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Essential Oils for Pest Control

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Clove Oil

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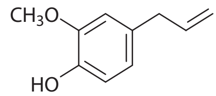

FIGURE 10.1
Chemical structure of eugenol.

10.1 Introduction

The family Myrtaceae comprises at least 132 genera and 5671 species distributed in tropical and subtropical regions worldwide, with centers of diversity in Australia, Southeast Asia, and South America, but with only poor representation in Africa. *Syzygium aromaticum* (L.) Merrill & Perry (with synonyms of *Eugenia caryophyllus* Bullock and S.G. Harrison, *Caryophyllus aromaticus* L., *Eugenia aromatica* [L.] [Baill.], and *Eugenia caryophyllata* [Thunb.] [Spreng.]), commonly known as clove, is a medium-sized tree from the Myrtaceae family. Its essential oil is isolated from dried flower buds, which are the source of its strongly smelling oil, popularly known as clove oil. The oil extracted from the stem and leaf is used in preparing high-grade eugenol and vanillin. The essential oil is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care as an antiseptic and analgesic. Previous studies have reported antifungal, anticarcinogenic, antiallergic, antimutagenic, antioxidant, and insecticidal properties. The chief constituent of clove oil is 70%–90% eugenol ($C_{10}H_{14}O_2$) (Figure 10.1), followed by β-caryophyllene and, in lesser amounts, α-humulene, caryophyllene oxide, and eugenyl acetate, although in different concentrations (Pandey and Chadha, 1993; reviewed by Razafimamonjison et al., 2013).

10.2 Botany of the Plant

The clove tree is a perennial tropical plant that grows to a height ranging from 10 to 20 m, having large oval leaves and crimson flowers in numerous groups of terminal clusters. The flowers are hermaphrodite, borne at the terminal end in small bunches, and produce a two-celled capsule (bud) containing the well-known hot aromatic fruit. The buds have a slightly cylindrical base and are surrounded by the plump ball-like unopened corolla, which is surrounded by four-toothed calyx. Production of flower buds, which is the commercialized part of this tree, starts after 4 years of plantation. Flower buds are collected in the maturation phase before flowering. The leaves are lanceolate on the branched tree and possess plenty of oil glands on the lower surface. The branches are semierect, the limbs are more often brittle with a grayish bark, and the head is bushy and dense (Board, 2010; reviewed by Cortes-Rojas et al., 2014).

10.3 Methods of Extraction of Oil

10.3.1 Conventional Extraction

Essential oils are defined as products extracted from natural plants by physical means only, such as hydrodistillation, steam distillation, dry distillation, cold press, headspace analysis, liquid CO_2 extraction, and solvent extraction. The composition of the extracted oil may vary from one extraction method to another. Headspace analysis offers a potentially rapid method to extract essential oils and requires very little plant material, but complete recovery occurs only for highly volatile materials. Steam distillation and solvent extraction as conventionally applied result in severe losses of volatile materials because the liquid in which the oil is collected must be subsequently removed by evaporation. Solvent extraction results in the recovery of nonvolatile compounds. While liquid CO_2 extraction can give reliable and efficient recovery with little or no decompositional changes induced by the extraction process, this process is expensive and used only on a limited basis in commerce. Moreover, similar results can be obtained by hydro- or steam distillation (reviewed by Charles and Simon, 1990).

These processes are not expensive, but they can induce thermal degradation, hydrolysis, and water solubilization of some fragrance constituents. Extracts obtained by solvents contain residues that pollute the foods and fragrances to which they are added. These disadvantages have attracted recent research attention and stimulated the intensification, optimization, and improvement of existing and novel "green" extraction techniques. All these techniques are appropriately applied with a careful consideration of plant organs and the quality of the final products (reviewed by Li et al., 2014).

These conventional extraction techniques could typically extract essential oils from plants ranging from 0.005% to 10%, which are influenced by the distillation duration, temperature, operating pressure, and most importantly, type and quality of raw plant materials.

There are some extraction methods to obtain clove oil, like the other essential oils. The most common ones are steam distillation and supercritical fluid extraction (SFE) methods, which are official approved methods for the isolation of essential oils from plant materials (Guan et al., 2007; reviewed by Li et al., 2014).

In steam distillation, the plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. The vapor laden with essential oils flows through a "swan neck" column and is then condensed before decantation and collection in a Florentine flask. Essential oils that are lighter or heavier than water form two immiscible phases and can be easily separated. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100°C, so that the volatile components with boiling points ranging from 150°C to 300°C can be evaporated at a temperature close to that of water. Furthermore, this technique can also be carried out under pressure, depending on the essential oil's extraction difficulty.

Supercritical fluid extraction has been established as an environmentally benign technique for separating essential oils from the vegetable. Moreover, extracts obtained by SFE can retain the organoleptic characteristics of the starting spice material (Guan et al., 2007).

From these two methods, there are other methods, like hydrodiffusion, hydrodistillation, and Soxhlet extraction (with the solvents of methanol, acetone, and chloroform) (reviewed by Li et al., 2014).

