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Essential Oils for Pest Control
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Pennyroyal Oil as a Green Pesticide

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N.C. Basantia and Hamir Singh Rathore

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22.1 Introduction

Pennyroyal (*Mentha pulegium* L.) is known by several common names, such as pennyroyal, mint, and poleo. Pennyroyal is native to Ireland, across southern and central Europe, and in the Ukraine (Tutin et al. 1976). A European folk name for the plant is "grows-in-the-ditch" (Polonin 1969). Repeatedly introduced in North America since European settlement, pennyroyal is now found naturalized in wildlands throughout the world (Grieve, 1959). These plants also thrive in Asia, Iran, the United Arab Emirates, Ethiopia, Brazil, Tunisia, and Portugal. This plant also grows spontaneously in humid parts of Iran. The worth of *Mentha* is evident, as its essential oils (EOs) and dried and fresh plant material are in daily use in confectionary, beverages, bakeries, cosmetics, pharmaceuticals, and pesticides (Shaikh et al., 2014). Essential oils of this herb have been used in traditional medicine in many countries. The oils are also reported to have potential for use to prevent oxidation of unsaturated fatty acids and increase a product's shelf life. Pennyroyal is a good alternative for synthetic antioxidants and is used as flavors in many foods. Besides food products, pennyroyal essential oils can be used in detergents and soaps, dental products, and insect and tick repellent agents.

22.2 Botany of the Plant

22.2.1 Taxonomic Classification (Shah et al., 2011)

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoleophyta
Class: Magnoliliopsida
Order: Lamiales
Family: Lamiaceae/Labiatae
Genus: *Mentha* L.
Species: *pulegium* (pennyroyal)
Botanical name: *Mentha pulegium* (pennyroyal)

22.2.2 Habitat and Distribution

M. pulegium L. is an herbaceous perennial plant group wildly found in humid areas of the plains and mountains. Pennyroyal is native to Ireland, across the southern and central European countries, in North Africa, Ethiopia, Brazil, Tunisia, Portugal, and the Arabic countries. Spain and Morocco are main pennyroyal oil producer countries. This plant also grows spontaneously in humid parts of Iran.

22.2.3 Botanical Description of Plant

M. pulegium L. is an aromatic perennial herbaceous plant reaching up to 40 cm. These plants are characterized by a uniformly prostrate to upright habit while flowering.

22.2.4 Leaves

The leaves of *M. pulegium* are petiolate, ovate to suborbicular, obtuse to cuneate, and entire to serrate.

22.2.5 Flower

The flower is of an inflorescence shape and verticillate, with four to many flowers.

22.3 Methods of Extraction of Oil

The common methods to extract essential oil from *M. pulegion* are (1) hydrodistillation (HD), steam distillation, and steam and water distillation; (2) Soxhlet extraction; (3) supercritical fluid extraction (SFE); (4) microwave-assisted hydrodistillation; and (5) ultrasound-assisted extraction (UAE).

22.3.1 Distillation

Distillation is the extracting oil process that converts volatile liquid (essential oils) into a vapor state and then condenses the vapor into a liquid state. In this process, the botanic material is completely immersed in water and the still is brought to boil. It is used to protect the oils to a certain degree since the surrounding water acts as a barrier to prevent overheating. When the condensed material cools down, the water and essential oil are separated, and the oil is decanted for use as an essential oil. As water distillation tends to be small, the operation takes a long time to accumulate much oil (Ranjitha and Vijiyalakshmi, 2014). In the steam distillation method, the botanical material is placed in a still and steam is forced over the material. The hot steam is used to release the aromatic molecules from the plant material. The steam forces the pockets open, and then the molecules of these volatile oils escape from the plant material and evaporate into the steam. The steam containing the essential oil is passed through the cooling system to condense the steam, which forms a liquid form of essential oil. Then, water is separated. The major advantage of steam distillation is that the temperature never goes above 100°C, so temperature-sensitive compounds can be distilled. The disadvantage is that not many compounds can be steam distilled—usually only aromatic ones (Masango, 2005; Ranjitha and Vijiyalakshmi, 2014). The hydrodiffusion method is similar to the steam distillation process. The main difference between these two methods is that the steam is introduced into the still. In the case of hydrodiffusion, steam is fed into the top, onto the botanical material, instead of from bottom, as in normal steam distillation. The steam containing the essential oil is passed through a cooling system to condense the steam, which forms a liquid of essential oil, and then water is then separated. The main advantages of this method are that less steam is used, the processing time is shorter, and there is a higher yield (Ranjitha and Vijiyalakshmi, 2014).

