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Nanoemulsion strategies in controlling fungal contamination and toxin production on grain corn using essential oils

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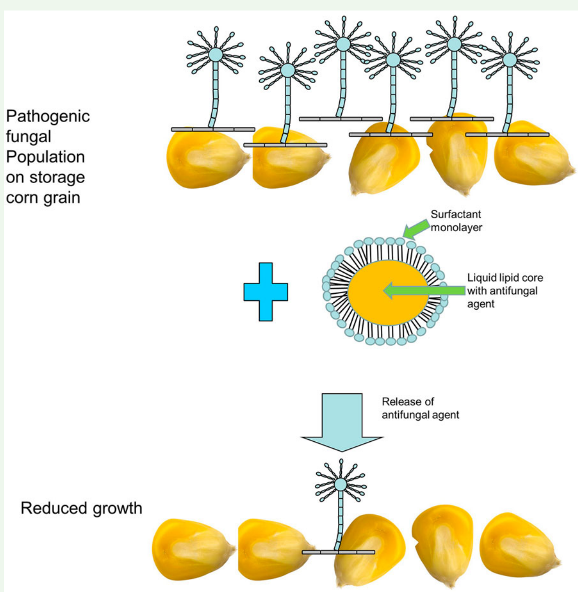
ABSTRACT

Malaysia is currently experiencing a feed shortage, and compounded with the contamination of feed, especially corn, a further shortage of feed is expected in the near future. Nanoscience-based technologies have recently become a hot topic in the agricultural sector as a way to improve the quality of agricultural products. Nanoemulsion is a major thrust in this field because it is easily formulated with a variety of ingredients and equipment. Oil-in-water nanoemulsions, in particular, are being used as delivery methods for a variety of hydrophobic compounds for use in agricultural products, including essential oils. The use of essential oil in the form of nanoemulsions as a fungal inhibitor to reduce fungal growth and fungal toxin production is the way forward for use in corn grain storage. The focus of this review is to compile and analyze the current body of information on the use of nanoemulsion-based delivery systems containing essential oils to control storage grain pathogens in general and corn grain in particular. As the use of essential oil in the form of a nanoemulsion for controlling mycotoxin contamination in corn grain storage is poorly reported, the information from this review will be useful for such work in the future.



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Introduction

Mycotoxigenic fungi are fungi that produce secondary metabolites called mycotoxins, which can contaminate a variety of food crops throughout the food chain, including grains, vegetables, and fruits. Asian countries, including Malaysia, have expressed grave concern about the presence of these mycotoxigenic fungi in stored grains and agricultural areas, particularly paddy-growing areas. Because environmental factors and storage conditions can influence mycotoxigenic fungi growth and development, understanding how they work is critical. The most common mycotoxigenic fungi found in food contamination are *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., which frequently destroy grain and maize crops both in the field and during storage. (*1*). Due to this issue, various studies have been conducted on the production and development of fungicide technology to control and inhibit the growth of Aspergillus flavus by using synthetic chemicals and fungicides. However, most fungicides are produced using chemicals and synthetic materials, which have various disadvantages in terms of toxicity, effectiveness, and cost. Therefore, fungicide products based on a safe green chemistry approach should be developed to replace synthetics and chemicals. Plant-based products such as essential oils are now gaining attention as natural products for controlling fungal and microbial diseases because they contain bioactive components. Essential oils such as citronella oil (from *Cymbopogon nardus*), manuka oil (from *Leptospermum scoparium*), clove oil (from *Eugenia caryophyllata* (Myrtaceae), cinnamon oil (from *Cinnamomum zeylanicum*), lemongrass oil (from *Cymbopogon citratus*), and kaffir lime oil (from *Citrus hystrix*) have been reported to have anti-microbial components (*2*). The strength and effectiveness of essential oils in controlling fungal infections are evaluated in this review using nano-emulsion production strategies. Nano-emulsions are effective bioactive material delivery systems that contain a variety of components that can be combined in a single formulation. These nano-emulsion applications have broad biocidal efficacy because they disrupt the outer membranes of bacteria, viruses, and fungi. Nano-emulsions are particularly well suited for the strategy of controlling fungal growth using essential oils because they can be used to cover, protect, and deliver a variety of natural substances with anti-microbial activity, such as essential oils and other phytochemicals (*3*, *4*).

Method

A literature analysis on the use of nanoemulsion strategies in controlling fungal contamination and toxin production on grain corn using essential oils was compiled for June 2022. Mycotoxin, fungus, grain (corn or maize), agriculture, essential oil, nanoemulsion, biocontrol, field studies, and Malaysia represent the primary focus of all searches. Google Scholar, Scopus, Science Direct, Web of Science, and PubMed were all included in the database search published until June 2022. More than 200 published manuscripts were covered. In the end, about 100 primary papers were selected based on the inclusion and exclusion criteria, which were as follows:

Entry criteria

The current research drew on articles published as primary research in peer-reviewed scientific journals and focused on the use of nanoemulsion strategies in controlling fungal contamination and toxin production on grain corn using essential oils.

Exclusion criteria

Emulsion or nanoemulsion formulation using chemicals were excluded in this review.

Data collection process

The first and second authors initially looked over all of the input papers, judging them just on their titles and abstracts. The final conclusion was reached only after the corresponding author pored through the entire paper.

Grain corn as a major crop

Corn (*Zea mays* L.) is a cereal crop that is widely cultivated in a variety of agro-ecological conditions around the world. It is a member of the Poaceae (Graminae) family, which also includes rice, wheat, and millet. This plant typically grows to a height of 2.5 m (8 ft) and, in certain wild strains, can reach a height of 12 m (40 ft) (*5*). The kernel of corn is also referred to as the seed of the plant or caryopsis. The endosperm accounts for the majority of the kernel's volume and weight. In Malaysia, corn is one of the most important crops which is valued for its nutritional properties as food and feed. More than 3 million tons of grain maize worth Malaysian Ringgit (MYR) 3.09 billion were imported by Malaysia and amounted to 14 percent of the country's agricultural commodities imports. Grain maize is the primary ingredient in animal feed, particularly in the poultry business, which has generated an average value-added of MYR 10 billion over the past five years (*6*).

Corn cultivation potential in Peninsular Malaysia is divided into two categories. The first category includes perennial crops as well as double-cropped rice areas. Meanwhile, the rain-fed areas, peat swamp forests, forest areas, and idle farmland fall into the second category. Planting during the drier seasons, particularly in Terengganu, Kelantan, Kedah, and Perlis, is usually necessitated by irrigation. According to previous records and experiences, drought is a serious concern in maize farming and has a significant impact on grain output reduction in Malaysia. Currently, corn production does not meet demand, despite being one of the world's most important cereal crops, including Malaysia. This is because most Malaysian farmers are interested in planting sweet corn (*7*) instead of grain corn because of its lower cost. Besides, corn production is also influenced by the climate and environmental factors during the planting and harvesting processes (*2*). This includes moisture stress, waterlogging, sun and temperature, relative humidity, and soil condition. All these factors may lead to other possibilities, including the presence of fungi, bacteria, rodents, infection by pests, and physiological plant disorders (*7*).

Fungal contamination of grain corn

Generally, all types of corn or maize are possible hosts for many fungi. A lot of studies have proven that various fungi from the field may contaminate cereal grains, including grain corn (*8*). Mycotoxins can arise at any time throughout maize production, but especially during the pre-harvest, harvest, drying, and post-harvest periods. The severity of the mycotoxin problem varies greatly depending on the environmental conditions at each stage. Fungi that produce toxins are also very adaptable, meaning they can thrive at a variety of times of the year. As plant pathogens, certain species are more invasive than others and can spread illness before harvest (*9*). The common name for these fungi is 'field fungi.' Although it is possible for them to keep growing and producing mycotoxins all through harvesting and drying, this often stops once the grain is stored due to the drying process resulting in low grain moisture content (*10*). The majority of toxigenic fungi in corn come from Fusarium species. It is common to refer to Aspergillus and Penicillium species as 'storage fungi' due to the fact that these fungi are not aggressive pathogens and typically do not infect plants in the field, with the exception of damaged kernels. Physical injury to kernels during harvesting and drying might trigger infection and mycotoxin generation by these fungi (*9*).

Fusarium spp. is the most important fungus responsible for causing the red and pink rots on corn. The production of toxins from these fungi may cause contamination of corn, affecting the yield and quality of corn grain. Other common symptoms that usually show up on corn are the common leaf rust, brown spot, leaf spot, gray leaf spot, and eyespot, which are caused by *Aspergillus* spp. Fungi could cause 50 to 80% damage to farmers' corn if the conditions are favorable for their development during the storage period. During storage, several types of fungi can become attached to corn seeds, causing them to deteriorate or remain viable to infect germinating seedlings. The typical genera found in stored grains are Fusarium, Aspergillus, and Penicillium. The growth of these fungi can be affected by a number of things, such as the amount of moisture, the length of time it has been stored, the temperature, and the activity of insects and mites. These things can also affect the level of contamination and the spread of fungi (*11*).