Some studies on the extraction of essential oil of cloves have concluded a yield of 8.6% on a steam distillation process of clove (*S. aromaticum*) for 8 h with the physical properties of essential oils that meet SNI 06-4267-1996. In the supercritical CO_2 extraction to get the yield 19.6% and 58.77% of eugenol, concentration at a pressure of 10 MPa and 50°C for 2 h are used. Extraction of essential oil clove buds by the steam distillation process for 8–10 h results in a yield of 10.1%; hydrodistillation for 4–6 h, 11.5%; and Soxhlet extraction for 6 h, 41.85%. On the extraction of essential oil of cloves with *E. caryophyllata*, using the hydrodistillation method for 150 min results in a yield of 5.06%; microwave-assisted extraction (MAE) for 10–30 min, 7.42%; and microwave steam distillation (MSD) for 10–30 min, 16.25% (reviewed by Daryono, 2015).

10.3.2 Green Extraction

The principles of green extraction can be generalized as the discovery and design of extraction processes that could reduce the energy consumption, allow the use of alternative solvents and renewable and innovative plant resources to eliminate petroleum-based solvents, and ensure safe and high-quality extracts or products. Turbo distillation, ultrasound or microwave-assisted extraction, instantaneous controlled pressure drop technology and household espresso machine method are used (reviewed by Li et al., 2014; Just et al., 2016).

10.4 Methods of Analysis of Oil

The analysis of clove oil is commonly performed with gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) equipment. GC is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture (Donald et al., 2006).

GC-MS is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can identify trace elements in materials that were previously thought to have disintegrated beyond identification (Donald et al., 2006).

Clove oil can be analyzed by GC-MS practically, and its components can be documented with the help of a device library.

10.5 Composition of Oil

The chemical composition of clove oil and the relative concentration of bioactive components may vary, depending on a range of factors, including the extraction method used, geographical location where the plant was cultivated, time of harvest, storage method, and part of the plant used. Clove represents one of the major vegetal sources of phenolic compounds as flavonoids, hidroxibenzoic acids, hidroxicinamic acids, and hidroxiphenyl propens (Table 10.1). Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9,381.70 to 14,650.00 mg/100 g of fresh plant material. With regard to the phenolic acids, gallic acid is the compound found in higher concentrations

TABLE 10.1

Composition and Percentage of Clove Essential Oil (*Eugenia caryophyllata*) Obtained with GS-MS Analysis

No.	Compound[a,b]	Kovats Index[c] (HP-20M)	Percentage (%)
1	Eugenol	2151	88.58535
2	Eugenyl acetate	2263	5.62086
3	β-Caryophyllene	1595	1.38830
4	2-Heptanone	1172	0.93232
5	Ethyl hexanoate	1232	0.66098
6	Humulenol	2265	0.27527
7	α-Humulene	1668	0.19985
8	Calacorene	1918	0.11437
9	Calamenene	1828	0.10538
10	2-Heptanol	1304	tr
11	Menthyl octanoate	1384	tr
12	2-Nonanone	1392	tr
13	Ethyl octanoate	1429	tr
14	α-Cubebene	1459	tr
15	Copaene	1491	tr
16	2-Nonanol	1499	tr
17	Linalool	1548	tr
18	2-Undecanone	1588	tr
19	Menthyl benzoate	1619	tr
20	Ethyl benzoate	1647	tr
21	Menthyl chavicol	1669	tr
22	α-Amorphene	1675	tr
23	α-Terpinyl acetate	1695	tr
24	α-Muurolene	1711	tr
25	Benzyl acetate	1714	tr
26	Carvone	1731	tr
27	γ-Cadinene	1756	tr
28	2-Phenyiethyl acetate	1826	tr
29	(E)-Anethole	1827	tr
30	Benzyl alcohol	1861	tr
31	Caryophyllene oxide	1976	tr
32	Menthyl eugenol	1985	tr
33	Humulene oxide	1986	tr
34	Cinnamic aldehyde	2018	tr
35	Ethyl cinnamate	2072	tr
36	Benzyl tiglate	2103	tr
Total identified			98.2769

Source: Chaieb, K. et al., *Phytother. Res.*, 21, 501–506, 2007.
Note: tr, trace (<0.1%).
[a] Order of elution on HP-20M capillary.
[b] Identified by comparison of the mass spectral and Kovats index data.
[c] Kovats indices on HP-20M column.

(783.50 mg/100 g fresh weight). However, other gallic acid derivatives, such as hydrolizable tannins, are present in higher concentrations (2375.8 mg/100 g). Other phenolic acids found in clove are the caffeic, ferulic, elagic, and salicylic acids. Flavonoids such as kaempferol, quercetin, and its derivate (glycosilated) are also found in clove in lower concentrations. Concentrations up to 18% of essential oil can be found in the clove flower buds. Roughly 89% of the clove essential oil is eugenol (Figure 10.1), and 5%–15% is eugenol acetate and β-caryophyllene. Another important compound found in the essential oil of clove in concentrations up to 2.1% is α-humulen. Other volatile compounds present in lower concentrations in clove essential oil are β-pinene, β-selinene, limonene, farnesol, benzaldehyde, 2-heptanone, ethyl hexanoate, humulenol, α-humulene, calacorene, and calamenene. The number of components of clove oil can reach up to 36 (reviewed by Pasay et al., 2010; reviewed by Cortes-Rojas et al., 2014; Girisgin et al., 2014).

