (insects) are becoming increasingly resistant to existing chemical pesticides. In order to grow healthy crops, the application of pesticides has to be decreased, while preventive and biological control should be intensified, *i.e.* biopesticides should be used instead of chemical ones. In addition to microbiological preparations, plant essential oils are also intensively used as biopesticides. The present study indicates that it is possible to use essential oils in organic agriculture as natural agents in the control of harmful insects. *In vitro* and *in vivo* studies indicate the existence of significant insecticidal effects of plant essential oils. Mono- and sesquiterpene components of essential oils are mainly neurotoxic to insects. However, despite this, the number of commercially available preparations is limited. It is necessary to better regulate the legislation on organic agriculture, as well as to additionally test the action modes of these oils.

Keywords: plant production; pests; biological control

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1. INTRODUCTION

Due to the application of pesticides, agricultural production has become intensive. There are many benefits of pesticide application in agriculture, but there are also a lot of adverse effects on the environment and human health. Because of these negative effects, many pesticides are no longer in use, which hinders crop protection. Synthetic pesticides have been an essential component of agricultural practice in recent years. To preserve and protect agroecological and natural systems in soils, it is necessary to analyse the physical and chemical properties of soils, and also to survey both agricultural and non-agricultural soils.

Insect damage in the process of cultivation, harvest, and storage has a detrimental impact on the world's food output. Furthermore, insects can transfer numerous diseases as they can act as hosts of some parasites that cause diseases. The total annual loss of crops due to pests and diseases is ~50% (approximately 35% in fields and 14% in storage) (Okwute, 2012). Pest infestation in food storage is one of the major problems in all countries as it causes considerable economic losses. A great number of insects, mites, and fungi feed on stored cereals. They cause damage and reduce the quality of the products. The net losses can range from 9% to 20% (Demeter et al., 2021).

Using chemical agents, such as pesticides, to protect plants is the most drastic way to protect yield against weeds, pests, and phytopathogens. Their heavy use results in their accumulation in the soil, where they influence plant nutrition, nitrogen, and carbon cycles, other biogenic elements, and beneficial microbes and other soil life forms. Pesticides from the soil enter groundwater and then enter human bodies through food chains. Moreover, pesticides have a toxic, mutagenic, and carcinogenic biological effect on fauna, flora, and human beings. A number of studies have shown strong links between pesticide exposure and the development of different cancers. For the reasons listed above, it is essential to decrease the use of pesticides and to increase the application of biological and preventive methods in crop protection. This implies biological ways of regulating plant pests, pathogens, and weeds in phytopharmacy, such as the application of biopesticides (Golijan Pantović et al., 2023).

As biopesticides, in addition to microbiological preparations, plant essential oils are also intensively used. Essential oils (EOs) are specific liquids that contain compounds from plants including volatile mono-, sesquiterpenes, and phenylpropane compounds. They are mainly metabolic products of higher plants (the families *Asteraceae, Lamiaceae, Apiaceae,* Rutaceae, *Myrtaceae* and *Lauraceae*). EOs are localized in different plant organs. The biological role of these oils is related to attracting insects, specific effects of the oil and inhibiting the germination of the seeds of other plants (autopathy), preventing the germination of one's seeds, and providing a living space for each individual, protecting plants from attacks by insects and animals, herbivores, as well as preventing development of microorganisms and by suppressing the spread of infection (phytoalexins). The aforementioned biological roles of EOs enable their successful application as biopesticides. *In vitro* and *in vivo* studies indicate the existence of significant insecticidal effects of EOs derived from plants. Mono- and sesquiterpene compounds of EOs are mainly neurotoxic to insects (Regnault-Roger et al., 2012).

The objective of the present study was to indicate the possibility of using EOs in organic agriculture as natural agents in the control of harmful insects.

2. EXTRACTION TECHNIQUES OF ESSENTIAL OIL

Numerous techniques employed to extract and produce EOs from plant substances have been described in the literature. Some of these techniques are ultrasonic-microwave assisted extraction, ohmic heating-assisted extraction, subcritical water extraction, solvent-free microwave extraction, and supercritical fluid extraction (Ahangari et al., 2021). Traditional techniques include cold compression, distillation, and solvent extraction techniques such as maceration, enfleurage, fermentation, percolation, hydro diffusion, and gravity extraction (Zin et al., 2020). The most often applied methods are steam distillation, solar distillation, distillation (hydro distillation), molecular distillation, an extremely low-pressure distillation process (Deng et al., 2020). Steam distillation is the most economical extraction method for EOs. However, there are a few drawbacks to this method. Due to the use of high temperatures and water, EOs could be chemically modified. When EOs are extracted using this approach, some water-soluble components and highly volatile components are lost. In addition, the obtainment of a solvent-free product is not possible if solvent extraction is used. Compared to hydrodistillation, headspace solid-phase microextraction (HS-SPME) is a simpler process by which the volatile fraction of aromatic/fragrant plants is extracted for a shorter period (Belliardo et al., 2006). It also needs a small quantity of material for the qualitative analysis of volatile compounds. This method was applied to characterize the chemical diversity of aromatic plants and to examine volatile fractions that are emitted by species without EOs. With the HS extraction, sampling of the volatiles is determined by the fibre affinity of each component (Benyelles et al., 2014).