Factors that influence fungal growth

Fungal diversity has increased throughout evolution, and it can be found in a wide variety of environments. Varied kinds of fungi present in the ecosystem may require different environmental conditions in order to develop at their best. Natural and anthropogenic variables have a substantial impact on fungal metabolism, which includes biological, chemical, and ecological circumstances (*12*). Therefore, toxin-producing fungi may invade at a certain stage and period, including the pre-harvesting period, harvest time, post-harvest handling, and storage stages. Fungi have three distinct groups: field fungi, storage fungi, and advanced deterioration fungi. Field fungi, including plant pathogenic fungi such as Fusarium, Alternaria, and Cladosporium, usually require a high value of water. In contrast, storage fungi such as Aspergillus and Penicillium require a low level of humidity for optimal growth. Meanwhile, the advanced deterioration fungi, such as Chaetomium, Scopulariopsis, Rhizopus, Mucor, and Absidia, required high moisture content (*13*). These conditions are influenced by environmental factors, as mentioned earlier. These factors play a crucial role in determining fungal species' prevalence, including their growth and mycotoxin production (*14*). Biological elements such as strain variability, inoculum source, and competing microflora, among others, are critical in the development of fungal growth. Having a large number of competing microorganisms, such as bacteria or other fungi, that interfere with fungal development and mycotoxin production is referred to as having a competitive microflora. For instance, *Trichoderma harzianum* produces an enzyme, chitinase, which exhibits broad antifungal activity against numerous fungal strains, including *Aspergillus niger* (*15*). *Bacillus subtilis* produces iturin A, which affects the osmotic pressure, energy metabolism, and metabolic transport of fungus cells and stops *Aspergillus carbonarius* from reproducing (*16*).

Mycotoxigenic fungi

Mycotoxigenic fungus creates secondary metabolites known as mycotoxins, which can infect a wide range of food crops and spread throughout the food chain (*17*). They are frequently linked to lower quality and quantity of marketable output such as corn, rice, peanuts, and other food crops. Mycotoxigenic fungi have become a big concern worldwide, notably in Asia Pacific countries such as Thailand, Indonesia, Japan, Korea, Taiwan, the Philippines, and Malaysia (Table 1). This is due to the formation of ambient and storage factors that promote mycotoxigenic fungus growth and development (*18*). *Aspergillus* sp., *Fusarium* sp., and *Penicillium* spp. are the most common mycotoxigenic fungi detected in mycotoxin contaminations (*19*).

i. *Fusarium* species

Fusarium is one of the most important mycotoxin-producing fungi in Malaysia, and it is capable of invading crops that are important to the country's economy. Phytophthora is a large genus of plant and soil fungi that can be found all over the world, including in Australia. It can be found in the natural microflora of commodities such as soybeans, beans, rice, maize, wheat, and oats in temperate and semi-tropical regions such as South Africa, China, and North America, as well as in temperate and semi-tropical regions such as the Mediterranean region (*25*). This species has the potential to reduce the quality and yield of a wide range of foods and feed products by up to 10% to 30%, depending on the food or feed product in question. Crops that are most commonly affected by *Fusarium* head blight disease are maize, wheat, and rice, which are all susceptible to the disease caused by *Fusarium* spp. (*26, 27*).

All stages of plant growth can be negatively impacted by *Fusarium* spp., a common soil-borne pathogen. Many different Fusarium species, including *F. proliferatum, F. verticillioides, F. graminearum, F. subglutinans, F. oxysporum* and *F. temperatum*, can infect maize at various times and places, from when the seed first germinates to when the stalk, ear, and root are fully developed. Brown discoloration of the seedlings, light yellow discoloration, and stunted seedling are prominent indications of seedlings infestation that can lead to seed rot at the seedling stage (*28–30*).

Many species of Fusarium, including *F. graminearum* (*Gibberella zeae*), *F. moniliforme*, *F. temperatum*, and *F. subglutinans*, cause maize stalk rot, a disease characterized by tan to pink or crimson staining and disintegration of the pith (*31*). Reduced growth, rotten leaf sheaths and internal stalk tissue, and brown streaks in the lower internodes are typical symptoms of stalk rot disease in maize. At maturity, the internal stalk pith tissues turn pink to salmon in color. Because of the disease Fusarium stalk rot, the host plant may die before its time, which will impede the transport of water and nutrients to the leaves and ears. Symptoms of a maize plant infection include wilting, a change in color from bright to dull, and a lower stalk that has dried out to the point that the pith tissues look torn (*29*). Distinct symptoms of stalk rot caused by *F. graminearum* include tan to dark brown discoloration of the lower internodes and pink to reddish discoloration of the pith tissue, whereas F. verticillioides causes brown streaks on the lower internodes and the rotted pith tissue may be whitish-pink to salmon in color Kanja *et al.*, (*31*). In Malaysia, a tropical country, *F. pseudograminearum*, a closely related species to

Table 1. Mycotoxigenic fungi that are present in agricultural crop commodities in Malaysia.

Species	Toxins produced	Crop	Reference
Monascus spp.	citrinin	Red rice	(*19*)
Penicilium chrysogenum	eleagrin, roquefortine, penicillin G and xanthocillin X		
Aspergillus niger	3-nitropropionic acid and ochratoxin A		
Aspergillus flavus	aflatoxins		
Fusarium oxysporum	fusaric acid, beauvericin, enniantin B, bikaverin, moniliformin, fumonisin, and trichothecenes	Corn and vegetable fruits	(*13, 20*)
Fusarium fujikuroi	fumonisins		
Fusarium proliferatum	fumonisin B1, moniliformin, beauvericin, fusaproliferin and fusaric acid		
Aspergillus flavus	aflatoxins	Peanuts and maize	(*21*)
Aspergillus flavus	aflatoxins	Wheat flour	(*22*)
Aspergillus spp.	aflatoxins	Milled rice	(*23*)
Eurotium spp.	mycotoxins		
Aspergillus flavus	aflatoxins	Corn-based product	(*24*)
Aspergillus flavus	aflatoxins	Rice and corn	(*25*)
Aspergillus niger	3-nitropropionic acid and ochratoxin A		

F. graminearum is most often reported species (30).

Increased plant disease is a result of the systematic and successful infection of seed and root, which then spreads to the internodes, stalk, and ear (15). Ear rots are a major cause of yield and quality loss due to Fusarium maize diseases. Corn ears are the primary target of ear rot disease, and it has been stated that *F. moniliforme*, now more often known as *F. verticillioides*, is the causative organism (25). According to reports, fusarium ear rot is the most prevalent maize disease in the United States (25). In recent years, *F. temperatum*, a closely related species to *F. subglutinans*, has been found and described as a novel pathogen causing ear rot in European maize (26, 27). A study in 2011 shows that there was a total of 220 Fusarium isolates isolated from out of 657 samples cultivated with corn plants exhibiting indications of Fusarium ear rot in 12 different locations around Malaysia . *F. proliferatum* was shown to be the most common Fusarium species across all of the sampling locations (out of F. *proliferatum*, *F. subglutinans*, *F. verticillioides*, and *F. nygamai*) (28).

Trichothecenes, fumonisins, and zearalenone are the three most poisonous and widely distributed fusarium poisons with significant economic impact. Disease symptoms including refusal to eat, vomiting, nausea, miscarriages, inflammation of the skin, blood disorders, hemorrhaging of internal organs, weight loss, suppression of the immune system, and alteration of the neurological system have been linked to trichothecenes in both people and animals (32). The fumonisin A, B, C, and P series are the four main classes of fumonisin analogs. These compounds are toxic and potentially cancer-causing (13). Most contaminated maize contains fumonisin B, which is the most significant and widely distributed fumonisin. Analogues of fumonisin B include the poisonous fumonisins B1, B2, and B3. While zearalenone (or F-2) is most often found in maize, it has been shown to infect different crops around the world. Countries such as South African and Nigerian have reported the contamination of this compound in food and animal feed (33). As endocrine disruptors, they are linked to a variety of health problems, including infertility in men and animals and the growth of various tumors (34).

