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The role of botanical treatments used in apiculture to control arthropod pests

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Abstract – Among pests of bees and beehives, arthropods make up a large and important group. Mites like *Varroa destructor*, *Acarapis woodi*, or *Tropilaelaps* spp., beetles (*Aethina tumida*, *Oplostomus* spp.), and lepidopterans (*Galleria mellonella*, *Achroia grisella*) decrease honey bee population and vitality, with subsequent significant colony production losses. Synthetic chemicals have been traditionally used to protect honey bee colonies from pests' infestations but they have often been of poor selectivity, consequent high toxicity to bees and humans, and resistance development by the targeted apiary pests. The current European policy encourages the usage of eco-friendly methods to combat bee pests and the international research highlights plant secondary metabolites as candidate alternatives of significance. In this review, we argue the potential of plant-derived substances in the protection of the bee colonies against their arthropod pests. The before mentioned major apiary arthropods are briefly described followed by the recent reports on the botanical extracts and notable constituent compounds exhibiting activity against them. We discuss the different ways the essential oils are reported to be applied to the bee or the apiary, along with the importance of the application method to the exhibited efficacy. We designate synergism issues of blends, attractants, and repellency cases, as well as selectivity and mode of action as reported for bees or insect pests.

bee products / apiculture / arthropod pests of bees / plant secondary metabolites / essential oils / *Varroa destructor*

Key Message

- Resistance issues of synthetic veterinary products lead to the plant-derived substances as candidate alternatives for the control of apiculture pests.
- Essential oils, terpenes, and other botanical extracts could represent active ingredients for the new-age veterinary apiary medicines.
- Research should focus on the selective beekeeping botanicals, synergistically acting blends, and the amelioration of application practices to enhance efficacy.

1. INTRODUCTION: XENOBIOTIC CHALLENGES IN BEEKEEPING—THE NEED FOR BOTANICALS IN VETERINARY MEDICINE

One out of three plants or plant products depend on bee pollination (Bradbear 2009) which fact depicts the primary role of honey bees, of importance for both the uncultivated land and agricultural areas, from an ecological and an economical point of view. A second major role

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of honey bees is the production of honey, the yearly yield of which reaches 1.8 million tons worldwide (García 2018; European Commission 2020). However, in recent years, honey bee populations have been in decline and their numbers are on global alert (Maggi et al. 2013; Hristov et al. 2020; Oberreiter and Brodschneider 2020; Steinhauer et al. 2021).

1.1. The threat of pesticides on honey bees

The indiscriminate use of pesticides resulted in honey bee populations being significantly reduced and their products polluted. The presence of toxic substances in honey is evident (Roszko et al. 2016; Solayman et al. 2016; Džugan et al. 2017). For this reason, honey bees are used as bioindicators of environmental contamination. In this context, Porrini et al. (2003) detected bee poisoning cases even with formulations withdrawn from the market. As honey bee population decreases worldwide (Bacandritsos et al. 2010; Ellis et al. 2010; Sánchez-Bayo and Wyckhuys 2019), insecticides are increasingly regarded as one of the most important factors for both their acute toxicity and endocrine disruption effects (Christen et al. 2018). The lethal and sub-lethal effects of plant protection products on bees may even intensify due to synergism in case of tag mixing before application (Thompson 2003; Mullin et al. 2010). Field-relevant insecticides may decrease egg laying and chemically destabilize the queens' mandibular glands. This may significantly disturb the pheromonal interactions between the queen and workers (Walsh et al. 2020). The exposure of bees to pesticides may even influence their subsequent infection by parasites. Pettis et al. (2012) showed that individual bees with undetectable levels of imidacloprid, after being reared in a sub-lethal pesticide environment within the colony, had higher *Nosema* spp. infection. Moreover, plant protection products (especially insecticides) that are applied on flowering crops may pollute the pollen (Ostiguy et al. 2019). The subsequent residues may be taken up by the honey bees during the collection of nectar and/or pollen.

Since plant protection products can be toxic to honey bees, the existing legislation clearly mentions that active substances used in these products can only be accepted if they are safe for bees. Most interestingly, following recent studies, indicating acute risks of certain insecticides (neonicotinoids and fipronil) for bees, the European Commission restricted their use and has taken further steps to improve the authorization process (Regulation (EC) No. 1107/2009; Regulation (EU) No. 781/2013). In specific, a risk assessment scheme and methodology for effects of plant protection products on bees has been requested by the European Food Safety Authority (EFSA) and new data requirements are assessed on the potential effects on honey bees. The EU has set maximum residue levels (MRLs) for pesticide residues in the framework of Regulation (EC) No. 396/2005.

1.2. The problems of synthetic veterinary medicinal products in apiculture

Apart from pesticides, bees are as well exposed to the xenobiotic substances used directly in beekeeping to treat various sanitary drawbacks. The ability of the beekeepers to recognize the apicultural pests' infestation as well as to select the best available method for colony protection is essential (Jacques et al. 2017). The chemical control of pests and pathogens in the apiary is done with veterinary medicinal products intended for use in food-producing animals. These products have to be scientifically evaluated according to human food safety requirements (Regulation (EC) No. 470/2009). As for pesticides, the EU has set MRLs in honey also for veterinary medicines, and these are listed in Regulation (EU) No. 37/2010 for residues of pharmacologically active substances in honey. For some substances, like amitraz and coumaphos, MRLs have been established, while for others (flumethrin, oxalic acid, and tau fluvalinate), the evaluation demonstrated that no MRL was required to protect food safety. In any case, products that have not been assessed as safe according

to these requirements can neither be authorized nor used otherwise for food-producing animals.

Nonetheless, the long-term use of synthetic veterinary medicinal products has led to the development of resistance to the bee pests' populations, the accumulation of residues in honey bee products, and an increased risk to human health (Martel et al. 2007; Rinkevich 2020; El Agrebia et al. 2020). Thus, there is an increasing demand for innovative, effective apiary tools that exhibit high efficacy and minimize adverse effects on honey bee fitness, with no leave or minimal residues in honey and wax, and with safety margin to the customer (Brascesco et al. 2017). Resistance issues of *V. destructor* to major synthetic acaricides have been, since long, reported and discussed (Feldlaufer 1999; Pettis 2004; Wakeling et al. 2012; Rinkevich 2020). Pyrethroid, organophosphate, and formamidine formulates have been used to control *Varroa* mite populations globally, since the 1980s. To date, populations of *Varroa* mites have developed resistance to the pyrethroid, Apistan (824 mg of tau-fuvalinate/strip, Vita Europe), the organophosphate, Checkmite (1300 mg coumaphos/strip, Bayer) (Currie et al. 2010), and the formamidine miticide, Apivar (500 mg of amitraz/strip, Veto Pharma) (Rinkevich 2020). In an attempt to manage the resistance to the synthetic miticides, organic acids such as formic acid, oxalic acid, and essential oil (EO)-based miticides are used in rotation (Bahreini et al. 2020). Actually, at the present, in Europe, there are various commercial veterinary medicinal products based on natural substances like lactic acid, acetic acid, formic acid, oxalic acid, thymol, and linseed oil (*Linum usitatissimum*: Linaceae).

