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Plants essential oil and extract: Inhibitor the growth of bacteria and fungi

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Authors:
Fatemeh Bidarnamani[1*]
Maryam Kamali[2]

1. Assistant Professor, Agriculture Institute, Research Institute of Zabol, Zabol, Iran
2. Assistant Professor, Department of Medical Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

**** Corresponding author:***
Fatemeh Bidarnamani

E-mail:
f.bidarnamani65@uoz.ac.ir

ABSTRACT

Pathogens are any organism that can produce disease. They can cause disease in human as well as plants, and animals. Pathogenic agents are combated by various fungicides and bactericides. All of these toxins, after eliminating pathogens, mostly remain in the cycle because they are not decomposed and cause many problems. Due to the consumption of plants and animals in human nutrition, diseases, especially types of cancer, have become common in recent years. In order to prevent these problems, the consumption of toxins should be reduced to a minimum level. One of these ways is to use plant extracts and essential oils to eliminate pathogens. Different extracts and essential oils have different properties, and according to various research, have positive inhibition effects on the pathogens growth and reproduction process. Research in biocontrol of pathogens will lead to a bright future in terms of eliminating chemical toxins for humans. In this review antibacterial and antifungal effects of extract and essential oil of some plants is given below. Several examples of the use of plant essential oils and extract in the inhibition of plant pathogens, food industry, and extension of post-harvest life of fruits instead of chemical additives were reported.

Keywords: Antibacterial, Antifungal, Medicinal plants, Pathogen, Post-harvest

Introduction

Plants are a large group of multicellular eukaryotes and are generally attacked by various pathogens. Plant diseases have become a severe risk that significantly reduce quality and quantity of agricultural products (Pascale et al., 2020). In recent years, agricultural product safety has become an important global issue, in which product problems caused by pathogenic microorganisms have been mainly considered (Hou et al., 2022; Benali et al., 2020). The Food and Agriculture Organization (FAO) estimates that plant diseases and pests cause significant losses in global crop production, ranging from 20% to 40% annually. These losses translate to an estimated economic impact of over USD 220 billion per year. However, most of the chemical compounds used in agriculture are still of interest due to their low price and effectiveness against phytopathogens. Using chemical compounds for a long time may lead to microbial resistance; on the other hand, it is a serious threat to human health (Chanprapai and Chavasiri, 2017; Matusinsky et al., 2015). Rosca et al. (2022) concluded (i) the role in agriculture and the actually annual production of 15 banned pesticides with high persistence (lindane, dieldrin, aldrin, endosulfan, parathion and parathion-metil, tributyltin oxide, DDT, pentachlorophenol, chlordane, heptachlor, paraquat, carbofuran, fenbutatin oxide, fipronil, and atrazine), (ii) its spreading in soil, water, air, and products, (iii) its toxicity on animals and humans and its fate in the environment compartments, and (iv) the ability of microorganisms to remove these compounds from the environment. excessive and irrational use of fungicides causes environment deterioration and has non-target effects on plants and animals. The fungicides are responsible for residue problems, resistance development in pathogens and different health hazards to human beings and other living organisms. To avoid these non-target effects of fungicides, there is a need for judicious use of fungicides along with Integrated Pest Management (IPM) practices

(Kumar Goswami et al., 2018). Therefore, alternatives to chemical compounds have become common due to the increasing demand for sustainable agriculture and public concern about environmental pollution and human health (Chidi et al., 2020). Nowadays, serious efforts are needed to find new natural antimicrobial compounds as alternative, safe, eco-friendly, cheap and easily degradable compounds (Yang et al., 2010).

The antimicrobial effect of essential oils and extracts of various medicinal plants such as thyme, cloves, savory, garlic, licorice, and fennel has been proven and their active ingredient has also been identified. Researcher had been reported the possibility of introducing, formulating, and using essential oils and extracts or any components of them with antimicrobial properties, as an alternative method of using chemical toxins in the management of fungal and bacterial disease of plants can be provided (Samavat et al., 2022).

Fungi are significant destroyers of foodstuffs, grains, leaves, and fruits during storage and retard the nutritive value through deterioration and by producing metabolites (Pittet, 1998). Environmental conditions is more serious in tropical and sub-tropical regions, due to beneficial characteristics such as temperature, relative humidity, and moisture content which help the development of fungal population. Fungal infection in food items lead to their bio-deterioration increase in free fatty acids content, change in color, texture, and decline in nutritional value (Dhingra et al., 2001).

