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Essential oils: A boon towards eco-friendly management of phytopathogenic fungi

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Abstract

Since the era of the beginning of the eco-friendly pest management, various plant-based products showed promising actions, and amidst them, essential oils obtained from the leaves, barks, stems and different parts of the plants proved to be a boon towards effective control of various phytopathogens. Essential oils are odoriferous, volatile organic compounds, consisting of a broad spectrum of secondary plant metabolites. Besides their antibacterial, insecticidal, antimicrobial activities, they have enormous potential to reduce the damage due to phytopathogenic fungus. In about 3000 essential oils identified, only 300 found to be economical, and a narrow spectrum of them has been used to manage fungi. As for as their mode of action is concerned, in fungal pathogens, essential oils establish a membrane potential across the cell wall and disrupt ATP assembly, cell walls and their permeability, leading to cell wall damage. Moreover, improvement in formulations like nano and microemulsions and stabilizing formulations has led to a new paradigm towards efficient control of plant pathogenic fungi. Therefore, the present review depicts the involvement of various plant essential oils in fungicidal and fungistatic activities for controlling plant diseases and also emphasizes on the modes of action of various essential oil products and their formulations available. It also reveals the constraints regarding the essential oil formulations which we have to eliminate for further betterment in eco-friendly phytopathogenic fungi management.

Keywords: Eco-friendly, essential oil, fungicidal, fungistatic, phytopathogen

Introduction

Crops being one of the most basic livelihood materials can be seriously injured by pests like insects, weeds, fungus, pathogens, vectors etc. and pesticides are the chemicals that are used to control or prevent them. Among the most used control methods are synthetic fungicides, but with high costs and environmental and toxicological risks for farmers. Moreover, the ardent search for the preservation of environment together with better ecological awareness has inspired the need of naturally occurring products, mostly of plant origin, aiming at the alternative tool for the management of phytopathogens ^[1]. For mitigating the problems mentioned above, new products derived from the plant's secondary metabolites are potentially useful. Many plants with pesticidal activity have been found for use in plant disease control, and also the raw material for the formulation of new alternative products ^[2]. Being the secondary metabolites, essential oils (EOs) can be extracted from various spices and herbs. There exist many literature reports on the antimicrobial activities of essential oils obtained from plant extracts, and these effects can be solely due to the presence of bioactive components ^[3-4]. More interestingly, it has been found out that these activities may be enhanced many folds only if the essential oil is applied in the form of small droplets (preferably in nano-range). Currently, essential oils are regularly studied for their antimicrobial, insecticidal, antifeedant, antifungal, antiulcer, antihelminthic, repellent, cytotoxic, antiviral, ovicidal, anaesthetic, molluscicidal, immunomodulatory, antioxidant, antiinflammatory, larvicidal and antinociceptive properties as well as for their use as food preservatives ^[5]. Various literature studies depicted the use of essential oils as antifungal agents against different food deteriorating fungi and stored food fungi. But, here the current review aims to depict the fungicidal and fungistatic action of various plant-based essential oils against phytopathogenic fungi which may cause a huge loss to the farming community every year. It also denotes the new paradigms of type of EO based formulations available for controlling fungi as well as the hindrance behind broad-spectrum development.

Essential Oils

Any kind of volatile oil/s having aromatic components and can give a difference in flavour, odour or aroma to a plant can be defined as Essential oils. These are basically by-products of plant metabolism, also known as volatile secondary plant metabolites. "Essential Oil" term is supposed to be derived from the term Quinta essentia coined by the Swiss reformer of medicine Paracelsus von Hohenheim in the sixteenth century, which means an effective component of a drug. Most of the essential oils can be found in glandular hairs or secretory cavities of the cell wall in plants and are also present as fluid droplets in leaves, barks, stems, roots, flowers, and fruits in different plants. Each of the essential oils can be considered to be a complex mixture consisting of terpenes (e.g. monoterpenes, diterpenes, sesquiterpenes, and terpenoids, their oxygenated derivatives, like phenols, alcohols, ketones, esters, aldehydes, ethers, and oxides), along with phenolic and phenylpropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways, respectively.