3. INSECT CONTROL WITH ESSENTIAL OILS

The possible application of botanical and natural materials possessing insecticidal properties in pest control, for example, the use of EOs or their derivatives, has been growing considerably in the last 20 years, due to their lower toxicity to mammals and environmental friendliness (Robu et al., 2015). Conventional fumigants, applied to kill insect pests that cause damage to stored grain, adversely affect the environment and health. Their efficiency is reducing due to a different degree of utilization, precision, exposure time, conditions, and rates of use (Khaliq et al., 2020). EOs or their products are rather safe for mammals, birds, and fish, which makes them a natural alternative in the

Table 1. Mammalian toxicity of some essential oil compounds.

Compound	Animal tested	Route	LD_{50} (mg/kg)
2-Acetonaphthone	Mice	Oral	599
Apiol	Dogs	Intravenous	500
Anisaldehyde	Rats	Oral	1510
Trans- Anethole	Rats	Oral	2090
(+) Carvone	Rats	Oral	1640
1,8-Cineole	Rats	Oral	2480
Cinnamaldehyde	Guinea pigs	Oral	1160
	Rats	Oral	2220
Citral	Rats	Oral	4960
Dillapiol	Rats	Oral	1000–1500
Eugenol	Rats	Oral	2680
3- Isothujone	Mice	Subcutaneous	442.2
d- Limonene	Rats	Oral	4600
Linalool	Rats	Oral	> 1000
Maltol	Rats	Oral	2330
Menthol	Rats	Oral	3180
2- Methoxyphenol	Rats	Oral	725
Methyl chavicol	Rats	Oral	1820
Methyl eugenol	Rats	Oral	1179
Myrcene	Rats	Oral	5000
Pulegone	Mice	Intraperitoneal	150
γ- terpinene	Rats	Oral	1680
Terpinen-4-ol	Rats	Oral	4300
Thujone	Mice	Subcutaneous	87.5
Thymol	Mice	Oral	1800
	Rats	Oral	980

Source: Robu et al. (2015).

Table 2. Essential oils and their insecticidal actions.

Essential oil	Plant source	Target pest	Mode of action	References
Peppermint oil	*Mentha piperita*	*Sitophilus zeamais*, maize weevil	Contact toxicity, fumigant action	Nerio et al. (2010)
Citronella oil	*Cymbopogon nardus*	*Culex quinquefasciatus, Culex nigripalpus* mosquito larvae	Repellent, disrupts sensory receptors	Patel (2017)
Clove oil	*Syzygium aromaticum*	*Tribolium castaneum*, red flour beetle	Inhibits acetylcholinesterase	Isman (2020)
Neem oil	*Azadirachta indica*	*Spodoptera littoralis*, armyworm	Growth regulator, feeding deterrent	Mordue and Nisbet (2000)
Eucalyptus oil	*Eucalyptus globulus*	*Aedes aegypti*, mosquito	Repellent, neurotoxic	Pavela et al. (2021)
Garlic oil	*Allium sativum*	*Spodoptera littoralis*, cotton leafworm	Larvicidal action, reduces larval feeding	Giuliano et al. (2024)
Geranium oil	*Pelargonium graveolens*	*Drosophila suzukii*, spotted wing drosophila	Deterrent, repellent, and insecticidal effects	Bošković et al. (2023)
C. citriodora and S. terebinthifolius EOs	*C. citriodora and S. terebinthifolius*	*Sitophilus zeamais*, maize weevil	Repellents	Fouad et al. (2023)
Melaleuca alternifolia EOs	*Melaleuca alternifolia*	*Sitophilus zeamais*, maize weevil	Contact, ingestion, fumigant	Fouad et al. (2023)
EOs from *T. pallescens* and *C. citratus*	*Thymus pallescens de Noé and Cymbogon citratus Stapf.*	*Sitophilus zeamais Motschulsky, Tribolium castaneum (Herbst)*	Contact toxicity, disorder of energy metabolism in insects	Moutassemf et al. (2024)

suppression of insect pests. During the last few years, an average of 350 papers have been published annually about the bioactivity of EOs toward insects (Grieneisen and Isman, 2018). The purified terpenoid constituents of EOs exhibit moderate toxicity to mammals (Table 1). However, with few exceptions, the EOs themselves or their derived products are largely non-toxic to mammals, birds, and fish. This characteristic supports their classification as "green pesticides."

Since EOs are relatively non-toxic to mammals, they belong to the reduced risk products (Dey and Gupta, 2016). Furthermore, many experiments and clinical tests have been performed with EOs because they have been used as remedies and medications. The oral LD50 value of a large number of EOs, including chamomile, citronella, lavender, clove and eucalyptus EOs, ranges from 2,000 to 5,000 mg kg−1 in rats. However, Acorus calamus L. EOs were found nonmammalian toxic showing high LD50 (4877.4 μL kg−1) for mice (oral, acute). However, some EOs are slightly toxic to very toxic. For instance, the LD50 values of boldo, cedar and pennyroyal EOs amount to 130, 830 and 400 mg kg−1, respectively (Dey and Gupta, 2016).