Taheri et al. (2023) concluded that application of essential oils can be considered as an eco-friendly and safe alternative methods to be replaced with utilization of hazardous agrochemical which are noxious for both consumers and the environment. Essential oils destroy cell membrane of phytopathogens and inhibiting growth and reproduction of various pathogens.

Pantoea stewartii is a bacterium responsible for serious losses of corn crop in the world and especially in America, where it is native (Duong et al., 2017). This bacterium belongs to the Enterobacteriaceae family. *Pantoea stewartii* subsp. stewartii is a gram-negative bacterium that mainly causes disease in sweet corn (Walterson and Stavrinides, 2015).

The bacterium *Rathayibacter toxicus* (previously known as *Clavibacter toxicus*) is carried into the grass by a nematode, *Anguina funesta*, and produces toxins within seed galls, is the causal agent of annual ryegrass toxicity (ARGT). Annual ryegrass seed heads carrying these seed galls cause significant livestock losses because they contain neurotoxins known as corynetoxins, which can lead to animal mortality if consumed (Colegate et al., 2015).

Koli et al. (2024) reported that during the antibiosis assay, *Pantoea agglomerans* and *Pseudomonas cedrina*, among the endophytic/epiphytic bacterial species, produced compounds that significantly inhibited *R. toxicus* growth in vitro condition, with capacity values 22.7 ± 2.3 and 20.0 ± 2.0, respectively. In conclusion, this study's findings have significant implications for the management of annual ryegrass toxicity (ARGT), as they highlight the promising potential of *P. agglomerans* and *P. cedrina* as viable candidates for biological control strategies in ARGT management.

Yulia (2005) concluded that application of extracts and essential oils reduced the symptoms of anthracnose caused by the fungus, but their effects against *C. goleosporoides* were variable, some extracts were phytotoxic at high concentrations and this had the unwanted effect of making infection easier for some fungi. One such product of complementary medicine is tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant named *Melaleuca alternifolia* employed largely for its antimicrobial properties. TTO is incorporated as the active ingredient in many topical formulations used to treat cutaneous infections. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments. most bacteria are susceptible to TTO at concentrations of 1.0% or less, MICs in excess of 2% have been reported for organisms such as commensal skin *Staphylococci*, *micrococci*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. TTO is for the most part bactericidal in nature, although it may be bacteriostatic at lower concentrations (Carson et al., 2006). The activity of TTO against antibiotic-resistant bacteria has attracted considerable interest, with methicillin-resistant *Staphylococcus aureus* (MRSA) receiving the most attention thus far (Hada et al., 2001). A report showed that microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus mirabilis*, *Listeria monocytogenes*, *E. coli* and *P. aeruginosa* were significantly inhibited by the EO of *P. halepensis*. This effect would be related to their main ingredients, especially caryophyllene, which is recognized for its antibacterial properties yet (Bouyahya et al., 2019).

In field trials with *Nicotiniana glutinosa*, plants were sprayed with 100, 250, and500 ppm TTO or control solutions and were then experimentally infected with tobacco mosaic virus. After 10 days, there were significantly fewer lesions per square centimeter of leaf in plants treated with TTO than in controls. In addition, by applying TTO at different stages in the virus replicative cycle, TTO was shown to have the greatest effect on free virus (prior to infection of cells), although when TTO was applied during the adsorption period, a slight reduction in plaque formation was also seen (Schnitzler, P., K. Schon; J. Reichling. 2001). Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture (Schnitzler et al., 2001). Some activity against bacteriophages has also been reported, with exposure to 50% TTO at 4°C for 24 h reducing the number of SA and T7 plaques formed on lawns of *S. aureus* and *E. coli*, respectively (Chand et al., 1994).

The application of 20 plant extracts and 13 different oils indicated that the extracts of *Geranium gruinum*, *Datura stramonium*, and *Mentha spicata* were the best antibacterial to reduce the growth of *B. thermophilus* with inhibition zones equal to 40, 39, and 35 mm, respectively. In contrast, the best active oils for inhibiting the growth of *B. thermophilus* were *Mentha spicata* and *Ocimum bacilicum,* with 50 and 45 mm inhibitory zone, respectively (Yousef et al., 2023).