Source of EOs

Essential oils can be obtained through steam distillation of aromatic plants, especially those used as fragrances and flavourings in the perfume industries and food industries, respectively. And more recently they are being used for aromatherapy and herbal medicines. Plant essential oils are developed commercially on a large scale, usually from several botanical sources, most of which are the members of the mint family (Lamiaceae). Various sources of plants have been used conventionally for protection of the stored commodities, particularly in the Mediterranean region and in Southern Asia. Approximately 3000 EOs have been obtained from different plant species, but out of them, only 300 are found to be important economically and used in the sanitaryfragrance, pharmaceutical, food phytosanitary, and agricultural, industries ^[3]. There exists a long list of essential oils such as rose (Rosa damascene), patchouli (Pogostemon sandalwood (Santalum album), lavender patchouli), (Lavendula officinalis), geranium (Pelargonium graveolens), etc. that are well known for their perfumery and fragrance properties to be utilized in industry. Amidst other essential oils there are lemongrass (Cymbopogon winteriana), Eucalyptus (Eucalyptus globulus), clove (Eugenia caryophyllus), vetiver (Vetiveria zizanoides), rosemary (Rosemarinus officinalis), and thyme (Thymus vulgaris) etc. are famous for their pest management activities ^[6].

Chemistry and General Spectrum of Usage of Eos

EOs consist of some superior and inferior bioactive components which are mainly responsible for its biological activities. These kinds of effects can be attributed to either significant components or synergistic interaction between both superior and inferior chemical components. Chemically, essential oil comprises of 20-60 components out of which major components lie at relatively high concentration (20–70%), and rest are minor components present in trace amounts ^[3]. In most of the plants, the amount of essential oil found is in between 1 to 2%, but the widest range may vary from 0.01 to 10%. In certain plants or plant parts, one main essential oil constituent may predominate whereas in others it may be a cocktail of various terpenes or other metabolites. Terpenes consist of units of isoprene, which is a 5-carbon-base (C5) unit. Terpenes are actually the major building blocks of

monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) tetraterpenes (C40) and large sequences ^[4]. These oxygenated terpenes, i.e. terpenoids, constitute 80-90% components of EO and allow a large variety of structures. Generally, a varied range of secondary metabolites viz., terpenoids, acidic compounds (e.g., benzoic, cinnamic, myristic acids), alcoholic compounds (e.g., geraniol, menthol, linalool), aldehydes (e.g., citral, cinnamaldehyde, benzaldehyde, carvone camphor), phenols (e.g., ascaridole, anethole) and ketonic bodies (e.g., thymol, eugenol), and phenols (e.g., ascaridole, anethole) produce by aromatic and medicinal plants. Among these metabolites, terpenes (e.g., pinene, limonene, myrcene, terpinene, p-cymene), aromatic phenols (e.g., carvacrol, thymol, safrole, eugenol) and terpenoids (e.g., oxygen-containing hydrocarbons) are found to have key roles in the composition of several essential oils ^[6]. Derivatives of aromatic polyterpenoids and terpenoids are synthesized by the shikimic acid and mevalonic acid pathways. Some botanical ingredients such as azadirachtin, menthol, carvone, ascaridol, toosendanin, methyl eugenol, and volkensin have reported potential to act against several fungal and bacterial pathogens and also against insect pest [7-^{8]}. The aromaticity of essential oils can provide various functions for the plants like (i) attracting or repelling variety of insects, (ii) cold or heat wave protection and (iii) using oil's chemical constituents as defence materials. Many of other essential oils have different utilities such as food additives, flavourings, and components of cosmetics, soaps, perfumes, plastics, and also resins etc. Being liquid at average temperature these essential oils can turn into gaseous substances at a slightly higher temperature.

There are many EOs that are isolated and visible around the world. Equilibrium estimates and EO values are obtained using GC-MS^[9]. Differences in their structure result in different organoleptic properties of EOs. Similarly, the differential odour of betel leaf EO cv. Tamluk Mitha is because of the presence of low molecular weight chemical compounds that include chemicals, especially terpenes, terpenoids, and phenolic compounds ^[10]. The characteristic nature of EOs provides a clear view of their biological activity due to the presence of different bioactive chemical compounds.