Table 2 and Table 3 provide an overview of EOs from various plant species and their effectiveness in pest management. Insecticidal or antimicrobial traits of EOs facilitate plant protection against microbes and herbivores and their efficiency is affected by the plant maturity period, plant growing region, and the EO extraction technology. There are several methods of the development and production of plant EOs and EO-based products. The active ingredients in these products include: (1) a blend/combination of EOs; (2) a particular EO or a terpenoid component; (3) a combination of synthetically derived terpenoids that are similar to those in a plant EO; and (4) new (synthetic) terpene mixtures derived from different plants (Isman, 2020).

Studies have been conducted on the effectiveness and effects of both traditional and EO therapies. Dey and Gupta (2016) found that EOs effectively inhibited phytophagous/plant-feeding insects before and after harvest while they developed, grew, and emerged as adults. On the other hand, little is known about the mechanisms of their activity.

The majority of the toxic and sub-lethal behavioural effects linked to mono- and sesquiterpenoids from plant EOs that have been observed in arthropods and allied insects are certainly the result of neurotoxicity or interactions with one or more arthropod nervous system receptors. In arthropods and insects, the mono- and sesquiterpenoid components of EOs quickly perform as neurotoxins, most likely because they interact with a variety of receptor types in their nervous systems (Isman, 2020). It will be almost impossible to connect bioactivity *in vitro* with toxicity in vivo without systemic research, despite confirmations of interrelationships between these matters and a wide variety of potential receptors.

Different EOs have shown significant effectiveness against a wide range of insects. EOs of lavender and peppermint have insecticidal activity against rice pests, such as *Nilaparvata lugens*. Lavender oil (*Lavandula angustifolia*) effectively controls mosquitoes (*Culex pipiens*), particularly being effective against both adult forms and larvae (Bosly, 2022). Isman et al. (2008) mention that rosemary (*Rosmarinus officinalis*) oil shows effectiveness against aphids. Essential oils act as neurotoxins on insects, affecting their nervous system and causing paralysis. Singh and Pandey (2018) describes how compounds such as menthol and limonene, found in peppermint (*Mentha piperita*) EO, block nerve impulses in insects, leading to their death.

Although synthetic insecticides are more widely used, EOs provide a more environmentally friendly alternative with a lower risk of toxic residues in the environment. Essential oils have also been applied against disease-bearing and disease-spreading mites and nematodes. Lemongrass EO acts as an agent that kills itch mites (*Sarcoptes scabiei*) in their egg and adult stages (Li et al., 2020). The ovicidal effect of lemongrass EO has been observed against barber's pole worm (*Haemonchus contortus*) eggs. The half maximal effective concentration (EC_{50}), or the concentration of lemongrass EO required to prevent the hatching of 50% of eggs, was 0.14 mg/mL (Macedo et al., 2015). Ozdemir and Gozel (2017) performed the study with tomato plants to determine the toxicity of EOs of 10 plants (wormwood, bergamot orange, lemon-scented gum, St. John's wort, lavender,

Table 3. Essential oils and their action.

Essential oils of/ active compound	Action
Peppermint (*Mentha piperita*)	Repels ants, flies, lice and moth
Pennyroyal (*Mentha pulegium*)	Wards of fleas, ants, lice, mosquitoes, ticks and moths
Spearmint (*Mentha spicata*)	Warding off flies
Basil (*Ocimum basilicum*)	Warding off flies
Citronella *Cymbopogon nardus*/ citronellal	An insect and an animal repellent, larvicidal
Catnip (*Nepeta cateria*)/ nepetalactone	Repelling mosquitoes, bees and other flying insects
Ocimum sanctum. Satoreja hortensis, Thymusserpyllum and *Origanum creticum*	larvicidal *S. litura* larvae
Lippia alba	Induces growth inhibition induces growth inhibition
Anethum sowa/carvone, dillapiole	Insecticide synergistic properties
Tumernic *Curcuma longa*/ α-phellandrene	Growth inhibition and larval mortality against *Spilosoma obliqua*
Curcumene and ginger	Inhibition of the mycelial growth of *Rhizoctonia solani*
Thymol, citronellal and α-terpineol	Feeding deterrent against tobacco cutworm, *S. litura*
Carvacrol, carveol, geraniol, linalool, menthol, terpineol, thymol, verbenol, carvones, fenchone, menthone, pulegone, thujone, verbenone, cinnamaldehyde, citral, citronellal, and cinnamic acid	Oviposition inhibitors and ovicides
Lemon *Citrus limonum*/ Geraniol and eugenol	Attract thrips, fungus gnats, mealybugs

Source: Robu et al. (2015).

wild mint, basil, black pepper, wild thyme, and ginger) against the root-knot nematode (*Meloidogyne incognita*).