Essential oils and plant extracts have shown the potential to protect and enhance the quality of pre and postharvest fruits owing to their antimicrobial properties. Some of the essential oils and plant extracts can be used to be formulated as organic fungicides to control diseases in preharvest fruits.

Essential oils and extract against Bacteria

Generally, Gram-negative bacteria are more resistant to essential oils (Eos) than Gram-positive bacteria (Trombetta et al., 2005). In gram-positive bacteria cell wall allows hydrophobic molecules penetrate the cells easily

and act on both the cell wall and cytoplasm. Phenolic compounds, which are also present in the EOs, generally show antimicrobial activity against gram-positive bacteria (Tiwari et al., 2009). Some EOs, such as those found in basil, sage, hyssop, rosemary, oregano, and marjoram, are active against *E. coli*, *S. aureus*, *B. cereus*, and *Salmonella* spp. but are less effective against *Pseudomonas* spp. due to the formation of exopolysaccharides that increase resistance to Eos (De Martino et al., 2009). The EOs of lavender, roses, geraniums, cloves, and rosemary are also able to inhibit Quorum Sensing (QS), whereas orange (*Citrus sinensis*) and juniper (*Juniperus communis*) EOs appear to have no anti-QS properties (Zaki et al., 2013). Investigations into the effects of different EO components are in progress. One of the most well-studied EO components is cinnamaldehyde (Niu et al., 2006).

The reasons of antibacterial effect of essential oils

Lipophily: It has been shown in numerous studies that one of the primary antibacterial action mechanisms is connected to the dissolving of essential oils components in the lipids of the bacterial cell membrane and mitochondria because of their lipophilic nature. This disrupts the cell membrane and causes ions to flow out, which causes bacterial cell death (Ksouda et al., 2019).

Hydrophobicity: According to several reports, antibacterial properties of the essential oils might be related to their hydrophobicity, which causes a rise in cell permeability and the subsequent leakage of cell components. The disturbance of cell walls and membranes, modification of ATP generation and protein synthesis, pH disturbance, intracytoplasmic alterations, DNA damage, and inhibition of quorum sensing are the most well-known targets of essential oils in bacteria (Cox et al., 2000).

Phenolic compounds and disruption of the cytoplasmic membrane: Mohammed and Al-Bayati (2009) reported that essential oils of *Thymus kotchyanus* aerial parts had the best inhibitory effect against *B. cereus*, *Klebsiella* *pneumoniae*, and *Escherichia coli*, while *Escherichia coli*, *Proteus mirabilis*, and *Staphylococcus aureus* were restrained with higher concentration of the essential oils. In general, phenolic monoterpenes including carvacrol, eugenol, and thymol are highly present in EOs with potent antibacterial effects against microorganisms. It seems logical that their operation will be similar to that of other phenolics, which is generally thought to involve disruption of the cytoplasmic membrane, disruption of the proton motive force (PMF), disruption of electron flow, disruption of active transport, and disruption of the coagulation of cell contents (Burt et al., 2004). Thymol can also break down the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS), and improving the cytoplasmic membrane's permeability to ATP (Mohammed and Al-Bayati, 2009).

Destruction of bacterial membranes: In *E. coli*, detrimental effects on potassium homeostasis, glucose-dependent respiration, morphology, and ability to exclude propidium-iodide have been observed. All of these effects confirm that TTO compromises the structural and functional integrity of bacterial membranes (Cox et al., 2001).

Production of reactive oxygen: Oxidative stress may be caused by the antioxidant properties of *Hibiscus rosa scinensis* phytochemicals, which can increase ROS production in microbial cells. Cell death may result from damage to proteins, lipids, and DNA, as well as other cellular components, from this stress (Alherabi et al., 2024). Another study showed that *Hibiscus* extracts can increase the production of reactive oxygen species (ROS) in certain bacterial strains, especially when they are stressed. An important mechanism for the antimicrobial effect of *Hibiscus* was impaired microbial viability (Tran et al., 2020).

Antifungal effect of essential oil and extracts

A recent study demonstrated the potential of Hibiscus extracts in the treatment of fungal infections, as a 10% Hibiscus extract achieved a MIC of 2 mg/ml against *C. albicans* (Touba et al., 2012). Clove oil significantly inhibited the growth of mycelium of the *Fusarium* species and reduced germination parameters than pine oil (Grzanka et al., 2021). The volatile oils of Fennel, Coriander, and Anise presented good antifungal effects against *Candida albicans* at different concentrations of 1%, 0.5%, and 0.25% respectively (Hammer et al., 1999). The volatile oils of Geranium, Japanese mint, Cinnamon, Clove, Ginger grass, and Lemongrass at different concentrations (0.01–0.15%) showed strong antifungal activities against *C. albicans* (Hammer et al., 2011).