Penicillium species

Penicillium is a varied and cosmopolitan fungus where almost 350 species have been found within this genus. *Penicillium* species are common fungi found around the world in a variety of habitats ranging from soil to vegetation, air, interior spaces, and numerous food products. This species is recognized to play a vital role in the fermentation of food and the breakdown of organic materials. Penicillium produces a number of mycotoxins, including ochratoxin A and patulin, which are found in contaminated foods and feeds (19). Different species *Penicillium* may produce different types of mycotoxins such as *P. citrinum* (citrinin), *P. citreonigrum* (citreoviridin), *P. islandicum* (cyclochlorotine, islanditoxin, luteoskyrin, and erythroskyrin), *P. crustosum* (penitrem A) and *P. verrucosum* (ochratoxin A). These fungi and their mycotoxins have the potential to contaminate harvested seeds and cause agricultural commodity losses in many parts of the world. Besides plants, this fungal species may also cause toxins to affect animals and humans (19). Although the appearance of the mold produced by Penicillium species on maize kernels varies, it is often blue–green and powdery. Similar to Fusarium, the taxonomy of the genus Penicillium has undergone multiple revisions and now includes some species that were formerly considered to be different taxa. However, the most frequent Penicillium species in maize are not linked to significant mycotoxin issues, despite the fact that there are several toxigenic Penicillium species. In the field, ear rot is mostly caused by *Penicillium oxalicum*, but several species of Penicillium infect corn during storage (35). Field infections by Penicillium, like those by Fusarium and Aspergillus, typically affect kernels that have already been injured by insects or other agents. Some Penicillium species thrive in stored maize despite the low water activity they require for growth, such as *P. aurantiogriseum* and *P. viridicatum*. In temperate regions, Penicillium predominates over Aspergillus (11).

ii. *Aspergillus* species

Aspergillus is discovered by Pier Antonio Micheli in 1729 (36) and included in the species adapted to a varied range of environmental circumstances. *Aspergillus* species are saprophytic and grow with high moisture content. While most of them are often the common soil fungi, several of them can produce mycotoxins.

Corn kernels are susceptible to infection from several species of Aspergillus, especially during storage but also when growing. Mycotoxin production is highest in *Aspergillus flavus* and *A. parasiticus;* in the field, *A. flavus* is typically to blame for symptoms of kernel rot. It’s easy to spot

A. flavus because of its distinctive olive-green color and powdery texture. There are situations where widespread *A. flavus* infection in the field might lead to the accumulation of large amounts of aflatoxins prior to harvest. It appears that *A. flavus* infection can be exacerbated by bug infestation in corn grain storage (*37*). Insects that feed on stored grain produce water and damage the surfaces that *A. flavus* spores can adhere to and grow on. Several common insect pests, such as granary weevils, rice weevils, and maize weevils, start their lives inside the kernel of corn. Flour bugs and the Indian meal moth are examples of secondary pests that originate outside of the kernel, particularly cracked kernels (*38*). In Malaysia, grain infestation by the maize weevil *Sitophilus zeamais* (greater grain weevil) was reported as earliest as in 1976 (*39*).

Toxins produced by *Aspergillus* can be divided into three groups which are carcinogen (for example Aflatoxin B1), nephrotoxins (for example Ochratoxin A), and neurotoxins (for example Territrem B). The most significant mycotoxigenic species are *A. flavus*, *A. parasiticus*, *A. niger*, *A. fumigatus,* and *A. ochraceus,* which produce aflatoxins and ochratoxins as well as citrinin, patulin, sterigmatocystin, gliotoxin, cyclopiazonic, and other potentially toxic metabolites (*36*). In livestock, these *Aspergillus* species produce mycotoxins, which are toxic, mutagenic, and carcinogenic. They can also cause storage deterioration, plant illness, and invasive disease in humans and animals. For instance, *A. flavus*, *A. niger,* and *A. ochraceous* have been found in maize grain, groundnuts, and coffee (*18*, *40*), while aflatoxin produced from mold-contaminated peanut has caused death to more than 100,000 turkey poults (*12*).

In general, mycotoxigenic fungi that produce mycotoxin may have a variety of consequences for humans and animals. Mycotoxins can enter the human body through inhalation, ingestion, or skin absorption. This can lead to illness, poor performance, and mortality in both animals and humans. Mycotoxicosis is a condition that can occur as a result of consuming mycotoxin-contaminated food. It would become infected by either direct contact with the contamination or through indirect means such as the ingestion of animal products such as meat or milk that have been exposed to polluted feed. Furthermore, mycotoxin contamination can cause acute or chronic health problems in humans or cattle, such as equine leukoencephalia, human esophageal and liver cancer, and other disorders (*18*, *40*).

Methods to prevent fungal infection on grain corn

Mycotoxigenic fungi such as *Aspergillus flavus*, *Aspergillus paraciticus*, and *Fusarium* are the dominant species responsible for mycotoxins production. *A. flavus* and *A. paraciticus* are specifically liable for agricultural product contamination before harvest or during storage (*21*). Several factors that will cause contamination of grains by fungi include the relative humidity, temperature of the grain, moisture content of the grain, amount of broken and foreign materials present during storage, presence of insects and mites, and length of the storage period. Various strategies are being investigated in order to avoid fungal growth, the formation of more mycotoxin, and the contamination of food. This includes physical, chemical, and biological treatments that require complex technology, equipment, and expensive chemicals or reagents to be performed on the patient (*17*).

Physical method

Sun drying, mechanical driers, heat treatment, ultrasonic treatment, cleaning, mechanical sorting, and irradiation are all part of the drying process. Washing (with water or a sodium carbonate solution) reduces mycotoxin levels in grains (*23*). Natural drying approaches and mechanical dryer equipment are normally utilized in the drying process. Corn grain should be dried to at least 14% moisture content for storage at the recommended drying temperature of 100 to 110 °C (*24*). At this temperature, fungal microbes respire slowly, which inhibits growth. Radiation such as X-rays is also used against aflatoxin. They can emit a tremendous quantity of energy, causing the breakdown of stable molecular structures. This method has been known to reduce the production of Aflatoxin B1 and Aflatoxin G1 as they are the most sensitive to X-rays (*41*). The drying method is a mandatory process prior to all treatment methods. It is a very effective and low-cost method for reducing the impact of fungal infestation during a short storage period, but for normal feed and human consumption purposes, where a longer storage period is required, chemical or biological methods must be used.

Chemical method

Various chemical compounds are effective at preventing fungal infection and destroying mycotoxins. Acids, bases, salts, oxidizing and reducing agents, miscellaneous reagents, and chemical preservatives such as potassium sorbate and calcium propionate are among the chemicals employed (25). Nowadays, insecticides have become the major chemicals to deal with aflatoxigenic fungi, and the most effective reagents used are sodium bisulphate, ammonia, and propionic acid (42). The most frequent fumigants are methyl bromide and phosphine. Currently, propionic acid-based fungicide formulations are the most promising treatments because they are effective at controlling both fungal and mycotoxin development while having no detrimental impact on grain physical quality (43).

In addition, novel materials such as nanoparticles have been applied in the production of chemical antifungal compounds. Nanoparticles based on copper (CuNPs), silver (AgNPs), zinc oxide (ZnO-NPs), selenium (SNP) and nanomaterials such as nanogel, nanobinders, nanoclay, and nanodiamonds, for example, can adsorb and remove mycotoxigenic fungi, mycotoxin, or pathogens in food and feed (44). As an example, selenium nanoparticles (SNP) obtained from *Trichoderma harzianum* JF309 exhibit more inhibition of fungus and decrease the production of deoxynivalenol and fumonisin B1 (45). Their future use, especially with metal-based nanoparticles, must be cautious since there is a fine balance between efficacy and nontarget toxicity (45). The use of potassium sorbate, sodium benzoate, and calcium propionate, for example, is considered an economic approach to preventing fungi infestations after the drying stage. These agents, however, have drawbacks such as unpleasant tastes and odors, as well as mild toxicity, with rat LD_{50}s in the range of several grams per kg bodyweight, and can be degraded (to sorbate) by fungi such as Penicillium roqueforti (46).

Biological method

Mycotoxin contamination has been dealt with using biocontrol techniques in numerous major crops, such as corn, cotton, and peanut. Some researchers have investigated biological ways of controlling *A. flavus* growth or mycotoxin production by using bacteria, fungi, and yeast as competitors (47). The vast majority of studies have focused on interactions between mycotoxigenic strains and other naturally occurring fungi on grains for cultivated competition. The use of *A. flavus* atoxigenic strains, for example, decreases aflatoxin contamination in numerous crops. (36). The mechanism, however, still needs to be elucidated.

Natural products and essential oils have been presented as a second option for limiting fungal infection and mycotoxin release. Essential oil (EO) is a secondary metabolite produced by plants that exhibits a variety of functions, such as antifungal, antiviral, antibacterial, antioxidant, and anticancer (48, 49). They are considered safe, natural extracts with a diverse variety of structurally distinct beneficial ingredients. The majority of essential oils are Generally Recognized As Safe (GRAS) and pose little danger of developing resistance to harmful bacteria (50). More than 1340 plants have been discovered as possible sources of antibacterial chemicals that are not harmful to the environment or consumers. Aside from that, EOs are useful for managing post-harvest illness and are a great way to reduce synthetic chemicals in agriculture (42).

