Essential oils as eco-friendly biopesticides? Challenges and constraints

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Recently, a growing number of plant essential oils (EOs) have been tested against a wide range of arthropod pests with promising results. EOs showed high effectiveness, multiple mechanisms of action, low toxicity on non-target vertebrates and possible use of by-products as reducing and stabilizing agents for the synthesis of nanopesticides. However, the number of commercial biopesticides based on EOs is still low. We analyse the main strengths and weaknesses arising from the use of EO-based biopesticides. Key challenges for future research include (*i*) development of efficient stabilization processes (e.g. microencapsulation), (*ii*) simplification of the complex and costly biopesticide authorization requirements, (*iii*) optimization of plant growing conditions and extraction processes leading to homogeneous chemical compositions of EOs.

The phenomenon of essential oils

Essential oils (EOs) are synthesized through secondary metabolic pathways of plants as communication and defence molecules. Generally, EOs play important roles in direct and indirect plant defences against herbivores and pathogens, in plant reproduction processes through attraction of pollinators and seed disseminators, as well as in plant thermotolerance [1]. The synthesis and accumulation of EOs are associated with the presence of secretory structures such as glandular trichomes (e.g. Lamiaceae), secretory cavities (e.g. Myrtaceae and Rutaceae), and resin ducts (e.g. Asteraceae and Apiaceae) [2], which can be found in various plant organs. Substances contained in EOs are classified into two chemical groups based on the metabolic pathway of their synthesis: (*i*) terpenoids, which are mainly represented by monoterpenes and less commonly by sesquiterpenes, and (*ii*) phenylpropanoids with low molecular weight. The main metabolic pathways of EO synthesis are shown in Box 1, Figure I [1, 3, 6].

Besides their communication and defence roles, EOs are responsible for the specific flavour and scent of aromatic plants [3, 4]. These characteristics, together with their diverse biological activities [5], have attracted high interest from industry, including food processing, perfumery, and medicine [6]. Pesticides protect against many pathogens (e.g., [1, 7, 8]) and arthropod pests (e.g., [9, 10]), including insects of high medical and veterinary importance (see Supplementary Data Figure S1 online). Thus, many EOs are currently considered for the development of plant protection products.

Many studies are published every year (see Supplementary Data Figure S2 online), indicating great prospects of EOs as active ingredients for the production of botanical pesticides [11]. Nevertheless, only very few commercial products based on EOs have been marketed and the number of newly introduced products remains minimal. This paradox has been discussed recently [1, 12, 13] raising the two main questions: (*i*) What are the causes for this low rate of converting research results into practical application? (*ii*) Which direction should the research take in order to change this unfavourable development? Here we attempt to provide some answers to these questions, and at the same time, we outline key challenges and potential trends for this area of fast-growing research.

Botanical pesticides based on essential oils

Pesticides have become a regular part of our lives. The yield of annual crop production heavily relies on the application of pesticides [13]. However, frequent applications of some synthetic pesticides has led to several problems [14]. For example, residues of many pesticides can be detected in foods at concentrations above recommended limits with negative effects on human health [15, 16]. Also, the effect of pesticides on non-target organisms is important [17]. Lastly, the development of pathogen and insect populations resistant against one or more synthetic pesticides is a major problem [18].

The above-mentioned issues culminated in the second half of the 20th century and resulted in the current efforts to reduce the use of pesticides [19]. Eco-friendly alternatives include botanical pesticides (BPs) produced from plant metabolites [1, 9, 13]. BPs, based on EOs, exploit the toxicity of aromatic hydrocarbons contained in the oils. EOs, including their active substances (see Supplementary Data Figure S1 online), show good biological activity and provide insecticidal, nematicidal, ovicidal, fungicidal, and bactericidal effects against pathogens and pests that are important factors in agricultural yield [1]. In addition, EOs are able to inhibit growth, food intake, and oviposition in a number of important pests [1, 7-10, 20, 21]. EOs may be composed of several dozen (usually 20-60) active substances, which are characterized by two or three main compounds at high concentrations (20–85%) as well as other components, present at trace levels [5, 7]. Although little is known about the mechanism(s) of action of individual compounds, evidence so far indicates that effects of most compounds differ in the mechanism(s) of action. However, one common mode of action for EOS that has been observed is based on their ability to disrupt cell wall and cytoplasmic membrane of the bacteria and fungi, leading to lysis and leakage of intracellular compounds or reported increased uptake of PI and leakage of K^+ [22-26], able to cause structural alterations of the outer envelope without promoting the release of cellular content. These cell surface changes were sufficient to induce cell death. In addition, compounds from EOs can exert their activities on insects through neurotoxic effects involving several mechanisms, notably through GABA, octopamine synapses, and the inhibition of acetylcholinesterase (Table 1).