10.6 Physical and Chemical Properties of Oil

Physical and chemical properties of clove oil may vary, depending on the extraction method used, geographical location where the plant was cultivated, time of harvest, storage method, and part of the clove (bud, leaf, or stem) used. A comparative list of physical and chemical properties of clove oil is presented in Table 10.2. Also, analytical data and standards of clove are presented (Table 10.3).

10.7 General Uses of Oil

10.7.1 Antioxidant Capacity

Eugenol, the major constituent of clove oil, has many antioxidant properties. The antioxidant activity may occur via various mechanisms, such as scavenging the radicals and chelating metal ions. Eugenol reportedly participates in photochemical reactions and displays strong antioxidant activity and photocytotoxicity (reviewed by Chaieb et al., 2007).

TABLE 10.2

Physical and Chemical Properties of Clove Oil

	Clove Bud Oil	Clove Leaf Oil	Clove Stem Oil
Specific gravity at 25°C	1.038–1.5250	1.036–1.046	1.048–1.056
Refractive index at 20°C	1.5270–1.5350	1.5231–1.5350	1.5340–1.5380
Optical rotation at 20°C	–1030′ to 00	–20 to 00	–1°5′ to 0°
Solubility	1:2 in 70% EtOH	1:2 in 70% EtOH	1:2 in 70% EtOH
Phenol content (as eugenol)	Not less than 85% by volume	Not less than 84% and not more than 88% by volume	Not less than 98% and not more than 95% by volume

Source: Panda, H., *Essential Oils Handbook*, National Institute of Industrial Research, New Delhi, 2003, p. 219.

TABLE 10.3

Analytical Data and Standards of Clove Oil

	Range (%)	BPC Standards (%)	U.S. Standards (%)	Indian Standards (%)
Moisture	5–11.0	–	–	Max 12
Ash	4.5–7	7.0 (max)	7.0 (max)	Max 7
Water soluble ash	2.7–4.2	–	–	
Acid insoluble ash	0–0.3	1.0 (max)	0.5 (max)	Max 0.5
Volatile oil	14.5–20	15 (min): Whole 12 (min): Powder	15 (min): For volume of ethanol extract	Min 15
Fixed oil	6.2–10.1	–	–	–
Alcohol extract	13.5–15.5	–	–	
Crude fiber	6–10	–	10 (max)	
Nitrogen	0.9–1.2	–	–	
Foreign organic matter (e.g., fruits)	–	1.0 (max)	–	
Stalks for stems	–	5.0 (max)	5 (max)	
Quercitannic acid	–	–	12 (min)	

Source: Singhal, R., Kulkarni, P.R., and Rege, D.V., *Handbook of Indices of Food Quality and Authenticity*, Woodhead Publishing, Cambridge, UK: 1997, p. 402.

Note: BPC, British Pharmacopoeia.

Jirovetz et al. (2006) found that the antioxidant action of 0.005% clove oil was identical to that of standard butylated hydroxytoluene at a concentration of 0.01%. It manifests considerable chelating potential against Fe^{+3}, resulting in the prevention of the initiation of hydroxyl radicals (Jirovetz et al., 2006). Clove oil thus shows powerful antioxidant activity; moreover, it can be used as an easily accessible source of natural antioxidants and in pharmaceutical applications (reviewed by Chaieb et al., 2007).

Shan et al. (2005) conducted a study on the main phenolic compounds of 26 spices, which were identified and quantified by high-performance liquid chromatography, followed by *in vitro* antioxidant activity analysis by the ABTS method. Results showed a high correlation between the polyphenol content and the antioxidant activity. Clove (buds) was the spice presenting higher antioxidant activity and polyphenol content tetraethylammonium chloride (mmol of Trolox/100 g dried weight) and gallic acid (equivalents/100 g of dried weight), respectively. The major types of phenolic compounds found were phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol and acetyl eugenol), and tannins (reviewed by Cortes-Rojas et al., 2014).

Antioxidants are important compounds for the treatment of memory deficits caused by oxidative stress. Pretreatment with clove essential oil decreases the oxidative stress assessed by malondialdehyde and reduced glutathione levels in the brain of mice. This study concluded that clove oil could revert memory and learning deficits caused by scopolamine in the short and the long term as a result of the reduction in the oxidative stress (Halder et al., 2011). Memory and learning improvements of clove oil were observed in scopolamine-treated mice at doses of 0.025, 0.05, and 0.1 ml/kg when compared with a saline solution control group in an elevated plus maze test. These works prove the benefits of the employment of clove as a rich source of antioxidants for the treatment of memory deficits caused by oxidative stress (reviewed by Cortes-Rojas et al., 2014).