The second-stage juveniles of the root-knot nematode were treated with three concentrations (1%, 3%, and 5%) of the aqueous solutions of these EOs at four distinct application time intervals (12, 24, 48, and 72 hours). The interconnections between EO-time and EO-concentration were statistically significant. EOs of sweet yellow clover, wormwood, black pepper, bergamot orange and wild mint were the most toxic at all concentrations and times. It is believed that essential metabolic, biochemical, physiological, and behavioural processes in insects were inhibited by EOs. There are various EO modes of action against insects. In order to estimate the entomotoxicity of plant EOs and phosphine gas at different concentrations, singly and mutually/jointly, Khaliq et al. (2020) carried out studies with apple of Sodom, neem, river red gum, thorn apple, and cultivated tobacco. Results showed that the highest mortality (65% and 80%) was caused by cultivated tobacco EO and phosphine gas, respectively at their highest concentrations (15% and 500 ppm). EOs of cultivated tobacco and neem had the highest synergistic toxic effect at 500 ppm + 15% combination. Zaka et al. (2018) set up a trial to assess the effects of four plant EOs, two plant extracts, two herbicides, and two insecticides on the confused flour beetle (*Tribolium confusum*). The findings demonstrated that the insecticides cypermethrin and abamectin increased mortality rates more quickly. Neem EO and the citrus extract came after these two pesticides. The dual combination of limonene and citral and geranyl acetate and citral (components of lemongrass EO) exhibited a mutual cytotoxic effect on the ovarian cell line of the cabbage looper (*Trichollusiani*).

Benelli et al. (2018) estimated the impacts of EOs derived from seeds of cumin (*Cuminum cyminum*) and anise (*Pimpinella anisum*) on two pests, green peach aphid (*Myzus persicae*) and Egyptian cotton leafworm (*Spodoptera littoralis*), two insect vectors, house fly (*Musca domestica*) and the nematode causing elephantiasis (*Lymphatic filariasis*) and Zika virus vector *Culex quinquefasciatus*. EOs expressed remarkable effects against all targeted pests. Anise EO was more efficient on larvae of southern house mosquito (*C. quinquefasciatus*) (LC_{50} = 25.4 μl L^{-1}) and Egyptian cotton leafworm (*S. littoralis*) (LD_{50} = 57.3 μg $larva^{-1}$). However, cumin EO was more effective against adults of house flies (*M. domestica*) (LD50 = 31.8 μg adult1) and green peach aphids (*M. persicae*) (LC50 = 3.2 ml L^{-1}). According to Kumar et al. (2008), the bael (*Aegle marmelos*) EO considerably decreased the oviposition and progeny emergence of pulse beetle (*Callosobruchus chinensis*) in treated cowpea seeds. Ganapathy and Karpagam (2016) carried out studies with bael and detected some phytochemicals, such as tannins, alkaloids, saponins, carotenoids, terpenoids, flavonoids, and coumarins. Numerous substances detected in bael leaves, including D-limonene, aegelin, marmesin, and ethyl-p-cumurate, have demonstrated pesticidal properties. It has been determined that many insect pests of agricultural and stored grain were repelled and killed by extracts of this plant (Kumar et al., 2008).

Stenger et al. (2021) established that Surinam cherry (*Eugenia uniflora* L.) EO at the concentration of 0.75% had expressed the insecticidal effect on the bronze bug (*Thaumastocoris peregrinus*) eggs, nymphs, and adults, and that it had been less toxic to *Cleruchoides noackae* (egg parasitoid of this bug) when used one and seven days after the bug parasitized the host than prior to it. Alkaloids with antibacterial properties, phenolic chemicals, and sesquiterpenes have been detected in Surinam cherry EO. Certain terpenes present in EOs affect the insects' central nervous system. If the enzyme acetylcholinesterase (AChe) is inhibited, these terpenes exhibit insecticidal effects on leafcutter ant species (*Atta laevigata*). They inhibit oviposition and the development of larvae of melonworm (*Diaphania hyalinata*) and are toxic to fruit flies (*Drosophila melanogaster*) (Bošković et al., 2023).

Castillo et al. (2017) evaluated the oviposition-deterrent, repellent, adulticidal, and pupicidal properties of EOs derived from the following plants in the laboratory environment: bushy matgrass, Colombian oregano, lemon-scented gum, lemongrass, or West Indian lemon grass, and *Cymbopogon flexuosus*, or East Indian lemon grass, sweet orange, canaga tree, tabog and sweet-scented marigold against the yellow fever mosquito. According to the same authors, all evaluated EOs exhibited insecticidal, repellent, and oviposition-deterrent properties against dengue-transmitting yellow fever mosquitoes. Colombian oregano EO, as an adulticide, caused the death of 100% of yellow fever mosquitoes at 300 and 1,000 ppm two minutes after the application of the treatment. Carvacrol, the main EO component of this plant species, caused this mortality rate at this time. Among many effects of carvacrol, a highly volatile monoterpenic phenol isomer, fumigant and repellent properties against mosquitoes stood out. The repellency rate of lemon-scented gum and Colombian oregano EOs against yellow fever mosquitoes was 100% when the exposure period was 0-2 minutes. According to Kalita et al. (2013) studied winged prickly ash, neem tree, and wild turmeric for their repellent activities against mosquitoes. Working with EOs from lemongrass and rosemary, Isman (2020) demonstrated that the interaction of all the main ingredients came from the increased absorption of toxicants by the insect's integument, instead of the suppression of detoxicating enzymes.

Demeter et al. (2021) examined the toxicity of EOs derived from 25 different plants: Siberian fir, garlic, camphor tree, tea tree, wild mint, nutmeg, lemon, copaiba balsam, cumin, lemon-scented gum, broad-leaved peppermint (blue peppermint), blue gum, American wintergreen, star anise, lavandin, German chamomile, myrtle, basil, holy basil, sweet marjoram, rosemary camphor chemotype, rosemary verbenone chemotype, thyme geraniol chemotype, vetiver and ginger on adult granary weevils. According to these authors, garlic EO was the most toxic. Lethal concentration 90 (LC_{90}), its computed value, was the lowest after 24 h as well as after 7 days. With regards to the toxicity to cost ratio, EOs of three plant species, American wintergreen, wild mint, and broad-leaved peppermint, were promising for the further production of biocides extracted from plants.

