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Practical Approaches to Pest Control: The Use of Natural Compounds

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Abstract

Food production is challenged by different factors: climate changes, market competitiveness, food safety, public demands, environmental challenges, new and invasive pests, etc. Intensive food production must be protected against pests, which is nowadays impossible with traditional techniques. The use of eco-friendly biopesticides based on essential oils (EOs), plant extracts (PE), and inert dusts appears to be a complementary or alternative methodology to the conventional chemically synthesized insecticides. The use of such biopesticides reduces the adverse pesticide effects on human health and environment. Biopesticides can exhibit toxic, repellent, and antifeeding effects. Development of bio-insecticides tackles the problem of food safety and residues in fresh food. Innovation within this approach is the combination of several types of active ingredients with complementary effects. Essential oils are well-known compounds with insecticide or repellent activities. New approaches, tools, and products for ecological pest management may substantially decrease pesticide use, especially in fruit and vegetable production. A win-win strategy is to find an appropriate nature-based compound having impact on pests, together with pesticide use, when unavoidable. Toxic or repellent activity could be used for pest control in the field conditions, as well as attractiveness of some compounds for mass trapping, before pests cause significant economic damage.

Keywords: insect, pests, essential oil, nature-based compound, metabolites, defense, antifeeding

1. Introduction

The current agricultural production, especially food production (whole production-market chain) in the fruit and vegetable sector, is challenged by climate changes, worldwide market competitiveness, food safety, environmental and public demands, new and invasive pests and diseases, etc. New invasive and destructive pests that recently appeared, especially in fruit and vegetable production, limited the use of chemical control agents because of their high persistence in the fresh food chain. For humans, fruits and vegetables are a rich source of vitamins, minerals, fibers, acids, sugars and secondary metabolites in biologically functional forms. Generally, a higher fruit and vegetable consumption is important in improving

human's health. Additionally challenged, by newer standards and climate changes, intensive food production is unthinkable without protection from pests and diseases, which is nowadays impossible using only commonly used plant protection techniques. Different approaches such as better hygiene, standards in production (e.g. GlobalG.A.P.), agro- and pomotechnical measures, prophylactic measures, beneficial insects, mechanical intervention, biocontrol products and less sensitive varieties have been developed. However, a wide use of pesticides is still necessary, but none of the pesticide control techniques, during the long-lasting history, developed against important economic pests has provided long-term protection against pest-resistant species [1, 2]. Also, it may result in higher residues on food and food products than the allowed maximum residue level (MRL) when produced under good agricultural practices (GAP), legally determined by regulations (e.g. EU regulation, WTO, CEFTA, etc.). Multiple pesticide residues were found in 48% of the analyzed apples, 55% of the peaches and 56% of the cherries in 2015 [3]. Additionally, pesticides have an impact on the environment. In several European countries, groundwater pesticide concentrations exceed the European quality standards. Increasing customers and consumers and society's concern about the effects of pesticide utilization on human health and the environment have led to continuous changes in exploring techniques for pest and plant disease management. Even though significant improvements have been made, there is a need for alternative methodologies to ensure a lower utilization of pesticides that have less impact on the environment and guarantee that fruits are practically free from pesticide residues.

The use of eco-friendly biopesticides based on essential oils (EOs), plant extracts (PE) and inert dusts appears to be a complementary or alternative methodology to the chemically synthesized insecticides. Within plant protection practices, modern environmental requirements impose the need for expanding the biological control measures. Investigations of biological activity of plant derivatives lead to this goal, and some researchers have demonstrated certain promising natural substances that can be used for this purpose [4–7]. Natural semiochemicals with low toxic potential which would not cause ecosystem disturbance due to the high mortality of the target insect population could become the predominant method of pest control in the future [8], relying on naturally acquired plant defense mechanisms. Antifeedant activities of essential oils or extracts of different plant species seem to interfere with insect chemoreceptors. Plants produce alkaloids, steroids, flavonoids, terpenoids and saponins that possess high antifeedant activities against different insects; therefore, these compounds could be used in certain formulations and products that would be suitable in integrated insect management programmes. Generally EOs and their components have been considered safer than other plant-derived chemicals like rotenone and pyrethrum, as well as the use of several inert dusts for pest and plant disease control [9–11]. Novel strategies are important and necessary, having in mind the challenges arising due to climate change (increased areas of pest species, number of generations, etc.), public demands and standards in production practice.

