Turkish Journal of Agriculture - Food Science and Technology, 6(1): 34-45, 2018

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X
www.agrifoodscience.com,
Turkish Science and Technology

Plant Essential Oils Used Against Some Bee Diseases

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ARTICLE INFO

Review Article

Received 11 September 2017
Accepted 30 November 2017

Keywords:
American foulbrood
Chalkbrood
Control
Essential oils
Varroa

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ABSTRACT

The most common honey bee diseases are American foulbrood (AFB) caused by the bacterium *Paenibacillus larvae*, Chalkbrood caused by fungus *Ascosphaera apis* and diseases caused by parasitic mites such as *Acarapis woodi*, *Varroa destructor*. These diseases and pests not only cause economic loss but also cause ecological problems related to the role of honey bees, as the most important pollinators on Earth. Synthetic acaricides and antibiotics are used to keep the diseases and mites in control. Use of the drugs lead to the development of drug-resistant organisms, detrimental effect on non-target organisms and the residue problem in bee products. For this reasons, the need for alternative control methods has become compulsory in recent years. It has been known that some plant oils used widely in perfumery and food industry for flavor and smell have been used as repellent to certain insects, bactericide and fungicide. Therefore, intensive studies have been carried out on plants with anti-mites, antibacterial and antifungal potentials and these studies are still going on. Recently, studies in this area have shown that essential oils of plants such as thyme, cloves, mint, lemon grass, cinnamon, grapefruit, rosemary, marigold, are lethal to some mites, bacteria and fungi. In addition, it has been reported that some components, isolated from these plants such as sanguinarine, thymoquinone, capsaicin, carvacrol, citral, eugenol, thymol, show these effects on the organisms. As a result, in countries rich in biodiversity due to endemic plant species, the essential oils used in control of these diseases should be favored instead of or in combination with conventional drugs in integrated the disease management programs because of the lack of harmful effects of essential oils on non-target organisms and environment.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 6(1): 34-45, 2018

Bazı Arı Hastalıklarına Karşı Kullanılan Bitkisel Esansiyel Yağları

MAKALE BİLGİSİ

Derleme Makale

Geliş 11 Eylül 2017
Kabul 30 Kasım 2017

Anahtar Kelimeler:
Amerikan yavru çürüklüğü
Esansiyel yağlar
Kireç hastalığı
Kontrol
Varroa

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ÖZET

En yaygın bal arısı hastalıkları, *Paenibacillus larvae* tarafından oluşturulan Amerikan yavru çürüklüğü, *Ascosphaera apis* tarafından oluşturulan kireç hastalığı ve *Acarapis woodi*, *Varroa destructor* gibi parazit akarların neden olduğu hastalıklardır. Bu hastalık ve zararlılar arı yetiştiriciliğinde sadece ekonomik kayıplara neden olmakla kalmaz, aynı zamanda Dünyanın en önemli tozlaştırıcıları olan bal arılarının rolü ile ilgili ekolojik problemlere de yol açar. Arıcılıkta, arı hastalıkları ve zararlılarının mücadelesinde sentetik akarisitler ve antibiyotikler kullanılmaktadır. Ayrıca, ilaçların bilinçsizce kullanımı, dirençli organizmaların gelişmesine, hedef olmayan canlılara karşı zararlı etkilere ve arıcılıktan elde edilen ürünlerde kalıntı problemine yol açmaktadır. Bu nedenlerden dolayı, alternatif kontrol metotlarına ihtiyaç duyulmaktadır. Parfümeri ve gıda endüstrisinde lezzet ve koku için yaygın olarak kullanılan bazı bitki yağlarının repellent, bakterisit ve fungisit olarak kullanıldığı bilinmektedir. Bu nedenle, akarisit, bakterisit ve fungusit etki potansiyeline sahip olan bitkiler üzerinde yoğun araştırmalar yapılmış ve hala yapılmaya devam etmektedir. Son zamanlarda bu alandaki çalışmalar, kekik, karanfil, nane, limon otu, tarçın, greyfurt, biberiye, kadife çiçeği gibi bitkilerin esansiyel yağlarının bazı akarlara, bakterilere ve mantarlara karşı öldürücü etkilerinin olduğunu göstermiştir. Ayrıca, bu bitkilerden izole edilen sanguinarin, timokinon, kapsaisin, karvakrol, sitral, eugenol, timol gibi bazı bileşenlerin organizmalar üzerindeki bu etkileri oluşturduğu bildirilmiştir. Sonuç olarak, endemik bitki türleri yönünden biyolojik çeşitliliği zengin olan ülkelerde, esansiyel yağların hedef olmayan organizmalara ve çevreye zararlı etkilerinin bulunmaması nedeniyle, hastalıklara karşı etkinliği belirlenmiş esansiyel yağların entegre zararlı yönetimi programlarında geleneksel ilaçların yerine veya birlikte kullanılması tercih edilmelidir.

DOI: https://doi.org/10.24925/turjaf.v6i1.34-45.1502

Introduction

Honeybees comprise mainly the species *Apis mellifera* L. (Hymenoptera: Apidae) found worldwide now. *A. mellifera* has been used by beekeepers to produce honey, wax and other apiary-related products, and has an important role in the pollination of native flora. Nowadays, eleven species are recognized with the genus, the other ones are less abundant ((Umpiérrez et al., 2011). Honey bees are an established component of country's agricultural system. Not only pollination by honeybees contributes significantly to global food production, but honeybees also produce other products that people use them as food, medicine, and commodity. Honey bees can be affected a wide range of viral, fungal and bacterial infection such as chalkbrood, AFB, which reduce performance, productivity and welfare of them (Alippi et al., 1996; Boudegga et al., 2010). They also host natural pests, such as *Varroa* mites (Weinberg and Madel, 1985).

The most common diseases of honey bees are AFB, chalkbrood and varroosis. In the treatment of these diseases, many synthetic drugs are used and the use of these drugs is limited due to reasons such as the development of resistance, contamination of apiary-products. The *Varroa* mites are an external parasite of honey bees and causes severe loss of honey bee colonies worldwide (Damiani et al., 2009). Application of synthetic acaricides is the most effective method against these pests. However, the intensive use of many chemical substances against mites has led to the development of resistance and the reduction of its effects (Delaplane and Hood, 1997; Milani, 1999). Furthermore, contamination of the hive products, especially honey and beeswax, lead to the idea of finding new and safer ways to control honey bee mites (Wallner, 1999). AFB disease is caused by the bacterium *P. larvae* (Alippi et al., 1996). Antibiotics have been used to keep the disease in control. But, the antibiotics can't eliminate the disease exactly (Reybroeck et al., 2012). Also, the use of antibiotics in the treatment of bee diseases has been banned in some countries (Genersch, 2010). Chalkbrood is a highly contagious disease of honey bees caused by *A. apis* that is a heterothallic spore-cyst fungus (Boudegga et al., 2010; Spiltoir, 1955; Spiltoir and Olive, 1955). Use of drugs to control the disease have been limited due to their effects on the vitality of the brood and longevity of the bees, and the development of resistance (Boudegga et al., 2010).

Demand for high-yield by the beekeepers and increased industrialization has led to increased stress factors which make the bees sick in the hives. Therefore, it reveals the necessity for development of new control methods (El Shafai, 2012). Synthetic drugs (pesticides, antibiotics) have significant disadvantages, including adverse consequences resulting from accidental contamination of honey, wax, and pollen (Boudegga et al., 2010; Isman, 2000). Despite the public awareness of the health and environmental effects of the long-term use of synthetic pesticides in Europe and North America, natural pesticides of both biological and plant origin have not been effective on the market. Biologic insecticides such as *Bacillus thuringiensis* and botanical insecticides such as pyrethrum, each command little more than 1% of the global insecticide market (Isman, 2000). These natural and low-risk drugs offer a highly desirable alternative to these synthetic products. There is an increase in the use of natural products due to the low toxic effect on the environment and mammals and the lack of residual problems in bee products (Bogdanov, 2006). Alternative methods to control these disease are the use of essential oils (Damiani et al., 2009; Fuselli et al., 2007; Kloucek et al., 2012).

Essential oils have been used in medicine, perfumery, cosmetic, and added to foods as part of spices or herbs. They are important for plant defense as possessing antimicrobial, antiparasitic, antiviral, antifungal properties (Hyldgaard et al., 2012). Some plant essential oils or components have a broad spectrum of activity against pathogenic bacteria and fungi (Kotan et al., 2008); tick infestation (Coskun et al., 2008); human lice and house dust mite (Williamson et al., 2007); insect vector (Chaiyasit et al., 2006); honey bee mites (Calderone et al., 1997; Ellis and Baxendale, 1997). Also, essential oils obtained by steam distillation of plant leaves and leaves of some aromatic plants (especially in Myrtaceae and Lamiaceae families, but also other plant families) are used to protect traditionally stored grain and legumes, and to repel flying insects in the house (Isman, 2000). The aim of this review is to state the studies carried out on the use of essential oils to control the most common diseases of the honey bees.