1.3. The promising role of botanical compounds in the apiary hygiene

The plant secondary metabolites may, in fact, be of possible interest in the development of eco-friendly veterinary medicinal products since in the last decades they have been intensively employed in the international research focus for agricultural pests' management and

have attracted the attention of many researchers (Damiani et al. 2015; Chowański et al. 2016; Ntalli et al. 2019; Suteu et al. 2020; Jan et al. 2020; Fowsiya and Madhumitha 2020; Dehsheikh et al. 2020). Many botanicals have low toxicity to mammals, are not considered pollutants for the environment, and are regarded safe by the society (Isman 2000). Several classes of plant-derived chemicals can be listed for plant protection, among which are pyrethrins, rotenoids, alkaloids, phenolic compounds, alcohols, aldehydes, fatty acid derivatives, terpenoids, EOs, and saponins. These substances reveal a wide range of biological activity and their mode of action varies (Spochacz et al. 2018). They are usually of short persistence, low toxicity against not-target organisms (Pavela et al. 2020) and in the case of complicated clusters of compounds, yielding extracts, they are difficult to build resistance against by pests (Senthil-Nathan 2019). Even in cases when they are not lethal, they may attract or repel pests and thus could be of interest for use in traps or to deter pests from inhabiting the hive (Annand 2008; Peng et al. 2015; Stuhl 2020). Next, plant secondary metabolites may cause significant sublethal effects, like limited hatching, lower longevity, or disturbed development (Ntalli et al. 2014). Most interestingly many plants produce volatile compounds, which are also found in honey (Soares et al. 2017) suggesting that they are non-toxic to bees. Recent reviews on natural botanicals of interest in beekeeping are those of Gregorc and Sampson (2019) summarizing studies on the efficacy of organic acids and EOs for integrated *Varroa* mite control in organic beekeeping; Flamini (2006) focusing on plant extracts as possible new leads of anti-acarid activity in veterinary medicine, and Umpiérrez et al. (2011) presenting data on EOs with anti-*varroa* activity.

1.4. The scope of the study

In our review, we focus on the major apicultural arthropods that are regarded as the most important pests in apiculture and the recent literature regarding their control with

plant secondary metabolites. We exclude the predatory hymenopterans because they do not inhabit hives. We discuss application methods and their effect on the exhibited efficacy, selectivity matters, sublethal activities, and mode of action of plant-derived substances of interest in beekeeping. We additionally report on the toxic cases of natural botanicals on bees and/or bee products, as these compounds should be excluded from the list of leads for veterinary medicine for use in apiculture. Last, we present the chemical structures, molecular formulas, and weights of plant secondary metabolites reported active against bee pest arthropods.

2. ARTHROPODS AS MAJOR PESTS OF BEES AND THE APIARY

The major apicultural pests include arthropods like *Varroa destructor* and *Varroa jacobsoni*, recently described as a parasite of not only *Apis cerana* but also *Apis mellifera* (see below), along with the trachea-inhabiting mite *Acarapis woodi*, and species belonging to the genus *Tropilaelaps*. Additionally, insects, like coleopterans and lepidopterans, inhabit hives. They may not cause as significant losses to the number of honey bees as mites, but they primarily pollute their products with their feces and cuticle fragments. They also feed on honey bee products (kleptoparasitism) and transmit viral, bacterial, or fungal pathogens, thus decreasing the incomes of the beekeepers (Smith et al. 2013). Extensive, precise, and comprehensive descriptions of the life cycles of bee pests, and their significance for the colony and the owner, as well as their distribution were previously described (Ritter and Akrotanakul 2006; Coffey and Mary 2007; Boncristiani et al. 2021). Hence, we present only crucial information relevant to their activity as pests along with the use of plant secondary metabolites to combat them in apiaries.

2.1. Mites

Varroosis is regarded as the most important arthropod-derived disease of bees. It is caused by the mite *V. destructor* (Acari: Varroidae). *Varroa destructor* was previously believed that they feed on hemolymph, but we now know that it feeds mainly on the fat body, which is of greater nutritional value (Ramsey et al. 2019). In addition, the mites can serve as vectors of important honey bee viruses. *Varroa destructor* was molecularly distinguished from the other important pest of bee species, that is *V. jacobsoni*—on the basis of DNA tests (Anderson and Trueman 2000)—and is regarded as a more damaging species. *Varroa jacobsoni* is regarded as a major pest of the Asian honey bee (*A. cerana*) while *V. destructor* is a pest of *A. cerana* and *A. mellifera*. However, due to the relatively recent discrimination between *V. destructor* and *V. jacobsoni* (Anderson and Trueman 2000), probably most of the trials carried out in Europe and America referring to *V. jacobsoni* before 2000 were in fact carried out with *V. destructor*. In any case, both species can be treated with the same methods of colony protection.

Contrary to *V. destructor*, *Acarapis woodi* (Acari: Tarsonemidae) is an internal parasite of honey bees that is very difficult to combat. It invades and inhabits the tracheal system of young honey bees (because of their soft cuticle), destroys the trachea, and feeds on honey bee hemolymph. The infestation may lead to significant sublethal effects, like decreased vigor and lifespan of honey bees. It also synergistically increases losses caused by *V. destructor* and in this case both pests should be combated (Downey and Winston 2001). As an internal parasite of the young honey bees, it cannot easily be treated with strong zoocides. Next, the miticide must enter the tracheal system of the honey bee, which makes obtaining the lethal concentration without harming bees extremely difficult (Eischen and Vergara 2004). *Tropilaelaps clareae* (Acari: Laelapidae) and the related but less spread species *T. koenigerum* and *T.*

mercedesae feed on hemolymph of capped immature honey bee stages. As a consequence, the infested colony may lose even half of its brood (Mortensen et al. 2014).

2.2. Insects

The small hive beetle, *A. tumida* (Coleoptera: Nitidulidae), feeds on pollen, honey, and immature stages of honey bees, and therefore can be considered a predatory species. *Aethina tumida* can cause significant harm to the colonies of European honey bees (Ellis Jr et al. 2003) and may destroy the colony within weeks (Chauzat et al. 2018). The beetle feces may induce fermentation of honey, due to the presence of the commensal yeast *Kodamaea ohmeri* in the beetle gut (Benda et al. 2008). The life cycle of *A. tumida* is divided between the hive-inhabiting period and the period spent outside a hive, making the pest especially difficult to combat.

Oplostomus haroldi and *Oplostomus fuliginus* are Scarabaeidae beetles that are not yet regarded as major pests of global significance (Torto et al. 2010; Fombong et al. 2012). However, there are suggestions of their possible invasive capabilities for example due to climate change (Oldroyd and Allsopp 2017; Abou-Shaara et al. 2021). The eggs, larvae, and pupae develop outside the hive. Adult beetles invade honey bee colonies and feed on broods, pollen, and honey (Donaldson 1989). Therefore, like *A. tumida*, they can be difficult to combat.

Achroia grisella (Lepidoptera: Pyralidae) is commonly known as the lesser wax moth. Pollution, diseases, or malnutrition provoke infestation by *A. grisella*, and may lead to a condition called “bald brood”. They also damage cappings, pollute honey bee pupae with feces, leading to their death, and make the bee products unfit for sale and use (Coffey and Mary 2007; Egelie et al. 2015). Currently there is no suitable chemical to combat wax moths (Jack and Ellis 2018).

The greater wax moth, *Galleria mellonella*, belongs to the same family as *A. grisella* (Lepidoptera: Pyralidae), hence having a similar way

of infestation. Both moths are secondary pests of the beehives. However, the body size of larvae and adults, 25-mm and 20-mm length, respectively, and the number of eggs laid per *G. mellonella* female, reaching 1500 eggs (Mikulak et al. 2018), make it more dangerous than the lesser wax moth. Just like *A. tumida*, the wax moths are rather “parasites of apiaries,” pests of hives, and stored frames, than parasites of bees. *Galleria mellonella* is mostly regarded as a kleptobiotic species (Breed et al. 2012).

Braulidae, known as bee lice, are wingless flies. The significance of these flies as pests is disputable. The infestation leads to sublethal effects, like the decrease of the number of laid eggs or the death of developing honey bees (Bailey and Ball 1991; Marcangeli et al. 1993). The flies are susceptible to miticides, so they can be eliminated during *V. destructor* treatment (Alfallah and Mirwan 2018).

