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**USE OF *CYMBOPOGON CITRATUS* ESSENTIAL OIL IN FOOD PRESERVATION:
RECENT ADVANCES AND FUTURE PERSPECTIVES**

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ABSTRACT

The economic burdens and health implications of food spoilage are increasing. Contamination of food sources by fungi, bacteria, yeast, nematodes, insects, and rodents remains a major public health concern. Research has focused on developing safer natural products and innovations to meet consumers' acceptance as alternatives to synthetic food preservatives. Many recent novel preservative techniques and applications of both natural and synthetic origin continue to proliferate in food and chemical industries. In particular, some essential oils of plant origin are potent food preservatives and are thus attractive alternatives to synthetic preservatives. This paper provides an overview of recent advances and future prospects in assessing the efficacy of the use of *Cymbopogon citratus* (lemongrass) essential oil in food preservation. The possible mechanisms of action and toxicological profile as well as evidence for or against the use of this

essential oil as an alternative to synthetic food preservatives in domestic and industrial applications are discussed.

Keywords

Lemongrass essential oil, shelf life, food spoilage, microbes

INTRODUCTION

Foodborne illnesses are highly prevalent worldwide. The majority have a known etiology, while others remain idiopathic (Tauxe et al., 2002). Contamination of food sources by fungi, bacteria, yeast, nematodes, insects, rodents, etc. remains a major public health concern. A substantial percentage of the general population in most countries has suffered from foodborne diseases at some point (Soares et al., 2013). For example, 25% of Americans are estimated to experience a significant foodborne illness annually (Tauxe et al., 2002). Thirty-one species of pathogens cause 9.4 million cases of food borne illness in the United States annually (Scallan et al., 2011); the incidence could be double or triple in most developing countries worldwide. For example, molds extensively damage foods, feeds, and other agricultural commodities in the field, during transportation, storage, and processing, leading to post-harvest losses. These molds also are known to destroy 10–30% of perishable crops in developing countries, reducing food quantity and quality (Agrios, 1997; Mataysoh et al., 2011). The activities of these pathogens result in the production of compounds toxic to humans and animals or compounds that devalue the physiochemical characteristics of foods including taste, smell, and texture. In agriculture, the market value and shelf life of stored foods depreciates, resulting in potential large-scale losses. The proliferation of emergent multidrug-resistant organisms warrants the urgent need to improve current food preservation techniques. Multidrug-resistant organisms acquire more virulent characteristics, making them immune to antibiotics; for example, the ability to form protective biofilms (Chiemielewski and Frank, 2003).

Food spoilage can also be caused by chemical and enzymatic auto-oxidation of lipids within the food substrate, resulting in food rancidity, especially in poultry, fish, meat, and dairy products (Dave and Ghaly, 2011). Large quantities of meat and poultry products are lost each year not only to microbial spoilage, but chemical deterioration as well (Kantor et al., 1997; Sanchez-Ortega et al., 2014). If a fraction of the amount of food lost to spoilage is preserved, it could meet the animal protein needs of a sizeable percentage of the global population (Cereveny et al., 2009). Many novel preservative techniques and applications of compounds of both natural and synthetic origin continue to proliferate in food and chemical industries. However, the increasing negative consumer perception of synthetic food preservatives, generally because of their salient toxic effects, is widening the search for alternative food preservation methods that are natural, safe, and less toxic, particularly preservatives derived from herbs and plant products, in order to replace the synthetic chemical compounds in current use (Hammer et al., 1999; Hervey, 2008) or even under restriction by relevant regulatory bodies (Guynot et al., 2003; Pacor et al., 2004; Wroblewska, 2009). The use of herbs and plant products as antimicrobial/antioxidant food preservatives has gained traction among researchers over the past few decades owing to their desirable characteristics including availability, fewer adverse effects, and diminished toxicological profile and systemic bioavailability compared to other available antimicrobial agents (Sokovic and Glamoclija, 2013). In particular, some essential oils of plant origin are potent food preservatives and are attractive substitutes to synthetic preservatives. Many of these essential oils are relatively easy to obtain, have low mammalian toxicity, and degrade quickly in water and soil, making them relatively environmentally friendly (Isman, 2000) and prompting their use in food preservation and processing. Among candidate essential oils with food-

preserving potential is that from *Cymbopogon citratus* Stapf (lemongrass) (Bakkali et al., 2008; Vazirian et al., 2012; Tyagi et al., 2014; Boukhatem et al., 2014). *C. citratus* is a perennial herb that grows worldwide, especially in the tropics and subtropics (Negrelle and Gomes, 2007; DOAFF, 2012).

C. citratus extract and essential oil have been used for decades due to their widely reported pharmacological and therapeutic activities (Nguefack et al., 2004a, 2004b, 2009; Ekpenyong et al., 2014a, 2014b). They have also been applied in food flavoring, cosmetics, and food and industrial preservation (Negrelle and Gomes, 2007; Millezi et al., 2013; Boukhatem et al., 2014).

Recent investigations suggest the possibility of the use of *C. citratus* essential oil (CCEO) as an effective inhibitor of biodegrading oxidation and storage-contaminating microbes. Accordingly, this paper reviews recent advances and future prospects in assessing the efficacy of CCEO in food preservation. The possible action mechanisms, toxicological profile, and evidence for and against the use of CCEO as an alternative to synthetic food preservatives in domestic and industrial applications will also be discussed.

Chemical Composition of CCEO

The bioactivity of any plant species is attributed to its physiochemical constituents. If these constituents are secondary metabolites, they are often essential in many processes affecting plant growth, development, and environmental interaction. Essential oils are among the many secondary metabolites of physiological importance isolatable from plant tissues. *C. citratus* bears an essential oil that is responsible for its rosy–lemony smell and taste (Ganjewala and Luthra, 2010). Essential oils can be obtained from various plant parts including the leaves, stalk, flowers,

and roots by using various extraction methods; however, they vary with respect to species and other factors (Ganjewala and Luthra, 2010). Many studies show that the chemical composition of CCEO varies by geographic region, genetic differences, the part of plant used for extraction, the method of extraction/experimental conditions, the age/stage of maturity, the season of harvest, and even the health status of the plant (Boruah et al., 1995; Chisowa et al., 1998; Ganjewa, 2009; Shah et al., 2011; Bharti et al., 2013; Ekpenyong et al., 2014a, 2014b). The major chemical constituent of CCEO is citral, which is a mixture of geranial and neral that constitutes approximately 70–85% of the total oil content (Opdyke, 1979). Other monoterpenes present include myrcene, geraniol, citronellol, limonene, 1, 8-cineole, nerol, α -terpineol, burneol, eugenol, elemicin, and geranyl acetate. Furthermore, fumesol, furfural, isopulegol, isovaleric aldehyde, L-linalool, β -caryophyllene, and valeric esters have also been isolated, and many others still under investigation (Opdyke, 1979; Akhila, 2010; Bharti et al., 2013). These essential oils are biosynthesized via the classical cystolic acetate-MVA and plastidic MEP pathways; the oils accumulate in the adaxial side of the leaf mesophyll, commonly adjacent to a non-photosynthetic tissue and between vascular bundles (Lewinsohn et al., 1998; Luthra et al., 1999). The synergistic activities of these essential oil constituents contribute to the overall bioactivity of *C. citratus* and its use in medicine and agriculture allied industries.

Food Contamination and Deterioration

Food products are rich nutrients sources that attract bacterial, viral, and fungal colonizers (Dijksterhuis et al., 2013) in addition to other pests such as insects and rodents. These organisms, particularly microbes, thrive in environmental conditions that favor their growth and virulence.

For example, in foodstuffs with low pH, low water activity, or high carbohydrate content, spoilage bacteria are usually not present, because such environments do not favor their growth (Krisch et al., 2011). However, food spoilage yeasts and molds can grow under these conditions and deteriorate various foods products such as fruit and vegetable juices and purees, soft drinks, pickled vegetables, dairy products, bread, dried fruits, sausages, etc. (Krisch et al., 2011). They also attack sprouted seeds and unpasteurized juices and milk. In addition, the activities of these spoilage microbes can cause pH changes, slime formation, structural component degradation, unpleasant odors, and appearance changes in meats (Krisch et al., 2011; Dave and Gharly, 2011).

Kumar et al. (2011) found that *Bacillus*, *Klebsiella*, and *Pseudomonas* are the dominant genera responsible for the spoilage of every category of food material. In fruits, vegetables, and meats, *Klebsiella* are potent spoilage bacteria. Furthermore, in meats, milk, and fatty products, *Pseudomonas* spp. is found in most of the spoilage. Kumar et al. also found that although *Klebsiella*, *Pseudomonas*, and *Bacillus* are dominant in spoilage, *Escherichia coli*, *Staphylococcus* spp., and *Micrococcus* spp. were also recovered from several categories of spoilage samples. Moreover, the reviews by Raybaudi-Massalia et al. (2009) and Barth et al. (2009) indicate that other bacterial genera and species such as *Salmonella*, *Listeria monocytogenes*, *Shigella*, *Xanthomonas*, and *Acidovorax* cause spoilage in both fresh cut fruits and fruit juice.