Essential Oils

Essential oils are the generic name for highly volatile plant compounds with a strong and characteristic odor. Essential oils are the concentration of hydrophobic liquid containing multiple volatile aroma compounds found in glands located in various parts of the aromatic plants: leaves, flowers, fruit, seeds, barks and roots (Bayala et al., 2014) and can be found in all parts of a plant (e.g. *Pinaceae*) or in certain parts of plant (e.g. rose flowers). They are found in almost all plant species, but only essential oils can be considered for plants that contain more than 0.1% (Imdorf et al., 1999). Essential oils have various functions in plants. Essential oils can act as an attractant for insects carrying pollen or repellent to protect plants against phytophagous insects (Ngoh et al., 1998). Many exhibit fungicidal and bactericidal activity to protect plants from microorganisms (Kotan et al., 2008; Mayaud et al., 2008).

The essential oil components of each plant species are different. However, the same plant species often produces essential oils with variable composition because of environmental and/or genetic factors; many species have varieties the so-called chemotypes (Flamini, 2003). For example, thymus (*Thymus vulgaris*) has at least seven chemotypes (Borugă et al., 2014). One group consists of the strong chemotypes containing higher concentrations of phenols such as thymol and carvacrol. However, the second group consists of the mild chemotypes containing high amounts of alcohols such as geraniol, linalool, and thujanol. The chemical composition of an essential oil depends on cultivation, climatic conditions and specific extraction process such as vapor distillation, cold pressing, chemical extractions (Imdorf et al., 1999).

The constituents of plant essential oils belong to one of the two groups of natural chemical classes called terpenoids and phenylpropanoids (Sangwan et al., 2001). Terpenoids represent the major components having about 90% of the total constituents of plant essential oils and belonging to different chemical classes (monoterpenoids, sesquiterpenoids, phenylpropanoids etc.) (Imdorf et al., 1999; Sangwan et al., 2001). Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids are thymol, carvacrol, linalool, linalyl acetate, citronellal, piperitone, menthol, and geraniol. The phenylpropenes constitute a relatively small part of essential oils, and those that have been most thoroughly studied are eugenol, isoeugenol, vanillin, safrole, and cinnamaldehyde (Hyldgaard et al., 2012). Monoterpenes which are the main terpenoid are volatile. Monoterpenes are found together with functional groups such as alcohols, phenols. But, in pure form, these compounds and many essential oils irritate eyes and mucous membranes and must be used with caution (Imdorf et al., 1999).

Essential Oils against Honey Bee Mites

Parasitic mites are one of the most important threats to the honey bee health. The mites causing colony problems in honey bees include species of Tropilaelaps (Laelapidae), the tracheal mite *A. woodi* (Tarsonemidae), and *Varroa destructor* Anderson and Trueman (Varroidae). Other mites encountered in honeybees are generally harmless or do not pose a great risk to honeybees (Umpiérrez et al., 2011). Among the diseases of honeybees, the parasitic mite *V. destructor* (Anderson and Trueman) is probably the most serious worldwide threat due to devastating effects to the beekeeping. *Varroa* mites damage bee colonies during larval and pupae form by feeding on haemolymph leading to decreased body weight, and shortened life span in honey bees (Rosenkranz et al., 2010; Akyol and Yeninar, 2008). Heavy infestations by *V. destructor* could also make the colony more prone to bacterial, fungal and viral diseases. In addition, bee populations dramatically decline and the colonies can die within three to six weeks if not treated (Damiani et al., 2009; Akyol and Yeninar, 2009). Therefore, the control of *V. destructor* has been an important part of maintaining the colony health. Synthetic acaricides including pyrethroids (flumethrine), formamidines (Amitraz), and organophosphates (Coumophos) have been the major effective method used for years in the control of *V. destructor* (Kanga et al., 2003).

Essential oils have antimicrobial, antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties (Turek and Stintzing, 2013). Essential oils were also reported to have repellent, deterrent and toxic effects on arthropods if applied via fumigation, topical use and ingestion (Umpiérrez et al., 2011). The mechanism of toxicity of essential oils on arthropods could be associated with cuticle disruption, molting and respiration inhibition, and reduction in growth and fecundity (Isman, 2000). They are generally considered to be safe for humans and also for bee populations. Therefore, use of these plant derived products is an emerging alternative method to control honey bee mites (Ebert et al., 2007; Turek and Stintzing, 2013). The main and pure components having biological activity against *Varroa* destructor and LC_{50} values of the essential oils derived from plant materials are presented in Table 1 and 2. A number of different essential oils were tested against honeybee mites. Essential oils have an important potential for controlling the parasite mites caused *A. woodi* (Calderone et al., 1997). Calderone et al. (1991) reported that clove oil, citronellal, and mixed terpenes had considerable acaricidal properties against *A. woodi*. In a study on the effects of monoterpenoids to tracheal mites, *A. woodi*, 7 monoterpenoids including citral, thymol, carvacrol, a-terpineol, pulegone, d-limonene, and menthol were applied as fumigants to mite-infested honey bees. Thymol and menthol were the most toxic compounds to honey bees, and a-terpineol was the least toxic. Menthol, citral, thymol, and carvacrol were more toxic to tracheal mites than to honey bees. Menthol was found to be about 19 times more toxic to mites than to bees. Menthol can be used in the control of these mites and is commercially available (Ellis and Baxendale, 1997). Calderone and Spivak, (1995) studied thymol, eucalyptus oil, and menthol and camphor mixture. The mean mite death rate was 96.7% in thymol-treated colonies and 27.5% in linanool treated colonies. Menthol is the only registered substance used against *A. woodi* in the United States. Eucalyptus oil contains citronellal which is shown to cause significant death in the tracheal mites (Calderone et al., 1991). A mixture of these products can provide control for both *varroa* and tracheal mite.

Thymol is the most frequently used essential oil component in beekeeping (Imdorf et al., 1999) due to the observation of high acaricidal effects which can be very well tolerated by the bees (Damiani et al., 2009). Thymol in different administration forms is reported to have 60-90% effectiveness against *varroa* populations. In addition, it is classified as non-toxic veterinary drug according to EU regulations without requiring maximal residue level in honey products (Imdorf et al., 1999). Damiani et al. (2009) studied the acaricide activity of essential oils of *Thymus vulgaris, Laurus nobilis, Lavandula officinalis* and *Lavandula hybrida* containing linalool, 1,8-Cineole and thymol as the main compounds in their composition against *V. destructor*. All essential oils caused mite mortality without severe harmful effects on adult bees. Colin, (1990) reported that essential oils of thyme, thymus vulgaris and sage, salvia officinalis effectively control *V. jacobsoni*. Gashout and Guzman-Novoa, (2009) evaluated some essential oils for their toxicity to the parasitic mite *V. destructor*. Essential oils of menthol, clove oil, origanum oil, and thymol were the most toxic products, causing 87, 96, 100, and 100% mite mortality, respectively. Islam et al. (2016) studied the effects of essential oils of Thyme (*Thymus linearis*), Lemon grass (*Cymbopogon citratus*), Rosemary (*Rosmarinus officinalis*), Mint (*Mentha longifolia*) and Formic Acid (65%) at 25, 50 and 100% concentrations on *V. destructor*. The essential oils tested at the highest concentrations were reported to control *Varroa* effectively. The mite mortality was the highest in formic acid treatment followed by lemon grass, thyme, mint and rosemary, respectively. Similarly, Abd El-Wahab et al. (2015) tested formic acid and essential oils of lemon

grass, cinnamon, thyme and anise at 50 and 100% concentrations. Essential oils at 100% concentration resulted in effective control in *Varrao* mites, and high honey yield compared to control. In another study, ten products, including cineole, clove oil, formic acid, margarine oil, menthol, thyme oil, oxalic acid, sage oil, thymol and wintergreen were studied to evaluate the toxicity on adult bee. Each product was tested in several concentrations in sugar syrup and daily dead bees were counted. Oxalic acid is the most toxic substance of the tested products. Menthol and sineole did not differ from controls fed with flat syrup after 8 days of treatment. In 14 days of treatment, wintergreen was the least toxic. Findings showed that the tested products can be used safely for oral treatment of bees if the dose in the hive is carefully managed (Ebert et al., 2007)

Lindberg et al. (2000) compared the effects of formic acid and Tau-fluvalinate used positive control in *Varrao* therapy with thymol, clove oil, Magic3, and methyl salicylate. According to the results of the study, it was found that the most selective treatment was Tau-fluvalinate, whereas thymol, carnation oil, magic3 and methyl salicylate showed equal or greater selectivity to formic acid. Thymol and Magic3 showed that the LD_{50} value is low in complete exposure applications, and that the mite mortality can be changed by steam or topical exposure. There was little or no effect of topical exposure to carnation oil. These results show that essential oil components alone may be useful in the control of *Varroa*, and should be a part of parasitic mite control approaches in honey bee colonies (Lindberg et al., 2000).

