

Nanoformulated essential oils against fungal and mycotoxin contamination of post-harvest commodities: A sustainable and consumer-friendly approach for food safety

Shikha Tiwari¹ and Nawal Kishore Dubey^{2*}

¹Department of Botany, S.S.S.V.S. Government Post Graduate College, Chunar, Mirzapur 231304, Uttar Pradesh, India

²Laboratory of Herbal Pesticides, Centre of Advanced Study (CAS) in Botany, Institute of Science, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

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Fungal and mycotoxin contaminations are one of the major threats to food security. Looking into the harmful effects of synthetic preservatives, consumers currently prefer to use safer alternatives for food preservation in order to enhance the shelf life of food commodities such as green preservatives. In this context, plant essential oils (EOs) and their bioactive components are gaining prime attention in food preservation to be used as green preservatives due to their broad bioactivity as well as biodegradable nature, favourable safety profile, diverse mode of action and recognition under Generally Recognized As Safe (GRAS) Category. The nanoencapsulation technique would effectively boost the large-scale application of essential oils as food preservatives through the controlled release of bioactive components. The present review presents the recommendations for essential oils and their nanoformulations as sustainable and consumer-friendly approach to ensuring food safety by reducing mould and mycotoxin contamination.

Keywords: Essential oils, Food safety, Green preservative, Mycotoxin, Nanoencapsulation

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Introduction

Food commodities are extremely important for human consumption in order to maintain their life activities. Different fresh fruits and vegetables provide all the necessary supplements like fibre and vitamins. However, several storage fungi pose a major threat to food security by deteriorating the quality of food products and feed, thus significantly affecting the socioeconomic status of the nation. Post-harvest challenges of fruits, vegetables and food commodities are mostly associated with microbial deterioration, which have currently become more intensified due to climate change, frequently promoting the emergence of new disease-causing strains¹. In many countries, food-borne illness caused by food-borne microbes and their toxic secretions is recognized as a major health hazard. Because of unsafe food consumption, millions of people become sick while hundreds of thousands die every year. As reported by the US Centers for Disease Control, it is estimated that food-borne illnesses result in around 48 million cases annually in

the US. Such food-borne infection results in a significant global burden, causing 33 million tons of losses daily². Filamentous fungi such as *Penicillium*, *Aspergillus* and *Fusarium*, as well as their associated mycotoxins such as ochratoxins, aflatoxins, patulin, zearalenone, fumonisins and trichothecens, are the major contaminants of food and feed³. Moulds penetrate host cells by producing lytic enzymes like pectinases and hemicellulases, which ultimately lead to biodeterioration of the food commodities, affecting the quality and sensorial properties of food due to secretion of health-hazardous mycotoxins⁴. Ingestion of mycotoxin-contaminated feed also causes metabolic disturbances in animals, ultimately reducing their productivity, egg production, hatchability of eggs, fertility, and growth rate⁵. Humans can be exposed to mycotoxins either directly through the consumption of contaminated food commodities like nuts, oilseeds, cereal-based products, cereals and spices or indirectly through the utilization of animal products like milk, egg and meat⁶. According to the Food and Agriculture Organization, nearly 25% (one-fourth) of global annual agricultural products are spoiled due to fungal

*Correspondent author
E-mail: nkubeybhu@gmail.com

and mycotoxin contamination, among which aflatoxins secreted by different species of *Aspergillus* are of prime concern because of their frequent occurrence in different food commodities, posing chronic toxicity and intense carcinogenicity⁷. In the United States, economic losses from wheat and barley contamination by *Fusarium* mycotoxins, along with related plant diseases, amount to \$ 2.9 billion annually, while managing crop contamination with aflatoxins costs \$1.4 billion/year⁸. Hence, management of post-harvest losses of food commodities due to fungal and mycotoxin contamination is not just important economically but also to save lives. Looking at the devastating effects of fungal and mycotoxin contamination, different synthetic preservatives have frequently been applied, such as grey preservatives.

It is undeniable that the use of synthetic preservatives has played an impressive role in preventing microbial contamination of food and enhancing its shelf life. However, in addition to causing sensorial damage to food products and adverse effects on human health, their indiscriminate application leads to the creation of resistant microbial strains⁹. Due to their non-biodegradable nature, such synthetic preservatives impose negative impacts on the sustainability of the environment by contaminating soil and water, as these additives consist of chemical substances; for example, sodium benzoate is not easily biodegradable. When these chemicals are flushed into waterways, they can affect aquatic life by altering water quality and potentially causing toxicity to fish and other organisms. Similarly, when pesticides are used in farming, they can leach into the soil, impacting plant growth and harming beneficial soil microorganisms, thus reducing soil fertility and overall ecosystem health¹⁰ and thereby exerting negative effects on the non-target organisms¹¹. Looking into the harmful effects of synthetic preservatives, consumers currently prefer to use safer alternatives for food preservation against fungal and mycotoxin contamination. Different plant-based preservatives have encouraged food and agricultural scientists to recommend their large-scale application as green preservatives because of their biodegradable nature, non-toxic profile, easy availability and traditional medicinal values. In this context, plant essential oils (EOs) and their bioactive components are gaining prime attention in food preservation due to their volatile nature and broad

bioactivity like antioxidant, antimycotoxigenic, antifungal, insecticidal and antibacterial. EOs are biodegradable, easily available, have diverse modes of action and are labelled under the Generally Recognized as Safe (GRAS) category by the Environmental Protection Agency and Food and Drug Administration, USA. For example, *Coriander* oil is biodegradable, easily available, and has a diverse mode of action, making it effective against microbes. It is commonly used as a natural preservative in food products and has been recognized by FDA, FEMA and Council of Europe. FDA has approved *coriander* oil as GRAS¹². It has been observed that the effectiveness of different edible coatings for packaging food commodities to protect them from microbial contaminations has been improved significantly by incorporating essential oils and their active components. The presence of several bioactive components in EOs also minimize the chance of resistant development in pests due to the synergistic mode of action¹³. In spite of such attributes as green preservatives, active components of different EOs are rapidly degraded by their exposure to environmental conditions such as high temperature, humidity and light that suppress their efficacy. Moreover, the high volatility and thermolability of EOs are also major challenges for their large-scale practical applicability. Thus, there is a need to develop some modern innovative approaches to solve such issues for the large-scale application of essential oils as sustainable and consumer-friendly approaches for food safety and to enhance the shelf life of post-harvest food commodities. The nanoformulations of the essential oils through nanoencapsulation techniques have effectively resolved the challenges associated with EOs through the controlled release of EOs components¹⁴.