Further research shows that besides bacteria-induced fruit and vegetable spoilage, various mold species including *Botrytis cinerea*, *Ceratocystis fimbriata*, and *Rhizoctonia solani* as well as various species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Eurotium*, *Phomopsis*,

Fusarium, *Mucor*, *Paecilomyces*, *Penicillium*, *Phoma*, *Phytophthora*, *Pythium*, and *Rhizopus* are involved in the fungal spoilage of fresh fruits and some processed derivatives including those thermally processed (Lund and Snowdon, 2000; Tournas et al., 2005; Raybaudi-Massalia et al., 2009). In addition, the occurrence of yeast species such as *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Aureobasidium pullulans*, *Candida diversa*, *Pichia fermentans*, *Pichia kluyveri*, *Pichia anomala*, and *Hansenula polymorpha* showed fruit juice spoilage potency in the study of Tyagi et al. (2014a). Similarly, the occurrence of yeast species such as *Saccharomyces* spp., *Cryptococcus* spp., and *Rhodotorula* spp. in fresh fruits as well as *Candida* spp., *Debaryomyces* spp., *Hanseniaspora* spp., *Pichia* spp., and *Zygosaccharomyces* spp. in dried fruits was reported by Jay et al. (2005). Several genera of yeast can be found on fruits and vegetables, because yeast grow faster in such environments than molds; moreover, yeast often initiate fruit spoilage, while molds finish the process of complex polysaccharide degradation in cells walls and rinds (Anonymous, 2014).

Stored grains such as rice, groundnut, sorghum, melon, cowpeas, maize, rye, wheat are more prone to attack by fungal species such as *Alternaria* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., and *Macrophomina* spp. (Adegoke and Odesola, 1996; Paraganama et al., 2003; Somda et al., 2007; Bankole and Joda, 2004; Mataysoh et al., 2011; Krisch et al., 2011) while tuber crops such as Irish potatoes, cassava, and yam are attacked mostly by mildew causing *Phytophthora* spp. and *Fusarium* spp. among others (Kabera et al., 2013). Ismail et al. (2012) isolated the phytopathogenic bacteria *Erwinia carotovora* subsp. *carotovora* associated with soft rot disease in girasole tubers, which could also affect other tubers. However, softening could also be caused by *Pseudomonas* spp. or even endogenous enzymes (Anonymous, 2014). *Penicillium*

species such as *P. commune*, *P. chrysogenum*, and *P. nalgiovense* are associated with spoilage in cheese, fermented sausages, and salamis (Frisvad et al., 2007; Krisch et al., 2011). However, this list of spoilage bacteria and fungi extends far beyond this short overview; many more have been reported in other studies such as Singh et al. (2011) and Dijksterhuis et al. (2013).

How do these microbes cause spoilage? Harmful microbes attack fresh, raw, and processed foods. Food spoilage microbes enter through existing cracks, wounds, or any open orifice and thrive in food substrates. Food spoilage microbes enter processed foods via multiple access routes either during processing, spicing, or even during storage. Many food substrates, especially fruits and vegetables, present ideal conditions for the survival and growth of many microorganism species. In fruits and vegetables, microbes, especially fungi, thrive owing to the rich nutrient medium and prevailing near-neutral pH, while natural acidity serves a barrier against some bacterial spoilage microbes (Barth et al., 2009). As these spoilage organisms explore their host, they also exploit them by using extracellular lytic enzymes that degrade host polymers. For example, fungi in particular produce an abundance of extracellular pectinases and hemicellulases (Miedes and Lorences, 2004). Colonization occurs after invasion. Fungi reproduce via spore/conidia production and dissemination; when airborne spores are released and come into contact with food, they form clusters of spores termed propagules. These propagules then enter foods where they cause damage (Dijksterhuis et al., 2013). Fungal colonization is initiated by the deposition of conidia on the product, which subsequently progress from an inactive (i.e., vegetative) to an active (i.e., reproductive) stage (i.e., germination). These transitions are encouraged by supportive environmental factors such as water, temperature, osmolarity, and nutrients such as phosphate, amino acids, and glucose (Thanh and Nout, 2004;

Thanh et al., 2005). Filtenberg et al. (1996) report that the germination of *Penicillium digitatum* conidia is stimulated by a certain combination of volatile chemicals surrounding wounded oranges, notably limonene, β -pinene, sabinene, β -myrcene, acetaldehyde, ethanol, and CO₂. Another biochemical, 1-octen-3-ol, produced by microbes such as *Aspergillus nidulans*, *Penicillium paneum* (Chitaria et al., 2004; Herrero-Garcia et al., 2011), and *Aspergillus niger* (Karlshoy et al., 2007), is hypothesized to act as a self-inhibitor that prevents the premature germination of conidia on conidiophores (Dijksterhuis et al., 2013).

Upon landing on a food substrate, if the environment is favorable, germ tube germination occurs, followed by radial or linear germ tube growth. Mycotoxins are produced by these harmful fungi, creating further damage and contamination, which can cause serious health hazards (Dijksterhuis et al., 2013; Pawlowska et al., 2012; Matasyoh et al., 2011). On the other hand, upon invading and infecting the food substrate, spoilage bacteria can cause various physiochemical changes, resulting in sliminess, souring, greening, or soft rot spoilage of the food substrates (Anonymous, 2014). The degree of spoilage depends on the type of food substrate and bacteria involved.

Besides microbes, certain insects exert strong qualitative and quantitative post-harvest destructive effects on both fresh and stored foods. Such insects bore into and thus reduce the quality of stored foods such as tubers and grains. Because of their voracious appetites, these insects disfigure the appearance of stored foods, thereby reducing both market value and shelf life. There are several such pests including specific species of borers, moths, beetles, worms, and weevils (Bode and Calvin 1990; Danielson et al 1991; 1993). In addition to microbial food spoilage, food rancidity is also attributed to the auto-oxidation of fat components and catalytic

activities of enzymes, which could lead to oxidative deterioration in meat and other fresh food, resulting in the emission of odors (Dave and Gharly, 2011). However, although most microorganisms have developed stress resistance mechanisms such as osmotolerance, intracellular protective compounds, and heat resistance (Dijksterhuis et al., 2013), known effective preservatives exert toxic effects on them. The food preservation mechanism of CCEO is discussed below

ANTIMICROBIAL FOOD PRESERVATIVE ACTIVITIES OF CCEO

Antibacterial Activity of CCEO

CCEO has been experimentally tested on both Gram-positive and Gram-negative bacteria in many studies. Some of these bacteria are sensitive to the antibacterial effect of CCEO, while others are resistant. The organisms include *Bacillus cereus*, *Bacillus subtilis*, *E. coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus*, *Salmonella choleraesuis*, *L. monocytogenes*, and *Pseudomonas fluorescens* (Adegoke and Odesola, 1998; Naik et al., 2010; Falcao et al., 2012; Singh et al., 2011; De Oliveira et al., 2010; Soares et al., 2013). Most studies show that CCEO more strongly affects Gram-positive bacteria than Gram-negative bacteria. The higher resistance manifested by Gram-negative bacteria may be associated with the presence of hydrophilic lipopolysaccharides on their outer membrane, which could create an impermeable barrier despite the lipophilic/hydrophobic nature of essential oils (Poole, 2002; Nakaido, 2003; Saha et al., 2008). CCEO added to other antibiotic vehicles (e.g., phenoxyethanol and stannous chloride) is effective against most multidrug-resistant bacterial strains such as *E. coli*, *S. aureus*, and *P. aeruginosa* (Onawunmi, 1988; Melo et al., 2001; Duarte et al., 2006).

Many recent studies aimed to elucidate the mechanism underlying the antibacterial activities of CCEO and the key components responsible for these actions. These antibacterial actions parallel cytotoxic activity, suggesting a common mode of action that is evidently membrane based. Many studies implicate the terpenoid components of the CCEO in its antibacterial action; in particular, citral, geranial (α -citral), and neral (β -citral) are effective (Onawunmi et al., 1984; Millezi et al., 2012), while myrcene did not exhibit observable activity alone but instead enhances the activities of geranial and neral when mixed together (Onawunmi et al., 1984). Others such as 1,8-cineole and eugenol, cinnamaldehyde, and linalool, which are also isolatable from *C. citratus*, also possess antibacterial activities (Gill and Holley, 2004; Gilles et al., 2010; Millezi et al., 2012).

The antibacterial action of CCEO targets bacterial cell membrane and cytoplasmic metabolism; these actions may include morphological changes, the inhibition of septum formation, spheroplast formation, the production of blisters or mesosomes, the development of abnormally shaped cells, and induction of lysis (Takaisi-Kikuni et al., 1996; Nguefack et al., 2004). The hallmarks of these activities could result in increased cell membrane fluidity and permeability, membrane protein destruction, inhibition of ATPase activities, inhibition of cell respiration, and ion transport/channel alterations, which are characteristic antibacterial actions of most essential oils (Fig. 1) (Cowan, 1999; Gill and Holley, 2004; Burt and Reinders et al., 2004).

Antibiofilm Activity of CCEO

Microorganisms on wet surfaces have the ability to aggregate, grow into microcolonies, and produce biofilms (Chimielewski and Frank, 2003). Bacterial biofilms are communities of bacteria enclosed in an extracellular matrix (Hanke and Kielian, 2007). In nature, biofilms

primarily comprise viable and nonviable microorganisms embedded in polyanionic extracellular polymeric substances anchored to a surface (Carpentier and Cerf, 1993; Wimpenny et al., 1993). The composition of the extracellular polymeric substances may include polysaccharides, proteins, phospholipids, teichoic and nucleic acids, and other polymeric substances hydrated to 85–95% water (Costerton et al., 1981; Sutherland 1983). Within a biofilm, the bacteria are protected from antimicrobials, environmental stresses, and immune responses from hosts or other external sources. Extracellular polymeric substances in the biofilm protect its inhabitants by concentrating nutrients, preventing access of biocides, sequestering metals and toxins, and preventing desiccation (Carpentier and Cerf, 1993; Chimielewski and Frank, 2003).