A number of factors could contribute to the overall effectiveness of any acaricide. The concentrations of the compounds, the duration of treatment, the mode of administration, the colony environment, the bee environment and the ambient temperature may affect the efficacy of a treatment (Calderone and Spivak, 1995). Temperature plays an important role in determining the effectiveness of the treatments, and significantly influences the activity of menthol as a control agent against *A. woodi* (Cox et al., 1989). Since the compounds exhibit a significant difference in the rates of volatilization in different temperature ranges, the effect of essential oils depends on the changing ambient temperature with respect to seasons (as in summer or autumn). For this reason, the development of a delivery system that is less sensitive to the temperature fluctuations is a priority (Calderone and Spivak, 1995).

In conclusion, the potential use of these selective and fully biodegradable compounds in the management of bee mites is encouraging. Essential oils could be an effective and a valuable tool to control the honey bee mites including *Varroa* spp. and *A. woodi* and should be integrated into the pest management approach in honey bee colonies. Studies show that such compounds may have important effects as commercial products when appropriate delivery systems are developed.

Essential Oils against American Foulbrood

AFB affects older larvae and young pupae, which are digested by enzymes secreted by the bacterium. The comb has a speckled appearance where infected larvae have been removed (Reybroeck et al., 2012). Oxytetracycline (OTC) hydrochloride or sulfathiazole have been used to control this disease. However, OTC- and sulfathiazole-resistance in *P. larvae* have become widespread (Genersch, 2010).

Table 1 Plant and Main Components of the Essential Oils. Essential Oils Having Biological Activity against *Varroa destructor*

Plant essential oils Botanical name	Components/Composition*	Reference
Thymus vulgaris	thymol (65.3%), carvacrol (5.4%)	
Laurus nobilis	1,8-cineole (29.5), linalool (22.1%), α-terpinil acetate (8.1%)	
Lavandula officinalis	linalool (53.47%), 1,8-cineole (6.82%), Terpinen-4-ol (7.64%), Camphor (8.43%)	(Damiani et al., 2009)
Lavandula hybrida	linalool (30.5%), Borneol (11.0%)	
Tagetes minuta	β-ocimene (62.8%), (Z)-ocimenone (10.2%), (E)-ocimenone (6.6%), limonene (5.8%), dihydrotagetone (4.2%).	(Eguaras et al., 2005)
Acantholippia seriphioides (A. Gray) Mold.	Thymol (29.2%), ortho-Cimene (23.3%), carvacrol (23.3%), γ-Terpinene (11.0%)	
Schinus molle L.	camphene (7.9%), myrcene (5.3%), β-phellandrene (28.3%), α-phellandrene (11.5%), caryophillene oxide (7.0%)	(Sergio Ruffinengo et al., 2005)
Wedelia glauca (Ortega) O. Hoffm. ex Hicken	α-pinene (23.2%), limonene (38.0%), β-pinene (23.4%)	
*Heterotheca latifolia***	borneol (31.5%), camphor (27.2%), limonene (7.2%), camphene (6.4%)	(S. R. Ruffinengo et al., 2002)
Zataria multiflora	-	(Ariana et al., 2002)
Saturea hortensis	-	
Mentha spicata	at least 50% carvone, l-limonene, pinene	
Heterotheca latifolia	-	(S Ruffinengo et al., 2001)
Tagetes minuta	-	
Syzygium aromaticum (L.) Merr. et Perry	eugenol (86.7%)	(Maggi et al., 2010)

*Composition in % vv-1, **Varroa Jacobsoni

Table 2 Plant essential oils and their pure components having biological activity against *Varroa* destructor and values of LC_{50} of the essential oils and the components.

Essential oil/component	LC_{50} (Lethal Concentration 50)	Reference
Citral*	10.1 µg/mL	(Ellis and Baxendale, 1997)
Thymol*	1.7 µg/mL	
Tau-ßuvalinate	-	(Lindberg et al., 2000)
Magic3	-	
Methyl salicylate	-	
Acantholippia seriphioides (A. Gray) Mold.	1.27 µL/cage 24 h	(Sergio Ruffinengo et al., 2005)
Schinus molle L.	2.65 µL/cage 24 h	
Wedelia glauca (Ortega) O. Hoffm. ex Hicken	0.59 µL/cage 72 h	
Acantholippia seriphioides	1.09 µL/cage 72 h	
Menthol	173.2 µg/vial,	(Gashout and Guzmán-Novoa, 2009)
Origanum vulgare	56.1 µg/vial,	
Thymol	56.1 µg/vial,	
Syzygium aromaticum	90.9 µg/vial,	
Syzygium aromaticum (L.) Merr. et Perry	0.59 µL/dish 24 h 0.36 µL/dish 48 h	(Maggi et al., 2010)

*Acarapis woodi

Essential oils from plant species showed the biological activity as growth inhibitors against all the *P. larvae* strains in many studies (Fuselli et al., 2008b; Gende et al., 2008; González and Marioli, 2010). Antimicrobial activities of essential oils were usually tested by minimal inhibitory concentration (MIC) using several *in vitro* techniques such as broth microdilution, tube dilution (Fuselli et al., 2009, 2008b; Gende et al., 2010; Maggi et al., 2011; Santos et al., 2014). The essential oil component of each plant species is different due to environmental and genetic factor and extraction method (Flamini, 2003; Imdorf et al., 1999). Therefore, their antibacterial activity is also different.

There are many *in vitro* research on the antimicrobial activity of essential oils obtained from many plants including *Eucalyptus cinerea* (González and Marioli, 2010), *Minthostachys verticillata* (González and Marioli, 2010), *Origanum sp.* (González and Marioli, 2010; Ozkirim et al., 2012; Roussenova, 2011), *Tagetes minuta* (Eguaras et al., 2005; Fuselli et al., 2005; González and Marioli, 2010), *Thymus vulgaris* (González and Marioli, 2010; Roussenova, 2011), *Cinnamomum sp.* (Ansari et al., 2016; Calderone et al., 1994; Gende et al., 2009; Gende et al., 2008; Roussenova, 2011), *Cymbopogon citratus* (Roussenova, 2011), *Ocimum basilicum* (Mărghitaş et al., 2011), *Citrus paradise* (Fuselli et al., 2006), *Acantholippia seriphioides* (Fuselli et al., 2006, 2005), *Lippia turbinate* (Fuselli et al., 2006), *Mintosthachys mollis* (Fuselli et al., 2007, 2006), *Syzygium aromaticum* (Calderone et al., 1994; Roussenova, 2011), *Salvia sclarea* (Roussenova, 2011), *Thymus capitatus* (Calderone et al., 1994), *Carapa guaianensis* (Santos et al., 2012), *Copaifera officinalis* (Santos et al., 2012), *Pimpinella anisum L.* (Gende et al., 2009), *Foeniculum vulgare* (Gende et al., 2009), *Eucalyptus globulus* (Gende et al., 2010), *Rosmarinus officinalis* (Maggi et al., 2011; Ozkirim et al., 2012), *Artemisia sp.* (Fuselli et al., 2008b), *Lepechinia floribunda* (Fuselli et al., 2008b), *Melaleuca viridiflora* (Fuselli et al., 2010), *Cymbopogon nardus* (Fuselli et al., 2010), *Carum carvi* (Kuzyšinová et al., 2014), *Azadirachta indica* (Melathopoulos et al., 2000), *Citrus sp.* (Fuselli et al., 2009), *Melaleuca alternifolia* (Santos et al., 2014), *Pimenta sp.* (Ansari et al., 2016; Calderone et al., 1994), *Litsea cubeba* (Ansari et al., 2016), *Trachyspermum ammi* L. (Ansari et al., 2016), *Mentha sp.* (Ansari et al., 2016), *Illicium verum* (Ansari et al., 2016), *Myristica fragrans* (Ansari et al., 2016) and *Urtica dioica* (Mărghitaş et al., 2011) against different strains of *P. larvae.* MIC values of Plant Essential Oils and some their pure compounds against *P. larvae* showed in Table 3.