The present review highlights the detrimental effects of mould and mycotoxin contamination on food security and the future perspective of essential oils and their nanoformulations as an exciting, sustainable, and consumer-friendly approach to ensuring food safety and reducing mould and mycotoxin contamination.

Methodology

This literature review utilized original research articles (primarily peer-reviewed) and review papers. Books from PubMed were also reviewed to incorporate concepts or fundamentals. A comprehensive

investigation on fungal and mycotoxin contamination of post-harvest products: A sustainable and consumer-oriented method for ensuring food safety was conducted by reviewing literature related to fungal and mycotoxin contamination, essential oils, nanoencapsulation, and food preservation using various online databases like Web of Science, Willey, Science Direct, and Google Scholar.

Challenges with synthetic preservatives in the food industry

Food preservatives are natural or synthetic antimicrobial substances that prevent unpleasant chemical alteration or decomposition of food products, which most probably occur due to lipid oxidation or microbial contamination¹⁵. Hence, preservatives play a crucial role in enhancing the shelf life of stored food products by preventing microbial contaminations. There are numerous synthetic preservatives which are frequently recommended by the food sector. Sorbic acid, sodium benzoates, sulfur dioxide, nitrites, sodium and potassium nitrate and propionates are some common antimicrobial food preservatives. In addition, there are different synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) commonly used in food industries, Ethoxyquine is also reported as a synthetic antioxidant used for animal feed although it is not allowed for human food¹⁶. However, the undesirable side effects comprising short and long-term health threats continuously rise consumer awareness towards synthetic chemicals used as grey preservatives¹⁷ (Fig. 1). Several synthetic preservatives are reported to impose health problems such as hyperactivity, nervous system damage, asthma, allergy, sleeping disturbances, irritability, restlessness and

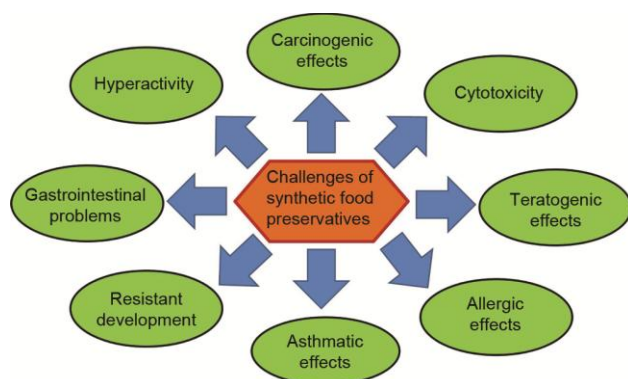


Fig. 1 — Some challenges associated with synthetic preservatives.

inattentiveness¹⁸. Potassium sorbate is one of the common food preservatives for sauces, soft drinks, fish products, pickles and cheeses and has been reported to have mutagenic and genotoxic effects against human blood lymphocytes¹⁹. High doses of sodium benzoate are reported to cause neuromodulatory defects through reducing glycine as well as inducing reactive oxygen species-mediated damages, which occurred due to mitochondrial dysfunctioning²⁰. Sodium and potassium benzoates are also reported to promote teratogenic effects on their consumption. Karwowska and Koniuk mentioned that nitrates and nitrites cause different types of cancers, including gastric cancer, oesophageal cancer, thyroid cancer, breast cancer, colorectal, adult glioma and renal cell carcinoma because of their conversion to carcinogenic compound nitrosamine²¹. Propionates are reported as an important antifungal food preservative. However, exposure to sodium propionates has several dreadful health effects. Xu *et al.* reported that exposure of zebrafish to sodium propionate caused cognitive defect, depressive behaviour, as well as a hyperglycaemic condition through accelerating the triglyceride content, total cholesterol and total blood glucose level²².

Moreover, exposure to sulphites, one of the common food preservatives, to sensitive persons induces several health problems, including diarrhoea, abdominal pain, urticaria, dermatitis and severe allergic and asthmatic effects²³. Despite of having a fabulous role as a shelf life enhancer of foodstuff, different synthetic antioxidants such as PG, BHA, BHT, and TBHQ have now become a matter of great concern for researchers because of their safety issues. BHA has been reported to promote carcinogenicity and cytotoxicity, while BHT and TBHQ cause DNA damage, tumour formation and excessive utilization of PG, which manifests liver toxicity and neurodermatitis problems in allergic persons²⁴. Besides having detrimental health effects, synthetic chemicals also induce destructive effects on the environment, negatively affect non-targeted organisms and induce resistant development in microbes, thereby affecting the sustainability of the environment. One of the food spoilage yeast, *Zygosaccharomyces bailli*, has been reported to develop resistance against weak acid preservatives like sorbic acid and acetic acid, which might be due to efflux pump-mediated removal of preservatives and their degradation or metabolism²⁵. *Aspergillus niger*

develops resistance against sorbic acid through a pad-decarboxylation system, which converts sorbic acid into volatile compound 1,3pentadiene²⁶. This decarboxylation-mediated resistance development against sorbic acid was also reported in *Penicillium* sp, some yeast sp and *Trichoderma*. Hence, hazardous effects of synthetic preservatives as coatings on health and environment have attracted researchers and food industries to search some eco-friendly and safer alternatives.