According to the accessible literature, a mixture of EOs derived from aniseed, basil, peppermint, and lavandin and pure compounds (E)-anethole, citral, geraniol, linalool, farnesol, and (Z)-jasmone have good repellent and toxic effects against bird cherry-oat aphid, green peach aphid and potato aphid (Pascual-Villalobos et al., 2017).

Sabadilla, rotenone, azadirachtin, pyrethrin, ryania, and nicotine are phytochemicals with insecticidal properties. These botanical insecticides are plant products, and are still difficult to purchase in the market. EOs extracted from neem tree and Dalmatian chrysanthemum were the first EO-based bioinsecticides developed and used. Azadirachtin and pyrethrin, the compounds of terpenoid, are the key constituents of both insecticides (Devrnja et al., 2022).

Despite being utilized in the USA for over a decade and in the EU for the last seven to eight years, EO-based bioinsecticides are still not commercially viable worldwide. The main botanical use in India was based on EOs extracted from neem trees. Neem extracts were registered in several European countries (Austria, Germany, Italy, Spain, and The Netherlands) (Prasanna et al., 2018).

The natural insecticide azadirachtin, a highly oxidized triterpenoid, has been extracted from the tropical neem tree. Triterpenoids include limonoids with a very complex molecular structure. Azadirachtin is important for organic agriculture worldwide. There are no alternative products available for control of some important pests, except for azadirachtin, which is very efficient in Whiteflies, spider mites, and other small, soft-bodied insects, including aphids management of the rosy apple aphid (*Dysaphis plantaginea*), a very harmful pest in organic fruit production. Many studies, both scientific and practical, have been performed mainly in the countries of the Indian subcontinent because this plant originated from this region. The studies were carried out with the aim of setting up programs for the use of these extracts, intended for exportation to Europe, especially for plant protection of organic crops. The initial studies showed a high antifeedant effect of azadirachtin against insects, particularly desert locusts (*Schistocerca gregaria* Forsskal). After many years of research, its effects on more than 200 insects have been proven (Devrnja et al., 2022).

The majority of commercialized plant-based insect repellents include EOs extracted from one or more of the following plants: neem, peppermint, citronella, eucalyptus, geranium, lemongrass, perennial soybean, and cedar (Choochote et al., 2007).

Due to its phytochemistry, insecticidal activity, and mode of action, the genus Piper (family *Piperaceae*) is the most observed genus. There are more than 1000 species in this genus. More than 600 active chemicals have been isolated and identified from different portions of the species, and over 100 taxa have been evaluated for pesticidal efficacy (Okwute, 2012).

When used as solution sprays, extracts from Ashanti, Indian, and Balinese long peppers are effective against cowpea weevil, variegated grasshopper, and mosquito larvae, with the mortality rate ranging from 96 to 100% in 48 hours (Dyer et al., 2004).

4. POSSIBILITIES FOR THE APPLICATION OF ESSENTIAL OILS IN ORGANIC AGRICULTURE IN SERBIA

The use of essential oils in organic agriculture in Serbia presents a promising alternative to conventional synthetic plant protection products, in line with the principles of sustainability and ecological safety. Due to their natural origin, biodegradability, and low environmental impact, essential oils are increasingly recognized as viable components in integrated pest and disease management strategies within organic farming systems.

According to the provisions of Article 21 of the Rulebook on Control and Certification in Organic Production and Methods of Organic Production (Official Gazette of the Republic of Serbia, Nos. 95/20 and 24/21), plant protection against pests, weeds, and pathogenic microorganisms in organic crop production must primarily be achieved through the application of various agrotechnical measures. These measures include the careful selection of plant species and varieties that are resistant to diseases and pests, proper crop rotation planning, appropriate soil cultivation methods, and the use of thermal processes. In addition, natural biological mechanisms are used, such as allelopathic relationships between plants (where plants chemically influence each other), planting protective plant barriers, and encouraging the activity of natural enemies of harmful organisms.

In situations where the aforementioned preventive measures are not sufficient to protect crops, and when there is an imminent threat to the harvest, the use of plant protection products is permitted by the Rulebook. However, even in such cases, actions must comply with the Law on Organic Production (Official Gazette of the Republic of Serbia, Nos. 30/10 and 17/2019) – amended law, and the use of such products is limited only to those preparations that are clearly defined and permitted under the Rulebook.

Table 4, Table 5, and Table 6 provide a list of approved plant protection products that may be used in organic production, specifically relating to insecticides. Furthermore, if traps and dispensers containing plant protection products (excluding pheromone dispensers) are used in organic production, it is mandatory to undertake measures to prevent these substances from entering the environment or coming into contact with cultivated plants, thereby further safeguarding the integrity of organic production.