22.3.2 Soxhlet Extraction

Soxhlet extraction is a general and well-established technique that surpasses other conventional extraction techniques in performance, except for limited fields of application, for example, the extraction of thermolabile compounds. Most of the solvent extraction units worldwide are based on Soxhlet principles with recycling of solvents. Basically, the equipment consists of a drug holder extractor, a solvent storage vessel, a reboiler kettle, a condenser, a breather system, and supporting structures like a boiler, a refrigerated chilling unit, and a vacuum unit (William, 2007). This technique is based on the choice of solvent coupled with heat or agitation. In this process, the circulation of solvents causes the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix. This method maintains a relatively high extraction temperature and no filtration of extract is required (Shams et al., 2015).

However, the limitation of this technique is that there is a possibility of thermal decomposition of thermolabile targeted compounds because the extraction usually occurs at the boiling point of the solvent for a long time.

22.3.3 Microwave Extraction Method

Solvent-free microwave extraction is used to separate the essential oil from plant material. The method involves placing the sample in a microwave reactor without any addition of organic solvent or water. The internal heating of the water within the sample distends its cells and leads to rupture of the glands and oleiferous receptacles. This process frees essential oil, which is evaporated by the *in situ* water of plant material.

A cooling system outside the microwave oven continuously condenses the vapors, which are collected in specific glassware. The excess of water is refluxed back to the extraction vessel in order to restore the *in situ* water to the sample.

The microwave isolation offers a net advantage in terms of yield and better oil composition. Furthermore, it is environmentally friendly. In this method, low-boiling-point hydrocarbon compounds undergo decomposition (Marie et al., 2004; Ranjitha and Vijiyalakshmi, 2014).

22.3.4 Supercritical Fluid Extraction

Supercritical fluid extraction is used for the extraction of flavors and fragrances. SFE is a separation technology that uses supercritical fluid as solvent. Every fluid is characterized by a critical point, which is defined in terms of the critical temperature and critical pressure. Fluids cannot be liquefied above the critical temperature regardless of the pressure applied, but may reach the density close to the liquid state. A substance is considered to be a supercritical fluid when it is above its critical temperature and critical pressure. Several compounds have been examined as SFE solvents (e.g., hexane, pentane, butane, nitrous oxide, sulfur hexafluoride, and fluorinated hydrocarbon).

However, the main supercritical solvent used is carbon dioxide. Carbon dioxide (critical condition 30.9°C and 73.8 bar) is cheap, environmentally friendly, and generally recognized as safe. Supercritical carbon dioxide is also attractive because of its high diffusivity and easily tunable solvent strength. Another advantage is that carbon dioxide is gaseous at room temperature and ordinary pressure, which makes analyte recovery very simple and solvent-free (Taylor, 1996; Ranjitha and Vijiyalakshmi, 2014).

22.3.5 Ultrasound-Assisted Extraction

The mechanical effect of ultrasound accelerates the release of organic compounds within the plant body due to cell wall disruption, mass transfer intensification, and easier access of the solvent to the cell content. Ultrasound-assisted extraction (UAE) is reported to be one of the important techniques for extracting valuable compounds from the vegetable material (Vilkhu et al. 2008). General ultrasonic devices are the ultrasonic cleaning bath and ultrasonic probe system.

The efficiency of UAE depends on various factors, such as the nature of the tissue being extracted, the location of the component to be extracted, the treatment of the tissue prior to extraction, the effect of ultrasonics, the surface mass transfer, and intraparticle diffusion.

UAE can extract analytes under a concentrated form and free from any contaminants or artifacts. It also demonstrates advantages in terms of yield, selectivity, operating time, energy input, and preservation of thermolabile compounds (Shams et al., 2015).