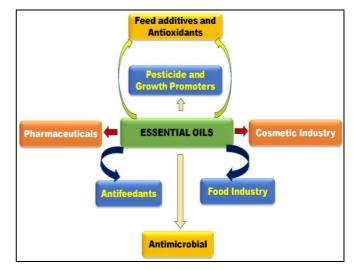


Fig 1: Spectrum of uses of Essential Oils

Antifungal Effects of EOs

Essential oils and their derivatives are used to manage the

wide range of plant pathogenic microbes. Essential oils found in several plants such as basil, fennel, citrus, lemongrass, rosemary, oregano, and thyme have depicted effective antifungal potential against a wide variety of flora pathogens ^[11]. Arora and Kaur ^[12] found that garlic and clove ingredients inhibited the proliferation of Candida acutus, Candida tropicalis, Candida apicola, Candida catenulate, Candida albicans, Candida inconspicua, Saccharomy cerevisiae, Rhodotorula rubra, and Trigonopsis variabilis. Ultee and Smid^[13] have reported that the essential oils of oregano and thyme contain phenolic composites (carvacrol and thymol) as key constituents, disrupting the fungal membranes and proving to be excellent antifungal agents. The antifungal properties of essential oils and their availability in cell function, plant growth, and mycotoxin production capacity have been studied ^[14]. Isothiocyanate found in onions and garlic has been found to be effective against Botrytis, Fusarium, fungus, and Cladosporium ^[15]. Nguefack et al. (2004)^[16] have shown complete inhibition of mycelial growth and conidial germination of three fungi (Fusarium moniliforme, A. fumigatus, A. flavus) in cornmeal agar using Ocimum anodissimum (African basil), Cymbopogon citratus (lemongrass) and Thymus vulgaris (thyme) EOs at 800, 1200, and 1000 ppm, respectively. Vági et al. (2005) [17] investigated the ineffable properties of Origanum greatana L. EO against three food allergens (A. niger, Trichoderma viride, Penicillium cyclopium). Viuda-Martos et al. (2008) [18] studied on complete mycelial inhibition of A. niger and A. flavor at a dosage of 0.94% lime, orange, mandarin and grapefruit EOs. They also reported high efficacy of grape EOs against Penicillium verrucosum and P chrysogenum, orange EO against A. niger and mandarin EO against A. flavus. Complete inhibition of hyphal growth and H⁺-ATPase activity of A. flavus using Lippia rugosa (Gossolhi) EO with 1000 ppm concentration was reported by Tatsadjieu et al. (2009) ^[19]. Prakash et al. (2010) ^[20] reported a mycelial growth reduction of A. flavus at 0.7 µl / ml of Piper betle L. cv. Magahi EO. The incredible growth of five food moulds such as A. flavus, A. oryzae, A. niger, Alternaria alternata was completely prohibited using a concentration of 5 µl / ml of Cicuta virosa L. var. Latisecta Celak EO^[21]. Combrinck et al. (2011) ^[22] reported thyme oil (when added to concentrations at 1000 µl / l and below), completely inhibits all bacteria except Penicillium strain and it also stood as a potentially effective inhibitor out of 18 essential oils when tested against five fungal strains (Lasiodiplodia theobromae, Alternaria citrii, Colletotrichum gloeosporioides, Botrytis cinerea and Penicillium digitatum). While, except for L. theobromae from avocado essential oil of Lippia citriodora was effective against all pathogens. Growth of P. expansum has been reported to be inhibited by using pomelo EO as compared to the four different EOs from citrus fruits such as orange, mandarin, pomelo, and lime ^[23]. Tao et al. (2014a) ^[24] established complete inhibition of P. italicum and P. digitatum at 2,5 and 40 µl / ml concentration of Citrus reticulata Blanco (ponkan) EOs. Kohiyama et al. (2015) [25] evaluated the antifungal effect of Thymus vulgaris EO against A. flavus. Inhibitory Concentration and ergosterol Minimum biosynthesis of A. flavus was restricted to 100 and 250 µg / ml of thyme EO. The antifungal activity of five EOs of anisum, Thymus vulgaris, Pimpinella Pelargonium odoratissimum, Rosmarinus officinalis and Foeniculum vulgare have been tested against specific pathogenic fungal strains of cereals e.g. Oculimacula yallundae, Microdochium

nivale, Zymoseptoria tritici, Pyrenophora teres and Fusarium culmorum ^[26]. Li *et al.* (2016) ^[27] reported that fumigation of *Litsea cubeba* essential oil with minimum inhibitory concentration and minimum fungicidal concentration of about 0.5 and 1.0 µl/ml can effectively control *A. flavus.* The antifungal activity of clove, thyme, oregano, lemongrass essential oil vapour was demonstrated against *A. flavus, A. parasiticus,* and *A. clavatus* by Božik *et al.* (2017) ^[28].