Complex mixtures of substances contained in EOs, with different mechanisms of action and often exhibiting mutual synergistic relationships [27-29], may be efficient in preventing the development of resistant pathogen and pest populations. This is one of several important benefits of BPs based on EOs (Box 2).

So why is the number of commercial products so low?

Although being very efficient as potential active ingredients, only few commercially manufactured BPs based on EOs exist. In our opinion, this is due to four main reasons: (i) Many published studies, but only few practical results; (ii) Strict legislation; (iii) Low persistence of effects; (iv) Lack of quality and sufficient quantity of materials for affordable prices.

Many published studies, but only few practical results

As noted by Isman and Grieneisen [12], most of the published studies of biological efficacy of EOs are based on screening the efficacy of one or more EOs against one or more target organisms. Most studies are thus only the first step in the development of new BPs. Only very few studies have dealt with the effect of EOs on non-target organisms, while such researches are important for the development and authorization of BPs [30-32]. For example, EOs may have a significant effect on insects even in sublethal doses [33]. However, this phenomenon has been rarely studied. In addition, the number of studies focusing on the mutual relationships of individual compounds contained in EOs is low [27-29], although this information is crucial for the development of standardized BP formulations. We believe that closer cooperation between scientific centres and potential manufactures in research is missing – i.e. a type of cooperation that would lead from taking *in vitro* data on the verification of biological efficacy all the way through to field experiments on pests attacking plants and livestock.

Strict legislation

The BP authorization processes are complex and costly, as well as the authorization of any newly synthesized compound with no history of use in the food, cosmetics or pharmaceutical industry. Authorizations in EU states require safety documentation through appropriate toxicological studies. However, in many cases, such studies do not exist and their preparation is too expensive for local manufacturers. The manufacture of BPs, often of only local importance, is usually at low scale, because production is restricted by limited availability of active substances. BP manufacturers therefore try to market such products outside the scope of the authorization process, for example, as fragrances or fertilizers with a secondary pesticidal benefit. However, this practice is desolating for many manufacturers, because they cannot openly declare the efficacy against pests on the label, which usually result in low sales. BP manufacturers in the EU look with certain hope towards Regulation (EC) No. 1107/2009 (http://eur-lex.europa.eu/eli/reg/2009/1107/oj), which has introduced a new term – "basic substances" (BS). This regulation [(EC) No. 1107/2009 Article 23] provides criteria for the approval of BS with specific provisions to ensure that such active substances can be legally used in the EU, as far as they do not have an immediate or delayed harmful effect on human and animal health nor an unacceptable effect on the environment. However, this regulation may not be easily applicable for the use of EOs, because the concept behind the regulation was that various food additives, without any further formulation adaptations (e.g. without the use of emulsifiers or additives), could be authorized as BSs. Therefore, the process for approval of some active ingredients, such as EOs, could be more complicated, because for their application the use of emulsifiers is necessary. Specifically, any products deviating from the definition of Regulation (EC) No. 1107/2009 Article 23, including the ones containing, for example, an already approved "BS" but contain a co-formulant will then be considered as plant protection product and no longer qualifies as BS [34]. These strict criteria have been set particularly to protect human health against risky pollutants. Among others, this was motivated also by the finding of high food contamination with pesticide residues in EU states, detected in analyses performed at the beginning of the 21st century (see, http://www.pesticide-residues.org/food.html).