2. Plant-pest interactions

2.1 Defensive mechanisms of plants under insect infestations

In all natural ecosystems, plants are exposed to stressful situations caused by biotic and abiotic factors that are largely responsible for significantly reducing crop productivity. For these reasons, plants produce secondary metabolites that protect them in adverse conditions [12]. When it comes to biotic stress, there are three basic

strategies that plants use to defend their enemies: [1] direct defense, [2] indirect defence and [3] tolerance [13]. These strategies are similar to those described by Berryman [14] who stated that plants either may tolerate attack or will use defence mechanisms. Which plant defence strategy will be used depends on the insect species that is causing the damage [15]. During the co-evolution of plants and insects, plants have developed certain responses to attacks of herbivores: changes in the chemical composition of their leaves, as well as their different morphological and physiological properties [16]. Considering the abiotic stresses, for example, the lack of water can significantly affect the choice of the plant defence mechanism. Lack of water in a negative sense causes physiological and morphological changes on plants [17]. The represented defence mechanisms in plants are directly related to the origin and intensity of stress, and it can be classified as indirect and direct defence mechanisms. As stress increases, the number of possible defence scenarios is decreasing.

Indirect defence mechanisms include all plant features that increase the attraction of pest natural enemies [13] or prevent pest oviposition [18, 19]. In contrast, direct defence mechanisms are morphological (e.g. thorns, hairs) or chemical in nature (primary and secondary metabolites), or as their combination, the leaves of some plant species have hairs that directly adversely affect herbivores and, in addition, glands that secrete secondary metabolites [20] and often have a toxic effect (e.g. alkaloids, terpenoids, phenols) and may also inhibit digestive enzymes [21] forcing them to detoxify, causing poorer growth and development of herbivores. If the level of biotic stress is of lower intensity, tolerance is represented. Tolerance is considered when a plant may lose tissue by the herbivore while continuing its further development [22].

The defence mechanisms of direct and indirect defenses can be further divided into passive or constitutive and dynamic or induced defence described in the following paragraphs.

2.1.1 Constitutive defence

Constitutive defence is a passive type of defence of a plant against herbivores and other pathogens and is recognizable by the use of accumulated secondary metabolites under favorable conditions for defensive purposes, caused by the resulting stress [16, 17]. It is a characteristic of perennial plants and is effective in fighting generalists such as the gypsy moth—*Lymantria dispar* L. (Lepidoptera:Erebidae). This type of defence is based on carbon and is present in plants growing under conditions that cause chronic excess of carbon, which provokes accumulation of carbon-based allelochemicals: lignin, tannins and other phenolic compounds, terpenes and resins. These herbal compounds that have negative effects on the growth, development or survival of another organism are considered as toxins. Plants that endure stressful situations by constitutive chemical defence must at the same time be able to sustainably synthesize and accumulate toxic substances without negative consequences on their physiology [23].

However, insects and other plant-borne pathogens have developed various mechanisms to respond to plant toxins [23] and often use them to identify plants as hosts for nutrition and oviposition [24]. Hilker and Meiners [25] consider that the presence of a particular insect species, which has developed adaptability to biochemical mechanisms to the toxic effects of plant secondary metabolites, enhances plant defence in the event of a subsequent herbivore attack. Nevertheless, constituent secondary metabolites having antifeeding action protect plants from most unadapt insects [26] and at high concentrations adversely affect specialized insects [27].

2.1.2 Induced defence

Induced defence in plants is based on their secondary metabolites (terpenes, phenols) and physical structures (cell lignification) as well as a reduction in the production of essential substances to attract herbivores in response to their attack [14]. The type of plant response depends on the balance between primary and secondary metabolites [28]. If the current reserves are reduced by stressful conditions (drought, nutrient deficiency), the presence of herbivore populations is more pronounced. Increased plant resistance reduces the presence and the harmful effects of insects. The minimal length of latency for a plant depends on the rate of decline of plant resistance (e.g. time needed for the plant to recover from defoliation) [29]. The response of plants to the harmful effects of insects is measurable over time (evolutionary time), ranging from a few minutes to a longer period [28].