22.4 Composition of Oil

The essential oil contains a complex mixture consisting mainly of oxygenated monoterpenes such as menthone, pulegone, neomenthol, and 8-hydroxy-∂-4(5)-p-menthen-3-one. In some studies of *M. pulegium* essential oil, however, pulegone does not occur as a major constituent. In the essential oil of *M. pulegium* from Austria, piperitone was found to be the main component, followed by limonene, menthone, and neomenthone, while pulegone was not even detected. This type of oil is sometimes described as the piperitone–piperitenone chemotype. Chemotypes are defined as individual plants producing distinctive dominant secondary compounds (Santesson, 1968; Keefover-Ring et al., 2009). These are usually qualitative designations and arbitrary. According to the essential composition, the existence of nine chemotypes has been reported for *Mentha* plants. The main components of the nine chemotypes of the genus *Mentha* are (1) pulegone/isopulegone, (2) piperitone/piperitone oxide, (3) geraniol and/or geranyl acetate, (4) linalool and/linalool acetate, (5) carvone/dihdrocarvone, (6) menthofuran, (7) menthone/isomenthone/menthol isomers, (8) piperitone oxide/piperitone oxide, and (9) pulegone/menthone/isomenthone.

There is great variability in the chemical composition of *M. pulegium* essential oil among the studies performed so far (Pino et al., 1996). The variation of yields and chemical composition of essential oils depend on several factors, such as the method of extraction, the used plant parts, the products and reagents used for extraction, the environment, the plant genotype, the geographical origin, the harvest period of the plant, the degree of drying, the drying conditions, the temperature and drying time, and the presence of parasites, viruses, and weeds (Karousou et al., 2005; Kelen et al., 2008). The major components of pennyroyal oil (*M. pulegon*) reported in different studies are given in Table 22.1.

22.5 Properties of Pennyroyal Oil

22.5.1 Physicochemical Properties

The volatile oil obtained by steam distillation of *M. pulegium* is a light yellow liquid having a mint-like odor. As per the Committee on Food Chemicals Codex (2004), the physicochemical properties of pennyroyal oil are as given in Table 22.2.

TABLE 22.1

Major Components of Pennyroyal Oil (*M. pulegon*)

Serial No.	Compound	Origin	Parts of Plant	Sample Period	Extraction Method	Content %	Reference
1	Pulegon	Iran	Aerial parts	Summer	Hydrodistillation	37.8	Aghel et al., 2004
		Jammu and Kashmir, India	Aerial parts	July 2001–2003	Hydrodistillation	42.9–45.4	Agnihotri et al., 2005
		Austria	Plant	nd	nd	25.1	Zwaving and Smith, 1971
		Algeria	Aerial parts	nd	Hydrodistillation	4.4–87.3	Beghidja et al., 2007
		Turkey	nd	Summer, 1993	Steam distillation	20.5	Mueller-Riebau et al., 1995
		Portugal	Flowers and leaves	July	Hydrodistillation	78.3–80.9	Reis-Vasco et al., 1999
		Greece	Aerial parts	nd	nd	0.1–90.7	Kokkini et al., 2004
		Morocco	Leaves	nd	nd	6.5	Elhoussine et al., 2010
		Egypt	nd	nd	nd	88.1	Aziz and Craker, 2009
		Brazil	nd	nd	nd	28.4–61.4	Oliveira et al., 2011
2	Piperitone	Iran	Aerial parts	Summer	Hydrodistillation	0.0–97.2	Aghel et al., 2004
		Jammu and Kashmir, India	Aerial parts	July 2001–2003	Hydrodistillation	0.1–26.7	Agnihotri et al., 2005
		Austria	Plant	nd	nd	70.0	Zwaving and Smith, 1971
		Algeria	Aerial parts	nd	Hydrodistillation	0.1–19.2	Beghidja et al., 2007
		Turkey	nd	Summer, 1993	Steam distillation	nd	Mueller-Riebau et al., 1995
		Portugal	Flowers and leaves	July	Hydrodistillation	nd	Reis-Vasco et al., 1999
		Greece	Aerial parts	nd	nd	0.1–39.8	Kokkini et al., 2004
		Morocco	Leaves	nd	nd	35.6	Elhoussine et al., 2010
		Egypt	nd	nd	nd	nd	Aziz and Craker, 2009
		Brazil	nd	nd	nd	nd	Oliveira et al., 2011