In addition to the existing Lists of registered plant protection products and Lists of registered fertilizers and soil improvers used in organic production-compiled in accordance with the Rulebook on control and certification in organic production and methods of organic production (Official Gazette of the Republic of Serbia, Nos. 95/20 and 24/21), specifically Annexes 1 and 2-

Table 4. List of active substances contained in plant protection products permitted for use in organic production: Substances of plant or animal origin.

Name	Description, composition requirements, usage conditions
Allium sativum (garlic extract) *	
Azadirachtin extract from *Azadirachta indica* (neem tree)	-
Beeswax	For post-pruning protection
COS-OGA	-
Hydrolyzed proteins, excluding gelatin	-
Laminarin	Seaweed cultivated using organic production methods or harvested sustainably in accordance with organic principles
Maltodextrin	
Pheromones	Only in traps and dispensers
Vegetable oils*	All approved uses, except as herbicides
Pyrethrin	Only of plant origin
Quassia extract from *Quassia amara*	Insecticide, repellent
Repellents of animal or plant origin (e.g., sheep fat)	Must not be applied to edible parts of plants. Must not be used on plants grown for feeding goats or sheep.
Salix spp. bark extract*	
Terpenes (eugenol, geraniol, and thymol)*	

*Plant extracts and essential oils

Source: Rulebook on control and certification in organic production and methods of organic production (Official Gazette of the Republic of Serbia, No. 95/20 and 24/21).

Table 5. List of permitted plant protection products that may be used in organic production – Chemical plant protection products: Insecticides.

Product Name	Active Substance	Content	Manufacturer	Representative
Galmin 800	Paraffin oil	780 g/L	Galenika Fitofarmacija, Zemun	-
Letol EC	Paraffin oil	790 g/L	Chemical Agrosava, Belgrade	-
Herbos white oil EW	Paraffin oil	800 g/kg	Iskra, Croatia	Seles, Belgrade
Nitropol S	Paraffin oil	855 g/L	Nitrofarm, Greece	Agromarket, Kragujevac
Galmin	Paraffin oil	940 g/L	Galenika Fitofarmacija, Zemun	-
Ovitex	Paraffin oil	817 g/L	Belchim Crop Protection, Belgium	Belchim Crop Protection SRB, Belgrade
Ozoneem Trishul 1% EC	Azadirachtin*	10 g/L	Ozone Biotech, India	BioGenesis, Bačka Topola
Nimbecidine 0.03% EC	Azadirachtin*	0.3 g/L (azadirachtin A)	T. Stanes, India	Agromarket, Kragujevac
Laser 240 SC	Spinosad	240 g/L	Corteva Agroscience, Switzerland	Corteva Agroscience SRB, Novi Sad
Laser Super	Spinosad	480 g/L	Corteva Agroscience, Switzerland	Corteva Agroscience SRB, Novi Sad
Larissa	Spinosad	240 g/L	Hebei Veyong, China	Savacoop, Novi Sad

*Plant extracts

Source: Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, Plant Protection Directorate (2025).

Table 6. List of permitted plant protection products that may be used in organic production – Chemical plant protection products: Growth regulators.

Product Name	*Active Substance*	*Content*	*Manufacturer*	*Representative*
Biox M	Mint oil	948 g/L	Xeda International, France	Arum, Deč

Source: Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, Plant Protection Directorate (2025).

there is a growing use of formulations based on medicinal herbs, vegetables, and weeds.

These preparations exhibit insecticidal, bactericidal, and fungicidal properties. Natural products are made from medicinal, aromatic, and spice plants, vegetables, weeds, and other herbs. In a wheat crop where multiple formulations were applied, the highest mortality of aphids was observed following the application of tobacco-based (57.9%) and garlic-based (57.91%) preparations (Filipović et al., 2024).

One of the studies on the application of essential oils in organic agriculture in Serbia is the work by Tabaković et al. (2023). The results indicate that lavender and spearmint essential oils at a concentration of 0.02% stimulated germination and reduced seed dormancy in alfalfa, showing potential for improving seed quality in organic production.

Terzić et al. (2023) examined the use of essential oils of oregano, cinnamon, basil, caraway, mint, and lavender for plant protection in marshmallow (*Althaea officinalis*), as well as their effect on seed quality parameters. The most effective treatment was lavender essential oil at a concentration of 0.02%. Lavender oil increased seed germination and reduced seed infections in marshmallow. This study demonstrated that lavender essential oil has significant potential in seed processing within organic marshmallow production.

In a study by Kostić et al. (2021), the effectiveness of anise, dill, and fennel essential oils was tested against gypsy moth (*Lymantria dispar* L.) larvae. The results showed that these oils, especially at a 1% concentration, had a significant toxic effect, even surpassing the commercial insecticide NeemAzal®-T/S in certain aspects.

In the work of Ratajac et al. (2023), an experiment showed that essential oils of lavender, thyme, and mint have a pronounced acaricidal effect on *Dermanyssus gallinae*, especially when the mites were directly exposed to the oils. The highest mortality rate was recorded with thyme oil, which acted the fastest and most effectively. However, testing the residual activity (the oils effectiveness after a certain time) revealed that efficacy significantly decreases within a few hours, indicating the need for frequent application or the development of extended-release formulations.

Research conducted by Petrović et al. (2019) demonstrated that *Carum carvi* essential oil has significant toxicity toward adult individuals of *Tenebrio molitor* and *Tribolium confusum*. Additionally, changes were observed in the activity of enzymes responsible for oxidative stress, indicating the potential of caraway oil as a natural insecticide.