Plant essential oils in food preservation

Based on preservative and medicinal attributes, different aromatic plants have been used since ancient times to enhance the sensorial quality of food and also to protect them from oxidative and microbial damage. EOs are the aromatic secondary metabolites of plant that can be obtained from different plant parts such as buds, seeds, roots, barks, flowers and leaves through solvent extraction, steam or water distillation, subcritical water extraction and supercritical fluid extraction. The biological activities of EOs are due to the presence of bioactive compounds like terpene, terpenoids, ketones, aldehyde, and phenolic acids. Generally, the major bioactive components contribute to diverse modes of antimicrobial action of EOs, while minor components act synergistically with major components and enhance their efficacy²⁷. The bioactive compounds of EOs varied in their composition, quality and quantity with variations in soil composition, vegetative cycle stage, age, plant part and climatic condition, which ultimately affect their bio-efficacy²⁸. EOs have attracted food sectors towards their application as green food preservatives based on their non-toxic, cost-effective, renewable, eco-friendly nature and wide range of biological activities such as antifungal, insecticidal, antibacterial, anti-inflammatory, antiviral, cytotoxic, anti-helminthic, ovicidal, immunomodulatory, antifeedant, repellent, larvicidal, antinociceptive, antioxidant and molluscicidal²⁹.

At present, different EO-based preservatives are available in the market. EcoTrol® having rosemary EO “Talent” the antifungal formulation prepared of carvone (<https://www.keyplex.com/knowledge-base/ecotrol-plus-and-sporan-ec2-hit-the-sweet-spot-for-a-california-cannabis-grower/>), EcoPCOR® introduced by EcoSMART® technology comprised of eugenol used as insect controlling agent while “DMC Base Natural” composed of 50% Sage, Rosemary and

Citrus EOs and 50% glycerol are currently used on large scale as green preservatives due to its antimicrobial properties. It works primarily by reducing the water activity in food. The high concentration of glycerol draws water out of microbial cells, leading to dehydration and disruption of their cellular processes, thereby preventing spoilage. According to the FDA, glycerol is classified as Generally Recognized As Safe. Glycerol is a non-allergenic, biodegradable, and eco-friendly alternative to synthetic preservatives³⁰. There are several EOs which exhibit a significant role as food preservatives by preventing mould and mycotoxin contamination through different modes of action. Bocate *et al.* reported that *Allium sativum* EO (garlic) was an effective antifungal agent for stored corn kernels against *Fusarium verticillioides*, *Aspergillus parasiticus* and *Gibberella zeae* that secretes fumonisin B₁, aflatoxin B₁ and zearalenone respectively³¹. In addition, *Schinus molle*, *Eucalyptus globulus*, and *Thymus capitatus* EOs were also found to be significant inhibitors for spore germination and mycotoxin production (aflatoxin and fumonisin) by *Aspergillus parasiticus* and *Fusarium moniliforme*³². Hu *et al.* reported that EOs of seven spices, *i.e.* *Mentha hypocalyx* Briq., *Cinnamomum camphora* L. (camphor tree), *Pimpinella anisum* L. (aniseed), *Cinnamomum zeylanicum* L. (dalchini), *Cymbopogon nardus* (L.) Rendle (citronella grass), *Zanthoxylum bungeanum* Maxim (prickly ash) and *Syzygium aromaticum* L. have antifungal activity against three moulds, *i.e.* *Aspergillus ochraceus*, *A. oryzae* and *A. niger* isolated from bread³³. EOs are enriched with oxygenated monoterpenes (esters, aldehydes and ketones) and several of them *viz* *Anethum graveolens* L. (dill), *Mentha longifolia* L. (horsemint), *Galagania fragrantissima* Lipsky, *Hyssopus seravschanicus* (Dubj.) Pazij, *Achillea filipendulina* Lam. (Fernleaf yarrow) and *Ziziphora clinopodioides* Lam. are having remarkable antioxidant potential³⁴. This antioxidant property contributes to enhancing the shelf life of food along with maintaining their sensorial values.

An earlier study reported that some essential oil constituents, such as citral, citronellol, eugenol, farnesol, and nerol, could protect chili seeds and fruits from fungal contamination up to 6 months. *Ageratum conyzoides* EO successfully controlled blue mould rot in mandarins, extending their shelf life to 30 days³⁵. Das *et al.* assessed the effectiveness of *Coriandrum*

sativum EO and its nanoencapsulation effectiveness preventing fungal growth and aflatoxin B1 contamination in stored rice³⁶. Tabassum *et al.* reported extending the shelf life of freshly cut papaya by using EOs³⁷. An edible coating based on nanoemulsion was created utilizing alginate as the wall material with varying quantities of oregano EO (0.5, 1.0, and 2.0 mL). The findings showed that oregano EO was significantly effective in prolonging the freshness of sliced papaya for as long as 16 days. Similarly, Arabpoor *et al.* studied the impact of *Eryngium campestris* EO (ECEO) applied to cherry preservation at 4°C. The CHNP-ECEO coating enhanced the pH, firmness, and antioxidant capacity of cherries while minimizing microbial presence³⁸.

Arasu *et al.* reported that *Allium sativum* EO effectively inhibited the infestation of *A. niger* and *A. flavus* in plum fruit, preserving its quality up to 10 days of storage. In an *in vitro* assay, *A. niger* and *A. flavus* were fully suppressed at concentrations of 7.5 and 6.5 µg/mL, respectively; however, the *in vivo* activity of *A. sativum* EO required 1.5 times higher concentration than that needed for the same antifungal effectiveness observed *in vitro*³⁹. Similarly, Yassein *et al.* demonstrated that cumin and peppermint EOs effectively inhibited mycotoxigenic fungi in baby food like cerelac, cornflakes, and milk powder with suppressive effects on aflatoxin and ochratoxin production⁴⁰. According to Atif *et al.* *Ocimum*

basilicum and *Vetiveria zizanioides* EO showed strong synergistic efficacy in protecting stored jackfruits from bacterial and fungal spoilage⁴¹.

These plant essential oil-based botanical preservatives target different sites on the food-borne fungi, including cell membrane, cell wall, biochemical alteration and different cellular components, thereby exerting their antifungal activity. Some of the possible antifungal and antimycotoxigenic mechanisms of action (Fig. 2) rendered by essential oils are mentioned below.