Biofilms are highly organized and can exist in single or multiple species communities, form a single layer or a 3-dimensional structure, or take the form of aggregates such as flocs or granules (Allison et al., 1987; Bryers, 1987; Bagge et al., 2001; Chimielewski and Frank, 2003). Because of their antibiotic resistance, biofilms can cause significant problems in the environment and during the treatment of different infections. The growth of biofilms in food processing environments leads to increased opportunities for microbial contamination. This adversely affects the quality of food products as it reduces shelf life and promotes disease transmission. Foodborne pathogens and spoilage organisms can attach to and produce extracellular polymeric substance on many foods and environmental surfaces (Chimielewski and Frank, 2003). Pathogenic bacteria can coexist within a biofilm with other environmental microflora; an example of this is *L. monocytogenes* surviving in *Pseudomonas* biofilms (Chimielewski and Frank, 2003). Some undesirable microorganisms such as *Lactobacillus curvatus* and *Lactobacillus fermentum* persist on milk residue in cheese processing plants even after repeated

cleaning, subsequently contaminating products (Wong, 1998). *L. monocytogenes*, *Pseudomonas* spp., *Bacillus* spp., and *Salmonella* spp. are food spoilage bacteria generally found on food substrates and in food processing plants (Chae et al., 2001; Bagge et al., 2001; Oosthuizen et al., 2001; Fatemi and Frank, 1999; Jeong and Frank, 1994).

The mechanisms of biofilm formation and their activities have been studied extensively. The formation of a bacterial biofilm is a developmental process that begins when a cell attaches to a surface; but how does a bacterial cell know if it is on or near surface? The phase of this “swim or stick” switch is determined by a sensory transduction mechanism termed surface sensing, which involves the bacterial flagellum (Belas, 2014). Biofilm formation consists of initial attachment, microcolony and extracellular polymeric substance production, and maturation (Davey and O’Toole, 2000). Planktonic cells attach to a surface, biofilm genes are activated, and other cells are recruited through quorum sensing. Cells grow on the surface, forming microcolonies, which continue to grow to produce a mature biofilm structure with channels (Chimielewski and Frank, 2003).

Nutrient and water limitations, equipment design, and temperature control are important indices in biofilm control (Chimielewski and Frank, 2003). Biofilms are difficult to eliminate by using standard antimicrobial treatments because of their high antibiotic resistance relative to free-living cells; this is due to the various adaptive and protective mechanisms developed by the bacteria (Lynch and Roberston, 2008; Mah and O’Toole, 2001; Zhang and Mah, 2008; Kavanaugh and Ribbeck, 2012). Because of these bacterial features, which aid their resistance to antibiotics, and the damaging effects of these microbes on industry, there is increased demand for novel

strategies to effectively inhibit bacterial biofilm formation and activities. Among several interesting candidate procedures is the application of antimicrobial essential oils in the treatments of biofilms. The antibiofilm activity of CCEO has been documented in the literature (Taweekaisupapong et al., 2012; Adukwu et al., 2012; Khan and Ahmad, 2012; de Oliveira et al., 2010) against biofilms of *L. monocytogenes*, *S. aureus*, and *Candida albicans*. Other essential oils such as marjoram, lemon, and basil essential oils inhibit the biofilm formation and quorum sensing activity of various food-related microorganisms (Suppakul et al., 2003). CCEO exerts a potent disinfectant action on *L. monocytogenes* biofilms on stainless steel (de Oliveira et al., 2010). The possible mechanism could be its ability to penetrate these biofilms more effectively owing to the lipophilic nature of essential oils (Sikkema et al., 1995). Adukwu et al. (2012) observed extensive disruption of *S. aureus* biofilms following exposure to CCEO. Similarly, Khan and Ahmad (2012) report deformities in the 3-dimensional structures of biofilms formed in the presence of sub-minimum inhibitory concentrations of *C. citratus*. Like other essential oils that may have similar chemical composition, CCEO could prevent biofilm formation and inhibit the AHL-mediated quorum sensing mechanism. However, further studies are required, because it remains unclear why some biofilms are resistant to CCEO.

Antifungal Activity of CCEO

CCEO exhibits positive fungicidal and anti-aflatoxigenic activities on different food storage and spoilage molds and yeast (Paranagama et al., 2003; Matasyoh et al., 2011; Farhang et al 2013). According to Boukhatem et al. (2014), CCEO exhibits considerably better antimicrobial activity when used in its vapor phase than in its liquid phase; this finding is corroborated by Tyagi and

Malik (2010b). A possible reason why the vapor phase is more effective is that the lipophilic molecules in the aqueous phase associate to form micelles, which suppress the attachment of essential oils to the organisms, whereas the vapor phase allows for free attachment (Inouye et al., 2001; Inouye et al., 2003). However, compared with the robust literature demonstrating the effectiveness of essential oils in the liquid phase, the potential of essential oil vapors is relatively less researched, although interest is increasing (Laird and Philips, 2010).

Casella and Casella (2010) report that CCEO exhibits antifungal activity superior to that of tea tree or lavender essential oil; it was effective against many yeast species (i.e., *C. albicans*, *Candida oleophila*, *Hansenula anomala*, *S. cerevisiae*, *Schizosaccharomyces pombe*, *Saccharomyces uvarum*, and *Metschnikowia fructicola*) and fungi (i.e., *Alternaria alternata*, *Aspergillus* spp., *Fusarium oxysporum*, *Penicillium roqueforti*, *Phytophthora infestans*, *Phoma sorghina*, *Phaeoramularia angolensis*, etc.) that cause food spoilage (Irkin and Korukluoglu, 2009; Tchinda et al., 2013; Kabera, 2013; Bonzi et al., 2013).

Empirical studies show that rather than being isolatable fractions, citral, geranial, neral, myrcene, linalool, eugenol, geraniol, limonene, geranyl acetate, and β -caryophyllene among other bioconstituents of CCEO are integral and synergistically exert the antimycotic food preservative activity of this medicinal plant (Vazirian et al., 2012; Tchinda et al., 2013; Kabera, 2013). For example, linalool is reported to inhibit spore germination and fungal growth; the inhibition of sporulation is posited to be due to the respiratory suppression of aerial mycelia (Lahlou and Berrada, 2001). On the other hand, Bard et al. (1988) report that the action mechanism of geraniol involves the enhancement of the rate of extracellular potassium leakage. Further studies

corroborate the effective maximum activity of geraniol isolated from CCEO against tested microbes (Arputha et al., 2012). Meanwhile, citral is reported to attack the microtubules of target organisms in addition to possessing other activities leading to cell death (Chaimovitsh et al., 2010).

The possible primary site of action of these essential oils is the cell membrane. Their activities on the membrane could affect the transport of nutrients and ions, membrane potential, and the overall permeability of fungal cells. This invariably induces the microbe to possibly die despite favorable conditions. In addition, several signal transductions are implicated in conidial germination including calcium/calmodulin signaling as well as the ras/MAPK and cAMP/PKA pathways (Osheroov and May, 2006). Helal et al. (2007) report that CCEO causes plasma cell disruption, mitochondrial structure disorganization and reduce the hyphal diameter of *Aspergillus flavus* (Link). Fumigated mycelia also experience Ca^{2+} , K^{+} , and Mg^{2+} leakage. Furthermore, total lipid content decreases, while saturated and unsaturated fatty acid content increase. The efflux of small ions is not necessarily indicative of the complete loss of membrane function as it can be observed in viable cells showing inhibited growth, because the cell uses energy for repair or survival rather than proliferation (Bouhhid et al., 2010). This loss of ions could indirectly affect cell membrane signal transduction and ultimately conidial germination. As CCEO is reported to be fungistatic (i.e., it inhibits fungal growth and reproduction without destroying them), making fungal pathogens inactive or dormant (Fig. 2).

Besides inhibiting conidial germination, CCEO inhibit mycotoxin production (Fig. 2). CCEO inhibits aflatoxins B₁, B₂, G₁, and G₂ and fumonisin production (Fandohan et al., 2004; de Souza

et al., 2005; Helal et al., 2007; Hegazy, 2011). The mycotoxins produced by *Aspergillus* spp. include ochratoxin A, malformins, fumitoxins, and gliotoxin (Hof and Kupfahl, 2009), which are reported to be carcinogenic in living organisms. The mycotoxins produced by fungi can cause serious health hazards including carcinogenic, immunologic, teratogenic, neurotoxic, nephrotoxic, and hepatotoxic effects as well as Kashin–Beck disease (Pawlowska et al., 2012). According to Alpsoy (2010), many inhibitors of aflatoxin biosynthesis may act at 3 levels: (1) the modulation of environmental and physiological factors affecting aflatoxin biosynthesis; (2) inhibition of signaling circuits upstream of the biosynthetic pathway; and (3) direct inhibition of gene expression or enzyme activity in the pathway. While the mode of action of most inhibitory compounds including CCEO is unknown, there is some evidence of effects of compounds on gene transcription or individual steps of enzyme activity in the biosynthetic pathway (Holmes et al., 2008). Nevertheless, it seems more apt to infer that the active components of CCEO can alter known environmental and physiological modulators of aflatoxin biosynthesis or signaling transduction pathways in the upstream regulatory network. Although the anti-aflatoxigenic activities of most essential oils may be related to the inhibition of the ternary steps of aflatoxin biosynthesis involving lipid peroxidation and oxygenation (Bluma et al., 2007), further research focusing on CCEO is required for clarification.