Additional research has been focused on increased concentrations of secondary metabolites, induced by the attack of insects or other pathogenic organisms. Terpenoids are considered to be the most abundant and diverse metabolic class of plant bioactive products (more than 40,000 structures). They have antifeedant, repellent and toxic effects and can act as regulators of insect development [30]. Bioactive natural products such as alkaloids possess well-known metabolic effects on mammals (e.g. caffeine, nicotine, morphine, strychnine and cocaine) and have probably evolved as a defence against herbivore insects [31]. It is known that the feeding of autumnal moth, *Epirrita autumnata* (Borkhausen) (Lepidoptera:Geometridae), with birch leaves increases the content of phenolic compounds [32]. Gypsy moth (*L. dispar*) feeding increases the content of tannins in oak leaves [33], while after the attack of bark beetles, terpenes and phenolics levels rise in the phloem of attacked trees [34]. Defensive proteins that act on insect digestive enzymes have also been identified in plants. For example, protease inhibitors [21] play a special protective role against insects and microorganisms, in addition to their primary role in the regulation and control of endogenous protease activity, and serve as reserve proteins [35]. The synthesis of protease inhibitors is a part of the induced defence of plants from insect attack. Thanks to the advances in genetic engineering, there is possibility to grow plants with increased levels of protease inhibitors with herbivore defence mechanisms.

2.2 The role of secondary metabolites in insect-plant interactions

Secondary metabolites are organic compounds including terpenes, phenols, alkaloids, proteins and enzymes. They are not directly involved in the development or reproduction of plants (as primary metabolites), but they are often represented in plant defence mechanisms. Usually found in only one plant species or genus, with limited distribution, their production in plants impairs plant growth and reproduction [36]. These compounds are considered as waste products of metabolism without essential function in plant survival [37].

Plants produce different chemical compounds that can be toxic or indigestive for animals [38]. Plant chemical defence is classified into two categories:

1. Quantitative defence, with massive production of indigestible substances; and
2. Qualitative defence, with limited production of toxic substances [39].

By the theory of apparency, plants with their organs are classified into apparent or unapparent [39]. The theory on the balance of growth and differentiation (plant's

“dilemma” for the determination between cell growth or division and differentiation) that creates specialized organs and compounds for defence has also been proved [38].

The presence and availability of nutrients in soil significantly contribute to the level of constituents and induced allelochemicals in plants [40, 41]. There are numerous examples for such actions [42]. Nitrogen fertilization affects the increase in induced poplar resistance after continuous feeding of gypsy moth caterpillars for only 72 h. The composition and concentration of secondary metabolites indicate the interspecies variation is not the case with the primary metabolites. Significant variation was observed between genotypes within the same species, different ages and different branches of one tree and between leaves of different ages on one branch.

2.2.1 Terpenes

Terpenes are the largest class of secondary metabolites (over 22,000 compounds described) and occur in all plants and are classified by the number of isoprene units: monoterpenoids (two units), isoprene sesquiterpenoids (three units), diterpenoids (four units) and triterpenoids (six units). Isoprene (C₅H₈) is the simplest terpenoid to protect cell membranes from damage under adverse conditions (high temperatures). The primary components of essential oils are monoterpenoids and sesquiterpenoids. They are volatile, and their aromas are characteristic of certain plants. They are toxic to insects and pathogens. Monoterpenoids can be used as insecticides, for example, pyrethrins (a compound from *Chrysanthemum*) acts as a neurotoxin to insects. Synthetic analogues of pyrethrin are pyrethroids, a chemical group of pesticides with a large number of commercial insecticides. Alpha- and beta-pinenes are known for repellent action. They are found in pine resin and are known as potent repellents. Monoterpenoids can also be used as spices and perfumes while being relatively harmless to humans. Diterpenoids may have antifungal and antibacterial properties such as gossypol, which is a component of cotton. Triterpenoids are similar in their molecular structure to plant and animal sterols and steroid hormones, which are imitations of insect-coated hormones. For example, azadirachtin is a limonoid isolated from Indian wood (*Azadirachta indica*) that has antifeedant activity and causes sterility. Limonoids also include citronella essential oil isolated from *Cymbopogon citratus* and in the United States is popular as a mosquito repellent for its low toxicity [43]. In addition to defence against the harmful insects and microorganisms, they have a role as a signal in attracting pollinators [44].