Medeiros et al. 2016, ^[29] performed a study against two wood decay fungi (Gloeophyllum trabeum and Trametes versicolor) to show the effectiveness of two species i.e. Brazilian savannah (Lippia origanoides Kunth and L. lacunosa Mart. & Schauer) and from clove [Syzygium aromaticum (L.) Merr. & L. M. Perry]. Results revealed that in any concentration the essential oil from L. origanoides showed the highest fungicidal activity against *G. trabeum* and *T. versicolor*. Gucwa et al. 2018, ^[30] reported that several essential oils (EOs) such Citrus limonum, Thymus as graveolens, vulgaris, Ocimum basilicum, Pelargonium Cinnamomum cassia, and Eugenia caryophyllus which are distributed by the company Pollena Aroma(Nowy Dwór Mazowiecki, Poland) were studied against a group of 76 clinical isolates of of C. glabrata and 183 of C. albicans. All of these oils revealed both fungistatic and fungicidal activity toward C. albicans and C. glabrata isolates. Farzaneh et al. (2015) [31] showed that essential oils of three Satureja species (S. hortensis, S. spicigera, and S. khuzistanica) could have fungicidal and/or fungistatic effects on postharvest pathogens of strawberry. They showed that in fungicidal activity, S. khuzistanica was the most potent oil. Against B. cinereal. S. hortensis oil was more effective than S. spicigera, and S. spicigera oil showed stronger fungicidal activity against R. stolonifer. Further, Laurus nobilis EO has antifungal activity against C. albicans probably due to monoterpenes and sesquiterpenes in its composition ^[32]. Recently a study was conducted to reveal fumigant antifungal activity of essential oils of 10 Lamiaceae plants against two phytopathogenic fungi, Raffaelea quercus-mongolicae, and Rhizoctonia solani. Among the tested essential oils, summer savoury (Satureja hortensis) and thyme white (Thymus vulgaris) essential oils exhibited the strongest fumigant antifungal activity against the phytopathogenic fungi via generation of reactive oxygen species (ROS) [33]. In a report of the antifungal and antiaflatoxigenic activity of Rosmarinus officinalis L. essential oil (REO) against Aspergillus flavus Link showed that REO reduced the mycelial growth of A. flavus at a concentration of 250 µg/mL by reducing the size of conidiophores and thickness of hyphae ^[34]. Moreover, the essential oils of Amazonian aniba, A. canelilla and A. parviflora. containing 1-nitro-2-phenylethane and linalool, have displayed remarkable antifungal effects against Aspergillus flavus, Aspergillus niger, Fusarium oxvsporum. Fusarium solani, Alternaria alternata. Colletotrichum gloeosporioides, Colletotrichum musae and Colletotrichum guaranicola [35]. Again, Mentha haplocalyx Briq. and Schizonepeta tenuifolia Briq. essential oils (EOs) were found effective against Panax notoginseng (Burk.) F.H. Chen disease [36]. Antifungal activity against synthetic fungicide resistant fungi were also evidenced as oregano and clove essential oils and their main components carvacrol (thymol, and eugenol) against Penicillium digitatum, as well as against isolates of Penicillium italicum and Geotrichum candidum resistant to imazalil or guazatine [37]. The effect of cinnamon, palmarosa,

orange, and spearmint EOs was shown to be the most potent in inhibiting the growth of *Fusarium* fungi as well as reducing the concentration of mycotoxins in maize seed as reported by Perczak *et al.* (2019) ^[38]. Geographical location also can help in differing the fungicidal activity of essential oils of the same species. Such an example like a study compared crucial oils obtained from fennel fruits cultivated in Poland (FEOPOL) and Egypt (FEO-EG) and tested *in vitro* against ten species of pathogenic fungi. The best result for FEO-POL was achieved against *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Botrytis cinereal*, whereas the antifungal activity of FEO-EG against tested fungi was weak or none ^[39].

The presence of volatile elements makes EO more efficient in the vapor phase than the liquid phase, making them a natural fumigant that can be used in the protection of stored products. The preliminary test used to measure the antifungal activity of EOs and plant-specific genes prior to the more detailed studies is the disc diffusion and disc volatilization assay ^[40]. EOs can have a static (inhibitory) or cidal (killing) effect on a range of microorganisms. The MIC of EO varies from one microbe to another, and its appearance of antifungal activity depends on the extent of the active microorganism. Various test principles were adopted and adjusted by various investigators to suit their specific conditions. Many factors such as methods used to extract EOs, inoculum volume, inoculum collection, the culture used, intermediate pH and inclusion affect the result of a test that detects Minimum inhibitory concentration (MIC)^[3].