10.7.2 Anesthetic Activity

Eugenol is used in a wide range of applications, such as a local anesthetic in dentistry and as an ingredient in dental cement for temporary fillings. It is relatively user-friendly and can be used in lower concentrations than other local anesthetics, and it is rapidly metabolized and excreted, thus requiring no withdrawal period. It has been shown to be effective in anaesthetizing fish such as rainbow trout, *Oncorhynchus mykiss*, and channel catfish, *Ictalurus punctatus*. Eugenol at 65 mg/L was shown to safely and effectively induce all stages of anaesthesia in juvenile and subadult tambaqui fish within the desired time. Further research needs to focus on assessing its efficacy in other tropical species, as well as investigating its lethal dosage (reviewed by Chaieb et al., 2007).

10.7.3 Antimicrobial Activity

The antibacterial activity of different clove oil extracts has been demonstrated against pathogenic bacteria, including *Campylobacter jejuni*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Escherichia coli*, and *Staphylococcus aureus* (reviewed by Chaieb et al., 2007; Hemalatha et al., 2015). A study reported that the growth rates of *Listeria monocytogenes* strains observed at 15°C and 5°C were significantly reduced by treatment with 1% and 2% clove oil (Mytle et al., 2006), and furthermore, Ogunwande et al. (2005) found that the essential oil of the fruit exhibited strong antibacterial activity against *S. aureus*, while the leaf oil strongly inhibited the growth of *Bacillus cereus*, with a minimum inhibitory concentration (MIC) of 39 μg/ml. The oil was also active against 26 strains of *Staphylococcus epidermidis* isolated from dialysis fluids, 3 human pathogenic Gram-positive cocci, 2 Gram-negative bacilli, and 1 Gram-positive bacillus (diameter of inhibition zone, 11–15 mm). In contrast, the oil was ineffective against *P. aeruginosa* ATCC 27853 (diameter of inhibition zone, 9 mm). These results are in agreement with those of another study reporting that clove essential oil exhibited antibacterial activity against a large number of methicillin-resistant *S. epidermidis* and *S. aureus*. However, the oil also appears to be effective against both Gram-positive and Gram-negative microorganisms, contrasting the results found in some other studies (reviewed by Chaieb et al., 2007).

Sofia et al. (2007) tested the antimicrobial activity of different Indian spice plants, such as mint, cinnamon, mustard, ginger, garlic, and clove. The only sample that showed a complete bactericidal effect against all the food-borne pathogens tested, *E. coli*, *S. aureus*, and *B. cereus*, was the aqueous extract of clove at 3%. At a concentration of 1%, clove extract also showed good inhibitory action (reviewed by Cortes-Rojas et al., 2014).

In addition to the wide spectrum of activity of eugenol against bacteria, a study showed that eugenol and cinnamaldehyde at 2 μg/ml inhibited the growth of 31 strains of *Helicobacter pylori*, after 9 and 12 h of incubation, respectively, being more potent than amoxicillin and without developing resistance. The activity and stability of those compounds was checked at low pH values since *H. pylori* resides in the stomach (Ali et al. 2005; reviewed by Cortes-Rojas et al., 2014).

10.7.4 Anticancer–Antitumor Activity

Clove essential oil has been reported to show anticarcinogenic and antimutagenic potential. Volatile oils display cytotoxic action toward the human tumor cell lines PC-3 and Hep G2, and in a recent study, eugenol was shown to induce apoptosis of human cancer cells (Namiki, 1994), with the major antimutagenic compound being identified as dehydrodieugenol. More recently, the antimutagenic activity of cinnamaldehyde was reported in human-derived hepatoma cells, where it suppressed the frequency of micronuclei induced by various heterocyclic amines (reviewed by Chaieb et al., 2007).

Eugenol isolated from clove oil was investigated using human promyelocytic leukemia cells (HL-60) and might be a potent agent in cancer therapy. After treatment with eugenol, the HL-60 cells showed hallmarks of apoptosis, such as DNA fragmentation and formation of DNA ladders in agarose gel electrophoresis. Apoptotic cell death was induced via generation of ROS, inducing a mitochondrial permeability transition, reducing the anti-apoptotic protein bcl-2 level, and inducing cytochrome C release to the cytosol (reviewed by Buchbauer, 2010).

10.7.5 Fungicidal Activity

The phenolic component of eugenol is known to possess fungicidal characteristics, including activity against fungi isolated from onychomycosis (Gayoso et al., 2005). The main antifungal action appears to be exerted on the cellular membrane (Cox et al., 2001). Eugenol has shown antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (Tampieri et al., 2005). Núñez et al. (2001) demonstrated that the mixture of clove oleoresin with concentrated sugar solution produced a strong fungicidal effect by reducing fungi inoculum size. Scanning electron microscope (SEM) micrographs showed significant morphological damage, with cellular deformity to *Saccharomyces cerevisiae* cells by clove oil (Chami et al., 2005). The fungicidal activity of clove essential oil has also been reported on several food-borne fungal species, and it was observed in a study that the essential oil of clove even inhibited the growth of *Aspergillus niger* (Pawar and Thaker, 2006; reviewed by Chaieb et al., 2007).