Natural substances face a number of opponents among the European Commission members responsible for authorization processes, being viewed by some even as more hazardous than synthetic compounds. These opponents use precisely the lack of relevant toxicological data for natural products as an argument against their use. In particular, there are fears of potential mutagenic or genotoxic adverse effects and negative effects on the human endocrine system. However, according to available information, most of EOs and their main compounds have been reported to be not mutagenic/genotoxic [5]. However, the genotoxic response may be affected by the experimental model chosen and the range of concentrations assayed, and this could be due in part to the induction of oxidative stress. However, some of them can be metabolically activated, such as cinnamaldehyde, or they can be metabolized to a substance without genotoxic activity, such as in the case of linalool [35]. Most negative effects of EOs appear with high dosages, application of undiluted EO concentrates or upon long-term exposure. In terms of toxicology, it should be noted that most EOs show only low acute toxicity, >2 g/kg for both oral and dermal application (Table 2). Given that any residues on plants are minimal (given the fumigation and degradation nature of EOs) [36], a number of EOs cannot be considered as risky substances.

Low persistence of effects

Essential oils are composed of lipophilic and highly volatile secondary plant metabolites, reaching a mass below a molecular weight of 300. Terpenoids tend to be both volatile and thermolabile and may be easily oxidized or hydrolyzed depending on their respective structure [37]. Thus, the chemical composition of essential oils is dependent on the conditions during processing and storage of the plant material, upon distillation as well as in the course of subsequent handling of the oil itself [36, 38]. Thus, these features may significantly reduce the efficacy of Eos against pests. Although the contact effect of EOs is very good, rapid fumigation into the environment and gradual biodegradation of active substances occur upon application on the plants with low persistence of the effect. High attention should be given to the development of suitable EO formulations as active ingredients of BPs that would show higher persistence of efficacy. However, this research is only beginning, and mostly focused on suitable encapsulation methods. Encapsulation is a process in which an active component is entrapped or coated by a matrix wall. This matrix isolates the bioactive molecule from the surrounding environment until its release in response to external conditions (pH, pressure, temperature, etc.) [36]. The wall material can be selected from a wide range of natural or synthetic polymers according to the desired characteristics of the final delivery system [39]. Although a number of EO encapsulation methods exist, developed predominantly for food industry and pharmaceutical purposes [40], especially inexpensive encapsulation methods are needed for the application of EOs as BP. Among the existing methods, coacervation, also known as phase separation, seems the most suitable solution. In terms of the use of EOs for BPs, simple coacervation is suitable, which uses one polymer, such as gelatine or ethyl cellulose [41]. The use of cyclodextrins (CD) may be another suitable method. CDs are cyclic glucose oligomers having six, seven or eight glucose units linked byα-1,4-glucosidic bonds, called, respectively, α-,β-and γ-CD [42]. The use of CD-complexation is widespread in pharmaceutical applications, foods, cosmetics, and toiletries. CDs may be considered as nanoencapsulating agents and the complex formation is equivalent to molecular encapsulation. The bioactive EO molecules are isolated from each other and dispersed on a molecular level in an oligosaccharide matrix [40].

Suitable encapsulation methods, as well as some nanoparticle synthesis methods – AgNP [43, 44] or understanding of the synergistic relationships [27-29], may finally result in an increase of biological activity of BPs based on EOs and thus to an extension of their persistence of efficacy, which is a very important part of research for manufacturers.