The results of studies on the insecticidal and repellent activity of plant extracts and essential oils have shown variable efficacy depending on the insect species, type of treatment, and concentration of the preparation. The most pronounced insecticidal effect on *Sitophilus oryzae* was observed with basil (*Ocimum basilicum*) essential oil, which at concentrations of 1% and 2% caused mortality ranging from 65% to 100%. The extract of *Erigeron canadensis* also exhibited significant contact insecticidal activity against *S. oryzae*. The extract of *Morus alba* caused mortality of *S. oryzae* in contact tests ranging from 5.01% to 64.7% after 48 hours. None of the tested extracts or essential oils demonstrated significant insecticidal activity against *Tribolium castaneum*.

Regarding repellent activity, the essential oils of *Lavandula angustifolia* and *Ocimum basilicum* at concentrations of 0.5% to 2% showed a clear repellent effect against *S. oryzae*. Basil also exhibited repellent activity against *T. castaneum* at concentrations of 1% and 2% (Mezei, 2016).

In the study by Bošković et al. (2023), a biopesticide formulation was developed combining essential oils of geranium (*Pelargonium* graveolens), dill (*Anethum graveolens*), and pine (*Pinus sylvestris*). Experiments demonstrated that this formulation significantly reduced the number of *Drosophila suzukii* larvae in treated fruits compared to control groups. It was also found that the formulation had a repellent effect, deterring females from laying eggs. The biopesticide showed satisfactory stability and efficacy under laboratory conditions, suggesting potential for practical application.

In a study conducted by Gvozdenac et al. (2021), lemongrass (*Cymbopogon citratus*) essential oil, cultivated in Serbia under controlled conditions, was tested against four major stored-product pests: *Plodia interpunctella* (larvae), *Sitophilus oryzae*, *Acanthoscelides obtectus*, and *Tribolium castaneum* (adults). The results showed that the oil had a strong repellent effect, particularly at higher concentrations, with the most pronounced activity observed against *T. castaneum*. The effect lasted up to 48 hours, indicating the potential use of this oil as a natural means of protecting stored products without synthetic pesticides.

Research conducted in Serbia has demonstrated that various essential oils—including those of lavender, basil, mint, thyme, oregano, cinnamon, dill, fennel, caraway, geranium, pine, and lemongrass-exhibit insecticidal, fungicidal, bactericidal, and repellent properties. These oils have proven effective against a wide range of pests. Furthermore, some essential oils have shown positive effects on seed germination and quality, suggesting additional roles beyond pest control-such as enhancing plant vigor and resistance.

The practical application of essential oils in organic production, however, faces several challenges. These include the need for standardization of formulations, variability in efficacy depending on concentration and target species, rapid degradation and limited residual activity, as well as regulatory and cost-related constraints. Despite these limitations, their integration into organic crop protection programs-particularly through direct spraying, seed treatment, and development of biopesticides-offers substantial potential.

In conclusion, with continued research, improved formulation technologies, and supportive regulatory frameworks, essential oils can become a key component of Serbia's organic agriculture sector, contributing to environmentally responsible crop protection and higher quality organic products.

5. STABILITY OF ESSENTIAL OIL-BASED INSECTICIDAL FORMULATIONS IN FIELD APPLICATIONS

The stability of EO-based insecticidal formulations is a key factor for their practical application in agriculture. While EOs have demonstrated significant insecticidal potential under laboratory conditions, their effectiveness in field applications depends on various environmental factors, such as light, temperature, humidity, and exposure to air (Isman, 2020). These factors contribute to the rapid degradation of active compounds in EOs, reducing their efficacy and limiting their commercial use. This part of the study examines the challenges and potential solutions for improving the stability of EO-based insecticidal formulations in field conditions.

5.1. Environmental factors affecting stability

5.1.1. Light exposure

Ultraviolet (UV) radiation from sunlight is one of the most significant factors causing the degradation of EOs in field applications. UV rays break down the chemical structure of volatile compounds in EOs, leading to a loss of insecticidal activity (Regnault-Roger et al., 2012). This issue is particularly prominent in oils such as citronella, eucalyptus, and lavender, whose active components are highly susceptible to photodegradation (Pavela and Benelli, 2016). Prolonged exposure to sunlight may require frequent reapplication of insecticides to maintain efficacy, increasing costs and limiting their large-scale use in agriculture.

5.1.2. Temperature and humidity

Temperature fluctuations present another challenge to the stability of EOs. High temperatures can cause the evaporation of volatile components, thereby reducing the concentration of active ingredients in the formulation. Conversely, low temperatures may increase viscosity or solidify oils, affecting their efficacy and application. Humidity also influences the evaporation rate of EOs - higher humidity can prolong the duration of action, while lower humidity can accelerate their volatilization (Mahanta et al., 2021). Najafian (2016) investigated changes in the composition of lavender essential oil during storage at different temperatures. The results showed that at room temperature there was a reduction in components with lower boiling points, while storage at lower temperatures, especially in the refrigerator, maintained the quality of the oil.