10.7.6 Antiviral Activity

In general, viruses are highly sensitive to the components of essential oils. Satisfactory results have been yielded in some studies on the *in vitro* antiviral activity of clove oil. For example, Hussein et al. (2000) found that *S. aromaticum* extract was highly active at inhibiting replication of the hepatitis C virus (≥90% inhibition at 100 μg/ml). Kurokawa et al. (1998) isolated and identified an anti–herpes simplex virus (HSV) compound, eugeniin, from the extracts of *S. aromaticum*, which showed specificity in inhibiting HSV-1 DNA polymerase activity (reviewed by Chaieb et al., 2007). The antiviral activity of eugeniin, isolated from *S. aromaticum* and *Geum japonicum*, was also tested against herpes virus strains, being effective at 5 μg/ml, and it was deducted that one of the major targets of eugeniin is viral DNA synthesis by inhibition of the viral DNA polymerase (reviewed by Cortes-Rojas et al., 2014).

10.7.7 Antinociceptive

The employment of clove as analgesic has been reported since the thirteenth century, for toothache and joint pain and as an antispasmodic, with eugenol being the main compound responsible for this activity. The mechanism involved has been attributed to the activation of calcium and chloride channels in ganglionar cells. The voltage-dependent effects of eugenol in sodium and calcium channels and in receptors expressed in the trigeminal ganglia also contributed to the analgesic effect of clove. Other results show that the analgesic effect of clove is due to its action as a capsaicin agonist. The peripheral antinociceptive activity of eugenol was reported by Daniel et al. (2009), showing significant activity at doses of 50, 75, and 100 mg/kg (reviewed by Cortes-Rojas et al., 2014).

10.8 Pesticidal Uses of Oil (Including Public Health)

10.8.1 Advantages as a Pesticide

Environmental problems caused by overuse of pesticides have been the matter of concern for both scientists and the public in recent years. It has been estimated that about 2.5 million tons of pesticides are used on crops each year, and the worldwide damage caused by pesticides reaches $100 billion annually. The reasons for this are (1) the high toxicity and nonbiodegradable properties of pesticides and (2) the residues in soil, water resources, and crops that affect public health. Thus, one needs to search the new highly selective and biodegradable pesticides to solve the problem of long-term toxicity to mammals, and on the other hand, one must study the environmentally friendly pesticides and develop techniques that can be used to reduce pesticide use while maintaining crop yields. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. The move toward green chemistry processes and the continuing need for developing new crop protection tools with novel modes of action make discovery and commercialization of natural products as green pesticides an attractive and profitable pursuit that is commanding attention. The concept of green pesticides refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. They are safe and ecofriendly. They are more compatible with the environmental components than synthetic pesticides (reviewed by Koul et al., 2008).

Clove oil is one of the most effective pesticides compared with other essential oils, is liquid at room temperature, and is easily transformed from a liquid to a gaseous state at room or slightly higher temperature without undergoing decomposition. Low concentrations can be effective on pests and used as a pesticide. Thus, production of a pesticide from clove oil or any of its components may not be expensive.

10.8.2 Limitations as a Pesticide

The toxicity of essential oils is relatively well studied experimentally and clinically because of their use in human and veterinary medicine. The mammalian toxicity of essential oils is generally low. Most essential oils, including clove, have an oral LD_{50} value ranging from 1500 to 5000 mg/kg in rats (reviewed by Regnault-Roger, 2013). Clove oil is generally recognized as safe substance when consumed in concentrations lower than 1500 mg/kg. The World Health Organization (WHO) established that the daily acceptable quantity of clove is 2.5 mg/kg of weight in humans. It can cause dermal toxicity on mammals as an irritant. Eugenol is easily absorbed when administrated by the oral route, rapidly reaching plasma and blood, with mean half-lives of 14.0 and 18.3 h, respectively. A cumulative effect has been hypothesized and associated with relieve of neuropathic pain after repeated daily administrations (reviewed by Cortes-Rojas et al., 2014).

Because risk includes both hazard and exposure, the use of clove oil requires that the applicators carefully follow the labeling recommendations. Essential oils are most often delivered by spraying or fogging that may induce a dermal or respiratory exposure. The need for suitable equipment for handling essential oils products and the treated plant must be observed to avoid accident or chronic intoxication. The essential oil risk, however minimal it may be, must not be ignored simply because essential oils are natural products (reviewed by Regnault-Roger, 2013).

10.8.3 Essential Oil–Based Insecticides

There are several studies on the effect of clove oil or its components on several pests and parasites. Clove oil, like some other essential oils, not only acts as a poison or neurotrope on the nervous system of insects, but also can intervene in cellular breathing either by inhibiting cellular oxidation through transfer interruption in the respiratory chain or by asphyxiation through the formation of an impermeable film insulating insects from the air. Moreover, clove oil may also have an inhibitory power to enzyme activity and insect growth with respect to adults, larvae, and eggs (Li et al., 2014).

Clove oil or its component eugenol has been tested as a pesticide on ticks, mites, mosquitoes, cockroaches, and grain pests. Satisfactory results have been obtained from studies on both adults and larvae. Examples presented here are classified according to pest type.

10.8.4 Ticks

Usage of environmentally friendly pesticides on animal ectoparasites has been a matter of concern to both scientists and the public in recent years. An acaricide should not leave residue in the meat and milk of both farm animals and poultry flocks. The acaricide or repellent potential of clove oil has been tested on ticks.