2.2.2 Phenols

Phenols are also a large class of plant secondary metabolites and comprise a wide range of compounds (flavonoids, anthocyanins, phytoalexins, tannins, lignins, furanocoumarins). They have different effects on harmful organisms. Tannins have a toxic effect on insects by binding to proteins and salivary digestive enzymes, including trypsin, leading to protein inactivation. By ingesting a large amount of tannins, herbivorous insects do not gain weight and finally die. Lignins are entrenched in the cell walls of plants and provide an excellent physical barrier against pathogens. Furanocoumarins are produced by a wide variety of plants in response to pathogens and are activated by UV light; they are toxic to vertebrates and invertebrates due to integration into DNA and affect at the cellular level [43].

2.2.3 Alkaloids

Alkaloids are a large class of bitter-tasting nitrogen compounds and are found in many vascular plants (caffeine, cocaine, morphine, nicotine). They are derived from the amino acids aspartate, lysine, tyrosine and tryptophan. They have powerful effects on the physiological processes of animals. Caffeine is toxic to insects and fungi and also inhibits seed germination in the vicinity of other growing plants (allelopathy). Nicotine is produced at the root of the tobacco plant and is transported in leaves where it is stored in vacuoles and in the presence of herbivores is released and has toxic effects. Plants that produce cyanogenic glycosides also produce enzymes that convert these compounds into the hydrogen cyanide, including glycosides that are stored in separate cells, and toxic cyanotic hydrogen is secreted by these tissues [43].

2.2.4 Proteins

In contrast to the simple chemicals such as the terpenoids, alkaloids and phenols, proteins require a large expenditure of energy from plants and are formed in significant amounts after the attack of pathogens. Once activated, the defence proteins and enzymes effectively inhibit fungi, bacteria, nematodes and herbivorous insects. Defence against herbivores is obtained by forming an enzyme complex which leads to enzyme inhibition. They include defensins, amylase inhibitors, lecithins and proteinase inhibitors. Defensins have broad antimicrobial activity. First isolated from barley endosperm (*Hordeum vulgare* L., Poales:Poaceae) and wheat (*Triticum aestivum* L., Poales:Poaceae), they are widely distributed and found in most plants. They are most prevalent in seeds but can be found in almost all plant tissues. In addition to inhibiting the growth and development of many fungi and bacteria, they inhibit the digestive proteins of herbivores and impair the cellular balance of ions. Proteins are inhibitors of digestive enzymes and block the normal process of digestion and absorption of nutrients in vertebrates and invertebrates of herbivores. Alpha-amylase interferes with starch digestion, lecithin has a wide range of functions including impaired digestion in insects and blood cell disintegration in vertebrates, and ricin (toxin) produced in castor (*Ricinus communis* L., Malpighiales:Euphorbiaceae) is a highly potent toxin and inhibits protein synthesis. Plants in response to the attack of herbivores produce proteases that inhibit digestive enzymes including trypsin and chymotrypsin and are widespread in nature [43].

2.2.5 Enzymes

A special group of proteins, enzymes, are produced in plants in response to the presence of pathogenic organisms and often accumulate in extracellular spaces where they degrade the cell walls of pathogenic fungi. Chitinases are enzymes that catalyze the degradation of chitin, a cellulose-like polymer present in the cell walls of fungi. Glucanases are enzymes that degrade glycosidic bonds, a class of cellulose-like polymers present in the cell walls of many oomycetes, while lysozyme is a hydrolytic enzyme capable of degrading bacterial cell walls [43]. Chitinase and glucanase enzyme activity lyses pathogen cells [45].

2.2.6 The effects of secondary plant metabolites on harmful insects: state of the art

There is strong public pressure for the production of health food, i.e. food without pesticide residues. For these reasons, extensive testing is being carried out such as the use of secondary metabolites as an alternative to pesticides, the creation of

resistant varieties, the application of nanoparticles, the joint cultivation of cultivated plants with plants that would be confusing on harmful insect and other research.

Plants have created many strategies during co-evolution with insects for effective protection. The most important defence mechanism in plants is the synthesis of biologically active compounds, the so-called secondary metabolites, which can act directly as insecticides or affect indirectly the behaviour of insects—these are called allelochemicals. Allelochemicals are divided into four subgroups, allomones, kairomones, synomones and anemones, and can be used in plant protection.