The inhibitory actions of natural products on mold cells generally involve cytoplasm granulation, cytoplasm membrane rupturing, damage to lipids and proteins, and inactivation and/or synthesis of intracellular and extracellular enzymes (Fig. 1). These actions can occur alone or in combination and culminate in the inhibition of mycelium germination (Cowan, 1999). Furthermore, in eukaryotic cells, essential oils can provoke depolarization of mitochondrial

potential, impact ionic calcium cycling and other ionic channels, which reduces pH gradient and affects proton pumps and ATP pool. Chain reactions from the cell wall or membrane invade whole cells, leading to widespread oxidative damage and bioenergetic failure following the leakage of free radicals, cytochrome C, and Ca^{2+} . Furthermore, the permeabilization of the outer and inner mitochondrial membrane leads to cell death (Ritcer and Schegel, 1993; Armstong, 2006; Bone and Mills, 2013).

Besides cytotoxicity, Dijoux et al. (2006) also posit that the essential oils of *C. citratus* and *Citrus aurantium dulcis* are phototoxic. In the postulated mechanism of phototoxicity, essential oils penetrate the target cells without damaging its membrane, proteins, or DNA. A radical reaction is elicited by the excitation of certain molecules when cells are exposed to the activating light, and energy transfer results in the production of an oxygen singlet; this causes cellular macromolecular damage and in some cases the formation of covalent adducts to DNA, proteins, and membrane lipids.

Antioxidant Actions of CCEO

The use of essential oils in food preservation tackles 2 main issues associated with food spoilage: the prevention of oxidation and microbial contamination. Hence, to maximize effectiveness, a food biopreservative should possess atoxic properties at low concentrations, antioxidant activity, and antimicrobial properties in addition to biodegradability (Soares et al., 2013). In fact, the antioxidant ability (the ability of the essential oil to inhibit the process of food oxidation) is essential to its activity as food a biopreservative: the higher the antioxidant activity of the oil, the greater its efficacy as a biopreservative (Soares et al., 2013).

The antimicrobial activities of CCEO have been discussed most extensively herein. This section focuses on antioxidant activity of CCEO as documented in literature. Soares et al., (2013) report that CCEO has greater antioxidant activity than the methanolic extracts obtained and similar antioxidant activity to that of the synthetic antioxidant butyrate hydroxytoluene (BHT). Because of its antioxidant properties, CCEO have been used experimentally to preserve different food substrates, including raw and cooked pork patties (Olorunsanya et al., 2010). Its antioxidant activity is attributed to the synergistic actions of monoterpenoid compounds such as α -citral, β -citral, myrcene (Ruberto and Barrata, 2000), and eugenol (Rathabai and Kanimozhi, 2013).

Balachandar et al., (2014) demonstrate that in addition to CCEO's DNA protective and antimicrobial activities, it possesses potential antioxidant activity that could be harnessed in food processing. Similarly, Nathchnok and Chiravoot (2012) evaluated the application of CCEO as replacement for the commercial antioxidant BHT in packaging for raw foods, especially because of the free radical-scavenging activity of eugenol. Others studies (Rathabai and Kanimozhi, 2013; Cheel et al., 2005) also demonstrate the antioxidant/free radical-scavenging activity of *C. citratus* extract and CCEO components, suggesting they can be used in food preservation at both the domestic and industrial levels.

ALLELOPATHIC EFFECTS OF CCEO

Effects on Seed Germination, Seedling Emergence, and Viability

“Allelopathy” (Greek: *allelo* and *pathy*, meaning “mutual harm” or “suffering”) was coined in 1937 by Austrian scientist Hans Molisch in the *Der Einfluss einer Pflanze auf die andere* -

Allelopathie (The Effect of Plants on Each Other) (Willis 2010). Commonly cited effects of allelopathy include reduced seed germination and seedling growth.

In recent years, much attention has been given to non-synthetic systems for seed treatment to protect them against seed-borne pathogens that can cause deterioration. Plant extracts play a significant role in the inhibition of seed-borne pathogens as well as the improvement of seed quality and field emergence of plant seeds (Renzen et al., 1996; Nwachukwu and Umechuruba, 2001; Bonzi et al., 2012). Bonzi et al. (2013) report CCEO significantly reduces the infection level of *P. sorghina* in *Sorghum bicolor* (L.) Moench seeds compared to untreated seeds. However, there are concerns that CCEO and its other extractable forms could have negative allelopathic effects on stored seed viability. However, this remains controversial.

For example, Adegoke and Odesola (1996), Paraganama et al. (2002), Bankole et al. (2005), Somda et al. (2007), and Adeji (2011) report no negative effects of CCEO or *C. citratus* powder on seed germination or viability. More specifically, Paraganama et al. (2002) report no adverse effect of CCEO treatment on stored cowpeas even after 30 days. Earlier, Adegoke and Odesola (1996) reported that a sample of cowpeas seeds treated with *C. citratus* powder and CCEO exhibited no physical deterioration or moldiness, while Adeji (2011) reports that CCEO treatment in fact increased germination and vigor of maize seeds.

On the contrary, studies by Dudai et al. (1999), Nwachukwu and Umechuruba (2001), Cruz et al. (2002), Li et al. (2005), Zeng et al. (2005), Sousa et al. (2010), Velarani et al. (2010), and Bonzi et al. (2013) demonstrate that CCEO applied to the seeds or soil (as a bioherbicide) inhibits the seed germination and viability of different species. In fact, Dudai et al. (1999) found that soil

application of CCEO inhibited the germination of both mono- and dicotyledonous plants species. Further studies implicate citral as a strong allelopathic compound in *C. citratus* that could inhibit seed germination and viability (Dudai et al., 1999; Chaimovitsh et al., 2010). Citral is a major component of CCEO and other lemon-scented plants (Opdyke, 1979). Furthermore, citral is also reported to be a strong inhibitor of seed germination in *Triticum aestivum*, *Amaranthus palmeri*, and *Brassica nigra* (Dudai et al., 1999). GC-MS-based analysis of seeds treated with citral shows the added presence of geraniol, nerol, geranic acid, and neric acid in the endosperms, which are presumed derivatives of citral generated by its detoxification in the seed (Dudai et al., 2000). In addition to its ability to induce seed germination inhibition, citral has antifungal (Yousef et al., 1978; Rodov et al., 1995), antibacterial (Asthana et al., 1992; Kim et al., 1995), and insecticidal (Rice and Coats, 1994) activities. The ability of citral to inhibit seed germination (Dudai et al., 1999) and its rapid toxic effect on seedlings are reminiscent of the effect of other herbicides of industrial value (Yasour et al., 2006; Chaimovitsh et al., 2010).

Chaimovitsh et al. (2010) provide further evidence suggesting citral is a potent and volatile anti-microtubule compound. They found that in the presence of micromolar concentrations of citral, microtubules were disrupted within minutes but F-actin remained intact. The effect of citral on plant microtubules was both time and dose dependent, and recovery only occurred several hours after a short exposure of several minutes to citral. Citral also disrupted animal microtubules albeit less efficiently. In addition, microtubule polymerization in vitro was inhibited in the presence of citral.

According to Sousa et al. (2010), although *C. citratus* has beneficial effects as a medicinal plant, it can incur serious problems and damage to cells with incorrect usage; this might be a limitation for its prolonged use as a preservative of seeds for agricultural purposes. Most studies in the literature indicate the use of CCEO in seed preservation prevents seed deterioration. However, it remains controversial whether its effect on seed germination could promote its use in agriculture. Importantly, its allelopathic effect due to its citral content could promote its use as a potent bioherbicide.

Anti-insecticidal Actions of CCEO

Essential oils from *Cymbopogon* and their major constituents function as allelochemicals. Allelochemicals are secondary metabolites that can also affect insect biology and behavior, and are therefore used in biocontrol as biopesticides (Ganjewala, 2009). Various studies show that *C. citratus* has relatively strong insecticidal and repellent action on different insects that hamper food storage (Parangama et al., 2002; Samarasekera et al., 2011; Kumar et al., 2013) even at the larval stage (Labinas and Crocomo, 2002; Li et al., 2005) compared to other commercial insecticidal products (Olivero-Verbel et al., 2010).

Sharaby (1988) assessed the anti-insect properties of different concentrations of CCEO against the lesser cotton leaf worm, *Spodoptera exigua* Hbn. The results show that CCEO inhibited egg laying, hindered the third instar larvae from feeding on treated leaf discs, was insecticidal, and inhibited hatchability of eggs. Thus, CCEO effectively controlled *S. exigua* by acting as an ovicidal and larvicidal agent. The insecticidal activity of CCEO against the khapra beetle (*Trogoderma granarium* Everts) was also evaluated on stored groundnuts in the study by

Asawalam and Igwe (2012). CCEO exhibited insecticidal activity. Therefore, the authors recommend using CCEO to control *T. granarium* in stored groundnuts.