Mode of Action

Essential oils can have the potential to destroy major fungal pathogens. Besides fungicidal and fungistatic effects, their mechanism of action varies in different cases as provided in most of the literature reports, the cytotoxic status of EOs in their environments is due to their ability to disrupt cell wall and cell formation, cytoplasmic stability, and the impact of cellular cell damage and macromolecule escape [3, 41]. The lipophilic properties of EOs allow them to enter through the cell wall and damage to the cytoplasmic membrane while disrupting various layers of fatty acids, polysaccharide, and phospholipids as well as increasing the permeability ^[40, 42]. Hydrophobic elements present in EO can alter the permeability of microbial cell membranes of strands such as H⁺ and K⁺, which alter protrusion, modulate cell pH and affect the chemical composition of cells and their function ^{[41,} ^{43]}. Essential oils can alter saturation and thus result in an imbalance in intracellular osmotic pressure, which in turn disrupts intracellular organelles, leaks cytoplasmic content and sometimes energy-retaining molecules (ATP) and ultimately disrupts the cell. EOs can deplete the mitochondrial membrane by reducing membrane potency affecting ionic Ca2 + cycling and other ionic channels and reducing the pH gradient of microbial cells [44]. In some fungi and other

malignant gram-positive microorganisms, being sensitive to iminazole and store in their cells unsaturated fatty acids, their arrangements of membrane structure leads to loss of cell function and, ultimately, lysis. Some of the essential oils like thymol, carvacrol etc. have a potential to inhibit the efflux pump as well as they may block the formation of beta-glucans and alter sterol biosynthesis pathway thereby breaking the integrity of cell walls and cell membranes inside fungi [45]. Han et al. (2019) ^[46] found that higher proportions of sesquiterpenes and monoterpenes essential oils of Chrysanthemum could be responsible for the inhibitory activity against Phytophthora nicotianae, by increasing mycelia membrane permeability and the content of mycelial malondialdehyde (MDA), finally resulting to cell death. In some study, scientists noticed that treatment of Mentha cardiaca L. essential oils with a rate of 1.25 and 2.5 µl/ml can enhance the leakage of Ca^{2+} , K^+ and Mg^{2+} ions from the A. flavus LHP-PV-1 cell membranes [47]. Rasooli et al. (2006) [48] have revealed that A. niger when treated with an inhibitory concentration of Thymus eriocalyx and Thymus x-porlock essential oils, brings drastic damage to the cell wall, cell membrane and cellular organelle mainly mitochondrial destruction. Some sort of serious morphological alterations in the hyphae of tomato late blight disease agent (Phytophthora infestans) were observed such as vacuolations, cytoplasmic coagulation, hyphal shrivelling and protoplast leakage when they are being exposed to volatile as well as contact phase of the EOs obtained from an aerial part of several aromatic plants ^[49]. Moreover, TEM images of A. *flavus* cells treated with Ageratum convzoides EO showed dramatic changes in ultrastructure of the endomembrane, especially plasma membrane and mitochondria ^[50]. They observed that the internal structure of mitochondria was disrupted showing a decreased ridge polarization in crystae whereas plasma membranes became rough, villiform, invaginated vesicles and sometimes decoupled from the cell wall of the treated mycelial cells. Some of the essential oils depicted complete autolysis of cell at the highest fungistatic concentrations. This kind of autolysis involved depletion of hyphal cytoplasm and membranous organelles including endoplasmic reticulum, nuclei, and mitochondria along with dishormonization of the cell structure. Further, SEM and TEM images of P. italicum treated with citral showed plasmalemma disruption and cytoplasmic disorganization along with collapsed and distorted hyphae^[51]. Recently, a study showed that *Thymus camphoratus* oil, rich in 1,8-cineole and α -pinene along with Thymus carnosus oil with high amounts of borneol and camphene were active against *Cryptococcus* highly neoformans and dermatophytes and very effective in inhibiting C. albicans germ tube formation as well as disruption of preformed C. albicans biofilms [52].