Clove leaf oil concentrations of 1%, 5%, 10%, and 15% have been evaluated *in vitro* for acaricidal activity against adults of *Argas* spp. soft ticks collected from poultry flocks. A 15% concentration of clove oil killed 80% of ticks in 24 h, with an LC_{50} = 3.15% and LT_{50} = 47.9 (Hasson and Al-Zubaidi, 2015).

10.8.5 Mites

Since *in vitro* and *in vivo* conventional acaricide resistance from human scabies mites has been reported, natural product extracts and compounds have become potential sources of alternative acaricides.

Clove oil and its components have been tested on permethrin-sensitive *Sarcoptes scabei* var. *suis* and permethrin-resistant *S. scabei* var. *canis*, separately at concentrations of 1.56%, 3.12%, 6.25%, 12.5%, and 25%. At all concentrations tested (1.56%–25%), contact with clove oil resulted in 100% mortality of permethrin-sensitive mites after 0.25 h. Permethrin-resistant mites died at the same time but required higher concentrations (≥6.25%) of clove oil. The acaricidal effect was faster in sensitive mites than in resistant mites. The acaricidal activities of eugenol, a major component of clove oil, and its minor component acetyleugenol, as well as the related analogues isoeugenol and methyleugenol, have been tested in contact bioassays against the two scabies mite populations. Mortality in the two mite populations at different concentrations of the compound have been plotted as dose–response curves, and EC_{50} values were derived after an hour of observation. For both mite populations, there was no significant difference between the activity observed for the positive control acaricide (benzyl benzoate) and the test compounds eugenol, acetyleugenol, and isoeugenol ($p > 0.11$). In contrast, methyleugenol had no acaricidal effect in sensitive mites after the first hour of observation. This compound only displayed activity in resistant mites after 24 h at the highest concentration tested (100 mM) (Pasay et al., 2010).

Three components (terpenes) found in essential oils (eugenol, geraniol, and citral) have been tested against the poultry red mite *Dermanyssus gallinae*. All provided 100% mortality in toxicity tests when undiluted. Even at 1% of this dose, eugenol was 20% effective against experimental pest populations, although the remaining terpenes have shown to be largely ineffective at this concentration. LC_{50} values confirmed the superior efficacy of eugenol (10%) over both citral (30%) and geraniol (40%) (Sparagano et al., 2013).

The eradication of house dust mites, *Dermatophagoides pteronyssinus*, by direct contact using the essential clove oil has been evaluated. For this, synthetic fibers have been immersed in 2% clove oil for 30 min, dried in a hot-air oven at 60°C for 2 h, after which 0.5 g of dust mites was exposed to these coated fibers placed in the Siriraj chamber. Ten mites were placed in the chamber and 10 μl of clove oil was pipetted or sprayed onto them. These latter two procedures were each carried out for three consecutive days at 0, 1, 3, and 6 months. The effectiveness of pipetting and spraying was 99% and 81%, respectively, while the placebo mortality was <5% (Mahakittikun et al., 2013).

Varroa destructor (Acari: Varroidae) is accepted as the most dangerous agent of honeybees (*Apis mellifera*) worldwide. Essential oil–based home-made or commercial acaricides are widely used to struggle against varroosis. The biological activity of the clove oil applied to *V. destructor* and *A. mellifera* has been evaluated in two laboratory tests. Mite lethality has been estimated using a complete exposure method test with the oil at different concentrations, and a systemic administration method of oil at different concentrations diluted in syrup has been placed in feeders for bees. The LC_{50} for the complete exposure method at 24 h was 0.59 ml/dish. The LC_{50} estimated at 48 h showed a slight decrease compared with that recorded at 24 h. Ratio selection (LC_{50} of *A. mellifera*/LC_{50} of *V. destructor*) for the complete exposure method was 26.46 and 13.35 for 24 and 48 h, respectively. Regarding the systemic administration method, mite's LC_{50} at 24 h was 12,300 ppm. *S. aromaticum* oil was found to be an attractant for *V. destructor* at 4.8% (w/w) concentration. The results have shown that oil toxicity against *V. destructor* differed depending on its administration. Nevertheless, the ratio selection calculated by this oil is expected to enable its application under field conditions with a good safety margin. This oil could also be used in combination with other oils in integrated pest management strategies in bee colonies (Maggi et al., 2010).

10.8.6 Mosquitoes

Recently, outdoor pest control has changed to using nonresidual and environmentally friendly alternative pesticides. Clove oil has been evaluated to determine mortality rates, morphological aberrations, and persistence when used against third and fourth larval instars of *Aedes aegypti* and *Anopheles dirus*. The oil has been evaluated at 1%, 5%, and 10% concentrations in mixtures with soybean oil. Persistence of higher concentrations has been measured over a period of 10 days. For *Ae. aegypti*, clove oil has caused various morphological aberrations, to include deformed larvae, incomplete eclosion, white pupae, deformed pupae, dead normal pupae, and incomplete pupal eclosion. All these aberrations have led to larval mortality. In *Ae. aegypti* larvae, there were no significant differences in mortality at days 1, 5, and 10 or between third and fourth larval instar exposure. In *A. dirus*, morphological aberrations were rare and *S. aromaticum* oil was effective in causing mortality among all larval stages. Oil was effective at producing mortality on days 1, 5, and 10, and had slightly increased LT_{50} rates from day 1 to day 10 (Soonwera and Phasomkusolsil, 2016).