Antibacterial effect is determined by the major component in the essential oil composition (Ozkirim et al., 2012). Essential oils contain compounds playing an important role in the treatment or prevention of AFB disease (Ansari et al., 2016; González and Marioli, 2010; Maggi et al., 2011). The main components of plant essential oils showing the antibacterial effects against *P. larvae* in *in vitro* experiments are cinnamaldehyde (Gende et al., 2008), eugenol (Ansari et al., 2016; Gende et al., 2008), limonene (Eguaras et al., 2005; Fuselli et al., 2009; Fuselli et al., 2008a; Fuselli et al., 2010), β-myrcene (Fuselli et al., 2009, 2008a; Maggi et al., 2011), thymol (Ansari et al., 2016; Calderone et al., 1994; Fuselli et al., 2006), p-cymene (Fuselli et al., 2007), carvacrol (Fuselli et al., 2007), γ-terpinene (Fuselli et al., 2007), camphor (Ansari et al., 2016; Calderone et al., 1994; Fuselli et al., 2008b), citronellal (Calderone et al., 1994; Fuselli et al., 2010), β-ocimene (Eguaras et al., 2005), (Z)-ocimenone (Eguaras et al., 2005), (E)-ocimenone (Eguaras et al., 2005), dihydrotagetone (Eguaras et al., 2005), (E)-anethole (Gende et al., 2009), 1,8-cineole (Fuselli et al., 2008b; Gende et al., 2010; Ozkirim et al., 2012), α-pinene (Fuselli et al., 2010; Gende et al., 2010), α-Thujone (Fuselli et al., 2008b), artemisia ketone (Fuselli et al., 2008b), camphene (Fuselli et al., 2008b), terpinen-4-ol (Fuselli et al., 2010), geraniol (Fuselli et al., 2010), azadirachtin (Melathopoulos et al., 2000), citral (Ansari et al., 2016), menthol (Ansari et al., 2016), menthone (Ansari et al., 2016), carvone (Ansari et al., 2016), β-

Pinene (Ansari et al., 2016) and trans-Anethole (Ansari et al., 2016) (showed in Table 4). In several *in vitro* studies, some of the pure compounds obtained from these plants have been tested for antibacterial activity against *P. larvae.* Authors have reported that pure compounds of plant origin including sanguinarine, thymoquinone, capsaicin, trans-2-hexenal, nordihydroguaiaretic acid, ouininin hydrochloride, naringenin, resveratrol, eugenol, thymol (Flesar et al., 2010) and carvacrol (Ozkirim et al., 2012) have strong antibacterial effect against *P. larvae.* The antibacterial effects of these compounds were examined *in vitro*. But, few studies have been conducted *in vivo* against *P. larvae*. Authors have reported that from the beginning of the treatments to the 24th and 31st day, cinnamon oil (*Cinnamomum zeylanicum*) reduced infected larvae percentages of 7.89% and 52.42%, respectively, in a field experiment (Gende et al., 2009). However, Albo et al. (2003) have reported that essential oils were not effective in the elimination of AFB clinical symptoms at any dose formulation or method ad administration. Results of in vitro experiments do not always correlate with results of field experiments as seen in the previous study.

Although physicochemical properties are similar in both the essential oils, the percentage of components shows certain differences according to their drying treatment. Authors have reported that oil toxicity against *P. larvae* differed depending on the drying treatment of the plant material (in air and oven conditions) before the distillation of essential oil (M. Maggi et al., 2011). Antibacterial activity can be increased by passing essential oils through different processes. Nanotechnology is a tool to improve the effectiveness of some materials on pathogen such as bacteria (Singh et al., 2008). Authors have reported that tea tree oil nanoparticles formed using nanotechnology show a higher *in vitro* antibacterial activity than tea tree oil against P. larvae strains. Showing that nanotechnology may increase the antibacterial activity of essential oils in the treatment or prevention of AFB (Santos et al., 2014). In another study, emulsifiers were used to enhance the antibacterial activity of essential oils. Results have shown that Propylene glycol, an emulsifier, has an additional inhibitory effect on *P. larvae* (Fuselli et al., 2005).

In vivo studies of every essential oil in which the antibacterial activity is determined in the laboratory should be performed. In addition, the activity of these essential oils should be increased using a variety of methods such as nanotechnology, emulsifier.

Essential oils against Chalkbrood disease

Chalkbrood is a highly contagious disease of honey bees caused by *A. apis* that is a heterothallic spore-cyst fungus (Boudegga et al., 2010; Spiltoir, 1955; Spiltoir and Olive, 1955). The larvaes of honey bee especially in the fifth instar are most susceptible to the disease. Adult honey bees are not sensitive to this fungal pathogen, but they cause transmission of the disease within the honeybee colony. Infections occur after incoming through the digestive tract of spores which can infect brood of the honey bee. The spores are very resistant to environmental conditions and can remain viable for 15 years. Young brood is contaminated by ingestion of spores carried in food or transmitted by the nurse worker bees and dissemination of this fungal pathogen between colonies occurs by contaminated hive material, in particular the transfer of the brood frames from infected colony to non-infected colony by the beekeepers (Aronstein and Holloway, 2013; Chantawannakul et al., 2003; Fries and Camazine, 2001). The infection is more common in areas with high humidity and in early spring due to rapid expansion of the colonies and the large number of young brood. The disease usually emerges when brood is under stress due to chemical, biological and physical factors and can cause about 80% of larvae deaths (Aronstein and Murray, 2010; Vojvodic et al., 2011).

A wide variety of drugs have been tested to control this disease. However, none of the tested compounds has provided the level of control required to fight the disease. Moreover, use of drugs to control the disease have been limited due to their effects on the vitality of the brood and longevity of the bees, and the development of resistance. Therefore, there is an increase in the study on an effective drug in treatment of chalkbrood disease. Essential oils obtained from some plants may show antimicrobial activity for the fungal pathogen (Boudegga et al., 2010; Hornitzky, 2001; Kloucek et al., 2012; Nardoni et al., 2017).

There are many *in vitro* research on the fungistatic effect of essential oils obtained from many plants including *Coriandrum sativum*, *Tagetes minuta*, *Lavandula x intermedia*, *Laurus nobilis* (Damiani et al., 2014; Eguaras et al., 2005; Larrán et al., 2001), *Cinnamomum glandulifera*, *Ocimum basilicum*, *Rosmurinus offacinalis*, *Eucalyptus globulus* (Larrán et al., 2001), *Eugenia caryophyllum*, *Piper betel*, *Illicium verum*, *Cinnamomum cassia*, *Acorus calamus* (Chantawannakul et al., 2003; El-enain et al., 2009; Jatisatienr and Jatisatienr, 1999), *Thymus vulgaris*, *Satureja montana*, *Origanum vulgare* (Colin et al., 1989; Kloucek et al., 2012), *Eucalyptus citrodora*, *Leptospermum petersonii*, *Leptospermum scoparium* (Davis & Ward, 2003), *Punica granatum*, *Psidium guajava*, *Callistemon viminalis*, *Cinnamomum zeylanicum* (El Shafai, 2012), *Litsea cubeba*, *Croton bonplandianus*, *Mentha spicata*, *Matricaria chamomilla*, *Piper betle*, *Daucus carota*, *Cuminum cyminum*, *Syzygium aromaticum* (Ansari et al., 2016), *Juglans regia* L. (Garbaczewska et al., 2014; Wianowska et al., 2016), *Armoracia rusticana*, *Cymbopogon flexosus*, *Allium sativum*, *Origanum compactum*, *Thymus satureoides*, *Cinnamomum aromaticum* (Kloucek et al., 2012), *Origanum onites* (Korukluoglu et al., 2009) *Melissa officinalis*, *Cymbopogon spp.* (Gochnauer et al., 1979), *Litsea cubeba*, *Pelargonium graveolens* (Nardoni et al., 2017), Propolis (Cinnamic acid, pinocembrin) (Voigt and Rademacher, 2015). Minimal Inhibitory Concentration (MIC) used to determine the *in vitro* activity of this plant essential oils showed in Table 5.

Table 3 MIC values of Plant Essential Oils and some their pure compounds against *Paenibacillus larvae.*