Many studies have proven that essential oils and natural products may inhibit the growth of mycotoxigenic fungi such as *Fusarium* species, *Aspergillus* species, and *Penicillium* species. According to (51), lemongrass, cinnamon, oregano, clove and palmarose oils have anti-fungal agents that will disrupt the release of fumonisin B1 by *F. proliferatum* in irradiated maize grain. The essential oils from *Origanum vulgare* and *Cinnamomum zeylanicum* exhibit antifungal activity against *A. flavus* while Eucalypyus (*Eucalyptus globules*) inhibits the growth of *A. parasiticus* and *A. flavus* in storage grain (52). The increasing trend for the usage of plants' essential oils to control fungal pathogens is due to various reasons, such as the nontarget toxicity of chemical-based pesticides, the increasing resistance of pathogens to chemical-based pesticides, and conformations to the United Nations sustainability development goals (SDGs) (53). Even though biological controls are more expensive than chemical methods, they are appealing features of this emerging new technology due to their safety and sustainability. Even though biological controls are more expensive than chemical methods, they are appealing features of this emerging new technology due to their safety and sustainability.

Application of essential oil in controlling fungal infection

In general, the oil composition of the plant varied according to the location of the plant's sections. When it comes to cinnamon oil, for example, the oil extracted from the leaves has a distinct flavor profile from the oil extracted from the bark. Most research has determined that the primary component of cinnamon oil derived from the leaf is called eugenol and that the predominant constituent of cinnamon oil derived from the bark is called cinnamaldehyde (54). To better reflect the usage of bacterial biomass in dye biosorption, a Scopus keywords search focused on the topics of 'dye,' 'biosorption,' 'microorganisms,' and 'dye' yielded 70 journal articles. The VOSViewer software is used to create the keyword co-occurrences of the documents. Similar terms were merged using the

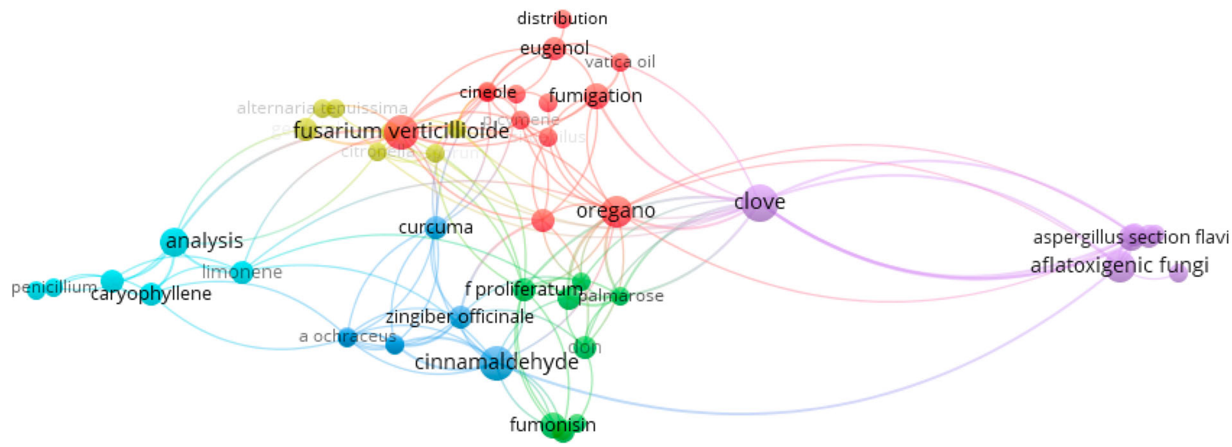

Figure 1. Density visualization of bibliometric keyword search results with the topics of 'essential oil', 'grain' and 'spoilage'.

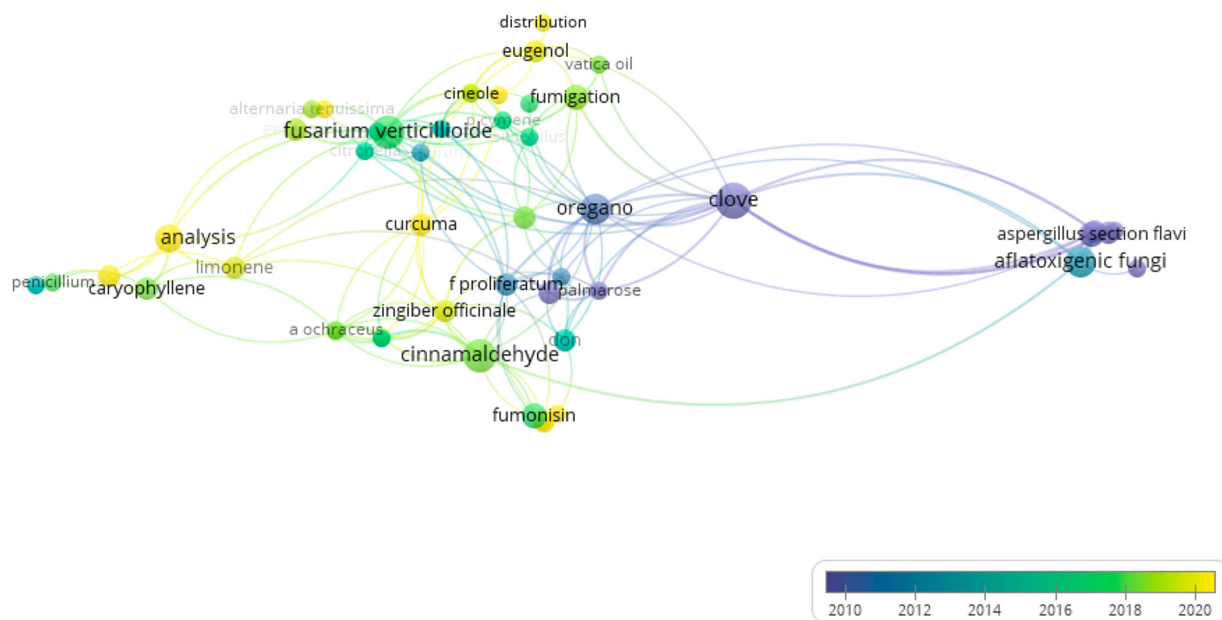

Figure 2. Overlay visualization of bibliometric keyword search results with the topics of 'essential oil', 'grain' and 'spoilage'.

thesaurus function. The study's goal is to conduct a complete bibliometric assessment of the research landscape of biological control of grain products using plant essential oils. Seven clusters are discovered. It appears that of the essential oils currently being used, cinnamon oil in the form of cinnamaldehyde is the most reported, as well as being increasingly utilized in recent publications (Figures 1 and 2). Cinnamaldehyde exhibits one of the most potent agents in reducing fungal colony infestations, leading to the improvement of the quality and safety of numerous grains (*55*), and this is probably the reason, along with commercial abundance, for this trend.

Steam distillation, hydro-distillation, and solvent extractions are all procedures for extracting essential oils. Essential oils can be derived from dry, fresh, or partially dehydrated plant sources. The steam distillation technique allows for the separation of mildly volatile, water-immiscible compounds at low temperatures, which is especially useful when the components boil at high temperatures (over 100 °C) and are prone to breakdown below this temperature. The steam flow enters the stoma, breaks it, and eventually carries the essential oil. The combination is steamed with a direct current of steam water, which heats the mixture and lowers the boiling point temperature due to the high pressure of steam tension that water presents to the volatile chemicals in essential oils. Meanwhile, hydrodistillation is recognized as one of the simplest and least expensive distillation techniques utilized in the production and extraction of essential oils. The plant material is cooked in this method, either by immersing it in boiling water or by steaming it (56). Another alternative to the above processes is solvent extraction, which has been used in certain studies for essential oil extraction. For instance, the study conducted by (57) utilized the solvent extraction method using acetone to extract agarwood oil. Furthermore, essential oils from flower leaves such as roses, jasmine, and violet can be extracted using solid solvents and high-quality fat. The quality of essential oils is determined by plant maturity, the portions of the plant used for extraction, the stage of the vegetative cycle, and the impacts of climate (58). Because essential oils differ in chemical composition, they may have different applications. Certain essential oils, including those from lemon myrtle, eugenol, clove, cinnamon, lavender, and thyme, may act as antimicrobial and antifungal agents in controlling bacterial and fungal growth in foodstuffs and agricultural products (59–62). Other applications of essential oils are stated in Table 2.

Mechanism of essential oil inhibition on fungi

The mode of action or mechanisms involving essential oils are still unclear, even though numerous modes of action have been suggested and explored. Nevertheless, a general condition associated with essential oils is their hydrophobic characteristics, which allow them to enter the plasma membrane, disturbing the structure, inhibiting enzymes of the ergosterol biosynthesis pathway, disrupting membrane fluidity, and ramping up penetrability, resulting in the leakage of cell contents such as fuel source (ATP), ions, and other important biomolecules (73). Different essential oils act in several ways against different microorganisms due to their composition, the functional groups present in the active compounds, and their synergistic effects.