Destruction of cell wall and cell membrane

The cell wall of the fungi is made up of chitin that comprises a covalent linkage of β-1,4 linked N-acetylglucosamine to β-1,3 glucan, which is responsible for the physical strength and structural integrity of the cell wall. Eucalyptus EO and its component 1,8 cineole target chitin synthase and cause inhibition of β-1,3glucan synthesis in the cell wall, ultimately leading to fungal cell death⁴². In addition, these EOs also target fungal plasma membranes through inhibition of membrane synthesis enzymes, destructing membrane, membrane fluidity and permeability or degrading membrane protein⁴³. Huang *et al.* reported that *Listea cubeba* EO inhibited the growth of *Colletotrichum scovellei* by disrupting cell wall integrity and causing a decrease in ergosterol content, leading to damaged membrane permeability⁴⁴. Shahina *et al.* also reported that *Cinnamomum zeylanicum* bark EO exhibited disruption of cell wall

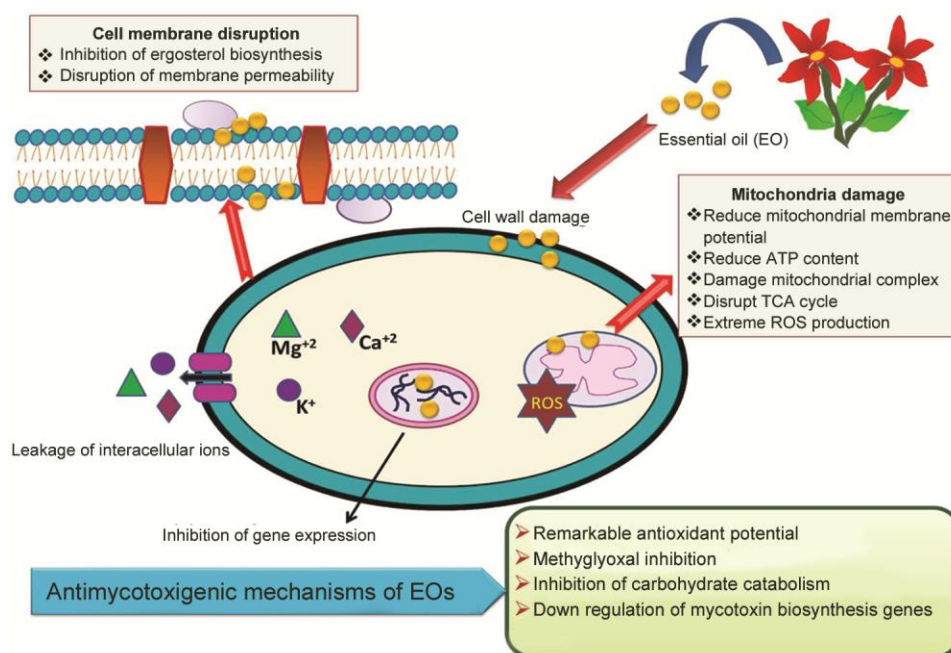


Fig. 2 — Possible antifungal and antimycotoxigenic modes of action of EOs.

integrity and cell surface damage in *Candida albicans*, suggesting botanicals as effective antifungal agents⁴⁵. Khan *et al.* reported that *Ocimum sanctum* L. EO and its active principles linalool and methyl chavicol are promising antifungal substances that inhibited the growth of azole-resistant strains of *Candida albicans* through damaging plasma membranes and reduced ergosterol content⁴⁶. Based on their lipophilic nature, EOs easily pass through the lipid membrane and interact with ergosterol or reach inside the nucleus to regulate biosynthetic genes. Citral showed antifungal activity against green mould *Penicillium digitatum* by inhibiting the ergosterol biosynthesis through down-regulating expression of ergosterol biosynthetic genes, *i.e.* ERG₃, ERG₅, ERG₆, ERG₇ and ERG₁₁⁴⁷. Helal *et al.* reported disruption of fungal cell membrane leading to leakage of cellular ion like K⁺, Mg⁺² and Ca⁺² that prompted cell death⁴⁸.

Mitochondrial damage

Mitochondria are known as the powerhouse of the cells as they make the availability of the energy currency ATP (adenosine triphosphate) through the tricarboxylic acid cycle (TCA) and oxidative phosphorylation. Mitochondria is one of the prominent sites for antifungal agents, and its damage ultimately causes cell death. Li *et al.* reported that tea tree EO induces severe mitochondrial damage leading to disruption of TCA cycle, enhancement of ROS and suppression of action of several enzymes such as ATPase, malic dehydrogenase, citrate synthetase, succinate dehydrogenase and α -ketoglutarate dehydrogenase resulting into death of *Botrytis cinerea*⁴⁹. Similarly, Ji *et al.* also reported the antifungal activity of oregano EO against *A. flavus* through enhancing oxidative stress and declining mitochondrial activity and ATP production⁵⁰. In addition, citral is also reported to inhibit the growth of *P. digitatum* via, inhibiting mitochondrial membrane potential, glutathione, and intracellular ATP content, causing adverse effects on mitochondrial complexes, ultimately leading to damage oxidative phosphorylation and supporting enormous ROS accumulation⁵¹.

Antimycotoxigenic mechanism

EOs effectively prevent mycotoxin contamination in food products by inhibiting the growth of fungi or altering mycotoxin synthesising genes. Castro *et al.* reported *Cymbopogon martini* (Roxb). Wats., *Cinnamomum zeylanicum* Blume and *Zingiber*