Plant essential oil/ Compound,	Minimal Inhibitory Concentration (MIC)	Reference
Cinnamomum zeylanicum	25-100 µg/ml	(Gende et al., 2008; 2009)
Citrus paradisi	385.0 µg/mL	(Fuselli et al., 2008a)
Acantholippia seriphioides	200–250 µg/mL	(Fuselli et al., 2006)
Lippia turbinata Griseb.	800–1000 µg/mL	
A. seriphioides, (propylene glycol used as emulsifier)	200 µg/mL	(Fuselli et al., 2005)
Tagetes minuta	700-800 µl/L	(Eguaras et al., 2005)
Carapa guaianensis	1.56–25%	(Santos et al., 2012)
Copaifera officinalis	1.56–12.5%	
Pimpinella anisum L.	300 µg/mL	(Gende et al., 2009)
Foeniculum vulgare Miller	250 µg/mL	
Eucalyptus globulus (from different area)	600-700 µg/mL, 900-1,200 µg/ml	(Gende et al., 2010)
Origanum onites	78 µg/mL	(Ozkirim et al., 2012)
Rosmarinus officinalis	625 µg/mL	
Artemisia absinthium	416.7 µg/mL	
Artemisia annua	401.9 µg/mL	(Fuselli et al., 2008b)
Lepechinia floribunda	393.6 µg/mL	
Melaleuca viridiflora	331.4 µg/mL	(Fuselli et al., 2010)
Cymbopogon nardus	318.6 µg/mL	
Azadirachta indica	3000 µg/mL	
Azadirachta indica-(Azadirachtin-rich extract)	300 µg/mL	(Melathopoulos et al., 2000)
Citrus paradisi	336.31 µg/mL	(Fuselli et al., 2009)
Citrus sinensis	800.0 µg/mL	
Melaleuca alternifolia	0.18–6.25%	(Santos et al., 2014)
Melaleuca alternifolia (nanoparticle)	0.01–0.93%.	
Pimenta dioica (L.)	78.0 µg/mL	
Litsea cubeba Pers.	85.0 µg/mL	
Trachyspermum ammi L.	137.0 µg/mL	
Mentha arvensis L.	144.7 µg/mL	
Mentha spicata L.	145.6 µg/mL	
Illicium verum Hook.f.	278.6 µg/mL	(Ansari et al., 2016)
Myristica fragrans Gronov.	285.8 µg/mL	
Cinnamomum camphora (L.)	286.2 µg/mL	
Ocimum tenuiflorum L.	412.8 µg/mL	
Daucus carota L	482.0 µg/mL	
Zingiber officinale Rosc	488.0 µg/mL	
Pelargonium graveolens L.	495.4 µg/mL	
Sanguinarine	4 µg/mL	
Thymoquinone	8–16 µg/mL	
Capsaicin	32 µg/mL	
Nordihydroguaiaretic acid	32 µg/mL	
Ouininin hydrochloride	64 µg/mL	
Naringenin	64 µg/mL	(Flesar et al., 2010)
Resveratrol	64 µg/mL	
Eugenol	64-128 µg/mL	
Tymol	64-128 µg/mL	
Tylosin tartrate	0.016–0.031 µg/mL	
Oxytetracycline	0.016–0.031 µg/mL	
Carvacrol	156 µg/mL	(Ozkirim et al., 2012)

Table 4 The Major Component of Plant Essential Oils Exhibiting Antimicrobial Activities against *Paenibacillus larvae*.

Plant essential oils Botanical name	Components/Composition*	Reference
C. zeylanicum	cinnamaldehyde (79.3%), eugenol (11.9%),	(Gende et al., 2008)
C. paradise	limonene (69.9%), myrcene (9.6%),	(Fuselli et al., 2008a)
A. seriphioides	thymol (29.2%), p-cymene (23.3%), carvacrol (23.3%) γ-terpinene (11.0%),	(Fuselli et al., 2006)
T. minuta	β-ocimene (62.8%), (Z)-ocimenone (10.2%), (E)-ocimenone (6.6%), limonene (5.8%), dihydrotagetone (4.2%),	(Eguaras et al., 2005)
P. anisum L.	(E)-anethole (96.3%),	(Gende et al., 2009)
F. vulgare Miller	(E)-anethole, (92.7%),	
E. globulus	Eucalyptol (63%-79%), α-pnene (13%-6%),	(Gende et al., 2010)
A. absinthium	α-thujone (62.3%)	
A. annua.	artemisia ketone (36.3%), 1,8-cineole (31.5%),	(Fuselli et al., 2008b)
L. floribunda	1,8-cineole (27.5%), camphene (16.6%), camphor (12.9%),	
R. officinalis	1,8-cineole (46.9%),	(Ozkirim et al., 2012)
M. viridiflora,	terpinen-4-ol (29.09%), α-pinene (21.63%), limonene(17.4%)	(Fuselli et al., 2010)
C. nardus	limonene (24.74%), citronelal (24.61%), geraniol (15.79%)	
C. paradise	limonene (69.87%), b-myrcene (11.28%)	(Fuselli et al., 2009)
C. sinensis	limonene (74.42 %), b-myrcene (11.28%)	
P. dioica (L.)	eugenol (62.1%), methyl eugenol (22.9%)	
L. cubeba Pers.	citral (72%)	
T. ammi L.	thymol (43.7%)	
M. arvensis L.	menthol (45.7%), menthone (20.4%)	(Ansari et al., 2016)
M. spicata L.	carvone (65.10%), d-Limonene (16.11%)	
I. verum Hook.f.	trans-anethole (89.5%)	
M. fragrans Gronov.	β-pinene (11.69%), α-Pinene (10.06%), sabinene (41.7%)	
C. camphora (L.)	camphor (68%)	

*Composition in % vv-1,

Table 5 MIC values of Plant Essential Oils against *Ascosphaera apis.*

Plant essential oil/ Compound,	Minimal Inhibitory Concentration (MIC)	Reference
Coriandrum sativum	700 µg/ml	(Larrán et al., 2001)
Tagetes minuta	700-800 µ L/L	(Eguaras et al., 2005)
Ocimum basilicum	800 µ L/L	(Dellacasa et al., 2003)
Piper betel	3.5-7.5% (w/v), 300 µg/mL	(Ansari et al., 2016; Chantawannakul et al., 2003)
Illicium verum	10 % (w/v)	
Cinnamomum cassia	1.5–10% (w/v)	(Chantawannakul et al., 2003)
Eugenia caryophyllum	7.5 % (w/v)	
Punica granatum	0.625 % (w/v)	
Psidium guajava	1.25 % (w/v)	(El Shafai, 2012)
Callistemon viminalis	5% (w/v)	
Cinnamomum zeylanicum	2.5% (w/v)	
Litsea cubeba	0.025% 50 µg/mL	(Ansari et al., 2016; Nardoni et al., 2017)
Croton bonplandianus .	50 µg/mL	
Mentha spicata	100 µg/mL	
Matricaria chamomilla.	200 µg/mL	
Daucus carota	300 µg/mL	(Ansari et al., 2016)
Cuminum cyminum	400-500 µg/mL	
Syzygium aromaticum	400 µg/mL	
Foeniculum vulgare	700 µg/ml	
Foeniculum vulgare	700 µg/ml	
Pelargonium graveolens	0.025 %(w/v)	(Nardoni et al., 2017)
Origanum onites	8.5 µg/mL	(Korukluoglu et al., 2009)
Armoracia rusticana	16 µg/mL	
Cymbopogon flexosus	63 µg/mL	
Allium sativum	63-125 µg/mL	
Origanum compactum	125 µg/mL	
Thymus satureoides	125 µg/mL	(Kloucek et al., 2012)
Cinnamomum aromaticum	125 µg/mL	
Cymbopogon flexosus	63 µg/mL	
Thymus vulgaris	31-250 µg/mL	
Origanum vulgare	63 µg/mL	
Laurus nobilis	500 µg/ml	
Propolis (Cinnamic acid, pinocembrin)	0.5, 1.5 % (w/v)	(Voigt and Rademacher, 2015)

Antifungicide effect depends on the major component in the essential oil composition. Therefore, its important which of the major chemical components take significant role to effectiveness. Essential oils containing isothiocyanate, diallyl sülfide, carvacrol, citral, caryophyllene, *E*-cinnamaldehyde, eugenol, thymol have the strongest antifungicide effect (Ansari et al., 2016; Colin et al., 1989; Davis and Ward, 2003; Dellacasa, 2003; Kloucek et al., 2012). Beside them, there are potentially useful components such as 1,8-cineole, carvone, citronellol, menthol, a-bisabolol and tepinen-4-ol (Ansari et al., 2016; Kloucek et al., 2012).

Various natural products show promise for the control of *Ascosphaera apis*, the causative agent of Chalkbrood disease in honeybee (Boudegga et al., 2010; El Shafai, 2012). These data (information) need to be tested in field studies to evaluate the efficacy of the most active essential oil in hives. In addition, ways of application and residues in honey bee products should also be determined.

Conclusion

Essential oils could be an effective and valuable tool to control honey bee diseases including AFB, Chalkbrood and *Varroasis* due to their antibacterial, antiparasitic and antifungal effects. But, the great majority of the study on effects of essential oils is *in vitro*. Therefore, *In vivo* studies of essential oils which have the effects should be performed. In addition, the activity of these essential oils should be increased using a variety of methods such as nanotechnology, emulsifier. Taken together, in countries that have endemic plant species, the essential oils used in control of these diseases should be favored instead of or in combination with conventional drugs in integrated the disease management programs because of the lack of harmful effects of essential oils on non-target organisms and environment.

References

Akyol E, Yeninar H. 2008. Controlling of *Varroa desrtructor* (*Acari: Varroidae*) in honeybee: *Apis mellifera* (*Hymenoptera: Apidae*) colonies by using of Thymovar® and BeeVital. Ital. J. Anim. Sci., 7(2): 237-242. https://doi.org/10.4081/ijas.2008.237.

Akyol E, Yeninar H. 2009. Use of Oxalic Acid to Control *Varroa desrtructor* in honeybee (*Apis mellifera* L.) colonies. Turk J. Vet. Ani. Sci., 33(4): 285-288. DOI: 10.3906/vet-0712-16.