(*Continued*)

TABLE 22.1 (CONTINUED)

Major Components of Pennyroyal Oil (*M. pulegon*)

Serial No.	Compound	Origin	Parts of Plant	Sample Period	Extraction Method	Content %	Reference
3	Piperitone oxide	Iran	Aerial parts	Summer	Hydrodistillation	nd	Aghel et al., 2004
		Jammu and Kashmir, India	Aerial parts	July 2001–2003	Hydrodistillation	0.1–26.7	Agnihotri et al., 2005
		Austria	Plant	nd	nd	nd	Zwaving and Smith, 1971
		Algeria	Aerial parts	nd	Hydrodistillation	0.1–19.2	Beghidja et al., 2007
		Turkey	nd	Summer, 1993	Steam distillation	nd	Mueller-Riebau et al., 1995
		Portugal	Flowers and leaves	July	Hydrodistillation	nd	Reis-Vasco et al., 1999
		Greece	Aerial parts	nd	nd	0.1–39.8	Kokkini et al., 2004
		Morocco	Leaves	nd	nd	21.2	Elhoussine et al., 2010
		Egypt	nd	nd	nd	nd	Aziz and Craker, 2009
		Brazil	nd	nd	nd	nd	Oliveira et al., 2011
4	Isomenthone	Iran	Aerial parts	Summer	Hydrodistillation	nd	Aghel et al., 2004
		Jammu and Kashmir, India	Aerial parts	July 2001–2003	Hydrodistillation	3.8–4.0	Agnihotri et al., 2005
		Austria	Plant	nd	nd	nd	Zwaving and Smith, 1971
		Algeria	Aerial parts	nd	Hydrodistillation	nd	Beghidja et al., 2007
		Turkey	nd	Summer, 1993	Steam distillation	nd	Mueller-Riebau et al., 1995
		Portugal	Flowers and leaves	July	Hydrodistillation	nd	Reis-Vasco et al., 1999
		Greece	Aerial parts	nd	nd	4.3–28.6	Kokkini et al., 2004
		Morocco	Leaves	nd	nd	nd	Elhoussine et al., 2010
		Egypt	nd	nd	nd	5.8	Aziz and Craker, 2009
		Brazil	nd	nd	nd	nd	Oliveira et al., 2011

Note: nd, not determined.

TABLE 22.2

Physicochemical Properties of Pennyroyal Oil

Serial No.	Parameter	Value	Reference
1	Angular rotation	+18 to + 25°	FCC, 1996
2	Specific gravity at 20°C	0.928–0.940	FCC, 1996
3	Refractive index at 20°C	1.483–1.488	FCC, 1996
4	Solubility	1 ml is soluble in 2 ml of 70% alcohol	FCC, 2004

22.5.2 Biological Properties

Pennyroyal essential oil possesses various useful properties, which can be classified as follows:

1. Antimicrobial properties
2. Aromatic characteristics
3. Antioxidant properties
4. Insecticidal properties
5. Medicinal characteristics

22.5.2.1 Antimicrobial Properties

Antimicrobial properties of pennyroyal essential oil can be due to the existence of some groups like pulegone, menthone, and neo-menthone. The EO can disrupt the structure of different layers in polysaccharides, fatty acids, and phospholipids of the bacterial membrane by changing the permeability of the cellular membrane and damaging the bacterial cell wall (Nobakht et al., 2011; Teixeira et al., 2012). Different researchers have demonstrated that pennyroyal essential oil has a strong antimicrobial property against microorganisms such as *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, *Bacillus cereus*, *Clostridium perfrigens*, *Staphylococcus aureus*, *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Klebsiela pneumoniae*, and *Brochothrix thermosphacta.* Among these microorganisms, the most and least antimicrobial properties have been observed for *S. typhimurium* and *B. thermosphacta*, respectively (Teixeira et al., 2012).