3. BOTANICALS FOR APIARIES—APPLICATION CONSIDERATIONS

3.1. Chemical composition variability and efficacy results

Currently EOs or constituents of EOs occupy the mass part of the international bibliography on botanically based veterinary medicine for apiaries. Some of them like lactic acid, acetic acid, formic acid, oxalic acid, and thymol are already registered for use in the apiaries in Europe. Procedures used to obtain the EO may affect bioactivity via chemical composition differences. As shown in the studies of Maggi et al. (2011), the preparation of the plant material as for instance the involvement of drying may affect the contents of active ingredients, such as camphor, and the subsequent biological activity. In particular, it was demonstrated that Rosmarinus EO caused a 50% lethality of *V. destructor* when tested at the concentration of 16.94 μL per Petri dish for plants dried in the oven, while more than 20 μL were necessary (not estimated) if plants were dried in air. Both oils caused low mortality of honey bees ($\text{LC}_{50} > 20 \mu\text{L}$, not estimated). In

the same frame, when *Eupatorium buniifolium* winter leaves, summer leaves, and summer twigs were used to obtain EO to test for effectiveness against *V. destructor*, the best effective were the summer twigs causing 100% mortality (Umpiérrez et al. 2013). But the yielding EO's efficacy depends on its chemical stability and release resting on many external factors, including temperature, light, acidity, airflow, and level of oxygen (Turek and Stintzing 2013). The slow-release application through microencapsulation is used to surpass problems relevant to the use of plant-derived substances, such as poor water solubility, aptitude for oxidation, uncontrolled diffusion, small persistence, enhanced degradation, volatility, oxidation, and small storage stability (Spochacz et al. 2018). In that direction, microencapsulation was successfully found to prolong the retention of menthol and mint oil, improved properties of marjoram oil release, protected basil oil from being broken down, and affected camphor oil release (reviewed in Bakry et al. 2016).

3.2. Application through direct contact vs. evaporation/fumigation

The treatment mode of EOs and components, like for instance systemic, topical, or evaporation, can lead to variations in bioactivity too (Rosenkranz et al. 2010). In this context, Sabahi et al. (2017) studied the importance of continuously releasing natural miticides to achieve safe and high rates of mite control in hives. The carriers and solvents were used as treatment delivery systems of the formulations. Specifically, they performed three types of applications, that is (a) oxalic acid (5 g per hive) in a sucrose solution impregnated in cardboard that was placed on top of the brood chamber frames of each hive, (b) a mixture of oregano and clove oils (1 g each per hive) in an ethanol-gelatin solution impregnated in absorbent pads applied in hives, and (c) oregano oil (0.99 g of EO/day) via electric vaporizers. The respective *V. destructor* mite control rates for treatments were 76.5, 57.8, and 97.4, evidencing that the strategy of continuous release

as well as using vaporized oregano oil can be highly effective against *Varroa* mites while no differences appeared for honey bee mortality between control and treatments. On the other hand, according to the research of Ruffinengo et al. (2014), the direct contact of bees with the EO may be even more effective in controlling *Varroa* than exposure through evaporation, but unfortunately it is also more toxic for the bees. Specifically, they tested *Acantholippia seriphoides* and *Schinus molle* microencapsulated EOs in Petri dishes at the test concentrations of 0.25, 0.5, and 1 g in direct contact of the bees with the oil or through evaporation. Both encapsulated EOs caused dose-dependent and time-dependent mortality of *V. destructor* but *A. seriphoides* EO was much more effective than *S. molle*. With regard to bee toxicity, the microencapsulated oil of *A. seriphoides* provided high values of honey bee mortality for the doses tested while *S. molle* oil presented a moderate bee mortality rate when it was applied to 1 g for 72 h (Ruffinengo et al. 2014). On the contrary, Adamson et al. (2018) report that limonene revealed rather low contact toxicity to honey bees but its toxicity increased after inhalation of its vapors. The lethality of menthol and cineole was described as being “no different from controls” when distributed with syrup, as food for honey bees (Ebert et al. 2007; Damiani et al. 2014).

Fumigation—the use of volatiles in closed areas, in order to disinfect and kill pests—is a common way to treat hives for parasites according to many researchers. When *Thymus kotschyanus*, *Ferula assa-foetida*, and *Eucalyptus camaldulensis* were tested against *V. destructor*, under laboratory conditions, *T. kotschyanus* appeared the most potent fumigant ($LC_{50} = 1.07 \mu\text{L/L air}$) followed by *E. camaldulensis* ($LC_{50} = 1.74 \mu\text{L/L air}$), while the lowest activity was attributed to the EO of *F. assa-foetida* ($LC_{50} = 2.46 \mu\text{L/L air}$). Interestingly, the EO of *T. kotschyanus* had the lowest toxicity on *A. mellifera* ($LC_{50} = 5.08 \mu\text{L/L air}$) and is thus of potential practical importance for use as an alternative miticide in the management of *V. destructor* in apiaries (Ghasemi et al. 2011). In a study undertaken with *G. mellonella*, 3rd

and 5th instar larvae were employed as targets for the fumigant toxicity of *Thuja* EO and the mortality was evaluated after 24 h of irradiation at 5–25 μL for 3rd instar larvae and 80 μL for 5th instar. According to the results, the percent mortality of 3rd instar larvae reached 100% once exposed to the higher concentration of *Thuja* oil (25 μL) after 24 h, while considering the 5th instar larvae, the mortality was 73.3% at 80 μL (Hamza and Sayed 2019). Lin et al. (2020) calculated the maximal fumigant dosages (V_{max}) of eleven EOs inducing less than 20% honey bee mortality at 72 h. The V_{max} of Odoriferous rosewood (*Dalbergia odorifera*) oil was the highest ($V_{\text{max}} = 14.0 \text{ mL}$), indicating that it had the lowest toxicity to bees while that specific concentration showed significant acaricidal effects to *V. destructor* (72.0% control). Fennel (*Foeniculum vulgare*) oil (66.0%), Mint (*Mentha haplocalyx*) (28.0%), Cablin patchouli (*Pogostemon* spp.) (28.0%), Cao Guo (*Lanxangia tsao-ko*) (24.0%), and Manchurian wildginger (*Asarum* spp.) (24.0%) followed. Most importantly, all plant oils used at their V_{max} dosages did not show a significant fumigant influence on the survival of honey bees. In another study, when vapors of methyl salicylate, formic acid, clove, and basil oils were used against larvae of *A. grisella*, methyl salicylate was 8 times more effective than the next effective substance, formic acid (Abou El-Ela 2014). Ellis and Baxendale (1997) used 7 monoterpenoids as fumigants against *A. woodi* in a laboratory experiment, and assessed for collateral toxicity on *A. mellifera* L. According to the results, menthol, citral, thymol, and carvacrol were toxic to tracheal mites but thymol and menthol were also significantly toxic to honey bees. Pulegone, d-limonene, and α -terpineol were more toxic to honey bees than to tracheal mites. The EC_{50} values ranged from 1.7 to 17.1 $\mu\text{g/mL}$ of free space in the jars. Moawad et al. (2015) showed that fumes of clove and geraniol (0.5 $\mu\text{L}/50 \text{ mL}$) caused above 90% reduction in egg hatchability and life span of adult stages of *G. mellonella*. Interestingly, when oregano oil was delivered using electric vaporizers, to test the hypothesis that continuous release of miticides

increases the varroacidal efficacy of EOs, the mite control rate was 97.4% and there was no honey bee mortality (Sabahi et al. 2017). But in a study on the acute fumigant toxicities of menthol (50 g per experimental colony), amitraz, fluralinate, and cymiazole, Scott-Dupree and Otis (1992) demonstrated that the miticides provided none or marginal control of *A. woodi*. In another study, Eischen and Vergara (2004) reported that the smoke of creosote bush (*Larrea tridentata*) exhibited high mortality against adult *A. woodi* with a subsequent toxicity for honey bees calculated at 18%. However, the toxicity against immature mite stages was insignificant. The fumigation with coffee beans (*Coffea arabica*), corncobs (*Zea mays*), pine needles (*Pinus cembroides*), mesquite leaves (*Prosopis glandulosa*), and tobacco (*Nicotiana tabacum*) were tested in the same research. Crushed corncobs caused very high mortality of honey bees (71%) with the lethal effect for *A. woodi* being about a half. Last, an interesting toxicity/efficacy ratio was obtained for coffee beans: 0% mortality for honey bees and 51.9% mortality for mites. In another study, winter fumigation of honey bee colonies with formic acid led to the lethality of mites but did not reduce the ratio of infested honey bees (Underwood and Currie 2009). Interestingly, when citral, limonene, citronellal, and linalool were studied for effectiveness in knocking down *A. woodi* from infested honey bees, citral was the best effective (Elzen et al. 2000). But vapor-phase oil products have a high variation in oil volatility in the hive due to the fluctuating ambient temperatures which renders mite control with fumigation delivery system unpredictable.