Cinnamon oil is an essential oil with antibacterial characteristics that can prevent the growth of both Gram-positive and Gram-negative bacteria. Cinnamon essential oil can be produced from a variety of plant parts, including the leaves, bark, and stick. Each isolated portion has the potential to develop antimicrobial action against microorganisms. Cinnamaldehyde, eugenol, linalool, benzyl benzoate, and other chemicals are involved in the mechanism that is responsible for cinnamon's antibacterial effect. Cinnamon essential oil has been shown to suppress bacterial growth by inhibiting cell division, anti-quorum sensing actions, inhibition of adenosine triphosphatase (ATPase), membrane porin, biofilm formation, and motility, consequently modifying the lipid profile and affecting cell membrane integrity, causing the formation of lumps and self-aggregation (64).

The antifungal properties of eugenol, cinnamaldehyde, linalool, and other bioactive components' modes of action are through impeding extracellular enzyme synthesis and altering the fungus's cell wall structure, resulting in integrity damage, cytoplasm loss, and finally mycelial death. Phenolic substances are known to be reactive and capable of forming hydrogen bonds with active regions of the target enzyme, which accounts for eugenol's antibacterial action (74). Different chemical compositions can have a distinct function that is not limited to limiting mycelial growth.

Table 2. Plant species essential oils and their applications.

Plant species	essential oils	Plant parts	Applications	References
Angelica glauca Edgew.	root oil	root	Antioxidant, anti-fungal, anti-bacterial, and phytotoxicity	(63)
Cananga odorata (Lam.) Hook.f. & Thomson	ylang oil	flower	Hypertension, stress, anxiety,anti-depression	(59)
Cinnamon zeylanicum	cinnamon oil	bark or leaves	Antifungal, antibacterial, uterine stimulant	(64, 65)
Citrus limon (L.) Osbeck	lemon oil	fruit rind	Boost immune system, digestion, Blemishes, flu, skin, athletes foot, colds, corns	(66)
Dendranthema indicum var. *aromaticum*	spearmint oil	leaves	Cold, headache, mosquito repellant	(67)
Eucalyptus globulus Labill.	eucalyptus oil	leaves	Healing wounds, headaches, boost the immune system	(68)
Lavandula angustifolia Mill.	lavender oil	flowers	Anxiety, stress, insomnia	(68)
Melaleuca alternifolia Cheel	tea tree oil	leaves	Antiviral, antibacterial, anti-inflammatory	(69)
Ocimum basilicum	basil oil	leaves	Aedes	(70)
Rosa damascena Mill.	rose oil	flower	Astringent, sedative, digestive stimulant, anti-depressant, anti-bacterial, antiseptic, increase bile production	(59)
Rosmarinus officinalis L	rosemary oil	flower, stem and leaves	Antioxidant, digestive system	(71)
Skimmia laureola (DC.) Zucc. ex Walp., ver. Nair	leaf oil	leaves	Antioxidant, anti-fungal, anti-bacterial, and perfumery	(61)
Stevia rebaudiana Bertoni	stevia leaf oil	leaves	Anti-fungal, antiseptic, anti-viral, anti-bacterial cold sores, corns	(72)
Thymus serpyllum L.	whole-plant oil	whole plant	Antioxidant, anti-fungal, anti-bacterial, and phytotoxicity	(60)
Zanthoxylum alatum Wall	leaf, stem, root oil	leaves, stem and root	Antioxidant, anti-fungal, anti-bacterial	(62)
Zingiber zingiber H.Karst.	ginger oil	rhizome	Promotes sweating, expectorant, prevents vomiting, antiseptic, antispasmodic, carminative,	(59)

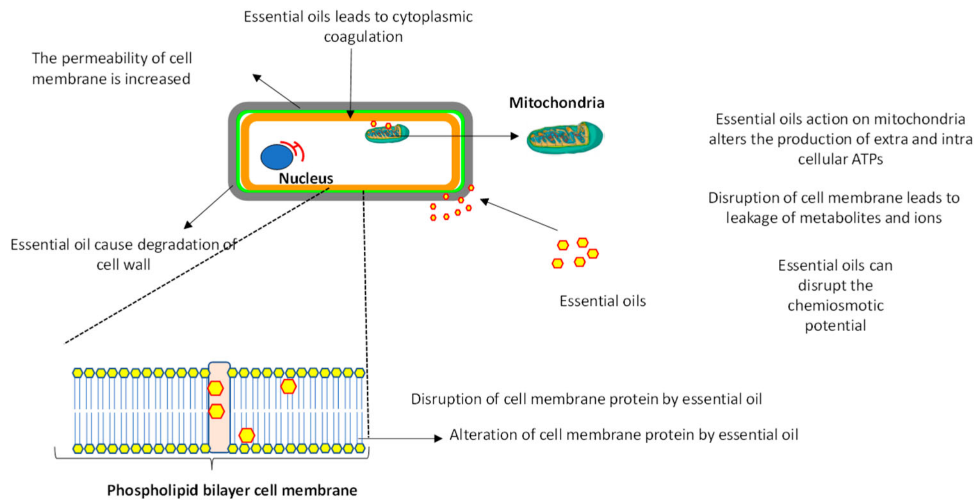

Figure 3. Mechanism of essential oil with anti-fungal properties (Adapted from (76)).

This impact is mostly due to the synergistic action of the molecules contained in the essential oil in question (3, 4). Even in cinnamon oil, eugenol can cause cell membrane damage and interact with ergosterol, whereas cinnamaldehyde can disrupt ATPase activity and cell wall biosynthesis, resulting in cytoplasmic coagulation and altering the fungal membrane structure (75) (Figure 3).

Besides cinnamon oil, clove essential oil is also one of the effective essential oils that act as an antifungal agent. Kalemba and Kunicks (77) show that the direct usage of essential oil of clove is helpful to inhibit the growth of *Aspergillus parasitica*, *Candida albicans* and *Cryptococcus neoformans*. Clove oil are effective in controlling dermatophytic fungi such as *Candida albicans*, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, *Microsporum audouinii*, and *Trichophyton rubrum* (78, 79). This is due to the presence of eugenol as the main compound responsible for clove aroma, which contains about 72-90% of its essential oil (78, 79). As previously stated, eugenol molecules may influence the level of ergosterol in the fungal cell membrane by interfering with the normal sterol biosynthetic pathway, resulting in a decrease in ergosterol biosynthesis (79). In general, different essential oils may have distinct mechanisms of action when it comes to antibacterial activity. Because of their hydrophobicity, they may partition into the lipid bilayer of the cell membrane, causing the cell membrane to become more permeable and the components of the cell to flow out. EOs, like other lipophiles, permeate through the cell membrane and cytoplasmic membrane, disrupting the composition of the different layers of fatty acids, polysaccharides, and phospholipids and causing them to permeabilize (73).

The advent of fast computing power has allowed for extensive in silico screening of potential fungal enzyme inhibitors from libraries of available plant essential oil bioactive compounds. The essential oil of *Trachyaspermum ammi* was tested for Candidapepsin-1, a fungal enzyme inhibitor. Cedrane was found to be the strongest binding compound to the fungal enzyme and may pose as a strong antifungal agent, subject to in vitro studies (80). In another study, the nanoemulsion chitosan fabricated *Myristica fragrans* essential oil (Nm-MFEO) is evaluated for its inhibitory effects on the aflatoxigenic strain of *Aspergillus flavus*. A molecular docking study between the major components of MFEO and Aflatoxin B1 in regard to the aflatoxin synthesizing genes (Ver 1 and Omt A) shows that elemicin and myristicin exhibited strong interactions with Ver 1 and Omt gene products, respectively (81). The acetyl derivative of monoterpene ester (4 *R*,5*S*)–4-hydroxy-7-tigloyloxycarvotanacetone, the most active constituent from *Blumea axillaris* (Lam.) DC (Asteraceae) against *Rhizoctonia solani* and *Aspergillus niger* shows strongly *in silico* docking interaction with similar interaction as the commercial fungicide carbendazim (82). A knowledge gap in *in silico* works of discovering essential oil mechanisms of pathogen inhibition is the lack of molecular dynamics simulation (MDS) works preferably in the >100 ns range to achieve better binding energy calculations using methods such as Molecular Mechanics – Poisson Boltzmann Surface Area (MM-PBSA) (83).