Lack of quality and sufficient quantity of materials for affordable prices

EOs are produced in 17,500 aromatic species of higher plants belonging mostly to a few families, including the Apiaceae, Myrtaceae, Lauraceae, Lamiaceae, and Asteraceae. However, only a small proportion (approximately 300 species) has found use in commercial application [1]. A great part of promising EOs originates from plants whose cultivation is expansive or disadvantageous due to low EO yields. Not even plants that are currently grown for commercial production of EOs can be cultivated easily. One of the reasons is that the physiological expression of plant secondary metabolism can differ at all developmental stages. Furthermore, the proportions of monoterpenes depend on temperature and circadian rhythm and vary according to the plant phenological phase [46, 47]. Finally, soil acidity and climate (heat, photoperiod, and humidity) directly affect the secondary metabolism of the plant [1] and EO composition. Therefore a standardized product, which is important for regulatory and marketing purposes, is a timely challenge [1, 5]. To address this challenge, elicitation products, genetic manipulations or new technologies of growing plants have been suggested, aimed at increasing the production and standardizing qualitative and quantitative parameters of EOs [45, 48, 49]. New methods for isolating EOs from plants have also been investigated. At present, EOs are isolated from plants using conventional/classical methods, i.e. using standard distillation of the plant material. Investiing in new technologies (e.g. ultrasounds, microwaves) in the last decades has led to the emergence of innovative and more efficient extraction processes (i.e. reduction of extraction time and energy consumption, increase of extraction yield, improvement of EOs quality) [50].

These new trends in the research of aromatic plants, together with the choice of suitable chemotypes showing high yields or better biological efficacy [33, 48], will open new prospects for the sustainable production and practical employment of EOs.

Concluding remarks and future perspectives

In our opinion, although a huge amount of studies have been published, focused on biological activity of EOs on target organisms, papers concerning toxicological studies and effects of EOs on non-target organisms are missing (see Outstanding Questions). Similarly, the mechanisms of action have not been fully clarified, including mutual relationships among individual substances in EOs and the effects of sublethal concentrations on target and non-target organisms. However, despite those shortcomings, based on available toxicological studies, we conclude that most EOs raise no concerns of their use in plant and livestock protection and can be considered as safe for environment and human health in common concentrations or doses (Box 2). Therefore, the existing legislation concerning authorization should be simplified and better cooperation should be established between research and BP manufacturers in order to put research results into practice. This is a key challenge, because BPs based on EOs have the potential to provide a significant improvement in the quality and safety of foods, including human health, which should be a priority for all food-producing nations worldwide.

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Disclosure

RP has been engaged in the development of botanical pesticides for more than 20 years. At present, he is the head of the team “Secondary Plant Metabolites in Crop Protection“. RP cooperates with several BP manufacturers in the development of new products, of which more than 10 are currently available on the market. RP has been awarded many prizes for his research of BPs including the highest award of the Government of the Czech Republic “Česká hlava” (“The Czech Head“).

GB is an entomologist focused on insect-plant interactions, and the development of novel control tools in the fight against arthropod pests. GB is cooperating with more than 80 researches worldwide and has published more than 150 researches. He has been involved in several international projects, including FP7 Project CoCoRo, GA 270382, ICMR Project 15200, and H2020 Project subCULTron, GA 640967FP7.

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Table 1. The most common mode of actions of essential oils.

Mode of action	Mechanism of inhibition	Examples of possible. compounds	Refs.
Inhibitor of cytochromes P450 (CYPs)	Inhibitors of insect P450 cytochromes responsible for phase I metabolism of xenobiotics, including insecticides.	Dillapiole from *Anethum sowa*, piperamides from *Piper* spp.	51
GABA receptors	Compounds bind to GABA receptors associated with chloride channels located on the membrane of postsynaptic neurons and disrupts the functioning of GABA synapse.	Thymol from e.g. *Thymus vulgaris*	52
Inhibition of cholinergic system	Inhibition of acetylecholinestrase (AChE)	Fenchone from e.g. *Foeniculum vulgare*, S-carvone from e.g. *Mentha spicata*, linalool from e.g. *Citrus* spp.	53, 54
Modulators of ectopaminergic system	They act through the octopaminergic system by activating receptors for octopamine, which is a neuromodulator.	Eugenol from e.g *Syzygium aromaticum*, α-terpineol from e.g. *Pinus sylvestris*.	55, 56

Table 2. Acute toxicity of some essential oils on non-target vertebrates[a]