3.3. Impregnating botanicals in hives

Another application mode of the EOs in apiculture comprises the impregnating of strips, papers, card boards, or floral foam bricks in test solutions and their subsequent placement in the hives or laboratory honey bee hoarding cages. The efficacy of *Salvia officinalis* EO against *V. destructor*, in the form of strips impregnated

with the mixture EO and twin, was 6.09% for the dose D1: 5%, 2.32% for D2: 15%, and 0.9% for D3: 20%. The chemical control Bayvarol (Flumethrin) gave a similar efficacy result, that is 9.97% (Bendifallah et al. 2018). The observed percent mortality of *V. destructor* after placing in the hive strips of staining paper drenched for 24 h in 5 mL dose with eucalyptus oil was 90.27% while lemon oil followed with 85.54% mortality, without affecting the colony strength of honey bees. The orange oil failed to furnish satisfactory control of *Varroa* mite (Bakar et al. 2017). In another study, the EO of *Siparuna guianensis* was efficient against the wax moths *G. mellonella* and *A. grisella*, and selective against *A. mellifera* being fivefold to tenfold more tolerant. For the treatments, moth larvae or honey bees were introduced into Petri dish arenas, with the bottoms covered with filter papers treated with the EO (Ferreira et al. 2017). In another study, card boards (1 × 1 × 0.2 cm) carrying 0.01 mL or 0.1 mL or 0.2 mL of clove oil, eugenol, basil oil, blue gum oil, or peppermint oil were introduced into dishes or glasses where *G. mellonella* larvae or adults were placed respectively. The LC₅₀ for clove oil, eugenol, basil oil, blue gum oil, or peppermint oil were calculated at 5.75, 6.57, 11.40, 23.92, and 48.10 μL/mL, respectively. These substances also affected oviposition, hatching, and eventually population growth (Owayss and Abd-Elgayed 2007). When *Eupatorium buniifolium* EO from the summer twig was twice applied (first application, 4.3 g per hive and second application, 8.6 g at day 12 of the assay) as an aqueous emulsion on Floral foam bricks that were placed on the top of the frames, it caused 100% mortality of *V. destructor* after 48 h of treatment with 0% mortality for *A. mellifera*. What is more, EOs from *E. buniifolium* showed lower risk ratio for honey bees compared to oxalic acid (Umpiérrez et al. 2013).

3.4. Other treatment modes

The topical application, injection in hemocoel, and food supplementing are other methods of treatment with the bee protective EOs and/or

Figure 1. Molecular structures along with forms and ascending weights of plant secondary metabolites reported active against bee pest arthropods

constituents. To avoid fumigant volatility in cold climates and to provide a more systemic route of exposure for the target pest, a direct application of EOs is used through direct feeding of bees. However, there must be a balance between toxicity to hive pests and toxicity (safety) to the bees (Ebert et al. 2007). Thyme (*Zataria multiflora*), savory (*Satureja hortensis*), rosemary (*Rosmarinus officinalis*), marjoram (*Origanum vulgare*), dillsun (*Anethum graveolens*), and lavender EOs applied topically with acetone as a carrier at the concentrations of 2 and 1% solvent mixture (w/w) caused a *V. destructor* mortality rate of around 95%. All EOs were not toxic to honey bees with the exception of dillsun causing 12% honey bee mortality (Ariana et al. 2002). In another research, 0.2 μL of EO from *Juniperus sabina* applied on the dorsal part of *G. mellonella* larvae caused 64% lethality. Additionally, the oil revealed a deterrent and anti-feedant effect when present in the larvae food (200 μL per 1.5 g of the diet) and suppressed the development of these insects (Elisovetcaia and Brindza 2018). When *Cannabis sativa* EO in PBS buffer added with 0.01% Tween 20 were injected into the *G. mellonella* hemocoel, through the last left proleg, the concentration of 1.56 mg/mL caused 50% mortality in the first 48 h (Zengin et al. 2018). Interestingly, the supplementation in a liquid protein diet was reported as an efficacious route for the incorporation of origanum oils in the fifth instar honey bee larvae targeted for invasion by *V. destructor* mites (Sammataro et al. 2009).

3.5. Efficacy vs. toxicity

As one can understand from the before mentioned studies, under all various treatment modes of EOs and/or constituents in apiaries, the efficacy against apicultural pests is always studied in parallel to selectivity issues. This is to tumble the main disadvantage of EOs, as



ethyl butyrate

$C_8H_{16}O_2$; MW: 16.16



carvacrol

$C_{10}H_{14}O$; MW: 50.22



thymol

$C_{10}H_{14}O$; MW: 50.22



camphor

$C_{10}H_{16}O$; MW: 52.23



eucalyptol/
cinoleol/1,8-cineole

$C_{10}H_{18}O$; MW: 54.25



linalool

$C_{10}H_{18}O$; MW: 54.25



isobutyl propionate

$C_7H_{14}O_2$; MW: 130.18



cinnamaldehyde

C_9H_8O ; MW: 132.16



limonene

$C_{10}H_{16}$; MW: 136.23



myrcene

$C_{10}H_{16}$; MW: 136.23



3-carene

$C_{10}H_{16}$; MW: 136.23



α -pinene

$C_{10}H_{16}$; MW: 136.23



phellandrene

$C_{10}H_{16}$; MW: 136.23



citral

$C_{10}H_{16}O$; MW: 152.23



pulegone

$C_{10}H_{16}O$; MW: 152.23



thujone

$C_{10}H_{16}O$; MW: 152.23



α -terpineol

$C_{10}H_{18}O$; MW: 154.25



borneol

$C_{10}H_{18}O$; MW: 154.25



citronellal

$C_{10}H_{18}O$; MW: 154.25



menthone

$C_{10}H_{18}O$; MW: 154.25



terpinen-4-ol/4-
terpineol

$C_{10}H_{18}O$; MW: 154.25



decanal

$C_{10}H_{20}O$; MW: 156.27



menthol

$C_{10}H_{20}O$; MW: 156.27



methyl octanoate

$C_9H_{18}O_2$; MW: 158.24



nicotine

$C_{10}H_{14}N_2$; MW: 162.23



eugenol

$C_{10}H_{12}O_2$; MW: 164.20



ethyl octanoate

$C_{10}H_{20}O_2$; MW: 176.26



methyl decanoate

$C_{11}H_{22}O_2$; MW: 186.29



citric acid

$C_6H_8O_7$; MW: 192.12



terpineol acetate

$C_{12}H_{20}O_2$; MW: 196.29



β -caryophyllene

$C_{15}H_{24}O$; MW: 220.35



azadirachtin

$C_{35}H_{48}O_{16}$; MW: 720.26

reported in the past, being the small difference between the lethal dose for mites and honey bees (Kraus et al. 1994). In that context, when the antiparasitic activity of *Humulus lupulus* EO was tested against the honey bee mite *V. destructor*, the mortality to honey bees was moderate to low while it was very toxic to the mite (Iglesias et al. 2020). In another study, ginger and mint EOs were selective for both *A. mellifera* and the pollinator *Trigona hyalinata* (Hymenoptera: Apidae) (da Silva et al. 2020), while Sabahi et al. (2018) showed that anethole and Cymbopogon oil control *Varroa* mites and are relatively safe for the larvae and adult honey bees. In all cases, the in vitro toxicity studies should be followed by further studies in the apiary to assess for effects on brood, queen mortality, and possible interference in beneficial microflora within the internal environment of the colony (Brasceso et al. 2017).