officinale Roscoe EOs to inhibit the growth of toxigenic food-borne fungi *Fusarium verticillioides* leading to suppression of the production of fumonisin B₁ and fumonisin B₂⁵². *Myristica fragrans* Houtt., *Cymbopogon flexuosus* (D.C) Stapf and *Satureja montana* L. EOs were also reported as promising alternatives of synthetic preservatives because of inhibition of the growth of *Aspergillus ochraceus* and *A. flavus* through damaging their cell wall; the synergistic action of bioactive components also prevented production of associated toxins ochratoxin A and Aflatoxin B₁ and Aflatoxin B₂ respectively⁵³. *Pimpinella anisum* L., *Foeniculum vulgare* Miller, *Rosmarinus officinalis* L., *Apium graveolens* L., *Thymus vulgaris* L. and *Elettaria cardamomum* (L.) Maton EOs were reported to have significant antimycotoxigenic efficacy against Ochratoxin A by suppressing Ochratoxin biosynthesis genes (*acOTApks*, *acpks* and *acOTAnrps*) and regulatory genes (*veA* and *laeA*)⁵⁴. Belasli *et al.* reported that *Laurus nobilis* L. EO significantly inhibited the growth of *A. flavus*, aflatoxin production and also exhibited prominent antioxidant property⁵⁵. The anti-mycotoxigenic activity of *L. nobilis* L. EO might be either due to its supreme antioxidant profile because ROS is reported as an inducer molecule for aflatoxin biosynthesis or due to its interference with some steps of the aflatoxin biosynthesis pathway. Phenolic compounds of EOs block the synthesis of acetyl Co-A, which is a precursor for aflatoxin synthesis through inhibiting the role of mitochondrial superoxide dismutase⁵⁶. Eugenol is reported to inhibit enzymes associated with glucose catabolism and lipid peroxidation, resulting in aflatoxin inhibition⁵⁷. Another anti-aflatoxigenic mechanism would be through inhibiting aflatoxin biosynthesis genes. Yahyaraeyat *et al.* reported that *Zataria multiflora* Boiss. EO inhibited Aflatoxin B₁, Aflatoxin G₁, Aflatoxin B₂ and Aflatoxin G₂ production by suppressing the expression of *ver-1*, *nor-1* and *omtA* gene⁵⁸. Further, Upadhyay *et al.* also reported that *Cistus ladanifer* L. inhibited methylglyoxal synthesis, a precursor molecule of aflatoxin biosynthesis, suggesting it as one of the possible anti-aflatoxigenic modes of action and future development of transgenic crops resistant to aflatoxin biosynthesis⁵⁹.

Nanoformulated essential oils as sustainable and consumer-friendly preservatives

In spite of having amazing food preservation potential, different factors such as high volatility,

unsuitable interaction of EOs with food components and high susceptibility towards oxidation and polymerization due to their lipophilic nature, thereby limit the large-scale practical applicability of EOs. Moreover, different extrinsic (gaseous composition of environment, type and concentration of microbes, temperature) and intrinsic factors (salt concentration, protein, water activity, pH and fat), as well as unpleasant sensorial alteration of food items due to the application of high concentration of aromatic EOs, also restrict their large-scale application in food sector⁶⁰. Antifungal and anti aflatoxigenic efficacy of *Lippia turbinata* Griseb var. *integrifolia* EO and *Peumus boldus* EO relied on water activity; antifungal activity of *P. boldus* EO was suppressed after its exposure to UV and light while antifungal activity of *L. turbinata* EO reduced after six months storage⁶¹.

Currently, nanoencapsulation technology has been applied to enhance the bioactivity and physical stability of EOs during food processing. Nanoencapsulation also improves bioavailability, solubility, absorption, and control of the delivery of bioactive compounds⁶². Nanoencapsulation refers to the entrapment of bioactive components inside a carrier matrix in a nanometric range (10^{-9} m)⁶³. Encapsulating material is called as capsule, outer cell, wall material, film, carrier, membrane and packing material while encapsulated substance are known as internal phase or payload, actives and core material. Encapsulating matrix for botanical preservatives must be biodegradable and non-reactive to food components as well and entrapped active substances should possess superior rheological quality even at higher concentration and potency to protect and preserve the bioactivity of entrapped bioactive components during storage and processing⁶⁴. A wide range of encapsulating biopolymers are applied, which can be categorized into polysaccharides (chitosan, cellulose, starch, alginate), proteins (wheat gluten, soy protein), lipid films (resin, fatty acids) and composite films. Chitosan and nanocellulose are highly preferred due to their abundance and versatility⁶⁵. Polysaccharides are the most favoured and well-known carriers for numerous bioactive substances in the food sector. *Pimpinella anisum* essential oil (PAEO) has been encapsulated by the chitosan biopolymer through an ionic gelation method to achieve nanoparticles with enhanced antifungal and anti-aflatoxin effectiveness against food-related moulds spoiling rice⁶⁶. Calderón-Santoyo *et al.*

reported the effectiveness of microencapsulated citral in Arabic gum and sodium alginate through spray drying. Consequently, microcapsules containing citral may act as an eco-friendly substitute for managing and reducing the post-harvest losses of banana⁶⁷. The essential oil of *Mentha spicata* L. and magnesium oxide are encapsulated within nanofiber mats created through an electrospinning process utilizing sodium caseinate-gelation (SC-GE) as a coating material. The nanofiber mats were created to assess their possible applications in the storage of fresh trout fillets in cold conditions⁶⁸. Analogous research demonstrated the effectiveness of encapsulated cinnamon essential oil within zein/ethyl cellulose electrospun nanofibers, which significantly hindered the decrease in weight and maintained the texture of *Agaricus bisporus* while also enhancing their quality throughout the storage duration⁶⁹. Different lipid materials utilized in food-related applications encompass fatty acids, glycerides, and waxes like carnauba wax, beeswax, paraffin, and phospholipids. Solid lipid nanoparticles incorporating *Zataria multiflora* essential oil have been developed using high-pressure homogenization and ultrasonication methods, employing glyceryl monostearate, Poloxamer 188, and Precirol® are utilized as surfactants. The nanoparticles are frequently spherical in shape, 100 nm in size, ensuring the regulated release of EO with excellent stability, protecting against enzymatic degradation of EOs, and being effective against fungal pathogens⁷⁰.

There are two types of emulsification approaches for encapsulation: top down approach, in which particle size declines and bottom up approach, in which particle size increases due to the self-assembly of molecules. Based on encapsulating material, nanoencapsulation strategies are of two types, *i.e.* polymer-based nanoencapsulation system (ionic gelation, complex coacervation, spray drying and freeze drying) (Table 1) and lipid-based nanoencapsulation system (liposome, nanoemulsion, solid lipid nanoparticles) (Fig. 3). In addition, nanoencapsulation can also be done by using special equipment, nanoparticles must be converted into a powdered form using special equipment such as electro-spraying, nano spraying dryer and electrospinning⁷⁷. Polymer-based nanoencapsulation creates nanosphere (core particle dispersed in the polymeric matrix) and nanocapsules (core particle surrounded by a polymeric matrix). Similarly, lipid based nanoencapsulation form biodegradable liposomes,