Nanoemulsion strategies in controlling fungal infection using essential oil

When it comes to managing fungal infections, the nanoemulsion approach was employed to boost the potency of essential oils. Due to the fact that nanoemulsions are an effective delivery technology for bioactive substances with a broad set of components that may be integrated into a single formulation, they are becoming increasingly popular in the pharmaceutical industry (84). The term 'nanoemulsion' is suitable since it conjures up pictures of nanoscale droplets, is short, and avoids any confusion with the term 'microemulsion.' Plant-based oils are now being researched for use in nanoemulsions, particularly due to their bioavailability and biocompatibility. This nanoemulsion application has broad biocidal action against a wide spectrum of pathogens by shattering the outer membranes of bacteria, enveloped viruses, and fungi (85). Nanoemulsions are particularly well suited for this strategy of fungal infection control using essential oils (for example, lemon oil) due to their ability to encapsulate, protect, and deliver a variety of natural ingredients with antimicrobial activity, such as essential oils (e.g. citrus fruits), phytochemicals (e.g. rosemary), and other natural ingredients with antimicrobial activity (such as oregano oil) (e.g. lycopene, lutein, and curcumin) (84).

Formation of nanoemulsion

In the formation of nanoemulsion, two-step processes are involved, where the first step is forming the macroemulsion, and then this is converted to nanoemulsion (86). The formation of nanoemulsion consists of two broad categories that require energy: energy-intensive methods and low-energy-intensive methods (87, 88). Generally, high-energy methods require large disruptive forces using microfluidizers, high-pressure homogenizers, and ultrasonicators. In contrast, in low-energy methods, there is no need for external force. This process involves breaking droplets into smaller ones, the adsorption of surfactants, and the collision of droplets. The stored energy of the system is usually used in the nanoemulsion preparation method by altering parameters such as the system composition and temperature. In the high-energy method, the energy is frequently obtained from a mechanical device or from chemical energy accumulated in the system over time. High-energy methods, in general, necessitate the employment of mechanical devices that produce significant disruptive forces, such as high-pressure homogenizers, microfluidizers, and ultrasonicators (Figure 4). Low-energy techniques, on the other hand, are sustainable and do not require the use of external force. The technique is classified according to whether spontaneous surfactant curvature is formed throughout the emulsion process. The process by which surfactant and/or solvent molecules rapidly migrate from the dispersed phase to the continuous phase without affecting the surfactant's spontaneous curvature is referred to as 'emulsification.' 'Phase inversion' approaches are used when the surfactant's spontaneous curvature varies throughout the emulsion stage. Droplets are broken down into smaller bits during this process, surfactants are absorbed, and droplets collide with one another. It is typical to practice using a system's stored energy in the nanoemulsion preparation process by altering variables such as system composition and temperature. Of the two, the high-energy methods are commonly used by researchers for the formation of nanoemulsions, and high-energy stirring and ultrasonic emulsification are among the most cited methods (87–89).

Alternatively, low-energy techniques frequently rely on the chemical potential of the constituents to provide energy input for the formation of nanoemulsions. The generation of nanoemulsions is governed by two low-energy mechanisms that are fundamental to the process. One way involves changing the

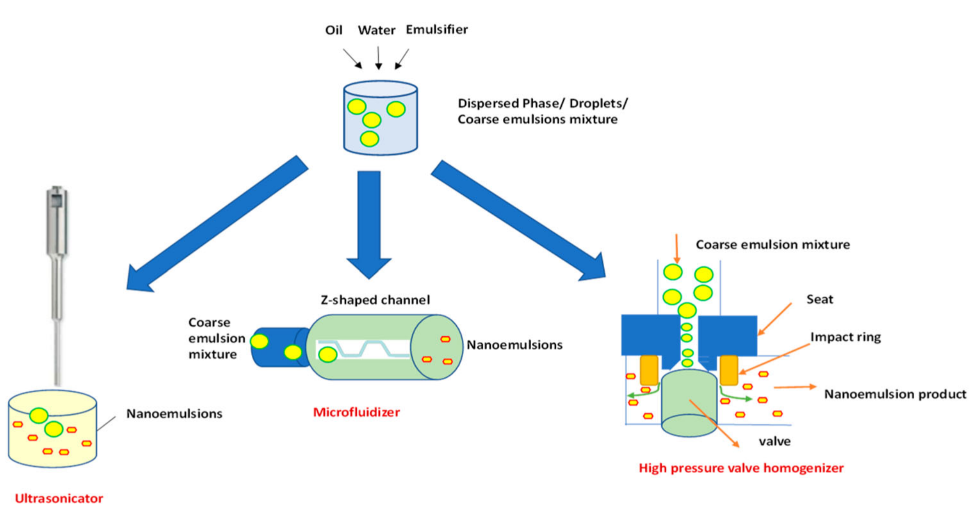

Figure 4. High energy methods such as high-pressure homogenization (HPH), ultrasonicator, and microfluidizer, which function in beak macroemulsions into smaller droplets. (Adapted from (89).

composition at a given temperature, whilst the other involves changing the temperature while using different compositions and interfacial qualities (interfacial qualities are the properties between two surfaces). Physicochemical parameters such as composition, solubility, and temperature all have an impact on these low-energy techniques. In order to explore these physicochemical characteristics, either the phase inversion temperature (PIT) method, the phase inversion composition (PIC) approach (Figure 5), or the solvent diffusion method is used (*87*, *90*). Both methods (low energy and high energy) need to be evaluated for their efficacy in producing optimal nanoemulsion properties for effective pathogen control, economy, and safety to non-target organisms. A summary of the various methods in the preparation of essential oil-based nanoemulsions is presented in Table 3.

Composition of nanoemulsion

For making a good emulsion, an evaluation system for satisfactory emulsion is needed, either oil in water emulsion or water in oil emulsion. It involves the use of two immiscible liquids and an emulsifier. One of the immiscible liquids must be oleaginous and aqueous in nature. At the same time, another immiscible liquid involved in the oil phase is made up of triacylglycerols, diacylglycerols, free fatty acids, non-polar essential oils, mineral oils, waxes, and various lipophilic components. However, the emulsion's stability and toxicity would be the most important topics to consider when creating a good formulation emulsion. These factors may influence the emulsifier choice by excluding certain types or groups of emulsifiers from further consideration.

Emulsifiers function as stabilizers in nanoemulsion preparation to conserve the nano-sized droplets that form. Emulsifiers reduce the interfacial tension between the two immiscible liquids and form small and stable nanoemulsions (*87*, *88*). In addition, emulsifiers aid in the prevention of collisions and coalescences between droplets, as well as the improvement of the kinetic stability of the emulsion. In nature, the emulsifier might be cationic, anionic, non-ionic, or zwitterionic, depending on the situation. Small-molecule surfactants, polysaccharides, phospholipids, and proteins are all examples of emulsifiers, as are other natural substances. The surfactant can be either ionic or nonionic, and both of these types of surfactants have distinct functions. Ionic surfactants suppress droplet agglomeration through electrostatic repulsion, while non-ionic surfactants suppress droplet agglomeration through steric hindrances, hydration contacts, and thermal oscillation interactions, among other mechanisms (*87*, *90*).

After all of the previously described components (oil, surfactant, and co-surfactant) have been investigated, the hydrophilic-lipophilic balancing system, abbreviated as the HLB system, can be implemented. The HLB system provides for the assignment of a number of components or a combination of ingredients for emulsification purposes. The precise HLB value of the surfactant used in the formulation is one of the most essential aspects of the development of stable nanoemulsions. It specifically acts as a scale for determining the suitable surfactants or surfactant mixtures required by the oil and aqueous phases, particularly mixed surfactants, because it reflects the ideal surfactant combination. This could help to create a more stable nanoemulsion recipe (*87*, *90*). Water in oil emulsion

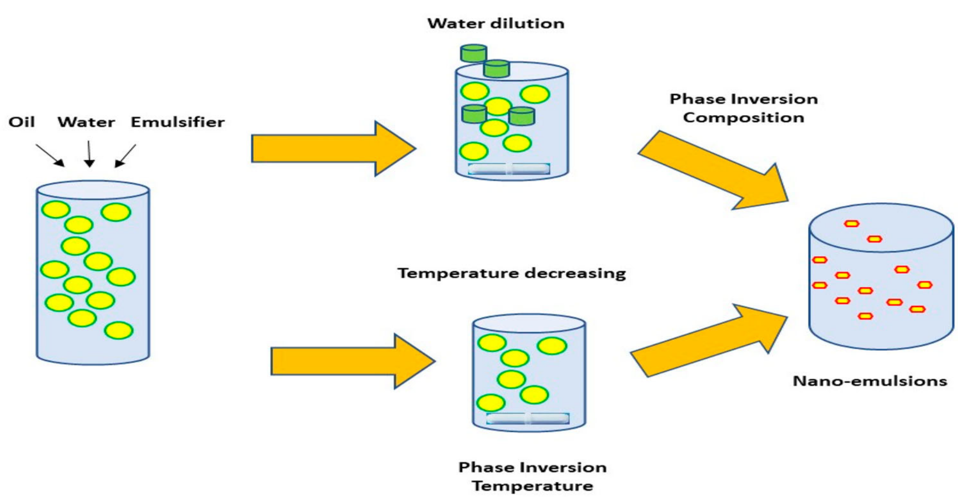

Figure 5. Low energy methods involve the breakdown of coarse macroemulsions during phase inversion (Adapted from (*89*)).