3.6. Synergism effects in plant secondary metabolites' blends

Most interestingly apart from the individual activity of EO or their constituents, there have been reported synergism events of blends. Ramzi et al. (2017) demonstrated the synergistic effect of *Thymus satureioides* and *Origanum elongatum* EOs blend on *V. destructor*. The blend of EOs yielding 55.35% of carvacrol and 20.60% borneol provided an efficacy rate of 93.94%. Synergistic combinations can decrease the risk of resistance developing (Harris 2002). Brasceso et al. (2017) performed in vitro miticidal tests on adult honey bees using thymol paired mixtures with phellandrene, eucalyptol, cinnamaldehyde, myrcene, and carvacrol. The hit mixture was thymol and phellandrene. Similarly, Calderone et al. (1997) used 1:1 (w/w) blends of thymol with cineole, citronellal, or linalool at the test concentrations of 12.5 or 25 g and applications performed at a 14-day interval. The mortality of mites in the colonies receiving thymol and cineole was 56.4 and 49.1% respectively. In similar research on non-honey bee pest mites, the addition of menthol

synergistically increased the toxicity of thymol (Mohammadi et al. 2015), while the mixture of thymol, camphor, eucalyptol, and menthol controlled *V. jacobsoni* and tracheal mites (Imdorf et al. 1999) as well as *A. tumida* (Ellis Jr et al. 2003). Most interestingly the EO constituents may synergistically increase the toxicity of synthetic insecticides, as reported for menthol increasing benthocarb efficacy through the activation of octopamine receptors and protein kinase A (Jankowska et al. 2019). Last, Costa-Júnior et al. (2016) found that some EOs may be more effective against organophosphate-resistant strains of *Rhipicephalus* parasite mites than against organophosphate-susceptible strains. Therefore, they may be used in those areas, where conventional pesticides are of limited value or are forbidden.

4. ESSENTIAL OILS FOR APIARIES—ATTRACTANCE, REPELLENCE, AND MODE OF ACTION

4.1. Attractant and repellent activity

However, EOs can not only serve as lead substances in veterinary products for use in the beehive, but may also serve to attract pests/parasites into traps, provided they have a significant attractive effect. In this direction, Komen et al. (2019) reported the attractant or repellent properties of *Ocimum kilimandscharicum* EO compounds against *A. tumida*. The *O. kilimandscharicum* EO contains linalool, camphor, and 1,8-cineole (Charles and Simon 1992). In another study, a blend of 40 µL ethyl propionate, 2 µL isobutyl propionate, and 2 µL ethyl butyrate in ethyl alcohol attracted *A. tumida* and was used in traps containing boric acid as an insecticide (Stuhl 2020). Camphor caused lethal effects and repellence to beetles (Obeng-Ofori et al. 1998; Cansian et al. 2015), while decanal, methyl octanoate, ethyl octanoate, and methyl decanoate were described as attractants for the greater wax moth. Interestingly, decanal is a major component of the larval aggregation

pheromone of the greater wax moth, *G. mellonella* (Türker et al. 1993; Kwadha et al. 2019). The attract-and-kill strategy may decrease lethality to honey bees and increase toxic effects against pests. The most important natural molecules of high and low toxicity to honey bees are shown in Figure 1 and Table I.

4.2. Mode of action

As regards the mode of action of plant secondary metabolites is in the focus of research in recent years but mainly considering agricultural pests. The mode of action of plant secondary metabolites on apiary pests is yet to be

Table I Bibliography compilation of natural compounds and plant sources of substances ordering according to honey bees toxicity

Substance/compound	Reference
High toxicity	
<i>Siparuna guianensis</i> essential oil	Ferreira et al. (2017)
Thymol	Glavan et al. (2020), Ellis Jr et al. (2003), Rosenkranz et al. (2010)
Menthol	Rosenkranz et al. (2010)
Pulegone, d-limonene, and α -terpineol, other terpenoids	Ellis and Baxendale (1997), Glavan et al. (2020)
Corncoobs (<i>Zea mays</i>) fumes	Eischen and Vergara (2004)
Azadirachtin	Peng et al. (2000)
Pyrethrins	Adamson et al. (2018)
Low toxicity	
<i>Thymus</i> spp.	Ghasemi et al. (2011), Owayss and Abd-Elgayed (2007)
Eucalyptus, lemon	Bakar et al. (2017)
<i>Zataria multiflora</i> , <i>Satureja hortensis</i> , <i>Rosmarinus officinalis</i> , <i>Origanum vulgare</i> , lavender	Ariana et al. (2002)
Thuja	Hamza and Sayed (2019)
<i>Siparuna guianensis</i>	Ferreira et al. (2017)
Cymbopogon	Sabahi et al. (2018)
<i>Eupatorium buniifolium</i>	Umpiérrez et al. (2013)
<i>Rosmarinus officinalis</i>	Maggi et al. (2011)
<i>Acantholippia seriphoides</i>	Rufinengo et al. (2014)
Odoriferous Rosewood, fennel	Lin et al. (2020)
Menthol, citral, thymol, carvacrol	Rosenkranz et al. (2010)
Coffee beans	Eischen and Vergara (2004)
Citral	Elzen et al. (2000)
<i>Laurus nobilis</i> , <i>Petroselinum sativum</i> , <i>Nigella sativa</i>	Zaitoun (2007)
<i>Plantago psyllium</i> , <i>Hordeum sativum</i>	Zaitoun (2007), Lalita and Yadav (2018)
<i>Raphanus sativus</i> , <i>Linum usitatissimum</i>	Lalita and Yadav (2018)
<i>Minthostachys verticillata</i> , <i>Baccharis flabellate</i>	Damiani et al. (2011)
<i>Swietenia</i> spp.	El Zalabani et al. (2012)
<i>Lepidium latifolium</i> , <i>Zataria multiflora</i>	Razavi et al. (2015)
<i>Punica granatum</i> , <i>Allium sativum</i> , <i>Majorana hortensis</i> extract, <i>Peganum harmala</i> , <i>Nigella sativa</i>	ELRoby and Darwish (2018)
Anethole	Sabahi et al. (2018)
Thymol, phellandrene	Brascesco et al. (2017)

reported. Only recently Li et al. (2017) studied the physiological reactions of *V. destructor* after treatment with *Syzygium aromaticum* EO and identified effects on the water-soluble protein content as well as on the activities of enzymes related to detoxification/protection that is Ca^{2+} - Mg^{2+} -ATPase, glutathione-S-transferase, and superoxide dismutase. Studies on agricultural pests' control with plant secondary metabolites report that if applied as fumigants, terpenoids differ in their selectivity and toxicity against insects of various orders. Cineole, l-fenchone, menthone, pulegone, and thujone were least selective; while carvacrol, menthol, citral, and citronellal showed different toxicity to the tested insects of even the same order (Lee et al. 2003).