Table 1 — Methods of encapsulation using various biopolymers

S. No.	Essential oil	Methods of encapsulation	Biopolymer used	References
1	<i>Cymbopogon martinii</i>	Ionic gelation	Chitosan	71
2	Clove oil	Ultrasonication	Whey protein	72
3	<i>Satureja montana</i>	Thin film hydration method	Lipid vesicles (niosomes)	73
4	<i>Cinnamomum cassia</i>	Spray-drying	Gum Arabic and maltodextrin	74
5	Lemon grass	Electrospinning	Cassava starch	75
6	Curry plant	Homogenization	Liposomes	76

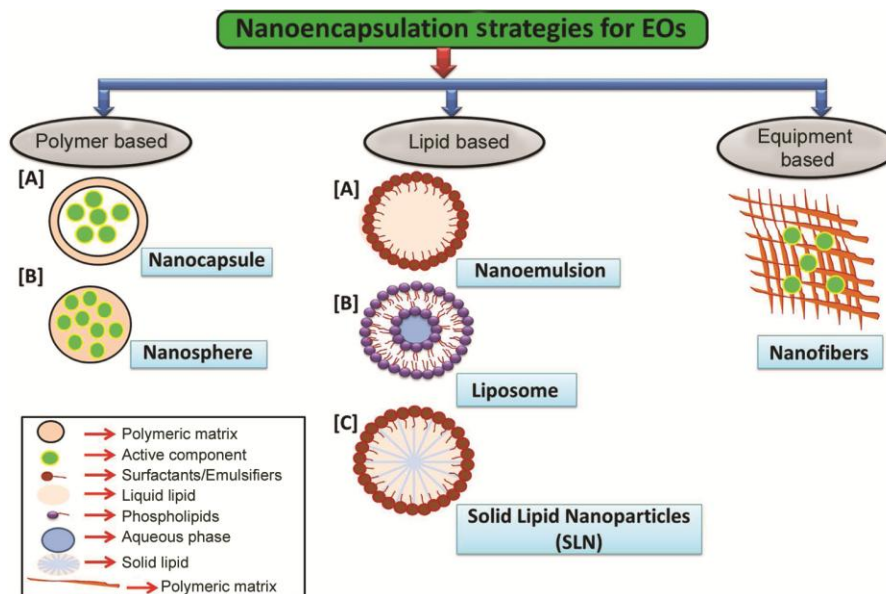


Fig. 3 — Nanoencapsulation strategies used for EOs.

nanoemulsion and solid lipid nanoparticles (SLN), however, specialized equipments such as electrospinning and electrospray lead to form nanofibres and nanofibrous scaffolds.

Nanosized EO-based green preservatives developed through nanoencapsulation can easily disperse in the food matrix due to increased surface area at the nano-range. Nanoformulations form stable and uniform colloidal suspension of water causing sustainable release of active substances. Moreover, nanoencapsulation also causes targeted release of effective bioactive compounds with amplified cellular absorption, suggesting their intensified bioavailability and bio-efficacy. In addition, nanoencapsulation of EOs also maintains the desirable sensorial values of food products by masking their intense aroma *via* sustained release and also limits their interaction with food components. There are many reports on the boosting antifungal and antimycotoxigenic efficacy of EOs and their bioactive components after nanoencapsulation (Table 2). Wen *et al.* reported that cinnamon EO nanofilm enhanced the shelf life of

strawberries without altering their flavour, suggesting EO nanoformulations as a consumer-friendly approach to preserve food for a longer duration by maintaining their sensorial qualities⁹³. Ghaderi-Ghahfarokhi *et al.* reported that thyme essential oil-loaded chitosan nanoparticles exhibited better preservative efficacy for beef burgers in comparison to free oil without alteration in organoleptic profile⁹⁴. Sindhu *et al.* reported *Curcuma longa* L. EO encapsulated inside chitosan as a promising antimicrobial agent against food-borne pathogens and mycotoxins, showing outstanding antifungal and antimycotoxigenic activity by completely inhibiting the growth of *Fusarium graminearum* and deoxynivalenol and zearalenone production at 0.75 $\mu\text{L}/\text{mL}$ ⁹⁵. Hasheminejad *et al.* reported nanoencapsulated emulsion of clove essential oil into chitosan matrix for improvement of antifungal efficacy in food system⁹⁶. Kujur *et al.* reported *Pelargonium graveolens* L. EO as an effective inhibitor of *Aspergillus flavus* and Aflatoxin B₁ production in stored maize through reducing carbon

Table 2 — Some nanoencapsulated EOs and their bioactive compounds recommended as possible green preservatives against food-borne fungi and mycotoxins