USE OF CCEO AS AN ALTERNATIVE TO SYNTHETIC FOOD PRESERVATIVES

Existing Knowledge

The effectiveness of most synthetic compounds used as food preservatives depends on their wide spectrum of antifungal, antioxidant, insecticidal, and antibiotic activities. However, these synthetic compounds possess salient adverse effects on body systems and the environment, hence the restrictions on their use (Matthew et al., 2005). For example, some of these synthetic compounds are reported to pollute the environment and even kill useful microorganisms when applied to the soil (Primentel and Levitan, 1986). In contrast, CCEO is considered “generally regarded as safe” for human consumption and has already been used on different substrates either domestically or industrially (Table 1). In fact, the components of CCEO are among the registered flavorings by the European Commission and the United States Food and Drug Administration (Hyldgaard et al., 2012). Furthermore, CCEO possesses a wide range of activities that favor its use (Fig. 3). CCEO is superior to synthetic fungicide such as agrosan DN, Dithane™ M-43, and copper oxychloride (Mishra and Dubey, 1994; Adegoke and Odesola, 1996). CCEO is considered an environmentally safe alternative to synthetic antioxidants such as BHT, which have been reported to cause hemorrhaging (Ndhalala et al., 2010).

Besides its lower toxicity, as a natural product, CCEO is biodegradable and hence ecologically and health friendly. It has significant broad-spectrum antimycotic potency that lasts for 210 days of storage (Tzortzakis and Economakis, 2007). It can be used on living/fresh and processed foods

such as fruits, meat, spawns, grains, fruit juice, cheese, etc. (Table 1). Furthermore, the shelf lives of various foods can be prolonged by the application of CCEO. It could also be applied in both pre-harvest (Velluti et al., 2003) and post-harvest conditions (Mataysoh et al., 2011). Unlike other synthetic food additives that tend to alter the physiochemical characteristics of food substrate they are applied to, modified CCEO could enhance the organoleptic properties of the food substrate, favoring its use as a natural food flavoring. This is evidenced by large market demands for such essential oils in the United States, Japan, Canada, France, Switzerland, and some other European countries where they are applied in pharmaceuticals, cosmetics, and flavoring (DOAFF, 2012). However, essential oils of plants including *C. citratus* are reported to cause significant functional damage to the liver and stomach of rats at higher doses per body weight (Fandohan et al., 2005). Concordantly, Ekpenyong et al. (2014) opine that although CCEO is considered safe for human consumption, high doses and prolonged usage of *C. citratus* and its active constituents should be discouraged and that further research on dose consistency is warranted.

The non-phytotoxicity of CCEO is disputed, as many studies provide conflicting results. As mentioned above, some authors report that CCEO possesses phytotoxic activity when applied to stored seeds by inhibiting the seed germination and when used as an herbicide (Nwachukwu and Umarchuba 2010; Chaimovitsh et al., 2010). On the other hand, others report that it has non-phytotoxic properties, as it does not affect the germination or physical characteristics of some stored seeds (Paranagama et al., 2002; Adeji 2011). Regardless, the dose and duration of exposure to the oil could affect its toxicity. Hence, regulated consumption and use should be encouraged.

Recent Advances

There have been some setbacks in the use of essential oils in food preservation, including their potency on raw foods and multi-resistant microbe strains, organoleptic properties, and consumer acceptability. Despite the demonstrated potential of various essential oils and their constituents in vitro, their use as food preservatives is limited by the fact that high concentrations are required to achieve substantial biopreservative effects. This is especially a challenge for their use in some raw foods, because the hydrophobic essential oil constituents are impaired by interactions with food matrix components such as lipids (Rattanachaikunsopon and Phumkhachorn, 2010), starch, and proteins (Gutierrez et al., 2008; Kyung, 2011; Hyldgaard et al., 2012). Furthermore, as the antimicrobial potency of essential oil constituents are dependent on their concentration and the cause of microbial contamination (Somolinos et al., 2010), one means of overcoming serious contamination by resistant strains is increasing the dose and concentration of the oil applied; however, this has resulted in negative organoleptic effects. This invariably affects consumer acceptance. Aiming to overcome these challenges, research on the use of CCEO as an effective biopreservative has recently yielded some innovations including the use of edible films or coatings, nanoemulsions, and synergistic combinations with other oils or other preservative methods. These aim to ensure the usage of CCEO in active packaging rather than as an active ingredient in the food itself.

The recent use of edible films and coatings as environmentally friendly innovations substantially improves the shelf life of many food products including fruits, vegetables, and animal-based foods (Valencia-Chamorro et al., 2011; Sanchez-Ortega et al., 2014). Edible films are thin films

prepared from edible material that act as a barrier to external elements (e.g., moisture, oils, gases, and vapors) to protect the product, extend its shelf life, and improve quality (Suyatma et al., 2005). Edible films can control moisture, oxygen, carbon dioxide, and flavor and aroma transfer between food components or the atmosphere surrounding the food (Du et al., 2011). The use of edible films in foods requires appropriate gas (i.e., oxygen), water, and physical stress barrier properties; good mechanical strength and adhesion; reasonable microbial, biochemical, and physicochemical stability; effective antioxidant, flavor, color, nutritional, and antimicrobial additive carrying abilities; safety for human consumption (i.e., free of pathogenic microorganisms and hazardous compounds); acceptable sensorial characteristics; low cost of raw materials; and simple technology for production (Lourdin et al., 1995; Debeaufort et al., 1998).

The choice of formulations for edible films is largely dependent on the desired function such as biodegradability, edibility, aesthetic appearance, and good barrier properties against oxygen (Cha and Chinnan, 2004). In addition, edible films can support antimicrobial, nutritional, and antioxidant substances (Gennadios et al., 1997). Accordingly, large amounts of antimicrobials have been incorporated into edible films according to their protein or polysaccharide component; for example, antimicrobial or antioxidant compounds incorporated into the polymer matrix of these edible films and coatings may prevent the growth of spoilage and pathogenic microorganisms, delay meat fat rancidity, prevent discoloration, and even improve the nutritional quality of coated foods (Gennadios et al., 2004; Soliva-Fortuny et al., 2012).

There are 3 categories of edible film components: hydrocolloids, lipids, and composites. Hydrocolloids include proteins and polysaccharides such as starch, alginate, cellulose

derivatives, chitosan, fruit puree, whey protein, soy protein, egg albumen, wheat gluten, sodium caseinate, and agar. Lipids include waxes, acylglycerols, and fatty acids. Meanwhile, composites contain both hydrocolloid components and lipids (Debeaufort and Voilley, 2009; Du et al., 2011; Valencia-Chamorro et al., 2011).

Edible films, coatings, and nanoemulsions containing CCEO have been developed. They allow CCEO to be used on post-harvest crops and in the preservation of seeds for planting, reducing spoilage in terms of the quality and quantity of seeds and fruits. Kim et al. (2013) developed a nanoemulsion containing CCEO to coat plums. This coating exhibited effective antimicrobial activities against *Salmonella* and *E. coli* O157:H7 and may therefore extend plum shelf life. Similarly, Mpho et al. (2013) found that CCEO plus modified atmospheric packaging significantly decreased the incidence of anthracnose (caused by *Colletotrichum gloeosporioides*), gray pulp, vascular browning, weight loss, and loss of fruit firmness; in addition, it demonstrated acceptable taste, flavor, texture, and higher overall acceptance after ripening at 25°C followed by cold storage at 10°C for 18 days. Furthermore, edible films were prepared from a mixture of partially hydrolyzed sago starch and arginate (SA). CCEO and glycerol were incorporated into the film to act as natural antimicrobial agent and plasticizer, respectively. The results of that study showed edible films containing CCEO were effective against *E. coli* O157:H7 at all test concentrations and that the addition of CCEO did not interact with the functional groups of the film as measured by Fourier transform infrared spectrophotometry (Maizura et al., 2007). Similarly, thermally stable microparticles containing *C. citratus* volatile oils have been made. Cyclodextrin and hydroxypropyl cyclodextrin were used as encapsulation materials; however, the use of cyclodextrin was most efficient (Weisheimer et al., 2010).

Microbial edible films and coatings have also been developed for use in preserving fish, meat, and meat products (Sanchez-Ortega et al., 2014). For example, CCEO (25% w/w) has been used in the preservation of sea bass slices against mesophilic and psychrophilic microorganisms, enterobacteria, and H₂S-producing bacteria LAB (Sanchez-Ortega et al., 2014).

Further applications of nanotechnology in the food industry brought about the development of food antimicrobial nanocarriers (Blanco-Padilla et al., 2014). Like other processes, nanoencapsulation incorporates antimicrobial/antioxidant agents into the food packaging materials. These innovations can overcome the water solubility constraints, evaporation, and sensory properties that limit the incorporation of antioxidant/antimicrobial essential oils such as CCEO in food products (Kim et al., 2013; Salvia et al., 2014). They also maximize the antimicrobial activity of citral, a major essential oil component of *C. citratus*, and the use of concentrations low enough to minimally alter the quality of the products but sufficient to inhibit microbial growth within the limits of food regulation standards (Maswal and Dar, 2014).