The data obtained from research on other arthropods showed plant secondary metabolite inhibition effects on acetylcholinesterase (Miyazawa and Yamafuji 2005)—one of the most

important neurotransmitter-breaking enzymes in synapses of both invertebrates and vertebrates, and a target enzyme for old synthetic insecticides: organophosphates and carbamates. In particular, among terpenoids with the highest AChE inhibitory activity are alpha-pinene 3-carene, and 1,8-cineole were found (Perry et al. 2000; Miyazawa and Yamafuji 2005). But it seems that the anticholinergic activity is not the only mode of action of botanicals on mites. For example, the acetylcholinesterase IC_{50} value of carvacrol against susceptible and resistant, to organophosphate and carbamate acaricides, strains of *Rhipicephalus* ticks was 0.04 and 0.28 mg/mL, respectively, while the same parameter for thymol was 0.93 and 0.13 mg/mL, respectively (dos Santos Cardoso et al. 2020), suggesting other additional modes of action. As regards thymol, the mechanism of the toxic activity is not well described. Like many other compounds found in EOs, it

Table II Mechanisms of action of natural molecules and plant sources of substance

Molecule/plant	Mechanism of action	Reference
<i>Syzygium aromaticum</i> EO	Effects on the water-soluble protein content; altered activities Ca^{2+} - Mg^{2+} -ATPase, glutathione-S-transferase, and superoxide dismutase	Li et al. (2017)
Alpha-pinene 3-carene, and 1,8-cineole	Inhibition of acetylcholinesterase	Perry et al. (2000), Miyazawa and Yamafuji (2005)
Thymol	Inhibition of acetylcholinesterase Modulation of GABA-mediated neurotransmission and influx of chloride anions to neurons Altered permeability of biological membranes Tyramine and octopamine receptors	Jukic et al. (2007), Askin et al. (2017) Jankowska et al. (2018) Lambert et al. (2001) Kostyukovsky et al. (2002), Enan (2005)
Carvacrol	Altered permeability of biological membranes	Lambert et al. (2001)
Menthol, linalool, methyl eugenol, and citronella	Malfunctions of neurotransmission through glutamate, GABA, acetylcholine, and dopamine synapses	Peana et al. (2008), Blenau et al. (2012), Li et al. (2020)
Junglone	Genotoxicity, promotion of oxidative stress	Altuntaş et al. (2020)
<i>Ryania speciosa</i>	Activation of the calcium channels of sarcoplasmic reticulum	Singh (2014)

inhibits acetylcholinesterase activity in synapses (Jukic et al. 2007; Askin et al. 2017) but it also modulates GABA-mediated neurotransmission and influx of chloride anions to neurons (Jankowska et al. 2018) and involves toxic activity using tyramine and octopamine receptors that belong to the G-protein coupled receptors (Kostyukovsky et al. 2002; Enan 2005). Thus, thymol may affect signal transduction through biological membranes and the activity of secondary messengers. What is more, thymol, as well as carvacrol, affects the permeability of biological membranes (Lambert et al. 2001).

Other effective monoterpenes, like menthol, linalool, methyl eugenol, and citronellal, are neurotoxic affecting neurotransmission through glutamate, GABA, acetylcholine, and dopamine synapses (Peana et al. 2008; Blenau et al. 2012; Li et al. 2020). In addition, they possess neurotoxic, insecticidal, and miticidal properties. These observations are in tune with the observations of altered bioelectrical activity to insect nerves after exposure to menthol (Jankowska et al. 2019). Some of the compounds found in EOs, like terpinen-4-ol, menthol, citral, or linalool, are competitive inhibitors of acetylcholinesterase, while others, like gossypol or camphor, are non-competitive ones. Also, many EO constituents, like camphor, carvone, menthol, alpha-terpineol, or thymol, affect facilitated diffusion of chloride anions through GABA receptors in the postsynaptic membrane (for review, see Jankowska et al. 2018). These two synapses are targets for numerous insecticides. Both acetylcholinesterase and GABA receptors differ between invertebrates (i.e., also arthropods) and mammals. Therefore, compounds that can selectively affect targets within non-mammalian synapses are interesting as possible insecticides used in the apiaries, with low toxicity to consumers. Last, the difference of size between the honey bees (10–20 mm) and their pests (*V. destructor* < 2 mm, *Tropilaelaps*: about 1 mm, *Acarapis*—about 0.1 mm, *A. tumida*—about 5 mm, and both wax moths being of a size comparable to *A. mellifera*, but much less robust) may affect respective toxicity of applied compounds in the apiary. The known modes of action of described substances is shown in Table II.

5. THE FREQUENTLY REPORTED THYMOL, MENTHOL, AND TERPINEOL AS FOR USE IN APICULTURE

Two of the most frequently studied phytochemicals in managing honey bee mites in literature are thymol and menthol, and many formulations have been developed based on thymol alone or blended with other compounds (Singh 2014). Thymol and menthol are suggested as alternatives to highly toxic synthetic insecticides used to fight *Tropilaelaps clareae* (Raffique et al. 2012; Pettis et al. 2017). Nelson and his group (1993) reported that 3 months of treatment with 30 and 60 g of menthol foam strips, paste, and menthol-dipped cardboard reduced the infestation of honey bees by the tracheal mites down to less than 1% of the initial value. Moreover, the residues of menthol in honey dropped down to the undetected values, in that time. Similarly, decreased prevalence of *A. woodi* and decrease of the menthol residues in honey were observed in *A. cerana japonica* colonies exposed to 30 g of menthol gas per hive (Maeda and Sakamoto 2016). Kevan et al. (1999) checked the effect of menthol delivered to honey bees with syrup. The substance was found in the hemolymph of honey bees and in consequence, in the bodies of mites that fed on honey bee fluid tissues. That method, also including microencapsulation of menthol, resulted in the mortality of tracheal mites (Kevan and Kevan 1997; Kevan et al. 2003).

Thymol, on the other hand, is a broad-spectrum pesticide (Isman 2006) that has been additionally widely accepted by the beekeeping industry in the fight against the *Varroa* sp. mite (Calderone 1999; Imdorf et al. 1999; Eguaras et al. 2004). The commercial miticide Apiguard® has been developed on the basis of thymol as an active ingredient (Coffey and Mary 2007). Gel formulations and slow-releasing thymol vapors appear to be rather effective in the control of *V. destructor* but with additional negative effect on colony development. After 2 weeks of treatment, the bees removed nearly 95% of all the applied products but the residues were relatively higher

in wax than in honey, because thymol is a fat-soluble ingredient (Floris et al. 2004). According to Mondet et al. (2011), honey bees respond to thymol gel formulations in an age-dependent manner. In particular, 2-day-old honey bees respond neutrally, foragers display strong avoidance while responses of 4-day-old honey bees are intermediate. These responses are triggered by olfactory receptors together with the contact chemoreceptors present in the antennae. Considering the toxicity of thymol on honey bees, Gashout et al. (2018) calculated the LD₅₀ value for a honey bee at 51.25 µg/bee and estimated the hazard ratio of thymol as lower than tau-fluvalinate or coumaphos, with a residual time in combs calculated in days, while the two synthetics residue for years. Glavan et al. (2020) compared the sublethal effects of long-term consumption of carvacrol and thymol on Carnolian honey bee workers (*A. mellifera carnica*) and according to the results they caused mortality only at the highest tested concentrations, that is 1% and 5% respectively. Both substances were toxic on *Varroa* at concentrations ten times lower than those causing significant honey bee mortality. However, the sublethal effects evidenced as an increased activity of acetylcholinesterase (AChE) and the detoxifying enzyme glutathione S-transferase (GST) were evident at the 0.05% of carvacrol and thymol. In fact, although thymol was found to be 10 times less toxic to honey bees than to *V. destructor* mites, the results on the nervous system of honey bees undoubtedly limit its usage (Glavan et al. 2020). In fact, Lindberg et al. (2000) suggested that the selectivity of thymol against *V. jacobsoni* is too low, to use it as a safe acaricide for hive protection. On the other hand, thymol was not very effective in provoking death to *A. tumida* (Buchholz et al. 2011) but with a significant repellent activity against the small hive beetle (Bisrat and Jung 2020). Thyme oil, rich in thymol, is toxic to *G. mellonella* larvae, and the LC₅₀ was estimated at 54.12 µL/mL (Oways and Abd-Elgayed 2007). The field varroacidal synergic efficacy of *Eucalyptus globulus* EO together with the constituent thymol confirmed the use of the mix as a viable alternative to the thymol-based commercial treatment (Atmani-Merabet

et al. 2018). The acceptable daily intake (ADI) value of thymol for humans is estimated at 0.03 mg/kg b.w. (European Commission 2016b).