The reasons of antifungal effect of essential oils

Changing cell permeability: Tee tree oil (TTO) alters the permeability of C. *albicans* cells. The treatment of *C. albicans* with 0.25% TTO resulted in the uptake of propidium iodide after 30 min (50), and after6 h significant staining with methylene blue and loss of 260 nm light absorbing materials had occurred. TTO also alters the permeability of *Candida glabrata*. Further research demonstrating that the membrane fluidity of *C. albicans* cells treated with 0.25% TTO is significantly increased confirms that the oil substantially alters the membrane properties of *C. albicans* (Hammer et al., 2004). scanning electron microscopy and transmission electron microscopy of C. gloeosporioides exposed to clove oil exhibited obviously deleterious morphological and ultrastructural alterations confirming the disruption of fungal cell wall and endomembrane system, which resulted in increasing in permeability and causing the loss of intracellular constituents (Wang et al., 2019).

Inhibition of respiration: As essential oils are generally lipophilic while plant extracts are typically extracted in organic solvents, the respiration rate of coated postharvest fruits may be significantly reduced due to the limited exchange of gases (Israfi et al., 2022). Tee tree oil inhibits respiration in *C. albicans* in a dose-dependent manner. Respiration was inhibited by approximately 95% after treatment with 1.0% TTO and by approximately 40% after treatment with 0.25% TTO. The 50% of respiration rate in *Fusarium solani* cultures is inhibited by a 0.023% concentration of TTO (Inouye et al., 1998).

Disruption the protein synthesis: Phenolic compounds in clove oil can inhibit the mycelial growth of *P. palmivora* by penetrating the fungal cell membrane and lipids. Hence, the compounds can access the cell's internal contents and disrupt the protein syntheses in the fungal cell (Ansari et al., 2013). Due to its ability to pass through the fungal cell membrane, limonene can disrupt protein synthesis in the fungus and subsequently inhibit fungal sporulation and germination (Phothisuwan et al., 2021).

Plant pathogenic

In the study of Samavati et al. (2022) EOs from *Cuminum cyminum*, *Lavandula angustifolia*, *Thymus daenensis*, *Trachyspermum copticum* and *Hyssopus officinalis* were evaluated for their inhibitory activities against *C. pseudonaviculata* Cy-08 in vitro. Quantitative and qualitative composition variations of the EOs were also analysed by GC–MS. Based on the high inhibitory efficacy and low related EC_{50}, MFC, and MIC values, *T. copticum*, *L. angustifolia*, and *Thymus Daenensis*, EOs can be mentioned as those providing extraordinary efficacy against Cy-08, respectively. Major chemical components of *T. copticum*, *L. angustifolia*, and *Thymus daenensis* EOs were thymol, linalyl acetate, and thymol respectively. Thymol and linalyl acetate may be the most effective compounds against *C. pseudonaviculata*.

Acorus calamus, *Ageratum conyzoides*, *Artemisia nilagirica*, and *Litsea cubeba* essential oil may be used as botanical pesticides for management of postharvest phytopatogenic fungal disease. However, further investigations are needed in light of pharmacological tests (Tripathi et al., 2016). The essential oil *of Cestrum nocturnum* L. had a remarkable effect on spore germination of all the plant pathogens with concentration and time dependent kinetic inhibition of *P. capsici*. The oil displayed remarkable in vivo antifungal effect up to 82.4 – 100% disease suppression efficacy on greenhouse-grown pepper plants. The results obtained from this study may contribute to the development of new antifungal agents to protect the crops from fungal diseases (Al-Reza et al., 2009).

The results of a study indicate that, the flower oil and leaf extracts of *M. liliflora* could be applicate as natural alternatives to synthetic fungicides to control the in vitro and in vivo growth of certain important plant pathogenic fungi (Bajpai and Kang, 2012).