Abd El-Wahab TE, Ebada, IMA, Zidan EW. 2015. Control of *Varroa* mite by essential oils and formic acid with their effects on grooming behaviour of honey bee colonies. J. Basic Appl. Sci. Res., 2(8): 7674–7680.

Albo GN, Henning C, Ringuelet J, Reynaldi FJ, De Giusti MR, Alippi AM. 2003. Evaluation of some essential oils for the control and prevention of American foulbrood disease in honey bees. Apidologie, 34(5): 417–427. https://doi.org/10.1051/apido:2003040.

Alippi AM, Ringuelet JA, Cerimete EL, Re MS, Henning CP. 1996. Antimicrobial activity of some essential oils against *Paenibacillus larvae*, the causal agent of American Foulbrood Disease. J. Herbs. Spices. Med. Plants., 4(2): 9–16. https://doi.org/10.1300/J044v04n02_03

Ansari MJ, Al-Ghamdi A, Usmani S, Al-Waili N, Nuru A, Sharma D, Khan KA, Kaur M, Omer M. 2016. In vitro evaluation of the effects of some plant essential oils on *Paenibacillus larvae*, the causative agent of American foulbrood. Biotechnol. Biotechnol. Equip., 30(1): 49–55. https://doi.org/10.1080/13102818.2015.1086690

Ansari MJ, Al-Ghamdi A, Usmani S, Khan KA, Alqarni AS, Kaur M, Al-Waili N. 2016. In vitro evaluation of the effects of some plant essential oils on Ascosphaera apis, the causative agent of Chalkbrood disease. Saudi J Biol Sci., 24(5): 1001-1006. https://doi.org/10.1016/j.sjbs.2016.04.016

Ariana A, Ebadi R, Tahmasebi G. 2002. Laboratory evaluation of some plant essences to control *Varroa destructor* (Acari: Varroidae). Exp. Appl. Acarol., 27(4): 319–327. https://doi.org/10.1023/A:1023342118549

Aronstein KA, Murray KD. 2010. Chalkbrood disease in honey bees. J. Invertebr. Pathol., 103(1): 20–29. https://doi.org/10.1016/j.jip.2009.06.018

Aronstein K, Holloway B. 2013. Honey bee fungal pathogen, *Ascosphaera apis*; current understanding of host-pathogen interactions and host mechanisms of resistance. In A. Mendez-Vilas (Ed.), Microbial Pathogens and strategies for combating them: science, technology and education (pp. 402–410).

Bayala B, Bassole IH, Scifo R, Gnoula C, Morel L., Lobaccaro JMA, Simpore J. 2014. Anticancer activity of essential oils and their chemical components - a review. Am. J. Cancer Res., 4(6): 591–607. https://doi.org/ajcr0001130

Bogdanov S. (2006). Contaminants of bee products. Apidologie, 37(1): 1–18. https://doi.org/10.1051/apido:2005043

Borugă O, Jianu C, Mişcă C, Goleţ I, Gruia AT, Horhat FG. 2014. Thymus vulgaris essential oil: chemical composition and antimicrobial activity. J. Med. Life., 7(3): 56–60. PMCID: PMC4391421

Boudegga H, Boughalleb N, Barbouche N, Ben Hamouda MH, Mahjoub El M. 2010. In vitro inhibitory actions of some essential oils on *Ascosphaera apis* , a fungus responsible for honey bee chalkbrood. J. Apic. Res., 49(3): 236–242. https://doi.org/10.3896/IBRA.1.49.3.02

Calderone NW, Bruce WA, Allen-Wardell G, Shimanuki H. 1991. Evaluation of botanical compounds for control of the honey-bee tracheal mite, *Acarapis woodi*. Am. Bee J., 131: 589–591. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US9136525

Calderone NW, Shimanuki H, Allen-Wardell G. 1994. An in vitro evaluation of botanical compounds for the control of the honeybee pathogens Bacillus larvae and *Ascosphaera apis*, and the secondary invader *B. alvei*. J. Essential Oil Res., 6(3): 279–287. https://doi.org/10.1080/10412905.1994.9698376

Calderone NW, Spivak M. 1995. Plant extracts for control of the parasitic Mite *Varroa jacobsoni* (Acari: Varroidae) in colonies of the Western Honey Bee (Hymenoptera: Apidae). J. Econ. Entomol., 88(5): 1211–1215. https://doi.org/10.1093/jee/88.5.1211

Calderone NW, William TW, Spivak M. 1997. Plant extracts used for control of the parasitic mites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). J. Econ. Entomol., 90(5): 1080–1086. https://doi.org/10.1093/jee/90.5.1080

Chaiyasit D, Choochote W, Rattanachanpichai E, Chaithong U, Chaiwong P, Jitpakdi A, Tippawangkosol P, Riyong D, Pitasawat B. 2006. Essential oils as potential adulticides against two populations of *Aedes aegypti*, the laboratory and natural field strains, in Chiang Mai province, northern Thailand. Parasitol. Res., 99(6): 715–721. https://doi.org/10.1007/s00436-006-0232-x

Chantawannakul P, Puchanichanthranon T, Wongsiri S. 2003. Inhibitory effects of some medicinal plant extracts on the growth of *Ascosphaera apis*. In III WOCMAP Congress on Medicinal and Aromatic Plants-Volume 4: Targeted Screening of Medicinal and Aromatic Plants, Economics & Law, 678: 183–189. https://doi.org/10.17660/ActaHortic.2005.678.26

Colin ME. 1990. Essential oils of Labiatae for controlling honey bee varroosis. J. Appl. Entomol., 110(1–5): 19–25. https://doi.org/10.1111/j.1439-0418.1990.tb00091.x

Colin ME, Ducos de Lahitte J, Larribau E, Boué T. 1989. Activité des huiles essentielles de Labiées sur Ascophaera apis et traitement d'un rucher. Apidologie, 20(3): 221–228. https://doi.org/10.1051/apido:19890304

Coskun S, Girisgin O, Kürkcüoglu M, Malyer H, Girisgin AO, Kırımer N, Baser KHC. 2008. Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus* (Ixodidae). Parasitol. Res., 103(2): 259–261. https://doi.org/10.1007/s00436-008-0956-x

Cox RL, Moffett JO, Wilson WT, Ellis M. 1989. Effects of late spring and summer menthol treatment on colony strength, honey production and tracheal mite infestation levels. Am. Bee J., 129: 547–549. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US8934566

Damiani N, Fernández NJ, Porrini MP, Gende LB, Álvarez E, Buffa F, Brasesco C, Maggi MD, Marcangeli JA, Eguaras MJ. 2014. Laurel leaf extracts for honeybee pest and disease management: Antimicrobial, microsporicidal, and acaricidal activity. Parasitol. Res., 113(2): 701–709. https://doi.org/10.1007/s00436-013-3698-3

Damiani N, Gende LB, Bailac P, Marcangeli JA, Eguaras MJ. 2009. Acaricidal and insecticidal activity of essential oils on *Varroa destructor* (Acari: Varroidae) and *Apis mellifera* (Hymenoptera: Apidae). Parasitol. Res., 106(1): 145–152. https://doi.org/10.1007/s00436-009-1639-y

Davis C, Ward W. 2003. Control of chalkbrood disease with natural products. RIRDC, a report for the RIRDC. Publication No. 03/107. Canberra, Australia, 23 pp.

Delaplane KS, Hood WM. 1997. Effects of delayed acaricide treatment in honey bee colonies parasitized by *Varroa jacobsoni* and a late-season treatment threshold for the south-eastern USA. J. Apic. Res., 36(3–4): 125–132. https://doi.org/10.1080/00218839.1997.11100938

Dellacasa AD, Bailac PN, Ponzi MI, Ruffinengo SR, Eguaras MJ. 2003. In vitro activity of essential oils from San Luis-Argentina against *Ascosphaera Apis*. J. Essential Oil Res., 15(4): 282–285. https://doi.org/10.1080/10412905.2003.9712143

Ebert TA, Kevan PG, Bishop BL, Kevan SD, Downer RA. 2007. Oral toxicity of essential oils and organic acids fed to honey bees (*Apis mellifera*). J. Apic. Res., 46(4): 220–224. https://doi.org/10.1080/00218839.2007.11101398

Eguaras MJ, Fuselli S, Gende L, Fritz R, Ruffinengo SR, Clemente G, Gonzales A, Bailac PN, Ponzi MI. 2005. An in vitro evaluation of *Tagetes minuta* essential oil for the control of the Honeybee pathogens *Paenibacillus larvae* and *Ascosphaera apis*, and the parasitic mite *Varroa destructor*. J. Essential Oil Res., 17(3): 336–340. https://doi.org/10.1080/10412905.2005.9698924

El-enain A, Aldel-Rahman MF, Abo-Elyousr KAM. 2009. Inhibitory activity of certain natural products on the growth of *Ascosphaera apis.* Ass. Univ. Bull. Environ. Res., 12(2): 99–106.