Metabolites from allomone subgroup represent a respectable group with the currently highest potential [46]. However, it is known that plant secondary metabolites (essential oils, alkaloids, saponins, glucosides, tannins, flavonoids, organic acids) are involved in the defence of harmful insects [4, 6, 7, 47, 48] leading to attempts for field application (spraying) of plant extracts. In recent decades, there are increased evidences of the diverse ecological, physiological and biochemical role of these compounds [37, 49, 50]. The antifeeding properties of plant sprays against harmful insects are thought to have no negative effects on predators or pollinators [51], thus providing an ideal opportunity for pest control [52]. Numerous secondary metabolites, plant extracts and essential oils have insecticidal properties [53, 54]. These substances have oral, contact or inhalation toxic effect to insects, together with antifeeding and repellent effects, which cause a decrease in reproductive potential and change in normal behaviour [55]. Plants produce a wide range of chemicals in various parts above and below ground that are used to defend against stress caused by biotic and abiotic factors but also for communication with other plants and organisms. On the other side, insects have developed strategies to avoid these chemicals [56] or effective detoxification systems specific to individual insect taxa [57], which can be very different between species feeding on the same plant [27, 58].

The insect's orienting abilities include receiving information about the spatial relationships of an organism, processing them and transmitting this information to effectors that can change the relationships. This can be redefined as the relationship between the input and output state of the system (insect/plant ratio); therefore, the chemosensory system allows insects to maintain a constant course, find a host or turn to a sexual partner [59]. Insects often use more than one substance to detect differences between host plants, and the use of secondary metabolites for these purposes is a consequence of evolution.

Dethier et al. [60] described the reactions of insects to chemical compounds:

1. Attractant: A chemical that causes the insect to orientate towards the source.
2. Repellent: A chemical that causes the insects to move away from the source.
3. Arrestant: A chemical that causes confusing action and slows the movement of an insect towards the source.
4. Feeding or ovipositional stimulant: A chemical that causes nutrition and egg laying (oviposition).
5. Deterrent: A chemical that causes an inhibition of nutrition and prevents egg laying (oviposition), and in that area the insect would otherwise feed and lay eggs.

This terminology is generally accepted in describing and considering the reaction of insects to chemical compounds that have been applied in the plant or targeted for protection against herbivores.

An essential biological characteristic of herbivores is nutrition, that is, whether they feed on a single plant species (monophagous), several plant species in one family (oligophagous) and various plant species (polyphagous) whose diet, oviposition and overall biological cycle unfold smoothly across different plant species of different families. In recent decades, extensive research has been done on the impact of secondary plant metabolites on harmful insects, regardless of which group they are classified in according to the nutrition classification.

Effects on stored product pests were widely investigated. Bioactive substances from *Myristica fragrans* Houtt. (Magnoliales:Myristicaceae) oil have been found to have repellent and antifeeding (contact and fumigant) activity and significantly affect offspring reduction in *Sitophilus zeamais* (Motschulsky) (Coleoptera:Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae) species [61]. *Elettaria cardamomum* L. (Zingiberales:Zingiberaceae) seed oil possesses contact and fumigant toxicity and antifeeding activity against *S. zeamais* and *T. castaneum* [62]. This essential oil causes reduction in the number of egg laying and egg hatching of *T. castaneum*. Extracts obtained from seeds of the *Basella alba* plant and leaves of *Operculina turpethum* and *Calotropis gigantea* act as inhibitors of *S. zeamais* development [63]. Essential oils obtained from the leaves of *Eucalyptus dunnii*, *E. saligna*, *E. benthamii*, *E. globulus* and *E. viminalis* (Myrtaceae) showed a pronounced insecticidal and repellent effect on *S. zeamais* [64, 65]. Somewhat weaker but also a very toxic and repellent effect on *S. zeamais* and *T. castaneum* showed the essential oil obtained from the leaves of *Cupressus sempervirens*, as well as cymene, the dominant component of the essential oils of *E. saligna* and *C. sempervirens* [65]. Both cinnamon extracts (*Cinnamomum zeylanicum*) and essential oils of the plants *Etingera elatior*, *E. pyramidospaera* and *Zingiber officinale* show strong repellent activity towards *S. zeamais*, while the moderate repellent activity is shown by the extracts of *Curcuma longa* and *Piper nigrum* [66]. Essential oils of *Ocimum basilicum* L. and *Salvia officinalis* L. caused significant mortality and repellent and anti-reproductive effect [67]. Examination of five ethanol extracts of medicinal aromatic plants for bean protection from weevil *Acanthoscelides obtectus* Say on repellent and toxic action as well as reducing F1 offspring showed a significant insecticidal activity of concentrated extracts of *Urtica dioica* L. and *Taraxacum officinale* L, while *Achillea millefolium* L. extract had repellent effect and caused a decrease in F1 offspring [68]. Similar tests on *A. obtectus* with the essential oils of *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Ocimum basilicum* L. and their dominant components (thymol, alpha-pinene, 1,8-cineol and linalool) showed that *T. vulgaris* EO and thymol have promising efficiencies and can be used as alternatives to synthetic pesticides [69].

Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say, Coleoptera: Chrysomelidae) is an oligophagous pest. The major components in the EOs of potato leaves responsible for the attractive action on potato sprouts have been identified and are referred as “volatile green leaves.” Basically, they are represented by a chain of saturated and unsaturated aldehydes and alcohols, formed by the oxidative degradation of plant lipids. The relative proportions of these end products (mainly alcohols and aldehydes) vary among different plant species within the same genus, as well as seasonally within one species, due to the aging and injury of the plants, all of which affect the degree of attraction of the CPB. It is reported what are the volatile components that attract potato gold: trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal, and linalool in the following ratios (expressed as a percentage): 100: 17: 7: 7: 4 [70]. Host attractiveness to insects related to secondary metabolites, based on the molecular interaction of CPB with plant species of the family Solanaceae, was investigated by Lawrence et al. [71].

The neem extract (i.e. azadirachtin) prepared against the third-stage larvae of *L. decemlineata* has significant antifeedant effect and low toxicity and can be used

to control oligophagous herbivores [4]. In biological studies of residual toxicity and antifeedant action of ethanolic derivatives of sage, *Salvia officinalis* L. (Lamiaceae) (essential oil, five fractions of the same oil F1–F5 and camphor), low toxicity was observed on the second-stage larvae and CPB adulthood, not affecting embryonic development, and the antifeedant activity on the larvae in the first 96 h was very significant for the subsequent activity declined [5]. The possibility of disturbing the attractive properties of the potato leaf on the female potato pollen in the olfactometer was investigated by applying an ethanolic solution of sage oil and five fractions (F1–F5) of this oil. The most pronounced impediments to the recognition of potato leaf are from the sage essential oil and the least expressed by fraction one (F1) [72]. Extracts of five plant species collected in Turkey (*Arctium lappa* L., *Bifora radians* M.Bieb., *Humulus lupulus* L. or *Xanthium strumarium* L. and *Verbascum songaricum* (Schrenk)) were used to investigate the antifeedant effect on *L. decemlineata* larvae. In the first 15 min, the interaction between the larvae and the leaf mass of the potatoes was significantly affected, and during the first 24 h, nutrition was reduced. Gökçe et al. [73, 74] observed that the toxic effect on CPB was obtained by the extracts of the dried rhizome of *Veratrum album* (CHCl₃, acetone and NH₄OH / benzene) and the compounds oxyresveratrol, b-sitosterol-3-O-b-D-glucopyranoside and jervine have the potential to be used as natural insecticides. Biological effects of 24 terpenes, commonly found in aromatic plants in the Mediterranean region, have been investigated to determine their antifeedant effect on CPB as well as allelopathic impact. Terpene (–) α -bisabolol possesses high antifeeding and low phytotoxic activity [44].

Gypsy moth is a polyphagous insect and belongs to the group of the most harmful butterflies. The caterpillar feeds on the leaves of almost all types of hardwoods, conifers and the green mass of many agricultural, fruit and vegetable crops. Protection against the damaging effect of gypsy moth must involve knowledge that secondary metabolites are involved in the defence of insect plants [4, 6, 8, 47]. Other EOs and their components have antifeeding activity against caterpillars: Kostic et al. [6] found that *Ocimum basilicum* EO and its dominant component linalool cause antifeedant activity against second-stage larvae, and Popovic et al. [8] found that fractions of *O. basilicum* EO also act as antifeedant on gypsy moth caterpillars of the second-instar (L2) as well as EOs of *Athamanta haynaldii* and *Myristica fragrans* [7]. Also, neem (0.09% azadirachtin, safer), shows good antifeedant activity against L2 and low digestive toxicity [4], which were confirmed in other investigations [6–8].