Utilization of essential oils or plant extracts for increasing shelf life of fruits

Various formulations of coatings with essential oils and extracts have previously been studied such as nano-emulsions and biopolymer-based coatings (Elkhetabi et al., 2022). Some essential oils and plant extracts have the potential to be formulated into organic fungicides to prevent stem canker in durian trees by inhibiting the growth of *P. palmivora* as tested in vitro condition. A recent study shows that the vapor of clove and citronella oils can slow down *P. palmivora* growth in vitro (Istianto and Emilda, 2021). Complete inhibition of crown rot disease in bananas was recorded when treated with a 250 mg/ mL concentration of Zimmu (*Allium* spp.)leaf extract without altering organoleptic properties. The extract treatment also was found to have better fungicidal activity than the benomyl in reducing crown rot severity (Sangeetha et al., 2013). Other research showed that cinnamon oil inhibited 100% conidial germination of *C. musae*, *Fusarium incarnatum*, and *Fusarium verticillioides* at concentrations of 1025, 950, and 9088 mL/L respectively (Kamsu et al., 2019).

Vilaplana et al. (2018) reported that bananas treated with 500 mL/L thyme oil showed a 46.4% decay reduction compared to the commercially available fungicide Imazalil, which showed only a 29.4% decay reduction. *Aloe vera* incorporated with garlic oil inhibited 87.7% mycelial growth and 91.2% spore germination when tested in vitro against *C. musae*. The mixture was also tested as an antimicrobial coating, which was then tested to decrease the occurrence and seriousness of anthracnose by 92.5% and 81%, respectively (Khaliq et al., 2019). Methanol extract of garlic, *A. sativum*, has similar fungicidal activity as chemical fungicides like carbendazim and kanamycin B against crown rot. Spraying emulsions of basil oil on bananas was observed to control anthracnose and crown rot in bananas stored for 21 days. Interestingly, no significant differences were reported compared to benomyl treatment. It also did not affect the physicochemical and sensory properties of treated bananas (Jahan et al., 2019).

application of 0.08% (w/w) orange oil vapor in a closed air system completely inhibited *Marasmius palmivorus* and Thieviolopsis sp. growth on salacca (*Salacca zalacca*) fruit due to the presence of limonene and subsequently extended the shelf life up to 28 days (Phothisuwan et al., 2021). Khaliq et al. (2019b) reported that the formulation of 100% *Aloe vera* and 10 mg/mL *Fagonia indica* extract could be applied as a coating to extend the shelf life and preserve sapodilla during storage.

Pathogens in the food industry

Bacteria: Bos- kovic et al. (2015) tested the antibacterial activity of *Origanum vulgare* (oregano) EO against four pathogenic bacteria (*Salmonella enteritidis*, *Bacillus cereus*, *Salmonella typhimurium*, and *Staphylococcus aureus*) using broth dilution assay with the MIC values of 160–640 µg/ml against all strains. Carvacrol alters the membrane potential, depletes the intracellular ATP pool, and makes the cytoplasmic membrane more permeable to potassium ions in *B. cereus* (Boskovic et al., 2015). Clemente et al. (2016) applicate broth dilution method to demonstrate the antibacterial efficacy of *Cinnamomum zeylanicum* essential oil against grampositive and gramnegative bacteria. The antibacterial action of *C. zeylanicum* essential oil was exhibited by the MIC ranges of 100–400 g/ml versus all strains evaluated. Purkait et al. (2020) tested the antibacterial activity of the EOs obtained from of *Syzygium aromaticum*, *Piger nigerium*, and *Cinnamomum*

zeylancium against *B. cereus*. All EOs tested showed significant antimicrobial activity with various levels of inhibition. Moghaddam et al. (2014) investigated the inhibitory effects of *Ocimum ciliatum* EO on 10 bacterial strains, including *Ralstonia solanacearum*, *Rhodococcus fascians*, *X. oryzae* pv. oryzae, *P. syringae* pv. syringae, *Brenneria nigrifluens*, *Xanthomonas citri*, *Agrobacterium vitis*, *P. stewartii* subsp. indologenes, *P. syringae* pv. Lachrymans, and *P. tolaasii*. The EO GC and GC-MS results showed that the EO of this plant is mainly contains methyl chavicol (87.6%), methyl eugenol (2.6%), and 1, 8-cineole (1.7%). The results of antibacterial test showed that the oil has antibacterial activity against all tested bacteria, including *P. stewartii* subsp. indologenes.

Fungi: Data show that a range of yeasts, dermatophytes, and other filamentous fungi are susceptible to TTO (Griffin et al., 2000).

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Declaration

Authors have no conflict of interest to declare.

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