Table 3. Various methods in the preparation of essential oil-based nanoemulsion.

Technique	E.g. EO-nanoemulsion	Advantages	Disadvantages
Emulsion inversion point method	Curcumin (91) and black pepper essential oil nanoemulsion (92)	the potential to accurately anticipate the rheological behavior of emulsions, which has applications in process modeling and the design of equipment used inside wells.	For a given system, a process can only be run once (concentration cannot be changed frequently)
High-pressure homogenization	Quercetin (93), peppermint oil (94), cinnamon (95)	The gold standard for making nanoemulsions. Nanoscale emulsions can form regardless of the hydrophilic-lipophilic balance of the components, allowing for the fabrication of stable, homogeneously sized particles with a narrow particle size dispersion.	Quite pricey, with even the cheapest model going for well over $10,000. Furthermore, each time the device is used, it must be thoroughly cleaned to avoid the spread of any contaminants. High-pressure homogenizers are notoriously cumbersome and cumbersome due to their size and weight.
Microfluidization	camellia seeds oil (96), curcumin (97), and β-carotene (98)	High-pressure homogenization is the most used technique, but this one is close behind. techniques like 'direct' emulsification, where the dispersed phase is injected directly into the continuous phase via microchannels, bypass the need for pre-emulsification. reduces the difficulty of creating a stable nanoemulsion with particles smaller than 160 nm. Because of its constant shear rate and fixed geometry, microfluidization is less prone to clogging and yields more consistent results. Procedure that is commonly used in the food business	There are a number of drawbacks to developing nanoemulsions for microfluidization using a two-step single channel as opposed to a single channel, including the need for additional energy and more expensive wastage of lipids and oils for making a coarse emulsion to be fed into the microfluidizer at the outset.
Phase inversion composition method	Ginger oil (99), neem oil (100), isoliquiritigenin (101)	The most often used technique after microfluidization and centrifugation High efficiency, simplicity, and yield are only some of the benefits of this low-energy approach. produces nanoemulsions at room temperature without the need for organic solvents or high temperatures, may be done with minimal overhead due to the absence of costly equipment, and has a low cost per unit of output.	needs a lot of surfactant, which could be harmful. synthetic surfactants are increasingly being phased out of use in the food business due to concerns over their temperature instability.
Phase inversion temperature method	Fisetin (102), cinnamon oil (103), and lemon oil (104).	Advantage similar to Phase inversion composition method	Since the oil/water emulsion, which is present at room temperature, must be inverted into a water/oil emulsion and the finely divided oil/water emulsion prepared by quick cooling, the mixture of the components must be heated to above the phase inversion temperature. One major drawback of the procedure is the high amount of energy needed for the heating and efficient cooling processes, which makes it extremely uneconomical.
Self-nanoemulsification method	Zedoary essential oil (105), essential oils of Cymbopogon citratus and Amomum compactum (106), cinnamon bark essential oil (107)	A number of advantages as compared to high energy and other low energy methods include protecting delicate chemicals from the harsh conditions of the high energy approach (particularly temperature and pressure), lowering the amount of surfactants used, and increasing thermal stability.	Some industries, such the food and feed industries, have stricter regulations on the use of co-surfactants because of safety concerns.
Ultrasonic Emulsification	orange peel essential oil (108), Nigella sativa L. essential oil (109), Cinnamon oil (110)	Nanoemulsions have improved stability, lower polydispersity, smaller droplets, and lower energy and emulsifier usage.	Due to the high temperatures generated, heat-sensitive chemicals may be altered during nanoemulsion preparation, which is limited to relatively small batches.

occurs when the HLB surfactant has a low value, but oil in water emulsion occurs when the HLB surfactant has a high value. The stabilization of oil in water nanoemulsions is best supported by surfactants exhibiting HLB value > 10. Oil in water emulsions is best supported by surfactants with HLB values from 8 to 18, while water in oil emulsions are best supported by surfactants with HLB values from 3.5 to 6 (*111*, *112*). In order to create a stable emulsion formulation with the requisite HLB, the surfactant or emulsifier's specific HLB value is required. The formation of best HLB-based outputs in the future can benefit from statistical optimization method such as Response Surface Method (*113*) and Artificial Neural Network (*114*).

Stability of nanoemulsion

Following the creation of a nanoemulsion formulation with the desired compositions, the stability of the nanoemulsion is the most significant issue to consider because it is a system that is capable of withstanding changes in its physicochemical properties over an extended period of time. The stability of the emulsion, on the other hand, is governed by the surfactants used, their composition, and the distribution of droplet sizes within it. Microdroplets are formed because of the action of surfactants in nanoemulsions on the interfacial tension between water and oil. Surfactants and other emulsifiers, in addition to their various functions, aid in the stabilization of heating, cooling, pH, ionic strength, and long-term storage. It also contributes to increased stability in a variety of ways. Ionic surfactants, for example, build a tight barrier that delivers an electrical charge, whereas non-ionic surfactants contribute to the formation of a steric barrier by containing bulky molecule groups in their composition (*89*).

In nanoemulsion formulation, the physicochemical stability of nanoemulsions is classified as kinetically stable systems because it degrades over time due to chemical instability and destabilizing physical phenomena such as coalescence, gravitational separation, and flocculation (Figure 6). The chemical instability of nanoemulsions is typically caused by the oxidation and hydrolysis of the chemicals involved. The nanoemulsion's small droplet size paired with its large surface area is one of the elements that make it vulnerable to chemical degradation (*89*). It's also worth noting that the transparency of nanoemulsions affects their chemical stability because clear nanoemulsions are easily harmed by UV or visible light. Aside from chemical deterioration, the different relative densities of the dispersed and continuous phases cause gravitational separation, which in turn activates the destabilizing physical mechanism of nanoemulsion. This approach has the potential to generate creaming or sedimentation in a nanoemulsion. Gravity influences the creaming of nanoemulsions, which occurs as a result of the movement of large particles and is unaffected by particle contact. Small droplets less than 70 nm in size, on the other hand, can generate Brownian effects that prevent creaming. Brownian motion produces the random movement of particles in a fluid as a result of collisions with other atoms or molecules, which is known as the Brownian effect or Brownian motion (*115*).

The size of the droplets in a nanoemulsion is proportional to the amount of colloidal interaction. It happens as a result of both attractive contacts between two neighboring droplets, such as van der

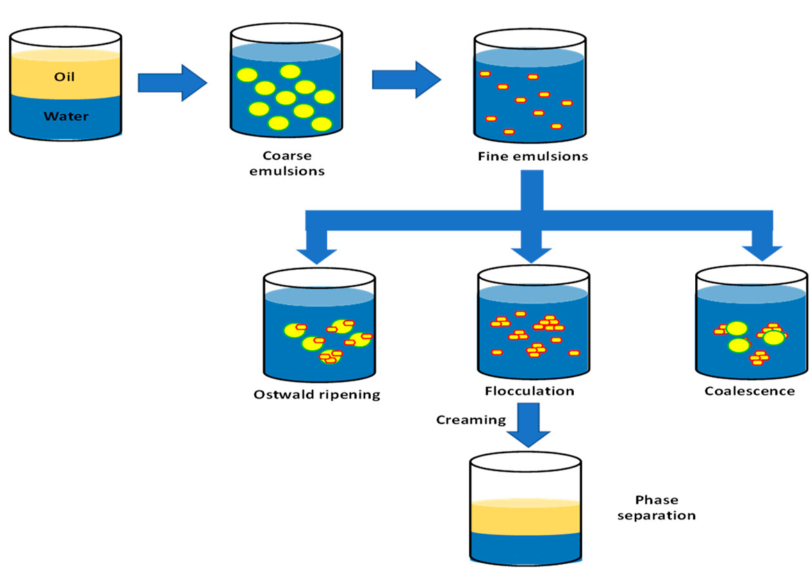

Figure 6. Nanoemulsion formulation and destabilising processes (Adapted from (*89*, *115*).

Waals interactions, and hydrophobic and repulsive interactions between two adjacent droplets, such as electrostatic and steric interactions (115). Nanoemulsions can destabilize at times, resulting in flocculation, coalescence, and Ostwald ripening, among other impacts. However, because nanoemulsions have small particle sizes, flocculation and coalescence are less likely to occur than in regular emulsions. Moreover, Ostwald ripening is typically followed by an increase in particle diameter and spacing, which is caused by the diffusion or transfer of solubilized oil molecules from tiny droplets to big droplets via a dispersed phase shortly after droplet size is increased (89, 115). Again, the use of RSM and ANN in the future can improve the stability of EO-based essential oil as are demonstrated in several studies (114, 116).