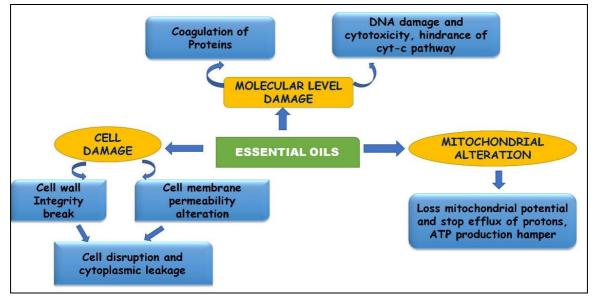


Fig 2: Mode of Action of Essential Oils on Fungal Strains

Recent Development in EO based Formulations

Encapsulating essentials oils using nano emulsified systems has emerged as a potential application in nano pesticides product delivery for microbial management in control released manner. Essential oil delivery using nano emulsified systems has a powerful application in the product of pesticide delivery systems as opposed to enabling extruded emissions. The various types of EO delivery are made up of two phases of nanocarriers, namely, (1) the formation of polymeric nanoparticulate, which has led to significant improvements in the important antimicrobial function, and (2) lipid carriers, including liposomes, strong lipid nanoparticles, efficient lipid particles, and nanoemulsions^[53]. Alternatively, there are a limited number of research studies reported in the current literature on the application of EOs based microemulsion for antimicrobial activity. Moreover, Basak and Guha (2017a) ^[54] promoted the antifungal activity of microemulsified EO of betel leaves against A. flavus in tomato paste. They also produced the same effects using betel leaf EO based microemulsion against P. expansum apple juice ^[55]. Recently, Ribes et al. (2018) [56] proposed a deduction in the amount of bark cinnamon EO by 25% to inhibit A. niger growth particularly when they are being used in combination with an antifungal agent (viz. Zinc gluconate or trans-Ferulic acid) in an oil-water emulsion to control spoilage of the strawberry jam. The Ultrasonication emulsion method has the advantages of producing clove oil nanoemulsions such as a green insecticide with a nanoemulsion droplet size of 43 nm^[57]. Ribes et al. (2017) [58] have shown enhanced antifungal activity of a combined cinnamon leaf containing emulsifiers such as whey protein isolate and polysorbate 80 against A. indication of niger. The antimicrobial EO-based nanoemulsion varies by emulsion formation. These models have some limitations, e.g., high power emulsion is required to prepare nanoemulsions and a large amount of solvent needed to form microemulsions. However, they are designed to control various plant pathogens, including insects, fungi, germs etc.

Major Constraints

Investigations on essential oils are carried out worldwide, which inspired researchers to focus on botanical antimicrobials. Essential oils are very effective in controlling

a broad number of pathogens, and the usefulness of these oils are broadly described. But some of the major hurdles are there to be sorted out for effective control pathogens. For example, very few of the essential oils are genotoxic and cytotoxic to non-target organisms which should be of great concern. Moreover, most of the natural products are structurally complex mixtures. Basically, isolation and separation of those complex mixtures using normal chromatographic techniques are not easy to do the job. Their sustainable and commercial uses have some drawbacks, such as their cost-effectiveness, photo and thermo-instability, difficulty in standardization etc. Furthermore, they have a less residual effect and need to be sprayed again and again. Farmers cannot handle such a huge number of spraying schedules, and less residual action added an extra disadvantage to be accepted by farmers. Methanol, eugenol, carvacrol, linalool are some of the essential oils have also considered causing lipid peroxidation, oxidative stress, cytotoxic and genotoxic damage to non-target organisms. Finally, essential oil formulations study for efficient control of broader spectrum of phytopathogenic fungi is deficient, and a further improvement in structural stabilization and commercialization is the need of an hour to proceed towards the permanent eco-friendly phytopathogenic fungi control.

Conclusion

The current review depicts the antifungal activities of the essential oils obtained from the plants. Essential oils are regarded as GRAS (generally regarded as safe) grade chemicals. The U.S. Food and Drug Administration (FDA) reported that; there are no toxicity issues associated with most of them. Additionally, because of their pleasant fragrance, they can even be used in food applications. But mere laboratory analysis and formulations cannot make up the demand for controlling pathogenic fungi in an eco-friendly way. Industrial manipulations and extensive manufacturing are needed. Furthermore, using botanicals and natural products along with essential oils can surely help in forming a suitable balance between green chemistry and pesticide chemistry. Again, enhanced efficacy, cost reduction as well as proper standardization of product spectrum and efficient regulatory approval of natural plant-based essential oils should be implemented for a green and sustainable world.

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