Another compound of interest is 4-terpineol and like α -terpineol it can be found in various plants: *Artemisia rupestris*, *Origanum majorana*, *Satureja montana*, *S. hortensis*, *Melaleuca alternifolia*, *M. leucadendron*, *Pimenta racemosa*, *Eucalyptus* spp., and *Bacopa caroliniana*. Either pure substances or EOs that contain these components revealed miticidal activity (Walton et al. 2004; El-Zemity et al. 2010; Tighe et al. 2013; Abdelgaleil et al. 2019) and were included in commercial medical products against the human skin mites *Demodex brevis* and *Demodex folliculorum* (Shovlin 2014; Gunnarsdóttir et al. 2016), a fact that argues for harmlessness for humans. According to the data presented to the ESFA (EFSA Panel on Additives and Products or Substances used in Animal Feed 2012), the safe use level of terpineols in food, for farm animals (poultry, pigs, and cows), is about 5 mg/kg complete feed. The high-test concentration of 5 mg/kg complete feed for linalyl acetate, linalyl butyrate, linalyl formate, linalyl propionate, linalyl isobutyrate, terpineol, α -terpineol, terpineol acetate, and 4-terpinenol is safe for all species with a margin of safety of 1.2 to 12. However, α -terpineol cannot be used against *A. woodi* according to Ellis and Baxendale (1997) because it is more toxic to honey bees than to the pest. α -Terpineol along with linalool, camphor, and geraniol was described as strong repellents, whereas limonene is an attractant of small hive beetles. Among the repellent substances, α -terpineol showed the strongest activity, with 100% effectivity at the concentration of 1000 ng/µL (Komen et al. 2019).

6. OTHER PLANT DERIVATIVES TO TREAT THE APIARY INFESTATIONS

Other than the EOs, not many plant extracts were studied against apiary pests. *Azadirachta indica* and *Vitex trifolia* extracts effectively controlled *V. destructor* in in vitro and field trials (Anjum et al. 2015) while azadirachtin, the

principal component of *A. indica*, is a widely used biopesticide, known as a chitin synthesis disruptor or development inhibitor in many insect species including pests of *A. mellifera*. The thickness of the 5th instar of *G. mellonella* larvae cuticle was reduced by 21% after consuming 498 ppm of azadirachtin. Other effects were hemolymph loss, darkening, and failure in molting (Ünsal and Güner 2016). Larvae forced to consume azadirachtin died after 27 days at the dose of 5 µg per larva. What is more, pupal development was not finalized after a treatment with 3 µg per larva and adult longevity decreased at all test concentrations ranging between 0.2 and 5 µg (Dere et al. 2019). Telles et al. (2020) reported mortality of *G. mellonella* larvae in the neem oil treatment. However, Peng et al. (2000) described the detailed activity of azadirachtin on both *A. mellifera* adult workers and larvae and the associated pest, *V. jacobsoni*. Azadirachtin was toxic to the adult healthy workers causing a dose-dependent mortality ($LC_{50} = 12.53 \mu\text{g/mL}$ after topical application) but was less toxic to mites ($LC_{50} = 35.43 \mu\text{g/mL}$). The highest toxicity was noted to the honey bee workers' larvae ($LC_{50} = 100.13 \text{ ng/mL}$). Neem oil containing azadirachtin caused a toxic effect on adult bees in the study of Peng et al. (2000). On the other hand, neem and canola oil showed contact toxicity against the phoretic *V. jacobsoni* and *A. woodi*. The mortality of the mite after fumigation with 10% neem oil was comparable to the lethality of 65% FA (Melathopoulos et al. 2000a, b). The research of Xavier et al. (2015) revealed that the short-term (< 14 days) exposure of honey bees to neem oil could not lead to massive mortality of honey bees, although sublethal effects were observed. Moreover, in the same research, the authors found that the syrup with neem oil was less attractive to honey bees than the control. Therefore, the application of neem oil may become not effective for the phoretic stage of *Varroa* sp. mites. The attractant and repellent properties of azadirachtin can also be used against *A. tumida* in traps (attractants), discourage the migratory stages to inhabit the hive (repellents), and help act as antifeedant (Mikami and Ventur 2008).

One of the most controversial plant-derived compounds used against insects is nicotine, present in the extract of *Nicotiana tabacum* (Solanaceae). Nicotine is very well studied on many animal models and its LD_{50} for the honey bee is equal to 315 ppm when applied topically, while for rabbits it is 50 ppm (Ryan 2002). Both *N. tabacum* extract and pure nicotine are studied as potential insecticides against many insect pests. The *N. tabacum* ethanolic extract LD_{50} value against *G. mellonella* larvae was calculated at 36.6 mg/mL after 24 h of exposure (Andjani et al. 2019), and was described as of very low toxicity to the adult honey bees and of no effect on the bee population growth (Telles et al. 2020). The study of Tsegaye et al. (2014) showed that tobacco smoking leads to a decrease of the number of infected combs with *G. mellonella*. However, the author's recommendation is rather to keep the colony healthy by supplementing the diet, so that it can fight the pest by itself rather than to use tobacco as a fumigant. The application of tobacco smoke should last no longer than 2–3 min with limited intensity, due to its possible effect on honey bees and their products. Honey bees were found to tolerate high concentrations of nectar nicotine which is metabolized in their bodies and excreted (du Rand et al. 2017). The bioactivity of *N. tabacum* extract may depend on the used solvent, i.e., an alcohol extract will be more effective than water extract, or may vary under different ways of application (oral or contact) (Cunha Pereira et al. 2020). In other studies, nicotine and other alkaloids like berberine deterred honey bees in a choice test (Detzel and Wink 1993). Alkaloids such as solasonine and *Solanum nigrum* extract containing solasonine can cause sublethal effects in *G. mellonella* larvae (Spochacz et al. 2021), but there are no data available on the effects of these substances on honeybees. Nicotine may appear in hives and honey as a result of collected pollen from the areas located close to tobacco crops. In such a case, the difference between nicotine content can be significantly higher in honey from those areas, compared to the control ones, and the amount of nicotine in honey can reach 2.98 µg/g (Balama et al. 2018). ADI value for humans is estimated

as 0.0008 mg/kg b.w./day (European Commission 2016a). That makes the ADI 70 kg for humans as low as 56 µg of nicotine per day. Such an amount can be found in 18 g of the honey. Since nicotine can be found in many Solanaceae plants, other than *N. tabacum*, additional treatment of hives with nicotine could increase the level of nicotine in honey bee products to unacceptable levels.

Walnut and yew extracts showed high toxicity against *V. destructor* (Garbaczevska et al. 2010). Junglone, present in walnuts, is not toxic

to humans, which makes it interesting for apiary protection. It was tested on *G. mellonella* larvae (LC₅₀ = 2.3 mg) and showed genotoxic properties and induced oxidative stress (Altuntaş et al. 2020).