CCEO has been experimentally encapsulated into a carnauba-shellac wax-based nanoemulsion by high-pressure homogenization (Kim et al., 2013; Jo et al., 2014) and alginate nanoemulsions by ultrasonication and microfluidization (Salvia et al., 2012, 2014). CCEO-loaded carnauba-shellac wax-based nanoemulsions decreased the total populations of *E. coli* O157:H7 and *L. monocytogenes* by 8.18 log CFU/g after 2 hours. After 5 months of storage, unloaded and CCEO-loaded carnauba-shellac wax nanoemulsions applied to apples decreased aerobic bacteria by 0.8 and 1.4 log CFU/g, respectively. The coatings also inhibited the development of yeast and molds. Moreover, the coatings inhibited the growth of *Salmonella typhimurium* and *E. coli* O157:H7 on

apples and plums, respectively. In addition, the application of nanoemulsions preserved various physicochemical qualities of fruits (Kim et al., 2013; Jo et al., 2014).

Similarly, CCEO nanoemulsions have been developed as coatings for berries to improve shelf life, reduce aging, and improve microbial safety. Coating berries with 3.0 g/100 g CCEO initially inhibited *S. typhimurium* and *E. coli* O157:H7 inoculated on the berries by more than 3.2 and 2.6 log CFU/g, respectively. The coatings did not significantly alter the flavor of the berries and actually improved their glossiness. The coatings also reduced losses of weight, firmness, phenolic compounds, and antioxidant activity as well as delayed increases in total anthocyanin concentration in berries (Kim et al., 2014).

In another series of studies, CCEO-alginate nanoemulsions demonstrated antibacterial effects against *E. coli*; however, the biological activity was dependent on the nanoemulsion production process. An investigation of the effects of microfluidization and ultrasonic processing on CCEO-alginate nanoemulsion revealed that while microfluidization enhanced antimicrobial activity, ultrasound diminished the activity. Nevertheless, additional studies are still in progress (Salvia et al., 2012, 2014). Other isolated components of CCEO, such as eugenol (Raybaudi-Massilia et al., 2008a, 2008b; Ghosh et al., 2014), limonene (Donsi et al., 2012), cinnamaldehyde (Raybaudi-Massilia et al., 2008b; Donsi, et al., 2012), citral (Maswal and Dar, 2014), and geraniol (Raybaudi-Massilia et al., 2008a), have been incorporated into edible coatings and nanocarriers.

However, the relatively high production cost and dearth of permissible production materials have proved to be limitations to use of nanoencapsulation techniques in the food industry (Fang and

Bhardi, 2010); these could also be possible limitations to use of CCEO-incorporating nanocarriers.

In the face of multi-resistant strains, CCEO has been shown to exhibit potent antioxidant and antimicrobial actions synergistically with other oils, antibiotics, or preservative techniques. A combination of CCEO and oregano essential oil is reported to exert potent antibacterial activities against *S. aureus* (Choi et al., 2012), while a combination of *C. citratus*, *Ocimum gratissimum*, and *Thymus vulgaris* essential oils is effective against *Penicillium expansum*, *A. alternata*, and *Bipolaris oryzae* (Nguefack et al., 2007; Tamgue et al., 2011). Similar positive results have been obtained with CCEO combined with the antibiotics amoxicillin and norfloxacin against *S. aureus* (Choi et al., 2012).

Adding CCEO and lime peel extracts to chicken patties enhances the antioxidant and antimicrobial actions of both extracts (Hayim and Ferial, 2013). Tyagi et al. (2014a) report that integrated thermal CCEO treatment exhibited superior performance over individual exposure (i.e., CCEO or thermal treatment alone) for fruit juice preservation against food spoilage yeasts. Therefore, these results collectively suggest that for multi-resistant strains, the use of other effective essential oils and procedures could complement or enhance the food preservative actions of *C. citratus*.

Thus, CCEO with the aid of edible films/coatings and nanocarriers as well as other essential oils and preservation techniques is effective for preserving fresh foods such as fruits, vegetables, seeds, and dairy and meat products.

Another interesting use of CCEO is as a preservative in the petroleum industry. The application of CCEO as a potential biocide for sulfate-bacteria growth control in petroleum reservoirs and consequently to prevent souring and/or as a coating against biocorrosion is of great interest for the petroleum industry (Korenblum et al., 2013).

Future Research Directions

Despite the wealth of knowledge about CCEO and its use in food and industrial preservation, much remains unknown. In addition, better options for its usage and extraction should be developed, warranting further research on the following issues:

- The possibility of extending its wide-spectrum antimycotic potency beyond 210 days
- Its extensive usage in other industries besides the food and petroleum industries
- Further investigation of the mechanisms of its antimycotoxin and antibiofilm actions
- Further research on the application and toxicity of its vapors
- Surmounting the limitations for its use in nanoencapsulating systems
- Improved and innovative methods for essential oil extraction
- Improvements in its combination with other antibiotics in order to reverse multidrug resistance
- The molecular basis of the synergistic interactions between CCEO and other essential oils, antibiotics, and even preservative techniques

- Clinical trials testing the use of CCEO in fungal treatments (none have been performed to date)

Despite the promising results of various pharmacological studies, most of which are animal based, there are some remaining concerns such as the stability, selectivity, and bioavailability of CCEO in the human body as well as possible adverse herb–drug interactions. In addition, the optimal ratio and dosing regimens should be explored to improve the efficacy and decrease the toxicity of CCEO upon consumption. The investigation of essential oils as a potential replacement therapy represents a new era of phytopharmaceuticals (Yap et al., 2014). Many more novel natural phytochemicals from plants combined with conventional antimicrobial food preservative agents are expected to bypass antibiotic resistance. Finally, more application-oriented research should be performed in order to fully utilize CCEO.

CONCLUSIONS

Current evidence indicates CCEO could function as a potent ingredient in food and chemical preservation albeit with some limitations. It possesses characteristics that could prolong shelf life and maintain the physiochemical characteristics of the substrates it is applied to (Fig. 3). Like other essential oils, CCEO is relatively easy to obtain, has low mammalian toxicity, and degrades quickly in water and soil, potentially allowing CCEO or its isolatable fractions to be applied in food preservation and processing. However, although its phytotoxicity and effects on seed germination remain controversial, these allopathic effects due to its citral content could promote its use as a potent bioherbicide. In addition, although it is considered safe for consumption, this

is rather subjective, as the dose and duration of exposure could affect its toxicity. Hence, regulated consumption and use should be encouraged.

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Table 1: Domestic, agricultural, and industrial use of *C. citratus* essential oil in food preservation

Food	Foodborne organisms	References
Fruits/fruit juice		
Strawberry	<i>Botrytis cinerea</i>	(Abd-alla et al., 2011)
Mango	<i>Colletotrichum gloeosporioides</i>	(Duamkhanmanee, 2008)
Papaya	<i>Alternaria alternata Fusarium semitectum Lasiodiplodia theobromae</i>	(Espitia et al., 2012)
Yellow passion fruit	<i>Colletotrichum gloeosporioides</i>	(Anaruma et al., 2010)
Stored table grapes	<i>Aspergillus flavus Aspergillus niger Aspergillus ochraceus.</i>	(Sonker et al., 2014)
Mixed fruit juice	<i>Saccharomyces cerevisiae Zygosaccharomyces bailii Aureobasidium pullulans Candida diversa Pichia fermentans Pichia anomala Pichia kluyveri Hansenula polymorpha</i>	(Tyagi et al., 2014)

Apple juice	<i>Aspergillus flavus</i> <i>Aspergillus ochraceus</i>	(Eissa et al., 2008)
Tomatoes	<i>Alternaria solani</i>	(Itako et al., 2013)
Grains/seeds/tubers		
Maize	<i>Fusarium proliferatum</i> <i>Aspergillus flavus</i> <i>Aspergillus fumigatus</i> <i>Macrophomina phaseoli</i> <i>Aspergillus niger</i> <i>Fusarium verticillioides</i>	(Adegoke and Odesola 1996; Vellutii et al., 2003; Fandohan et al., 2004; Matasoyoh et al., 2011; Atanda and Olopade, 2013).
Melon seeds	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Aspergillus tamaris</i> <i>Penicillium citrinum</i>	(Bankole and Joda 2004)
Stored rice	<i>Aspergillus flavus</i>	(Paranagama et al., 2003)
Cowpea	<i>Aspergillus flavus</i> <i>Macrophomina phaseoli</i> <i>Penicillium chrysogenum</i>	(Adegoke and Odesola, 1996)
Sorghum	<i>Colletotrichum graminicola</i> <i>Phoma sorghina</i> <i>Fusarium moniliforme</i>	(Somda et al., 2007; Bonzi et al., 2013)
Irish potatoes	<i>Phytophthora infestans</i> (Mont.)	(Kabera et al., 2013)

Groundnut	<i>Aspergillus niger</i> <i>Aspergillus flavus</i> <i>Aspergillus terreus</i> <i>Aspergillus fumigatus</i> <i>Penicillium citrinum</i> <i>Fusarium oxysporum</i> <i>Alternaria alternata</i> <i>Curvularia lunata</i>	(Manoorkar and Gachande 2014)
Others		
Farmer cheese (<i>Wagashi</i> cheese)	<i>Aspergillus niger</i> <i>Aspergillus terreus</i> <i>Aspergillus flavus</i> <i>Fusarium verticillioides</i> <i>Fusarium poae</i> <i>Penicillium citrinum</i> <i>Scopulariopsis brevicaulis</i>	(Sessou et al., 2012 a/b)
Yogurt	<i>Aspergillus flavus</i> <i>Aspergillus parasiticus</i> <i>Aspergillus ochraceus</i>	(Shaaban et al., 2010)
Spawns (<i>Pleurotus</i> spp.)	<i>Aspergillus flavus</i> <i>Aspergillus fumigatus</i> <i>Aspergillus niger</i> <i>Alternaria alternata</i> <i>Penicillium citrinum</i> <i>Curvularia lunata</i> <i>Trichoderma harzianum</i>	(Mahanta et al., 2012)