Pavela [46] found that *Foeniculum vulgare* EO has a very pronounced digestive toxicity to fourth-instar (L4) caterpillars of *Spodoptera littoralis*. Singh et al. [75] found that trans-anethole exhibited moderate digestive toxicity to first-instar (L1) *Chilo partellus* (Swinhoe) (Lepidoptera:Crambidae) caterpillars, whereas it showed significantly lower toxicity to second-instar caterpillars.

There are numerous positive properties that herbal extracts and EOs have compared to those of the conventional insecticides such as the absence of adverse environmental effects, the disturbance of biocenosis, the absence of nonspecific effects on predators and parasitoids, the minimal toxicity to mammals, the ease of detection and finally the inability to develop resistance. Some disadvantages must be overcome in order to make their application as efficient and easy as possible. The problems encountered in dealing with EOs are their high volatility, incoherence, inadequate formulation, limited shelf life and action on a very limited number of pests [76, 77].

When insects develop resistance to certain plant secondary metabolites, they also develop resistance to the associative molecules of these metabolites generating synergistic effects. For example, in oak leaves, the tannin-binding protein forms

complexes with tannins, difficult to digest. Fenny [39] concludes that tannins, as part of a wide range of defence mechanisms, have repellent, antibiotic and growth-inhibiting properties, via their effect on protein availability. However, for gypsy moth, tannic acid is an attractant, and the alkaline pH value of the digestive tract prevents the formation of tannin protein complexes. Insects often use more than one substance to detect differences between host plants, and the use of secondary matter for these purposes is a consequence of evolution. In recent decades, there has been increasing evidence of the diverse ecological, physiological and biochemical role of these compounds [49, 50].

2.3 Inorganic compounds

One of the alternative methods of crop protection and protection of stored agricultural products in warehouses has been the use of various inorganic dusts in recent years.

So far, diatomaceous earth (DE) preparations have been mostly registered and applied in agricultural practice. The diatomaceous earth was created by the fossilization of tiny aquatic algae (microscopic algae) by organisms called diatoms. The main constituent of their skeleton is called silica, which in contact with water and oxygen forms silicon dioxide. The compositions on the basis of DE consist mainly of an amorphous form of silicon dioxide (amorphous silicon dioxide) and a smaller part of the crystalline silicon dioxide (crystalline silicon dioxide). The first registered composition on the basis of DE was registered in 1960 in the United States for control of insects and mites. To date, over 150 preparations for various uses have been registered. They are used to counter bedbugs, cockroaches, crickets, fleas, ticks, spiders and many other pests. They have also found application in the protection of stored products, except in conventional agricultural production and in IPM and organic production [78].

In addition to DE, many other inorganic powders such as silicophosphate, rock phosphate, sand, kaolinite, clay, zinc oxide, titanium dioxide, vermiculite dust, zeolite, alumina, etc. have also been studied [9, 10, 79–82]. In addition to natural dusts, the possibility of obtaining and applying nano-dusts has been increasingly studied in recent years. The application of modern nano-methods yields nanopowders of improved properties (**Figure 1**) and efficiency and is more environmentally friendly (less toxic to mammals and plants, durability, eco-friendly, less harmful to the environment than the conventional) [9, 79, 80, 83, 84].

The mechanism of action of native and nano-dusts is not fully understood. Some authors believe that the particles of these preparations bind to the exoskeleton and

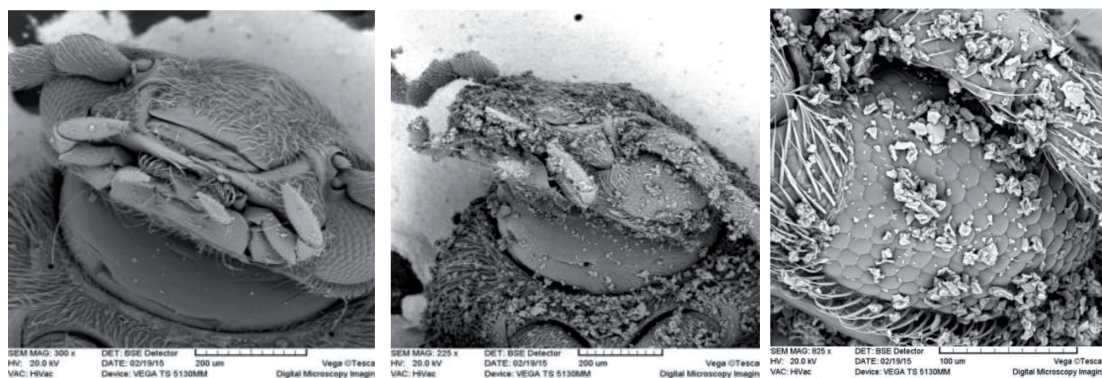


Figure 1. SEM images of untreated (left) insect treated with Al_2O_3 (middle) and enlarged image of insect treated with Al_2O_3 (right) [9].