In 2007, Zaitoun et al. published a paper about over 20 medicinal ethanolic plant seed extracts tested against *G. mellonella*, and *A. mellifera*. Among these plants, 6 effectively controlled *G. mellonella* larvae. *Abrus precatorius* and *Laurus nobilis* caused 100% mortality, *Petroselinum sativum* and *Plantago psyllium* 95%, *Hordeum*

Table III Natural substances toxic to major arthropod pests of apiaries

Pests	Botanical species	Pure substances
<i>Varroa</i> spp.	<i>Acantholippia seriphioides</i> , <i>Allium sativum</i> , <i>Anethum graveolens</i> , <i>Azadirachta indica</i> , <i>Baccharis flabellata</i> , canola, coffee beans, creosote bush, <i>Eucalyptus camaldulensis</i> , <i>Eupatorium buniifolium</i> , <i>Ferula assa-foetida</i> , <i>Ginkgo biloba</i> , <i>Gleditsia chinensis</i> , <i>Hordeum sativum</i> , <i>Lepidium latifolium</i> , <i>Linum usitatissimum</i> , <i>Laurus nobilis</i> , <i>Majorana hortensis</i> , <i>Minthostachys verticillata</i> , <i>Nigella sativa</i> , <i>Odoriferous Rosewood</i> , <i>Origanum elongatum</i> , <i>Origanum vulgare</i> , <i>Peganum harmala</i> , <i>Petroselinum sativum</i> , <i>Plantago psyllium</i> , <i>Punica granatum</i> , <i>Raphanus sativus</i> , <i>Rosmarinus officinalis</i> , <i>Salvia officinalis</i> , <i>Satureja hortensis</i> , <i>Schinus molle</i> , <i>Sophora flavescens</i> , <i>Swietenia</i> spp., <i>Teucrium chamaedrys</i> , <i>Thymus</i> spp., <i>Vitex trifolia</i> , walnut, yew, <i>Zataria multiflora</i>	Anethole, azadirachtin, borneol, camphor, carvacrol, cineole, citral, cymbopogon, eucalyptol, eugenol, fennel, geranial, lavender, lemon, menthol, orange, phellandrene, thymol
<i>Acarapis woodi</i>	Canola	Camphor, eucalyptol, menthol, thymol, thymol
<i>Tropilaelaps</i> spp.	-	
<i>Aethina tumida</i>	<i>Ocimum kilimandscharicum</i>	Camphor, eucalyptol, menthol, thymol
<i>Achroia grisella</i>	<i>Siparuna guianensis</i>	
<i>Galleria mellonella</i>	Blue gumm, <i>Cannabis sativa</i> , coconut soap, clove, <i>Juniperus sabina</i> , <i>Laurus nobilis</i> , <i>Nicotiana tabacum</i> , <i>Siparuna guianensis</i> , thuja, <i>Thymus</i> spp.	Azadirachtin, basil, geranial, junglone, pepper, peppermint
<i>Braula</i> spp.	-	-
<i>Oplostomus</i> spp.	<i>Ocimum kilimandscharicum</i>	

The table summarizes the species and compounds mentioned in the references cited in the text

sativum 78.3%, and *Nigella sativa* caused 45% of mortality. Extracts mentioned above caused the death of the pupal stage, with the exception of *L. nobilis* seed extract that caused the death of insects shortly after emerging from pupae. Interestingly, only *A. precatorius* extract caused 29.2% mortality of honey bee workers, while the other extracts of activity on *G. mellonella* larvae did not show significant mortality to honey bees. Similarly, Lalita and Yadav (2018) calculated the activity of acetone extracts of *P. psyllium* husk extract at 93.33%, and leaf extracts from *H. sativum* at 80%, *Raphanus sativus* (73.33%), *L. usitatissimum* (66.66%), *Cucurbita moschata* (46.66%), and *Vicia sativa* (46.66%) on *G. mellonella*.

In another study, three different *L. nobilis* extracts were tested on *V. destructor* and subsequent effects on *A. mellifera*. According to the results of 24-h post treatment, the ethanol extract was the most effective ($LC_{50} = 2.68 \mu\text{g}/200 \mu\text{L}$ of ethanol solution) followed by the EO ($LC_{50} = 64.68 \mu\text{g}$ on Petri dish) and last the hydrosol (a by-product of distillation) being not effective. When the same extracts were used against *A. mellifera*, the EO was less toxic ($LC_{50} = 108.12 \mu\text{g}$ on Petri dish) while the LC_{50} for the ethanol extract was not possible to estimate (Damiani et al. 2014). When the ethanol extracts of *Baccharis flabellata* and *Minthostachys verticillata* were sprayed on Petri dishes, they both caused mortality of *V. destructor* without harming *A. mellifera* (Damiani et al. 2011). Similar properties against *V. destructor* were demonstrated by El Zalabani et al. (2012) for the 90% ethanolic extract from leaves and stem bark of *Swietenia mahogani* and *Swietenia macrophylla*. The toxicity on honey bees was very low or none in both laboratory and field tests. The methanolic extracts of *Lepidium latifolium* and *Zataria multiflora* showed acaricidal activity in field tests against *V. destructor* with 100%, and 86.26% mortality after 12 days, respectively, when 500 ppm was applied. Both had no toxic effects on honey bee colonies (Razavi et al. 2015). ELRoby and Darwish (2018) demonstrated that fumigation with the extract from pomegranate peel (*Punica granatum*), garlic

bulb (*Allium sativum*), and marjoram whole plant extract (*Majorana hortensis*) reduced *V. destructor* infestation of honey bees at 94.6%, 89.8%, and 89.8%, respectively. The abovementioned extracts also decreased the infection of the brood, at the descending order of harmala seed extract (*Peganum harmala*), followed by pomegranate peel extract, black cumin seed extract (*Nigella sativa*), and garlic bulb extract. All of them provoked more than 88% reduction of infestation. More than 93% of effectiveness was obtained by marjoram, harmal, black cumin, and garlic extracts in powder formulation in the dusting method. Extracts used by these researchers were safe for *A. mellifera* and the honey productivity was high. The plants—sources of natural substances—as well as single compounds toxic to major arthropod pests of apiaries are summarized in Table III.

7. CONCLUSIONS

Apiculture is an important branch of livestock and the global economy. Honey production generates economically important incomes for both individual breeders and countries. Honey bees have an additional tremendous effect on plant production via pollination. Therefore, the health state of honey bee colonies is of major importance and needs a lot of attention.

Apicultural pests should be combated with eco-friendly tools that are not only effective but also selective for bees. Many EOs and other plant extracts have been studied to control the apiary mites and insects, and the application mode seems crucial for the exhibited bioactivity. Some single botanical compounds like thymol and menthol are already frequently used or registered (thymol) against pests in the apiaries but many more are still to be developed into new-age veterinary medicines in apiculture. As the review presents, menthol and thymol are active against the majority of hive pests. *Thymus* spp. are reported as active against *Varroa* spp. and *G. mellonella* while camphor basil (*O. kilimandscharicum*) controls effectively the

beetles of apiaries. Since the EOs of *O. kili-mandscharicum* contains, among others, camphor, and cineole, it may show activity against other pests, including *Varroa* spp. Using thymol, menthol, camphor, cineole, and essential oils containing these substances is of significant interest for the control of pests in apiaries. However, there are other natural substances that are promising tools in fighting the pests and their toxicity should be extensively studied, including methods of application and mode of action. For example, eucalyptol was reported as successful against *Varroa* spp., *A. woodi*, and *A. tumida*, which makes it a very interesting object of extended research. Next, plants belonging to the *Citrus* genus may be sources of useful EOs, as suggested by research on varroa mites.

Synergism of botanicals' blends as well as with synthetic miticides enhance efficacy while repellents and attractants may help develop behavioral tools like traps. This area seems to be an additional promising field of research. Using various substances in blends or in a planned order may significantly increase protection. Data concerning the selectivity of substances, interactions between them, and their modes of action is not full and the activity of natural substances needs further intensive research in that field. Therefore, there is a need for biochemical, cytological, and physiological basic studies that may be crucial for understanding the mechanisms of toxicity of natural substances, the ability of pests to gain resistance, and the reasons for selective toxicity against different pests, or between pests and bees. The comparison of toxicity on pests and bees is important because substances toxic to mites, moths, and beetles may be toxic to honey bees, too given that they belong to the same phylum. The next important field of studies, as shown in the present review, is the method of application. In many cases, fumigation seems to be an effective strategy. Next, long-term treatments with low concentrations of active substances may be of interest, as they may lead to increased efficacy values. The application mode is in fact crucial because it attracts attention of beekeepers. In all cases,

the high quality of products, with lower toxicity to consumers, should be a priority ensuring the health state of the human population and the environment.

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AUTHOR CONTRIBUTION

NN, MS, and ZA equally participated in planning, writing, and editing the manuscript, reviewed the literature, and provided discussion and conclusions to the manuscript.

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