S. No.	Nanoencapsulated essential oil	Remarks	References
1	<i>Valeriana officinalis</i> L. (garden valerian)	Nanoformulation of <i>V. officinalis</i> exhibited remarkable antifungal activity and aflatoxin B ₁ inhibition at 6.5 to 3.5 µL/mL, respectively, against <i>Aspergillus versicolor</i> , <i>A. niger</i> , <i>A. flavus</i> , <i>Fusarium oxysporum</i> and <i>Penicillium italicum</i> ; ultimately prevent nutritional deterioration of <i>Citrus sinensis</i> and maintained post-harvest quality. Superior activity of nanoencapsulated EO was due to its controlled release, ergosterol disruption, methylglyoxal inhibition and interaction of valerianol with Ver-1 protein.	78
2	Cinnamon, peppermint, thyme, clove and lemon grass	Nanoemulsion of the five selected essential oils showed enhanced antimycotoxigenic activity against 3-acetyl deoxynivalenol, deoxynivalenol and 15-acetyl deoxynivalenol which is a secondary metabolite of <i>Fusarium graminearum</i> . The superior anti-mycotoxigenic activity of EO-loaded nanoemulsions was attributed to the improved stability and solubility of EOs.	79
3	<i>Carum copticum</i> L. EO (ajwain) and <i>Peganum harmala</i> L. (harmala) extract	Nanoencapsulated <i>C. copticum</i> and <i>P. harmala</i> EOs combination showed promising <i>in vivo</i> and <i>in vitro</i> efficacy against <i>A. alternate</i> at 0.2 µL/mL.	80
4	<i>Bunium persicum</i> (Boiss) Fedtesch (black cumin)	Chitosan-based nanoencapsulated <i>Bunium persicum</i> EO was reported to have high thermal stability and antioxidant properties with promising antifungal and anti-mycotoxigenic efficacy by completely inhibiting the growth of <i>A. flavus</i> and aflatoxin B ₁ production at 0.3 µL/mL	81
5	<i>Cupressus sempervirens</i> L. (Italian cypress), <i>Pinus roxburghii</i> Wall. (chirpine), <i>Juniperus communis</i> L.	Nanoencapsulated synergistic formulation enhanced the shelf life of <i>Oryza sativa</i> by preventing <i>A. flavus</i> and aflatoxin B ₁ contamination at 4.0 and 3.5 µL/mL, respectively. The formulation also had a broad safety profile and preserved sensorial profile and minerals by preventing lipid peroxidation, recommending it as an innovative smart green approach for food security.	82
6	<i>Syzygium aromaticum</i> L. (clove)	Nanoencapsulated <i>S. aromaticum</i> EO prevented Fumonisin B ₁ and Fumonisin B ₂ contamination in maize kernels during storage. Nanocapsules were reported to have antifungal and anti-aflatoxigenic activities at 0.3 µL/mL.	83
7	<i>Cinnamomum verum</i> J. Presl (true cinnamomum)	Cinnamon nanoparticles exhibited prominent efficacy in controlling <i>A. flavus</i> and aflatoxins production at 1.56 µL/mL in beef burger patties, along with preserving its organoleptic characteristics.	84
8	<i>Cinnamomum glaucescens</i> (Nees) Hand.-Mazz. (sugandhkokila)	Nanoencapsulated EO showed significant efficacy against <i>A. flavus</i> (0.9 µL/mL) and aflatoxin B ₁ production (0.8 µL/mL). It also exhibited an acceptable sensorial profile, preservation of minerals and inhibition of lipid peroxidation along with a non-toxic nature, suggesting it is a novel green alternative to synthetic preservatives.	85
9	<i>Melaleuca cajuputi</i> Maton & Sm. Ex R. Powell	Essential oil-loaded chitosan nanoparticle promising effect in enhancing the shelf life of <i>Agaricus bisporus</i> . Further, nanoformulation also has a broad safety profile (LD50 >1200 mg/kg body weight) and enhanced antioxidant quality, along with maintaining the weight and organoleptic profile of the mushroom.	86
10	<i>Ocimum americanum</i> L. (hoary basil)	Nanoformulation of the EO prevented <i>A. flavus</i> growth and aflatoxin B ₁ production in stored <i>Setaria italica</i> at concentrations 0.2 and 0.175 µL/mL, respectively, against seed without affecting its sensorial or germinative properties. The interaction of citral with Ver-1 and Omt-A protein via <i>in silico</i> modelling suggests that it has an Aflatoxin B ₁ inhibitory action. The appreciative safety profile recommends its botanical-based nanoformulation as a safer green preservative for stored millet.	87
11	<i>Zataria multiflora</i> Boiss L. (wild marjoram)	Nanoencapsulated EO prevents <i>A. flavus</i> and aflatoxin B ₁ contamination in pistachio. Nanoencapsulated EO has depicted enhanced bio-efficacy, as free EO and nanoformulation showed 90.5 and 99% inhibition of Aflatoxin B ₁ production in pistachio, suggesting green nano fungicides as an efficacious approach for pistachio preservation.	88

(Contd.)

Table 2 — Some nanoencapsulated EOs and their bioactive compounds recommended as possible green preservatives against food-borne fungi and mycotoxins (*Contd.*)

S. No.	Nanoencapsulated essential oil	Remarks	References
12	<i>Ocimum viride</i> Willd.	Nanoencapsulated EO exhibited efficacy as an eco-friendly alternative to synthetic chemicals in preventing post-harvest deterioration of kinnow fruit through inhibiting <i>Penicillium digitatum</i> and <i>P. italicum</i> via ROS-generated hyphal distortion and suppression of spore-germination.	89
13	<i>Cymbopogon schoenathus</i> (L.) Spreng. (camel grass)	Nanoencapsulated EO showed superior antifungal activity with MIC value 0.6 µL/mL compared to free EO against <i>Aspergillus flavus</i> due to enhanced bioactivity of EO and thermal stability of nanoemulsion.	90
14	<i>Alpinia speciosa</i> Schum (shell ginger) and <i>Cymbopogon flexuosus</i> (D.C) Stapf	The nanoencapsulation of EO inside polymeric matrix strongly suggests its application as a safe food preservative because of inhibition of toxigenic food contaminating fungi <i>Aspergillus westerdjkiae</i> and <i>A. ochraceus</i> and ochratoxin A production at 3.25 and 29.54%, respectively.	91
15	<i>Cananga odorata</i> Hook.F. & Thomson (ylang-ylang)	Nanoencapsulated EO exhibited better antifungal, anti-aflatoxigenic and antioxidant potential compared to free EO, as it completely inhibited the growth of <i>A. flavus</i> and aflatoxin B ₁ production at 1.0 and 0.75 µL/mL, respectively. EO-loaded nanoformulation also prevented lipid peroxidation in <i>Arachis hypogea</i> without phytotoxic effect, suggesting application as nano green preservatives and as a shelf life enhancer for stored food commodities.	92

source utilisation, ergosterol inhibition, leakage of ions and dysfunctioning of ver-1 gene along with maintaining nutritional value and organoleptic profile⁹⁷. Recently, combinations of EO formulations have been applied in food sectors because of the synergistic mode of action and to obtain superior antifungal activity at low concentrations. Synergistic formulation of *Coriandrum sativum* L. and *Pimpinella anisum* L. nanoemulsion showed enhanced antifungal and anti-aflatoxigenic efficacy under both *in situ* and *in vitro* conditions over unencapsulated synergistic formulation. The synergistic mode of action of the nanoencapsulated EOs would not allow the development of new resistant races of food-borne microorganisms, which are frequently reported with synthetic preservatives. The *in silico* findings showed the interaction of linalool and anethol with nor-1 protein as one of the aflatoxin B₁ inhibitory actions. The formulation also prevented lipid peroxidation and maintained a sensorial profile, suggesting its application as an eco-friendly nano-green food preservative⁹⁸. Tiwari *et al.* also reported improved bio-efficacy in terms of antifungal, anti-aflatoxigenic and antioxidant properties of nanoencapsulated *Homalomena aromatica* Schott. EO over free oil along with favourable safety profile having high LD₅₀ value of 7150.11 mg/kg on mice and protecting spices from lipid peroxidation and maintaining their sensorial value⁹⁹. Besides, bioactive components of EOs also play a tremendous role in food preservation through inhibiting fungal and mycotoxin contamination (Table 3).