22.5.2.2 Aromatic Characteristics

Pennyroyal essential oils are cyclohexenes and aromatic. Pulegone is the original compound of these oils that has a specific mint aroma (from intense to balsamic and pungent). However, the quality and quantity of the aroma vary, depending on the variety, growth conditions, weather conditions, amount of sun, and lack of water. High temperature, lack of water, and intense sun in summers make the herb produce more monoterpenes (like menthone and isomenthone). Pulegone, besides its isomers isopulegone and piperiton, can have an important role in producing the mint aroma. Some other compounds, such as 3-octanol and noneone, have been observed in pennyroyal, so that both of them, and especially 3-octanol, have a huge role in the aromatic characteristics (Díaz-Maroto et al., 2007). The method of volatiles extraction has a considerable impact on the quality and quantity of the aroma. Pennyroyal essential oil extracted by supercritical fluid extraction is better than that extracted by the other methods (Reis-Vasco et al., 1999).

22.5.2.3 Antioxidant Properties

Pennyroyal essential oils have an antioxidant property due to the existence of phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes. The main components are pulegone and menthone. The essential oil of *M. pulegium* exerted a strong antioxidant activity that is almost equal to that of butylated hydroxytoluene (Ouakouak et al., 2015).

22.5.2.4 Insecticidal and Insect Repellency Properties

Essential oils show good potential against insect and mite pests. They have shown effectiveness by fumigation and topical application, besides having antifeedant and repellent properties (Regnault-Roger, 1997). The essential oil of pennyroyal wards off fleas, ants, lice, mosquitoes, ticks, and moths (Koul et al., 2008). Pennyroyal oil has effective insecticidal activity due to its chemical composition in general due to monoterpenes but also due to the synergestic effect of some minor consituents (Zekri et al., 2013). The components, mainly pulegone, piperitenone, thymol, limonene, and pionene, show important insecticidal activity against several pests (Frazios et al., 1997; Boughdad et al., 2011). The fumigant effect of this oil against adults of *Sitophilus oryzae* is due to pulegone (Zekri et al., 2013). The toxic effect of *M. pulegium* essential oil against *S. oryzae* is through inhibition of neurotransmitters (acetylcholinesterase and octopamine) (Price and Berry, 2006; López and Pascual-Villalobos, 2010; Zekri et al., 2013; Enan et al., 1998; Hollingworth et al., 1964).

Zekri et al. (2013) evaluated the insecticidal activity of *M. pulegium* essential oil against stored cereal pests. Pennyroyal oil has shown an important fumigant effect against *S. oryzae* (L.) adults. At 2 µl of essential oil per liter of air, insects have been totally decimated on the fourth day. The calculated lethal concentration (LC_{50}) and LC_{99} vary according to the time. They decrease gradually as the fumigation time increases. The extreme lethal concentrations vary from 2.65 to 0.044 µl/L of air and from 143.9 to 0.518 µl/L of air, respectively. The effectiveness of the pennyroyal oil may be attributed to its chemical composition generally and monoterpene particularly. The fumigant effect of this oil against adults of *S. oryzae* could be explained by the high content of pulegone and piperitenone without ignoring the synergistic role of the minor compounds. The essential oils extracted from the mint species *M. pulegium* and *Mentha spicata*, together with their main constituents, pulegone, menthone, and carvone, were tested for insecticidal and genotoxic activities on *Drosophila melanogaster* (Franzios et al., 1997). The EOs of both aromatic plants showed strong insecticidal activity, while only the oil of *M. spicata* exhibited a mutagenic one. Among the constituents studied, the most effective insecticide was found to be pulegone, while the most effective for genotoxic activity was menthone.

22.5.2.5 Medicinal Characteristics

Pennyroyal essential oil has been used as an abortifacient and may induce menstruation (Conway and Slocumb, 1979). It has been useful for treatments in some conditions and diseases. Pennyroyal can be consumed as an antiflatulent, antitussive, diuretic, or anti-inflammatory. It is used for the treatment of infant's chronic diarrhea, asthma, and candidiasis. It has also been applied as an anticonvulsive, antiemetic, antinausea, antistress, heart stimulant, or sedative medication in traditional medicine (Vallnce and Edin, 1955; Dietz and Bolton, 2010; Nobakht et al., 2011; Da Rocha et al., 2012).

The consumption of pennyroyal essential oil in high to very high doses in humans can cause disorders, such as gastritis, convulsions, disseminated intravascular coagulation, hepatotoxicity, pulmonary toxicity, renal failure, or central nervous system (CNS) toxicity; coma; and even death (Anderson et al., 1996; Sztajnkrycer et al., 2003; Da Rocha et al., 2012).