El Shafai AAF. 2012. *In Vitro* Control of *Ascosphaera apis* Fungus by Some Plant. Thesis, Islamic University Gaza Deanship of Graduate Studies Faculty of Science Biological Sciences Master Program Botany & Mycology.

Ellis MD, Baxendale FP. 1997. Toxicity of seven monoterpenoids to tracheal mites (Acari: Tarsonemidae) and their honey bee (Hymenoptera: Apidae) hosts when applied as fumigants. J. Econ. Entomol., 90(5): 1087–1091.

Flamini G. 2003. Acaricides of natural origin, personal experiences and review of literature (1990-2001). In Studies in Natural Products Chemistry, 28: 381–451. https://doi.org/10.1016/S1572-5995(03)80146-1

Flesar J, Havlik J, Kloucek P, Rada V, Titera D, Bednar M, Stropnicky M, Kokoska L. 2010. *In vitro* growth-inhibitory effect of plant-derived extracts and compounds against *Paenibacillus larvae* and their acute oral toxicity to adult honey bees. Vet. Microbiol., 145(1–2): 129–133. https://doi.org/10.1016/j.vetmic.2010.03.018

Fries I, Camazine S. 2001. Implications of horizontal and vertical pathogen transmission for honey bee epidemiology. Apidologie, 32(3): 199–214. https://doi.org/10.1051/apido:2001122

Fuselli SR, De La Rosa SBG, Gende LB, Eguaras MJ, Fritz R. 2006. Antimicrobial activity of some Argentinean wild plant essential oils against *Paenibacillus larvae* larvae, causal agent of American foulbrood (AFB). J. Apic. Res., 45(1): 2–7. https://doi.org/10.1080/00218839.2006.11101304

Fuselli SR, García de la Rosa SB, Eguaras MJ, Fritz R. 2008a. Chemical composition and antimicrobial activity of Citrus essences on honeybee bacterial pathogen *Paenibacillus larvae*, the causal agent of American foulbrood. World J. Microbiol. Biotechnol., 24(10): 2067–2072. https://doi.org/10.1007/s11274-008-9711-9

Fuselli SR, García de la Rosa SB, Eguaras MJ, Fritz R. 2008b. Susceptibility of the honeybee bacterial pathogen *Paenibacillus larvae* to essential oils distilled from exotic and indigenous Argentinean plants. J. Essential Oil Res., 20(5): 464–470. https://doi.org/10.1080/10412905.2008.9700060

Fuselli SR, García de la Rosa SB, Eguaras MJ, Fritz R, Ndagijimana M, Vannini L, Guerzoni ME. 2007. Efficacy of indigenous plant essential oil andean thyme (*Acantholippia* seriphioides A. Gray) to control American Foulbrood (AFB) in Honey Bee (*Apis mellifera* L.) Hives. J. Essential Oil Res., 19(6): 514–519. https://doi.org/10.1080/10412905.2007.9699319

Fuselli SR, Gende LB, García de la Rosa SB, Eguaras MJ, Fritz R. 2005. Short communication. Inhibition of *Paenibacillus larvae* subsp larvae by the essential oils of two wild plants and their emulsifying agents. Span J Agric Res., 3(2): 220–224. DOI: 10.5424/sjar/2005032-147

Fuselli SR, Maggi, M., García de la Rosa SB, Principal J, Eguaras MJ, Fritz R. 2009. *In vitro* antibacterial and antiparasitic effect of citrus fruit essential oils on the honey bee pathogen *Paenibacillus larvae* and the parasitic mite *Varroa destructor*. J. Apic. Res., 48(1): 77–78. https://doi.org/10.3896/IBRA.1.48.1.15

Fuselli SR, García de la Rosa SB, Eguaras MJ, Fritz R. (2010). *In vitro* antibacterial effect of exotic plants essential oils on the honeybee pathogen *Paenibacillus larvae*, causal agent of American foulbrood. Span J Agric Res., 8(3): 651–657. DOI: 10.5424/sjar/2010083-1261

Garbaczewska S, Cieniecka-Roslonkiewicz A, Michalczyk A, Kielczewska A. 2014. The use of components of the extract from the leaves of walnut *Juglans regia* L against the fungus *Ascosphaera apis*. CHEMIK, 68(2): 117–122.

Gashout HA, Guzmán-Novoa E. 2009. Acute toxicity of essential oils and other natural compounds to the parasitic mite, *Varroa destructor* and to larval and adult worker honey bees (*Apis mellifera* L.). J. Apic. Res., 48(4): 263–269. https://doi.org/10.3896/IBRA.1.48.4.06

Gende LB, Floris I, Fritz R, Eguaras M. 2008. Antimicrobial activity of cinnamon (*Cinnamon zeylanicum*) essential oil and its main components against Paenibacillus larvae from Argentine. Bull. Insectol., 61(1): 1–4.

Gende LB, Maggi, MD, Damiani N, Fritz R, Eguaras MJ, Floris I. 2009. Advances in the apiary control of the honeybee American foulbrood with cinnamon (*Cinnamomum zeylanicum*) essential oil. Bull. Insectol., 62(1): 93–97.

Gende LB, Maggi MD, Fritz R, Eguaras MJ, Bailac PN, Ponzi MI. 2009. Antimicrobial Activity of *Pimpinella anisum* and *Foeniculum vulgare* Essential Oils Against *Paenibacillus larvae*. J. Essential Oil Res., 21(1): 91–93. https://doi.org/10.1080/10412905.2009.9700120

Gende LB, Maggi M, van Baren C, di Leo L, Bandoni A, Fritz R, Eguaras M. 2010. Antimicrobial and miticide activities of Eucalyptus globulus essential oils obtained from different Argentine regions. Span. J. Agric. Res., 8(3): 642-650. https://doi.org/10.5424/sjar/2010083-1260

Genersch E. 2010. American Foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. J. Invertebr. Pathol., 103: 10–19. https://doi.org/10.1016/j.jip.2009.06.015

Gochnauer R, Boch R, Margetts VJ. 1979. Inhibiton of *Ascosphaera apis* by Citral and Geraniol. J. Invertebr. Pathol., 34(1): 57–61. https://doi.org/10.1016/0022-2011(79)90053-3

González MJ, Marioli JM. 2010. Antibacterial activity of water extracts and essential oils of various aromatic plants against *Paenibacillus larvae*, the causative agent of American Foulbrood. J. Invertebr. Pathol., 104(3): 209–213. https://doi.org/10.1016/j.jip.2010.04.005

Hornitzky M. 2001. Literature review of chalkbrood. Rirdc, 150(1): 1–17.

Hyldgaard M, Mygind T, Meyer RL. 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol., 3: 12. https://doi.org/10.3389/fmicb.2012.00012

Imdorf A, Bogdanov S, Ochoa RI, Calderone NW. 1999. Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie, 30(2–3): 209–228. https://doi.org/10.1051/apido:19990210

Islam N, Amjad M, Haq E, Stephen E, Naz F. 2016. Management of *Varroa destructor* by essential oils and formic acid in *Apis Mellifera* Linn. Colonies. J. Entomol. Zool. Stud., 4(6): 97–104.

Isman MB. 2000. Plant essential oils for pest and disease management. J. Crop. Prot., 19(8–10): 603–608. https://doi.org/10.1016/S0261-2194(00)00079-X

Jatisatienr C, Jatisatienr A. 1999. The fungicidal properties of extracts of clove (*Eugenia caryophyllus* Spreng.) and sweet flag (*Acorus calamus* Linn.). In II WOCMAP Congress Medicinal and Aromatic Plants, Part 2: Pharmacognosy, Pharmacology, Phytomedicine, Toxicology 501 (pp. 87-94). https://doi.org/10.17660/ActaHortic.1999.501.11

Kanga LHB, Jones WA, James RR. 2003. Field trials using the fungal pathogen, *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) to control the ectoparasitic mite, *Varroa destructor* (Acari: Varroidae) in honey bee, *Apis mellifera* (Hymenoptera: Apidae) colonies. J. Econ. Entomol., 96(4): 1091-1099. https://doi.org/10.1603/0022-0493-96.4.1091

Kloucek P, Smid J, Flesar J, Havlik J, Titera D, Rada V, Drabek O, Kokoska, L. 2012. *In vitro* inhibitory activity of essential oil vapors against *Ascosphaera apis*. Nat. Prod. Commun., 7(2): 253–6.