that they adsorb lipids from the cuticle and cause dehydration of the insect [80]. In contrast, other authors believe that dust particles can physically damage the cuticle and lead to dehydration, that they can ingest damage to the intestinal tract of insects, and that they can block the trachea and thus the insect's breathing [85, 86], like abrasion of the cuticle, absorption of the cuticular waxes from the epicuticle surface, damaging of the digestive tract, blocking of spiracles and tracheae, surface enlargement combined with dehydration and repellence caused by the physical presence of the dust. It is assumed that such chemically inert compounds attached to an exoskeleton are able to adsorb cuticular lipids, thus causing rapid dehydration of insects.

Mineral elements (macronutrients and trace elements) play an important nutritional role in plants and are necessary for the normal course of many cellular processes such as primary and secondary metabolism, defence, gene regulation, hormone perception, energy metabolism, reproduction and signal transduction [87]. A series of functions performed in plants can be affected by the increase in their resistance and protection against harmful organisms. According to Reynolds et al. [88], silicon (Si), which has been found to play a significant role in overcoming the various biotic and abiotic stress factors to plants, may have an indirect and direct effect on enhancing the defence capabilities of plants against harmful insects as part of the mechanisms of physical and induced chemical defence of plants. The physical defence mechanisms involving Si are mainly related to the deposition of Si, mainly in the form of opalina phytoliths, in the cell walls, especially in the epidermal cells of the plants, thereby increasing their firmness and abrasiveness, which in insects can lead to difficult nutrition and damage to the oral apparatus. Also, such a plant food for the insects is reduced digestibility which negatively affects the parameter growth and feeding insects and which is reflected in their reduced growth, length of life and fertility. The presence of Si in the plant may also initiate or accelerate a number of different chemical defence mechanisms that protect the plant from harmful insects. Si can cause a significant increase in defence enzymes such as peroxidase, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase involved in the processes of lignification and synthesis of suberin (peroxidase), increased production of phenolic compounds (PAL) and oxidation of phenolic compounds (polyolase) which increases the hardness of plant tissue and the production of compounds that have detergent and toxic properties while reducing the nutritional quality of food and the digestion of proteins. Also, silicon exerts a positive effect on the biosynthesis of volatile compounds such as jasmonic acid and salicylic acid, in which herbivore-invaded plants emit to attract the natural enemies (predators and parasitoids) of the insects that attack them. Silicon definitely may be considered as an environmentally friendly option in the concept of sustainable agriculture.

3. Conclusion

Intensive food production must be protected against pests and diseases, which is nowadays impossible with single and traditional techniques. However, a wide use of pesticides is still necessary, which may result in higher residues on food and food products than the allowed maximum residue level (MRL). The use of eco-friendly biopesticides based on essential oils (EOs), plant extracts (PE) and inert dusts appears to be a complementary or alternative method to chemically synthesized insecticides. The use of biopesticides may reduce the adverse effects of chemical pesticides on human health and environment. Biopesticides can exhibit toxic, repellent and antifeedant effects on different insect species. Investigations for developing a new bio-insecticide tackle the problem of food safety and residues in fresh food. Innovation within this approach is the combination of several types

of active ingredients with complementary effects. Essential oils are well-known for their insecticide or repellent activity. But so far their use in practice is limited due to their high volatility and short period of action. This problem could be solved by their encapsulation with natural coating materials. Regarding such formulation, their volatility should be prolonged, and EOs will have a chance to provide satisfactory efficacy against pests. New approaches, tools and products for ecologically improved pest management may substantially decrease pesticide use against pests, especially in the fruit and vegetable sector. A win-win strategy is to find an appropriate nature-based compound which will have a wide spectrum of impacts on pest populations. Toxic or repellent activity could be used to control their presence in the field conditions, combined with the use of attractants of some compounds for pest mass trapping, followed by pesticide use when unavoidable.

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