Another work evaluated the larvicidal activity of aqueous and methanolic extracts from clove, *E. caryophyllata* Thunberg (Myrtaceae), and its chemical component, eugenol, against malaria and dengue mosquito vectors. Bioassays have been carried out with these extracts and eugenol on *Anopheles darlingi* Root, 1926 and *Ae. aegypti* Linnaeus, 1762 (Diptera, Culicidae) third-instar larvae. The median lethal concentration values obtained with aqueous extract against *Aa. aegypti* (LC_{50} = 6.4 mg/ml) were higher than those observed against *A. darlingi* (LC_{50} = 99 mg/ml). Eugenol exhibited an LC_{50} value of 3.6 mg/ml against *Ae. aegypti* larvae (Medeiros et al., 2013). These findings show eugenol's potential as a larvicide against malaria and dengue vectors.

Clove oil is also used as a mosquito repellent by itself or in combination with some other essential oils, like citronella, hairy basil, catnip, and vetiver (reviewed by Tisgratog et al., 2016).

10.8.7 Grain Pests

Grain pests cause severe postharvest losses in all types of grains and legumes. The control of these insects by conventional insecticides may increase the risks associated with pests and hazards to human health and environmental contamination. Thus, protecting grains with alternative chemical control options is needed. Clove oil can be a good alternative instead of the conventional insecticides.

Nonfumigant applications of clove and cinnamon essential oils have been tested for insecticidal and repellent activities on the bean weevil, *Acanthoscelides obtectus*. Clove oil, which includes 92.94% eugenol, showed a toxicity of LD_{50} = 43.6 µl/kg beans, steadily decreased the growth rate of *A. obtectus* in a dose-dependent manner, and lost its insecticidal activity over time. Additionally, the clove oil delayed bean weevil emergence, whereas cinnamon oil repelled the bean weevil (Jumbo et al., 2014).

Similarly, clove oil has been tested at the doses of 35, 17.9, 8.9, 3.6, 1.8, 0.4, and 0.2 µl/g for maize weevil *Sitophilus zeamais* and bean weevil *A. obtectus* under laboratory conditions. The clove essential oil has caused a mortality of 100% for both species 48 h after treatment with concentrations of 17.9 and 35 µl/g. The LC_{50} for *A. obtectus* was 9.45 µl/g, against 10.15 µl/g for *S. zeamais* (Jairoce et al., 2016).

A grain pest population that is resistant to conventional insecticides might have distinct but possibly overlapping mechanisms to mitigate the actions of essential oils and conventional insecticides. This situation has been represented for clove oil on *S. zeamais* (Correa et al., 2015).

Another test was conducted on two-spotted spider mite, *Tetranychus urticae*, one of the most serious pests of crops, with cumin, clove, and spearmint oils. The total preadult developmental time was significantly shorter when treated with *Eugenia caryophyllata* oil than with control. The mean total fecundity (eggs/female) ranged from 31.08 for those treated with clove oil to 64.44 for the control. The highest ovicidal activity was recorded for cumin oil (LC_{50} = 7.65 µl/L air), followed by clove (LC_{50} = 8.73 µl/L air) and spearmint (LC_{50} = 9.01 µl/L air). According to repellency tests, by increasing the concentration of oils, the repellency effects were increased. The most potent repellency effect was recorded for clove, followed by spearmint and cumin oils (Kheradmand et al., 2015).

10.8.8 Cockroaches

Indoor use of conventional pesticides always has a risk of contamination of foods and hypersensitive allergic reactions in humans and animals. So, use of natural pesticides or repellents has become more of an issue.

Evaluation of the repellency and fumigant toxicity of clove (*S. aromaticum*) and sesame (*Sesamum indicum*) oils against the American cockroach (*Periplaneta americana*) has shown complete repellency (100%) against first-instar nymphs at a concentration of 2% for clove oil and 6% for sesame oil. The same result has been obtained against fourth-instar nymphs at a concentration of 10% of sesame oil after 48 h. Clove oil completely repelled all fourth-instar nymphs after 24 h at a concentration of 8%. For the adult stage, the greatest repellency percentages have been recorded by clove oil (90.00 ± 5.77%) and sesame oil (83.33 ± 3.33%) after 48 h at a concentration of 10% (Omara et al., 2013). Clove oil provided highly fumigant toxicity against nymphs and adults of *P. americana* after 24 and 48 h, respectively. Complete mortality (100%) has been recorded at a concentration of 7.5 µl/L of air for first-instar nymphs, 10 µl for fourth-instar nymphs, and 17.5 µl for adults after 48 h of fumigation (Omara et al. 2013).