5.1.3. Air exposure and oxidation

Oxidation is a critical factor that reduces the stability of EOs when exposed to air. Atmospheric oxygen reacts with the chemical compounds in oils, leading to the formation of degradation products that may be less effective as insecticides (Raut and Karuppayil, 2014). Certain EOs, particularly those containing terpenes (e.g., limonene or pinene), are especially prone to oxidation, which not only decreases their insecticidal potency, but may also alter their aroma, reducing their attractiveness to insects (Tripathi et al., 2009).

5.2. Strategies for improving stability

5.2.1. Encapsulation technologies

One of the most promising approaches for enhancing the stability of EO-based insecticidal formulations is the use of encapsulation technologies. Encapsulation involves enclosing EOs within a protective capsule, such as nanoparticles or microcapsules, shielding the oils from environmental factors like light, air, and temperature. These encapsulated formulations allow for controlled release of active ingredients over an extended period, thereby prolonging their efficacy and reducing the need for frequent applications. Research has shown that encapsulation significantly improves the shelf life and stability of EOs, making them more suitable for field applications (Maes et al., 2019).

5.2.2. Formulating with stabilizers

Adding stabilizers or co-formulants to EO-based insecticidal formulations can also enhance their stability. These agents can function as preservatives, antioxidants, or UV protectants, reducing the degradation of active components. For example, studies have explored the use of antioxidants such as vitamin E or butylated hydroxytoluene (BHT) in EO formulations to prevent oxidation and extend shelf life. Tocopherols, including vitamin E, act as antioxidants that protect these substances from oxidative degradation, thereby extending their shelf life and stability. The combination of oregano essential oil and BHT significantly reduces oxidation indicators, suggesting that the addition of BHT to essential oil formulations can enhance their resistance to oxidation and prolong their shelf life (Olmedo et al., 2019).

5.2.3. Improved application methods

Beyond formulation improvements, optimizing application methods significantly enhances the stability and efficacy of essential oil (EO)-based insecticides. Implementing controlled-release systems, such as slow-release granules or advanced spraying techniques that minimize direct exposure to environmental factors like sunlight, can extend the duration of insecticidal action. For instance, a study demonstrated that zedoary oil-impregnated sand granules provided complete larval mortality of *Aedes aegypti* mosquitoes for nine days, outperforming the efficacy of non-encapsulated oil, which lasted only three days (Champakaew et al., 2007). Additionally, microencapsulation techniques have been employed to protect volatile compounds in EOs from rapid degradation. Research on *Citrus grandis* oil-based formulations revealed that microencapsulation preserved the oil's stability and maintained over 80% protection against mosquitoes for an extended period, even after 12 months of storage (Misni et al., 2021). These findings underscore the importance of integrating controlled-release technologies and strategic application methods to enhance the longevity and effectiveness of EO-based insecticidal formulations in pest management programs.

6. CHALLENGES AND POTENTIAL OF ESSENTIAL OIL-BASED PLANT PROTECTION PRODUCTS IN SUISTANABLE AGRICULTURE

Research on the efficacy of plant protection products based on EOs shows great potential in the context of sustainable agriculture and the reduction of synthetic pesticide use (Pavela and Benelli, 2016). These products are increasingly being studied due to their biodegradability, non-toxicity to the environment, and the possibility of use in organic farming. However, although the research is promising, there are numerous challenges that limit their widespread application in commercial agriculture.

One of the main issues is the instability and rapid degradation of EOs under the influence of light, temperature, and other environmental factors (Bakkali et al., 2008). This leads to short-lived effects and the need for frequent applications, which can increase costs and reduce their effectiveness compared to conventional pesticides. Additionally, variability in the chemical composition of EO, depending on the plant species, growing conditions, and extraction methods, can lead to unpredictable results in application (Regnault-Roger et al., 2012).

Furthermore, the mechanism of EO action is often limited to contact activity, meaning that they do not have a systemic effect like some synthetic insecticides and fungicides (Tripathi et al., 2009). This may require more precise application methods and combining them with other plant protection strategies, such as biological control and agronomic measures.

Nevertheless, recent studies suggest the possibility of improving the stability and prolonged action of EOs through encapsulation in nanoparticles or microcapsules (Raut and Karuppayil, 2014). These technologies could allow for controlled release of active components and better protection of oils from degradation. Further research is needed to develop more effective formulations and determine optimal application methods in agricultural practice.

Plant protection products based on EOs represent a promising, but still developing alternative to synthetic pesticides. Their future application will depend on advancements in formulation technology, as well as comprehensive research that will confirm their effectiveness, cost-efficiency, and sustainability in various plant cultivation conditions.

7. CONCLUSION

There are currently very few commercially accessible EO-based products. Some of the reasons are numerous obstacles in their application, such as their selectivity, phytotoxicity, and effective concentration for each plant species and its pathogens without harmful consequences for the plant itself. Furthermore, numerous products that are successfully used in some countries do not exist or are not available in other countries. Therefore, it is necessary to better regulate the legislation in this field of plant protection and organic agriculture, especially due to the obvious resistance of numerous pathogens and pests to commercial pesticides. In the future, additional studies with the aim of estimating possible effects on non-targeted species, cost-effectiveness, as well as a deeper comprehension of the action modes are required.

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CONFLICT OF INTEREST

The authors declare that they have no financial and commercial conflicts of interest.

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