Plant	LD_{50} mg/kg	
	Orally administered	Dermally administered
Abies alba	>5,000 (rat)	>5,000 (rabbit)
Anethum graveolens	4,040 (rat)	>5,000 (rabbit)
Angelica archangelica	>10,000 (rat)	>5,000 (rabbit)
Apium graveolens	>5,000 (rat)	>5,000 (rabbit)
Cinnamomum camphora	3,730 (rat)	>5,000 (rabbit)
Citrus sinensis	>5,000 (rat)	>5,000 (rabbit)
Coriandrum sativum	4,130 (rat)	N.I.
Cymbopogon citratus	>5,000 (rat)	>5,000 (rabbit)
Elettaria cardamomum	>5,000 (rat)	>5,000 (rabbit)
Eugenia spp.	2,650 (rat)	>5,000 (rabbit)
Foeniculum vulgare	3,120 (rat)	N.I.
Lavandula angustifolia	4,250 (rat)	>5,000 (rabbit)
Melaleuca alternifolia	1,900 (rat)	>5,000 (rabbit)
Ocimum basilicum	>5,000 (rat)	>5,000 (rabbit)
Rosmarinus officinalis	>5,000 (rat)	>10,000(rabbit)
Thymus vulgaris	2 840 (rat)	>5,000 (rabbit)
Zingiber officinale	3,400 (mouse)	N.I.

[a]According to Safety Data Sheets of Sigma Aldrich, N.I. = not indicated

Figure Legend

Figure I. MVA and MEP pathways. Abbreviations: FPP, farnesyl diphosphate; DLG, D, L-glyceraldehyde; DMAPP, dimethylallyl pyrophosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; GA-3P, glyceraldehyde 3-phosphate; GGPP, Geranylgeranyl diphosphate; GPP, Geranyl diphosphate; GGPPS, Geranylgeranyl diphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; IPP, isopentenyl pyrophosphate; MEP, 2-C-methyl-d-erythritol-4-pphosphate.

Box 1. Pathways of terpenoid biosynthesis in aromatic plants

Monoterpenes are the most common group contained in essential oils. It is well known that the biosynthesis of terpenoids in plants takes place via two main pathways (see Figure I): the mevalonate (MVA) pathway in the cytosol and the methylerythritol phosphate (MEP) pathway in the plastids, which yields the 5-carbon precursors isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), which are condensed via geranyl pyrophosphate synthase to give the 10- carbon monoterpenes (not shown). Although isopentenyl pyrophosphate can move between compartments, the monoterpenes and diterpenes tend to be formed in the plastid, where unique cyclases produce the ring structures. Monoterpenes present in essential oils may contain terpenes that are hydrocarbons, alcohols, aldehydes, ketones, ethers, and lactones. The sesquiterpenes have a wide variety of structures with more than 100 skeletons, because the elongation of the chain to 15 carbons increases the number of possible cyclizations that are formed via the mevalonate pathway in the cytosol. Aromatic compounds are less common and are derived mainly from the shikimate pathway, for example, the phenylpropanoid dillapiole, but a few phenols, such as carvacrol and cuminaldehyde, are a rare group derived from terpene biosynthesis by desaturation.

Box 2. Essential oils as botanical pesticides: advantages and future challenges

Overall, the use of essential oils as botanical pesticides has shown a number of advantages including:

- High effectiveness against a wide number of pests and diseases of agricultural and medical importance.
- Multiple mechanisms of actions: due to the large number of active ingredients in each blends, the development of resistance is less likely
- Low toxicity against non-target organisms, including humans
- The production processes are relatively simple and cheap
- Low health risk during application due to low toxicity rates of residues

Key challenges for further research are:

- Simplification of the complex and costly authorization process to legitimize new botanical pesticides, based on plant extracts with proven history of use in the food industry, cosmetics or medicine;
- Avoid loss of efficiency against target pests in the field, highlighting the needing of efficient stabilization processes (e.g. encapsulation). Alternatively, the botanical-mediated synthesis of effective nanoinsecticides could help to avoid high levels of degradation of active compounds from essential oils.
- New production technologies that guarantee abundant quantities of raw essential oil sources with homogeneous chemical composition.