Korukluoglu M, Gurbuz O, Sahan Y, Yigit A, Kacar O, Rouseff R. 2009. Chemical characterization and antifungal activity of *Origanum onites* L. essential oils and extracts. J Food Saf, 29(1): 144–161. DOI: 10.1111/j.1745-4565.2008.00124.x

Kotan R, Kordali S, Cakir A, Kesdek M, Kaya Y, Kilic H. 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC. ex Benth. Biochem. Syst. Ecol., 36(5): 360–368. https://doi.org/10.1016/j.bse.2007.12.003

Kuzyšinová K, Mudroňová D, Toporčák J, Nemcová R, Molnár L, Mad'ari A, Vanikova S, Kožár M. 2014. Testing of inhibition activity of essential oils against *Paenibacillus larvae* - The causative agent of American foulbrood. Acta Veterinaria Brno, 83(1): 9–12. https://doi.org/10.2754/avb201483010009

Larrán S, Ringuelet JA, Carranza MR, Henning CP, Ré MS, Cerimele EL, Urrutía MI. 2001. *In Vitro* fungistatic effect of essential oils against *Ascosphaera apis*. JEOR., 13(2): 122–124. https://doi.org/10.1080/10412905.2001.9699633

Lindberg CM, Melathopoulos AP, Winston ML. 2000. Laboratory evaluation of miticides to control *Varroa jacobsoni* (Acari: Varroidae), a Honey Bee (Hymenoptera: Apidae) Parasite. J. Econ. Entomol., 93(2): 189–198. https://doi.org/10.1603/0022-0493-93.2.189

Maggi MD, Ruffnengo SR, Gende LB, Sarlo EG, Eguaras MJ, Bailac PN, Ponzi MI. 2010. Laboratory evaluations of *Syzygium aromaticum* (L.) Merr. et Perry essential oil against *Varroa destructor*. JEOR., 22(2): 119–122. https://doi.org/10.1080/10412905.2010.9700278

Maggi M, Gende LB, Russo K, Fritz R, Eguaras M. 2011. Bioactivity of *Rosmarinus officinalis* essential oils against *Apis mellifera*, *Varroa destructor* and *Paenibacillus larvae* related to the drying treatment of the plant material. Nat. Prod. Res., 25(4): 397–406. https://doi.org/10.1080/14786419.2010.481261

Mărghitaş L, Dezmirean D, Chirilă F, Fiţ N, Bobiş O. 2011. Antibacterial activity of different plant extracts and phenolic phytochemicals tested on *Paenibacillus Larvae* Bacteria. Scientific Papers: Animal Science and Biotechnologies, 44(2): 94-99

Mayaud L, Carricajo A, Zhiri A, Aubert G. 2008. Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett. Appl. Microbiol. 47(3): 167–173. https://doi.org/10.1111/j.1472-765X.2008.02406.x

Melathopoulos AP, Winston ML, Whittington R, Smith T, Lindberg C, Mukai A, Moore M. 2000. Comparative laboratory toxicity of neem pesticides to honey bees (Hymenoptera: Apidae), their mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae), and brood pathogens *Paenibacillus larvae* and *Ascophaera apis*. J. Econ. Entomol., 93(2): 199-209. https://doi.org/10.1603/0022-0493-93.2.199

Milani N. 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie, 30(2–3): 229–234. https://doi.org/10.1051/apido:19990211

Nardoni S, D'Ascenzi C, Rocchigiani G, Papini RA, Pistelli L, Formato G, Najar B, Mancianti F. 2017. Stonebrood and chalkbrood in *Apis mellifera* causing fungi: in vitro sensitivity to some essential oils. Nat. Prod. Res., 32(4): 385-390. DOI:10.1080/14786419.2017.1306703

Ngoh SP, Choo LEW, Pang FY, Huang Y, Kini MR, Ho SH. 1998. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). Pest Manag Sci, 54(3): 261–268. https://doi.org/10.1002/(SICI)1096-9063(1998110)54:3<261::AID-PS794>3.0.CO;2-C

Ozkirim A, Keskin N, Kurkcuoglu M, Baser KHC. 2012. Evaluation of some essential oils as alternative antibiotics against American foulbrood agent *Paenibacillus larvae* on honey bees *Apis mellifera* L. JEOR., 24(5): 465–470. https://doi.org/10.1080/10412905.2012.703504

Reybroeck W, Daeseleire E, De Brabander HF, Herman L. 2012. Antimicrobials in beekeeping. Vet. Microbiol., 158(1–2): 1–11. DOI:10.1016/j.vetmic.2012.01.012

Rosenkranz P, Aumeier P, Ziegelmann B. 2010. Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology, 103(1): 96–119. https://doi.org/10.1016/j.jip.2009.07.016

Roussenova N. 2011. Antibacterial activity of essential oils against the etiological agent of american foulbrood disease (*Paenibacillus larvae*). BJVM., 14(1): 17–24.

Ruffinengo S, Eguaras M, Bailac P, Torres J, Basualdo M, Ponzi M. 2001. Essential Oils in the control of *Varroa destructor* . An Evaluation in Laboratory. Proceedings of the 37th International Apicultural Congress, 28 October – 1 November 2001, Durban, South Africa, (November), 28–31.

Ruffinengo S, Eguaras M, Floris I, Faverin C, Bailac P, Ponzi M. 2005. LD50 and repellent effects of essential oils from Argentinian wild plant species on *Varroa destructor*. J. Econ. Entomol., 98(3): 651–655. https://doi.org/10.1603/0022-0493-98.3.651

Ruffinengo SR, Eguaras MJ, Cora D, Rodriguez E, Bedascarrasbure E, Bailac PN, Ponzi MI. 2002. Biological activity of *Heterotheca latifolia* essential oil against *Varroa jacobsoni*. JEOR., 14(6): 462–464. https://doi.org/10.1080/10412905.2002.9699921

Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS. 2001. Regulation of essential oil production in plants. Plant Growth Regul, 34(1): 3–21. DOI:10.1023/A:1013386921596

Santos RCV, Alves CF, Dos S, Schneider T, Lopes LQS, Aurich C, Giongo JL, Brandelli A, de Almeida Vaucher R. 2012. Antimicrobial activity of Amazonian oils against *Paenibacillus* species. J. Invertebr. Pathol.,109(3): 265–268. https://doi.org/10.1016/j.jip.2011.12.002

Santos RCV, Lopes LQS, Alves CF, dos S, Fausto VP, Pizzutti K, Barboza V, De Souza ME, Raffin RP, Gomes P, Takamatsu D, Morinaga Y, Boligon AA, Athayde ML, Felippi CC, de Almeida Vaucher R. 2014. Antimicrobial activity of tea tree oil nanoparticles against American and European foulbrood diseases agents. J. Asia Pac. Entomol., 17(3): 343–347. https://doi.org/10.1016/j.aspen.2014.02.003

Singh M, Singh S, Prasad S, Gambhir IS. 2008. Nanotechnology in medicine and antibacterial effects of silver nanoparticles. Dig. J. Nanomater. Biostruct., 3(3), 115–122.

Spiltoir CF. 1955. Life Cycle of *Ascosphaera apis* (*Pericystis apis*). BSA., 42(6): 501–508. Derived from: http://www.jstor.org/stable/2438686.

Spiltoir CF, Olive LS. 1955. A Reclassification of the Genus Pericystis Betts. Mycologia., 47(2): 238–244. DOI: 10.2307/3755414

Turek C, Stintzing FC. 2013. Stability of essential oils: A review. Compr. Rev. Food. Sci. Food. Saf., 12(1): 40–53. DOI: 10.1111/1541-4337.12006

Umpiérrez ML, Santos E, González A, Rossini C. 2011. Plant essential oils as potential control agents of varroatosis. Phytochem. Rev., 10(2): 227–244. https://doi.org/10.1007/s11101-010-9182-0

Voigt K, Rademacher E. 2015. Effect of the propolis components, cinnamic acid and pinocembrin, on *Apis mellifera* and *Ascosphaera apis*. J. Apic. Res., 59(1): 89–95. https://doi.org/10.1515/jas-2015-0010

Vojvodic S, Jensen AB, James RR, Boomsma JJ, Eilenberg J. 2011. Temperature dependent virulence of obligate and facultative fungal pathogens of honeybee brood. Vet. Microbiol., 149(1–2): 200–205. https://doi.org/10.1016/j.vetmic.2010.10.001

Wallner K. 1999. Varroacides and their residues in bee products. Apidologie, 30(2–3): 235–248. https://doi.org/10.1051/apido:19990212

Weinberg KP, Madel G. 1985. The influence of the mite *Varroa jacobsoni* oud. on the protein concentration and the haemolymph volume of the brood of worker bees and drones of the honey bee *Apis mellifera* L. Apidologie, 16(4): 421–436. https://doi.org/10.1051/apido:19850407

Wianowska D, Garbaczewska S, Cieniecka-Roslonkiewicz A, Dawidowicz AL, Jankowska A. 2016. Comparison of antifungal activity of extracts from different Juglans regia cultivars and juglone. Microb. Pathog., 100: 263–267. DOI:10.1016/j.micpath.2016.10.009

Williamson EM, Priestley CM, Burgess IF. 2007. An investigation and comparison of the bioactivity of selected essential oils on human lice and house dust mites. Fitoterapia, 78(7–8): 521–525. DOI:10.1016/j.fitote.2007.06.001