Only a few investigations have been conducted to survey pennyroyal essential oil's stability over time. The effects of various factors on essential oil in general are discussed here to understand the storage stability of pennyroyal oil.

- *Light*: Ultraviolet light and visible light are considered to accelerate the autoxidation process. Monoterpenes have been seen to degrade rapidly under the influence of light (Misharina et al., 2003).
- *Temperature*: Ambient temperature crucially influences essential oil stability in several respects. Generally, chemical reactions accelerate with temperature and the rate of reaction expressed by the Arrhenius equation. Hence, both autoxidation and decomposition of hydroperoxide advance with increasing temperature. Contrarily, lower temperature favors the solubility of oxygen in liquids, which in turn may negatively affect essential oil stability. Concerning hydroperoxide stability, it was revealed that at low or moderate temperature, hydroperoxide formation predominated the decomposition rate, while the opposite is true at 50°C (Aidos et al., 2002).
- *Oxygen availability*: Oxygen consumption during storage of different monoterpenes has been recorded, and changes in composition as well as physicochemical properties of essential oil were generally more pronounced in half-filled containers than when only little or no headspace was present (El-Nikeety et al., 1998). Oil oxidation accelerates with the concentration of dissolved oxygen, which in turn depends on oxygen partial pressure in the headspace, as well as ambient temperature.
- *Metal contaminants*: Upon distillation in primitive stills or during storage in metallic containers, impurities of metals can be released into essential oils. Equal to light and heat, heavy metals, especially copper and ferrous ions, are considered to promote autoxidation in particular if hydroperoxides are already present.

Altogether, reliable and comprehensive studies on pennyroyal essential oil storage are rarely found, and concrete specifications on appropriate storage conditions, as well as shelf life, have not been clearly defined to date.

22.6 General Uses

The aerial parts of this plant contain a wide diversity of secondary metabolites, such as tannins, resins, pectins, and essential oils. Fresh or dried leaves and flowering tops are commonly used for their healing and culinary properties.

22.7 Advantage of Pennyroyal Oil as a Pesticide

The constituents of pennyroyal oil are selective and have little or no harmful effect on the environment and the nontarget organisms. Essential oils have received much attention as potentially useful bioactive compounds against insects, showing a broad spectrum of activity. They have low mammalian toxicity, degrade rapidly in the environment, and are locally available. The aromatic characteristic of essential oils present a variety of functions for the plants, together with repelling or attracting insects utilizing chemical components in the oil as defense resources. Essential oil could be a useful alternative to synthetic insecticides in the production of organic food.

22.8 Constraints of Pennyroyal as a Pesticide

In terms of specific constraints, the efficacy of these materials falls short when compared with synthetic pesticides. Essential oils also require somewhat greater application rates (as high as 1% active ingredients) and may require frequent reapplication when used outdoors. The commercial application of plant essential oil–based pesticide has challenges, like sufficient quantities of plant material, standardization and refinement of the pesticide product, protection of technology, and regulatory approval. In addition, as the chemical profile of plant species can vary naturally depending on geographic, genetic, climatic annual, or seasonal factors, pesticide manufacturers have to take additional steps to ensure that their product will perform consistently. All this requires substantial costs, and smaller companies are not willing to invest the required funds unless there is a high probability of recovering the costs through some form of market exclusivity. Finally, once all these issues are addressed, regulatory approval is required (Mohan et al., 2011).

22.9 Conclusion

Pennyroyal oil consists of a diverse array of bioactive compounds and exhibits a wide range of activities, such as antimicrobial, antioxidative, and insecticidal activities. Therefore, this oil has great potential not only in food, pharmaceuticals, and cosmetics, but also in insect repellents, insecticides, and a variety of other ways to control a large number of pests. Pennyroyal oil has huge potential as an alternative to synthetic pesticides in stored cereal and crop protection, although the efficacy of these oil constituents is comparatively less than that of the synthetic pesticides. However, it is gaining momentum as far as environmental pollution and human health are concerned. It is expected that the innovative formulations of pesticides based on this essential oil will find their greatest commercial applications in urban pest control, vector control vis-à-vis human health, and pest control in agriculture, and will help in organic food production systems.

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