Safety aspects of nanoformulations for practical application

At present, the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have established regulatory methods for utilizing EOs in food products. These authorities have sanctioned numerous EOs within the high safety classification. Currently, there is a limited amount of information available regarding the commercialization of nanoformulations. This may occur due to these nanoparticles possess novel physiochemical attributes that are extremely tiny in dimension and thus may lead to toxicity in humans. Ribeiro *et al.* proposed that the encapsulation of *Eucalyptus citriodora* Hook. EO increased the toxicity in the tested mice relative to its non-encapsulation formulations¹¹⁰. Chaudhari *et al.* conducted a toxicity assessment using oral administration of a chitosan-loaded nanoemulsion from *Origanum majorana* on a mouse model. They noted that nanoemulsions showed a high safety profile, with a calculated LD₅₀ value of 11,889 µg/kg of body weight¹¹¹. The authors suggested that the nanoemulsion with a high median lethal dose value is strongly recommended for safely preserving stored maize grains. Kumar *et al.* reported that nanoencapsulated *Foeniculum vulgare* essential oil protected *Sorghum bicolor* seeds from fungal deterioration as well as lipid peroxidation without altering the sensory and nutritional properties¹¹². In a different study, they documented the acute oral toxicity of *Myristica fragrans* and *Anethum*

Table 3 — List of some nanoformulated bioactive compounds against fungal and mycotoxin contamination

S. No.	Nanoformulated Bioactive components	Remarks	References
1	Carvone	Nanoencapsulated carvone exhibited an efficacious role in the preservation of bread slices by improving its gas composition and sensorial qualities, as well as preventing <i>A. flavus</i> growth and aflatoxin B ₁ production.	100
2	Geraniol, citral and terpineol	Nanoformulated synergistic antifungal formulation exhibited enhanced antifungal and aflatoxin B ₁ inhibitory activity in comparison to its free form through impairing aflatoxin biosynthetic genes (<i>ver-1</i> , <i>nor-1</i>), cell membrane and biochemical activities.	101
3	Eugenol	Eugenol-loaded chitosan nanoemulsion exhibited a significant role in the preservation of the nutritional properties of rice by protecting it from <i>A. flavus</i> and aflatoxin B ₁ production.	102
4	Citral	Nanoencapsulated citral performs efficacious activity against <i>Fusarium oxysporum f.sp. lycopersici</i> , as the stability of citral enhanced due to its controlled release after nanoencapsulation.	103
5	linalool	Nanoencapsulated linalool showed controlled release, thus enhancing its bio-efficacy against <i>A. flavus</i> and aflatoxin B ₁ production. Moreover, it also prevents lipid peroxidation and maintains the sensorial properties of rice.	104
6.	α -terpineol	nanoencapsulated α -terpineol exhibited an efficacious role as an antifungal preservative as it prevents maize sample deterioration through aflatoxin and also maintains the organoleptic properties of maize.	105
7	Carvacrol	Nanoencapsulated carvacrol performed enhanced thermal stability, water solubility and antifungal properties against <i>Alternaria alternata</i> and <i>Botrytis cinerea</i> .	106
8	Thymol	Thymol-based nanoformulation depicted a promising role as a botanical fungicide because it effectively prevents the growth of <i>Botrytis cinerea</i> .	107
9	Methyl salicylate	Nanoencapsulated methyl salicylate performed remarkable antifungal activity against <i>A. flavus</i> and aflatoxin B ₁ production. Inhibition of omtA and ver-1 gene is the most probable anti-aflatoxigenic mechanism. Similarly, ergosterol content depletion and leakage of vital cellular ions contributed to antifungal activity	108
10	Eugenol, t-anethole, menthol	Nanoencapsulated synergistic formulations comprised of Eugenol, t-anethole, and menthol showed superior antifungal and anti-mycotoxigenic activity in comparison to individual components and also maintained sensorial properties of <i>Eleusine coracana</i> .	109

graveolens on examined mice, yielding calculated LD50 values of 9231.89 and 18, 714 $\mu\text{g}/\text{kg}$ of body weight, respectively^{113,114}.

Challenges associated with nanoformulated EOs

Besides having a tremendous role in food preservation, nanoparticles also have certain challenges related to their safety and toxicology. The large surface-to-volume ratio of nanoparticles enhanced its bioactivity and toxicity towards humans, although very few reports are available regarding this. Nanoparticles may accumulate inside the human body as well as in the environment coming from the excretory products of humans, synthesis units, the agriculture sector and packaging¹¹⁵. Carbohydrate nanoparticles that are not digestible exert adverse health effects by interacting with gut mycoflora¹¹⁶. Further, the ingestion of nanoparticles in food has also been reported to cause hazardous effects on the kidneys, spleen, and liver, as well as detrimental

effects on the environment through residue accumulation¹¹⁷.

Conclusion and future outlook

Looking towards the harmful effects of synthetic preservatives, plant essential oils and their bioactive components are gaining prime attention in food preservation as green preservatives against fungal and mycotoxin contamination of post-harvest food commodities due to their broad bioactivity like antioxidant, antimycotoxigenic, antifungal, insecticidal and antibacterial activities. Their biodegradable nature with favourable safety profile, diverse mode of action and labelling under the Generally Recognized as Safe (GRAS) Category by the Environmental Protection Agency and Food and Drug Administration, USA, strengthen their future perspectives for large-scale application in food packaging. Nanoencapsulation of EOs would enhance their bio-efficacy as well as stability and would boost

their large-scale application as a sustainable and consumer-friendly approach in the form of green preservatives to be used as food coatings against fungal and mycotoxin contamination and enhancing the shelf life of postharvested food commodities.

Conflict of interest

All authors declare no conflict of interest.

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