Cream filled cakes and pastries	<i>Staphylococcus aureus E. coli</i> <i>Candida albicans Bacillus cereus Salmonella typhimurium</i>	(Vezarian et al., 2012)
Corn meal agar	<i>Aspergillus flavus Aspergillus fumigatus Fusarium moniliforme</i>	(Nguefack et al., 2004)

Table 2: Antimicrobial activities of *C. citratus* essential oil (CCEO) and its isolatable components on different microbes

Microorganisms	Antimicrobial action	Inhibitory concentration	Reference
<i>Listeria monocytogenes</i>	Antibiofilm formation	100%	(De Oliveira et al., 2010)
<i>Candida</i> spp.	Antibiofilm formation Germ tube formation inhibition	MIC: 0.5 μ L/mL [0.4 mg/mL] MFC: 1.0 μ L/mL [0.8 mg/mL]	(Tweechaisupapong et al., 2012)
<i>Staphylococcus aureus</i> <i>Staphylococcus epidermis</i> <i>Escherichia coli</i> <i>Candida parapsilosis</i> <i>Candida tropicalis</i>	Antibacterial and antifungal action by CCEO	ND	(Soares et al., 2013)
<i>Phytophthora infestans</i>	Antifungal action by CCEO	400–600 μ L/L	(Kabera et al., 2013)
<i>Aspergillus flavus</i> (SGS-421)	Antifungal action by CCEO:	14% (w/w)	(Atanda and Olopade, 2013)

	inhibition of fungal growth and aflatoxin B ₁ production		
<i>Colletotrichum graminicola</i> <i>Phoma sorghina</i> <i>Fusarium moniliforme</i>	Antifungal action by CCEO: inhibition of radial growth	6%	(Somda et al., 2007)
<i>Colletotrichum coccodes</i> <i>Botrytis cinerea</i> <i>Cladosporium herbarum</i> <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i>	Antifungal action by CCEO: Fungal spore production inhibition (up to 70% at 25 ppm), retarded (up to 100% at 500 ppm); reduction in spore germination and germ tube length in <i>C. coccodes</i> , <i>B. cinerea</i> , <i>C. herbarum</i> , and <i>R. stolonifer</i> .	25–500 ppm	(Tzortzakis and Economakis, 2007)

	Accelerated spore germination (at 100 ppm) for <i>A. niger</i>		
<i>Proteus mirabilis</i> <i>Staphylococcus aureus</i> <i>Klebsiella pneumoniae</i> <i>Pseudomonas aeruginosa</i> <i>Candida albicans</i>	Antibacterial and antifungal action by CCEO	MIC: 0.78 mg/mL	(Nyarko et al., 2012)
<i>Salmonella typhi</i>	Antibacterial action by CCEO	ND	(Rathbai and Kanimozhi, 2013)
<i>E. coli</i> <i>Klebsiella pneumoniae</i> <i>Pseudomonas aeruginosa</i> <i>Proteus vulgaris</i> <i>Bacillus</i> spp. <i>Staphylococcus</i> spp.	Antibacterial action by CCEO	MIC: 50–150 mg/mL	(Hindumathy, 2011)
<i>Alternaria alternate</i> <i>Aspergillus niger</i> <i>Fusarium oxysporum</i> <i>Penicillium roqueforti</i>	Antifungal action by CCEO: inhibited growth and survival of fungal and yeast	MIC: 0.062 µL/mL MFC: 20 µL/mL	(Irkin and Koruluoglu 2009)

<i>Candida albicans</i>	species		
<i>Candida oleophila</i>			
<i>Hansenula anomala</i>			
<i>Saccharomyces cerevisiae</i>			
<i>Schizosaccharomyces pombe</i>			
<i>Saccharomyces uvarum</i>			
<i>Metschnikowia fructicola</i>			
<i>Phoma sorghina</i>	Antifungal action	(a) 8% (w/w)	(a) (Bonzi et al., 2013)
<i>Colletotrichum graminicola</i>	by CCEO: reduction of infection rates		
<i>Botrytis cinerea</i>	Antifungal action by CCEO: suppression of mycelial growth	MIC: 5–15 μ L	(Sirinpornvisal et al., 2009)
<i>Aspergillus flavus</i>	Antifungal action	(a) 1200 ppm	(Nguefack et al., 2004)
<i>Aspergillus fumigatus</i>	by CCEO	(b) 1200 ppm	
<i>Fusarium</i>		(c) 500 ppm	

<i>moniliforme</i>			
<i>Aspergillus flavus</i>	Anti-aflatoxin inhibition: 0.1 mg/mL	MIC: 1 mg/mL	(Paranagama et al., 2003)
<i>Alternaria alternata</i>	Antifungal action by CCEO: inhibition of mycelial growth (1.97%) and sporulation	50 µL	(Itako et al., 2013)
<i>Phaeoramularia angolensis</i>	Antifungal action	MIC: 400–500 ppm	(Tchinda et al., 2013)
<i>E. coli</i> <i>Candida albicans</i> <i>Bacillus cereus</i> <i>Salmonella typhimurium</i>	Antibacterial and antifungal action	MIC: 5 µL/mL MBC: 1 µL/mL	(Varizian et al., 2012)
<i>Aeromonas hydrophila</i>	Antibiofilm action: reduction of biofilm adhesion by 4.51 log CFU/cm ² at 25°C	MIC: 31 µL/mL	(Millezi et al., 2013)
<i>Aspergillus fumigatus</i>	Antifungal action	0.03% v/v	(Bansod and

<i>Aspergillus niger</i>	by CCEO		Dai,2008)
<i>Aspergillus fumigatus</i>	Antifungal action	MIC: 15–118 mg/mL	(Mataysoh et al., 2011
<i>Aspergillus niger</i>	by CCEO		
<i>Aspergillus parasiticus</i>			
<i>Aspergillus ochreus</i>			
<i>A. niger Aspergillus parasiticus</i>	Antifungal action	MIC: 5 µL/mL	Shaaban et al., 2013)
<i>Aspergillus ochreus</i>	by CCEO: Inhibition of growth, spore germination and synthesis of aflatoxin (4 µL/mL)		
<i>E. coli CY1PD</i>	Antifungal action	1 µL/mL 1 µL/mL 16	(Singh et al., 2013)
<i>ABY42 E382 CQ1</i>		µL/mL 32 µL/mL 16	
<i>Enterococcus faecalis</i>		µL/mL 32 µL/mL 1 µL/mL	
<i>SV7 SV20</i>		8 µL/mL 1 µL/mL 4 µL/mL	
<i>Streptococcus mobilis</i>		16 µL/mL 32 µL/mL 4	
<i>SV11 SV27NC</i>		µL/mL 32 µL/mL	
<i>Staphylococcus aureus SK10S2</i>			

<i>SK5S1 Bacillus</i> <i>coagulans CB1 CB6</i> <i>Klebsiella</i> <i>pneumoniae CP62</i> <i>M10 Edwardsiella</i> <i>tarda 26P IBCY</i>			
<i>Saccharomyces</i> <i>cerevisiae</i> <i>Zygosaccharomyces</i> <i>bailii Aureobasidium</i> <i>pullulans Candida</i> <i>diversa Pichia</i> <i>fermentans Pichia</i> <i>kluyveri Pichia</i> <i>anomala Hansenula</i> <i>polymorpha</i>	Antifungal action	MIC: 0.28–1.3 mg/mL MFC: 0.56–4.5 mg/mL	(Tyagi et al., 2014)
(a) <i>Salmonella</i> <i>typhimurium</i> (b) <i>E. coli</i> <i>O157;H7</i>	Antibacterial action by CCEO: inhibition by more than (a) 3.2 and 2.6 log CFU/g bacteria, respectively	ND	(Kim et al., 2014)

(a and b) <i>Listeria innocua</i> <i>Salmonella enteritidis</i> , <i>E. coli</i> (c) <i>Salmonella hadar</i> <i>E. coli</i> O157;H7	Antibacterial action by CCEO: (a and b) reduced >5 logCFU/mL (c) 50% reduction of bacterial population	(a) 0.2% (b) 0.5% (c) 0.097–0.079	(a and b) Raybaudi-Masselia et al., 2006 (c) Friedman et al., 2004
(a) <i>L. monocytogenes</i> (b) <i>S. hadar</i> <i>E. coli</i> O157;H7	Antibacterial action by citral: (a) 1.1–1.3 log CFU/mL reduction (b) 50% reduction of bacterial population	(a) 0.01% (b) 0.008–0.07	(a) (Ferante et al., 2007) (b) (Friedman et al., 2004)
(a) <i>Colletotrichum gloeosporioides</i> (b) <i>Fusarium</i> spp. <i>Rhizopus</i> spp. <i>Penicillium</i> spp. <i>Aspergillus flavus</i>	Antifungal action of citral: (a) reduced fungal growth by 70% (b) inhibitory action	(a) 0.5% (b) 1.0%	(Garcia et al., 2008)
<i>S. hadar</i> <i>E. coli</i>	Antibacterial action	0.02–0.05	(Friedman et

<i>O157:H7</i>	by eugenol: 50% reduction of bacterial population		al., 2004)
<i>Fusarium</i> spp. <i>Rhizopus</i> spp. <i>Penicillium</i> spp. A. <i>flavus</i>	Antifungal action by eugenol	MIC: 4%	(De Souza et al., 2005)
(a and b) <i>L. innocua</i> <i>S. enteritidis</i> , <i>E. coli</i> (c) <i>S. hadar</i> <i>E. coli</i> <i>O157:H7</i>	Antibacterial action by geraniol: (a and b) >5 log CFU/mL reduction (c) 50% reduction of bacterial population	(a) 0.02% (b) 0.6% (c) 0.0069–0.025%	(a and b) (Raybaudi- Masseli et al., 2006) (c) (Friedman et al., 2004)

ND, not defined; MBC: minimum bacterial count; MFC: minimum fungal count; MIC: minimum inhibitory concentration.