Nanoemulsion release

In addition, nanoemulsions loaded with bioactive and therapeutic chemicals can hide off-putting tastes, boost physical stability, improve targeting ability and specificity, shield biological components from chemical degradation, and regulate the rate of release. The nanoemulsion must deliver its loaded compound to the intended target, and release studies are critical to the success of a specific nanoemulsion formulation. The dialysis tubing method, with a common molecular weight cutoff of 12 kDa or about 2.4 nm, is one of the simplest and most commonly used methods in studying *in vitro* control release of nanoemulsion aside from United State Pharmacopeia (USP) apparatuses I to IV (117). Most of the release kinetics studies for essential oil-loaded nanoemulsions are carried out for human or animal drug delivery system studies, and very few studies have been done on agricultural scenarios. Despite this, some similar aspects of drug release studies can be used in future studies of essential oil release from nanoemulsions in agriculture. In one study, curcumin-loaded nanoemulsions were tested for encapsulation and stability using lecithin, Tween 20, and SMP (Sorbitan Monopalmitate) (118). According to their findings, nanoemulsions containing 2.0% w/w lecithin were stable for about 86 days, but nanoemulsions containing Tween 20 or SMP at the same concentrations were unstable after 5 days or around 24 h based on release studies. Indeed, the quickest release of curcumin was observed at 20 concentrations of 0.5% w/w. It appears that the smaller the size of the nanoemulsion, the better the release process. In one study, a formulation with a size of 15.3 nm showed about 10% greater release of drug than a nanoemulsion with a size of 324.1 nm, likely due to a greater surface area in the former (119). Essential oil-loaded nanoemulsion release kinetics can be described using four different kinetic models, namely the Hixson–Crowell, zero order, first order, and Higuchi models, while the release mechanism can be studied using the Peppas equation (120).

Nanoemulsions' thermodynamic stability tells us something about their physical and thermal stability both in the short and long term. When the temperature of a nanoemulsion is raised, the oil/water ratio decreases and the nanoemulsion becomes less viscous, both effects being caused by the intermolecular interaction in the continuous phase. Consequently, this can pave the way for the inner phase of the nanoemulsions' volatile components to be released and migrate to the environment (121). The energy of molecular collisions and the system's entropy both rise with temperature, which may explain why greater temperatures hasten the release of volatile components. A rise in temperature can boost a system's entropy, which in turn can make emulsions more unstable and hasten the diffusion of volatile components from the inner to the outer phase. This is due to the fact that at low temperatures, colloidal systems are at their most stable. The Stokes–Einstein equation describes how temperature and viscosity influence the release of volatile components (122).

From kinetic theory, Einstein derived his theory of Brownian motion. The equation suggests a one-to-one relationship between temperature and diffusion coefficient and an inverse relationship between viscosity and diffusion coefficient. It's also important to make it clear that the migration of volatile components from the inner phase to the outer phase of nanoemulsions is directly related to the diffusion coefficient of droplets in liquids. Because of their increased Brownian motion owing to heating, scattered droplets may have advanced toward each other and coalesced on exposure to high temperatures, lowering the efficacy of a nanoemulsion (123). This has to be taken into account when adding nanoemulsion to dried grain, as the use of heat for drying can affect the stability of the added nanoemulsion if the grain is not cooled enough before the nanoemulsion is added.

Identification and characterization of nanoemulsion formulation

The identification and characterization of the resulting nanoemulsion is a vital stage in the development of a nanoemulsion formulation. To accomplish this, analytical techniques that include separation and characterization, as well as imaging techniques, can be applied. Some of the techniques typically employed in nanoemulsion characterization approaches include nuclear magnetic resonance, zeta potential, differential scanning calorimetry, and dynamic light scattering. There are several imaging techniques available to identify and characterize the size of nanoemulsions, including Dynamic light scattering (DLS) is a physics technique that uses the light scattering from minuscule particles in suspension to calculate the size distribution profile of the particles. It's one of the quickest methods for assessing droplet size, polydispersity, and zeta potential, among other things. DLS is a technique that analyses the fluctuation in scattering intensity caused by the Brownian motion of particles in proportion to particle size. This approach, which is based on this measurement, is used to calculate the translation diffusion coefficient. Before determining particle size in dilute emulsions, particle interaction is removed. This is accomplished through the application of the Stokes–Einstein equation (*124*).

> This method can be used to determine a sample's average particle size. The polydispersity index (PDI) is a measure of the extent of the size distribution in suspension acquired from the cumulative analysis of the phenomenon of dynamic light scattering. For polydispersity, all information on nanoemulsion homogeneity, quality, and storage stability may be gathered in one spot. Low PDI nanoemulsions (less than 0.1) are typically regarded as having excellent monodispersed and quality attributes (*89*). Diluting samples is required to avoid many scattering effects. Other compounds, such as surfactant and co-surfactant, may have an effect on particle size as determined by DLS when present in the nanoemulsion (*115*).

i) **Transmission Electron Microscopy (TEM)**
The shape of droplets is an important component of an emulsion system. One of the most commonly utilized technologies for determining the droplet size of a nanoemulsion is the transmission electron microscope (TEM). The goal of TEM analysis is to track the surface morphology of emulsion droplets, as shown in images (*125*). Following digital image processing examination, the TEM discovers higher resolution images of the scattered phase. DLS can provide qualitative and quantitative data such as size diameter and size distribution. However, there are still limitations to using TEM for nanoemulsions. For example, during the preparation process, the sample's structure may change, and the electron beam of a TEM may cause damage to the sample (*125*).

Use of nanoemulsions in field trial works

The successful use of essential oils in controlling agriculture pathogens is an important starting point for the use of essential oils in the form of nanoemulsion to capitalize on the many advantages that nanosized particles carry (*81*, *126*, *127*). To date, there is no published literature, to the best of our knowledge, on the use of essential oils in nanoemulsions to control pathogens in grain corns. However, the existing examples of the use of nanoemulsions and essential oils in field trials to control agriculture pathogens other than grain corn will benefit grain corn research in the future. For instance, in Vietnam, one of the by-products of the curcumin industry is curcumin-removed turmeric oleoresin (CRTO). This by-product, previously thrown away and posing an environmental pollution threat, has now been evaluated as an active ingredient for the preparation of a botanical fungicide-based nanoemulsion to control anthracnose disease caused by *Colletotrichum gloeosporioides* in litchi, an important agriproduct. A field trial work using the nanoemulsion to treat infected plants at the preharvest stage is evaluated using water-diluted nanoemulsion at the volume of 800 L per hectare with doses from 5 to 10 mg/mL sprayed at the interval of 14 days. The results show that the CRTO nano-emulsion is successful in controlling the disease (*128*).

Another essential oil nanoemulsion prepared from crude Aniseed (*Pimpinella anisum* L.) and another formulation containing the compound (E)-anethole, the main component in *Pimpinella anisum* is tested against *Nasonovia ribisnigri* Mosley (Hemiptera: Aphididae); one of the most destructive aphid species to lettuce in the Southeast of Spain (Torre Pacheco, Murcia) to an open field plot at the preharvest stage. The field foliar treatments of lettuce at 0.2 and 0.4% concentrations infested with *N. ribisnigri* reduced the number of the aphid populations, while (E)-anethole nanoemulsion treatment gave similar results to the aniseed essential oil (*127*). Currently, our lab is carrying out an upscale study of the use of Agrozide™, an EO nanoemulsion in controlling mycotoxin (aflatoxin) production in stored grain corn (*129*). The formulation developed using the statistical optimization method Response Surface Method was found to be stable for at least six months (unpublished results), which is similar to several published results on the stability of nanoemulsions (*130*, *131*). Field trial works of developed nanoemulsion efficacy on controlling plant pathogen is seriously lacking and remains an important future study.

Conclusion

To reduce fungal contamination in the environment, nanoemulsions are being used to spread hydrophobic bioactive compounds such as antimicrobials and antioxidants. According to a number of studies, nanoemulsions can improve the properties of fungicides that are based on essential oils and are used to control bacteria and fungus on agricultural products. In many circumstances, nanoemulsions' small droplet size and large droplet surface area are related to a number of advantageous features. A variety of bioactive compounds, including hydrophobic, hydrophilic, and amphiphilic molecules, can be integrated into a single delivery system, increasing their efficacy via additive or synergistic effects. As a result, nanoemulsion-based systems including essential oil are appropriate for use in fungal treatment applications because they increase the physical and chemical stability of the formulation and, among other things, the preservation of high-quality agricultural goods. As the world is experiencing food shortage due to the current Ukraine-Russian scenario, methods to prolong grain corn storage are needed more urgent than before. Field trial work using nanoemulsion forms of essential oil to combat fungal growth in grain corn is an important future work that needs to be explored.

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Data availability

Raw data were generated at MARDI. Derived data supporting the findings of this study are available from the corresponding author (NAM) on request.

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