Surface contact toxicities of clove and rosemary, *Rosmarinus officinalis* L., oils have been investigated against the American cockroach, *P. americana* (L.), in the laboratory. Both clove and rosemary oils have shown variable mortality percentages according to concentration, exposure time, and stage of the insect. Clove oil induced highly significant contact toxic effects against *P. americana* nymphs and adults after 4, 24, and 48 h exposure. First- and fourth-instar nymphs were more sensitive to clove oil (LC_{50} values of 0.0001 and 0.0077 µl/cm^2, respectively) than rosemary oil (LC_{50} values of 1.92 and 2.25 µl/cm^2, respectively) after 24 h. Regarding adults, the effect of both oils was very weak after 48 h exposure. Clove oil had lower LT_{50} values than rosemary oil. For clove oil, the lowest LT_{50} values ranged from 14.10 to 4.85 h in the case of the first instar at a low concentration level of 0.0005–0.0020 µl/cm^2, and 20.00–5.00 h for adults at a high concentration level of 2.0–3.0 µl/cm^2. The LT_{50} was high for the fourth-instar nymphs and ranged from 43.67 to 26.68 h at moderate concentrations, 0.002–0.010 µl/cm^2, respectively (Sharawi et al., 2013).

10.8.9 Head Lice

Head lice, *Pediculus humanus capitis* De Geer, are an obligate ectoparasite of humans worldwide that causes pediculosis capitis, with high prevalence in children. *P. h. capitis* has been treated by methods that include the physical removal of lice, various domestic treatments, and conventional insecticides. Head lice resistance to conventional insecticides has increased, and new alternative topical therapies for head lice infestations are needed, especially those containing plant-derivative active ingredients. Moreover, absorption of synthetic chemicals from the skin of the head has the potential hazard of chronic diseases.

Two herbal shampoos based on *Alpinia galanga* and *S. aromaticum* against head lice have been tested on children, and they have been compared with malathion shampoo (1% w/v malathion) and baby shampoo in order to assess their *in vitro* efficacy. The results have revealed that all herbal shampoo at 6 µl/cm^2 is a more effective pediculicide than malathion shampoo and baby shampoo at 3 µl/cm^2. The highest pediculicidal activity was shown by *A. galanga* shampoo at a dose of 6 µl/cm^2, with 100% mortality at 3 min, an LT_{50} value of 0.4 min, and an LC_{50} value of 1.8 µl/cm^2. At a dose of 6 µl/cm^2, *S. aromaticum* shampoo showed 100% mortality at 10 min, an LT_{50} value of 0.9 min, and an LC_{50} value of 2.3 μl/cm². However, malathion shampoo showed low toxicity, with 34.5% mortality at 10 min and an LT_{50} value of 35.9 min, and baby shampoo was nontoxic to all head lice during the observation periods. All data in this study showed that two herbal shampoos based on *A. galanga* and *S. aromaticum* have high potential to alternative pediculicides for head lice treatments of children (Soonwera, 2015).

Another study was carried out to determine the pediculocidal activity using hexane flower bud extract of *S. aromaticum* against *P. h. capitis*, examining direct contact and fumigant toxicity (closed- and open-container methods) bioassay. The filter paper contact bioassay study showed pronounced pediculicidal activity in the flower bud hexane extract of *S. aromaticum*. The toxic effect was determined every 5 min in an 80 min treatment. The results showed percent mortalities of 40, 82, and 100 at 5, 10, and 20 min, and the LT_{50} value was 5.83 (0.5 mg/cm²); 28, 82, and 100 at 5, 10, and 30 min (LT_{50} = 6.54; 0.25 mg/cm²); and 13, 22, 42, 80, and 100 at 5, 10, 20, 40, and 80 min (LT_{50} = 18.68; 0.125 mg/cm²), respectively. The vapor phase toxicity was tested at 0.25 mg/cm². There was a significant difference in the pediculicidal activity of *S. aromaticum* extract against *P. h. capitis* between closed- and open-container methods. The mortality was more effective in the closed containers than in the open ones, indicating that the effect of hexane extract was largely a result of action in the vapor phase exhibiting fumigant toxicity (Bagavan et al., 2011).

10.8.10 Other Pests

Eugenol, eugenol acetate, and β-caryophyllene were effective in repelling red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae), with eugenol being the fastest-acting compound. Clove oil was also an effective spatial repellent for pestiferous social wasps *Vespula pensylvanica* and paper wasps, mainly *Polistes dominulus* (reviewed by Cortes-Rojas et al., 2014).

10.9 Conclusions

The development of essential oils as pesticides and biocides to control insects is an alternative or complementary approach to synthetic insecticides. They are environmentally friendly products; that is, they have natural origin and are biodegradable, and they have diverse physiological targets within insects that may delay the evolution of insect resistance. Thus, they are especially suited to organic farming, as well as to integrated pest management.

The essential oils have an attractive potential to substitute for synthetic insecticides because of their diversity and efficiency.

Clove oil is a good candidate for a safer control agent that may provide good antipest activity due to its low toxicity to mammals and easy biodegradability.

These features indicate that pesticides based on clove essential oil could be used in a variety of ways to control a large number of pests. However, more research is needed on the tested oils, such as the acaricidal effects of each major compound, and their modes of action and efficacies in field conditions.

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