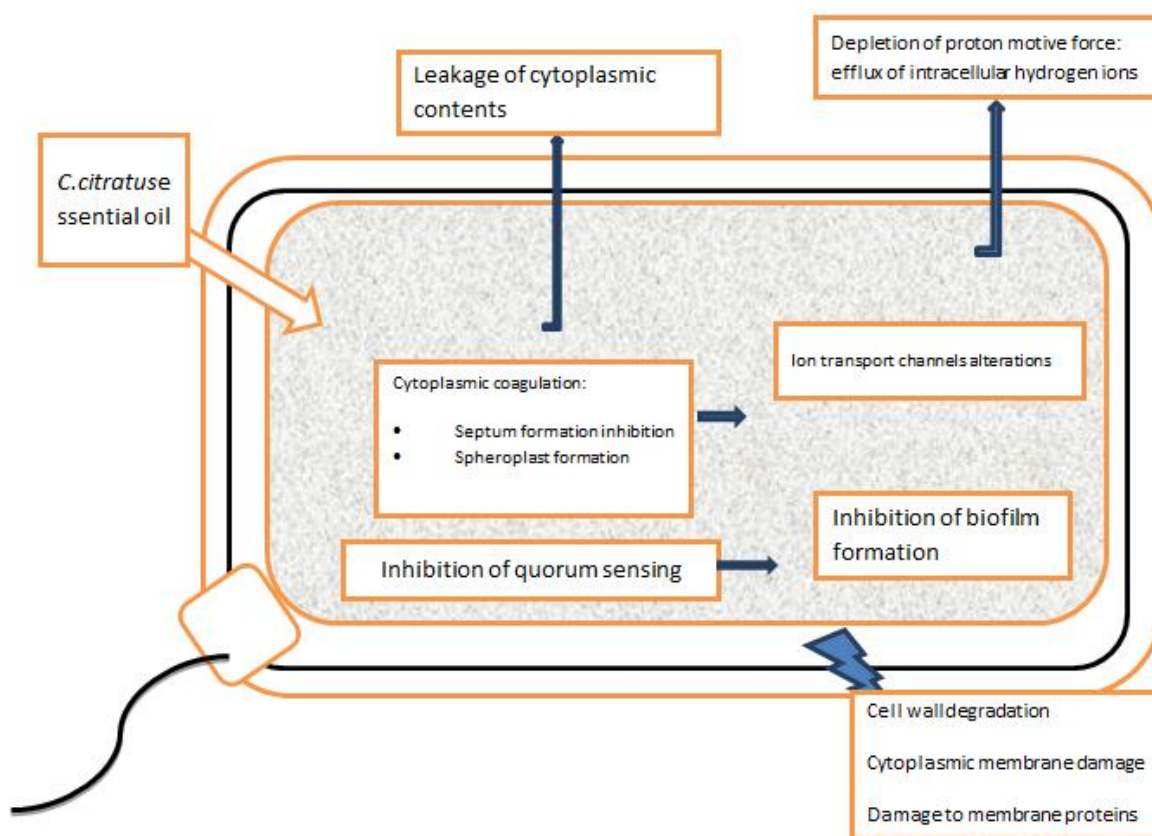


Fig. 1. **Possible antibacterial mechanism of action of *C. citratus* essential oil:** The interaction of CCEO and its fractions at the membrane, and the cytoplasmic levels in bacterial cell promotes a cascade of processes leading to cell immobility and mortality. These actions include cytoplasmic coagulation, membrane proteins degradation, and inhibition of biofilm formation.

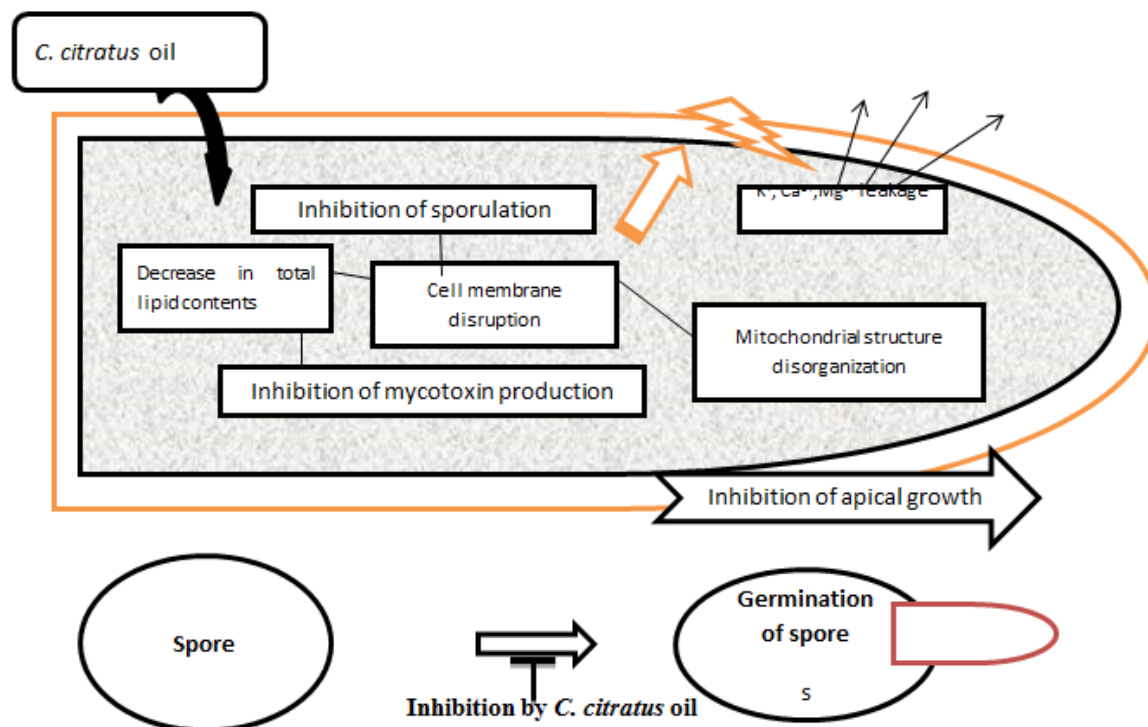


Fig. 2. Antimycotic mechanism of action of *C. citratus* essential oil and its active constituents: Due to hydrophobic and lipophilic nature of CCEO and its phytochemicals, it could interact with the fungal membrane, interfering with its integrity and leading to electrolytes leakage. Other actions include: inhibition of mycotoxin synthesis and mitochondrial structure disorganization, inhibition of apical growth and spore germination. These actions could lead to inhibition of cell growth or even death.

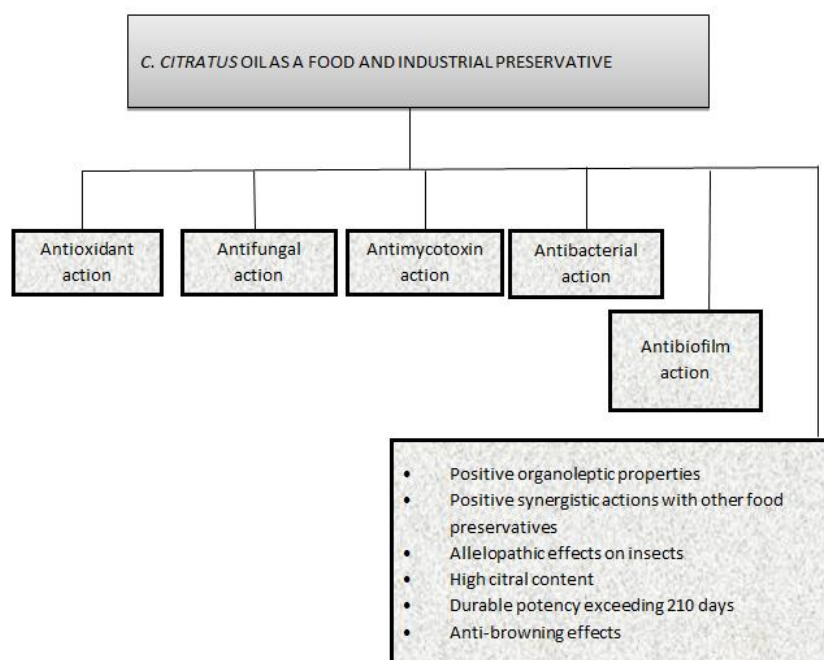


Fig. 3: Features of *C. citratus* essential oil promoting its application in food and industrial preservation