# Effect of plant products against seedborne fungal diseases

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## Abstract

Fungal pathogens of plants in different climates cause challenging problems in crop cultivation and economic threats. To control fungal pathogens due to serious side effects of artificial fungicides for human health such as carcinogenesis, allergies and genetic problems, lack of environmental balance and development of resistance to chemical fungicides in populations of fungal pathogens, identification of safe alternatives to control fungal pathogens such as natural plant materials are considered as one of the principled methods. Plant essential oils contain a wide range of antifungal secondary metabolites and play an important role in environmental adaptation and plant evolution. Plant essential oils reduce the spore germination of fungal phytopathogens, disintegration of fungal hyphae and formation of vacuoles in these structures, cytoplasmic coagulation, protoplast leakage, deformation of intracellular organelles of plant pathogenic fungi such as mitochondria, inhibition of enzymatic reactions in mitochondrial membranes and affected by the entry and exit of ions into the fungal cell, and finally reduce their pathogenicity on the host plants.

**Keywords:** Essential oils and plant extracts, Fungal diseases, Seeds

## Introduction

Seed fungal of plant seeds are very destructive pathogens due to production of mycotoxins and secondary metabolites, which are known as virulence factors leading to reduce yields, seed germination, and cause quantitative and qualitative damage, together with their toxicity for human consumption. These fungal pathogens are located on the surface or inside of the seeds and transmitted under suitable environmental conditions. Such fungal infections occur before and during harvest as well as during storage. Therefore, control of these pathogens is essential for production of organic products and also for crop protection. Exponential increase in the use of chemicals such as synthetic fungicides as the most common method of controlling fungal pathogens led to an increase in pathogenic isolates resistance to the fungicides [54] and accumulation of hazardous fungicides in food products [20]. Many antimicrobial chemicals are not easily converted to simple forms and eventually remain in the food chain for long periods of time, with toxic residues adversely affecting various mammalian species [38].

In order to meet the demand of consumers and producers for safe and organic foods without chemical preservatives, it is important to identify alternative methods for the management of plant fungal diseases. One of the environmentally friendly approaches to control these destructive pathogens is the use of natural products, especially the extracts and essential oils of many medicinal plants. These compounds are a rich source of biologically active components with antifungal effects for management of fungal diseases [19-46]. The natural origin and safety of plant essential oils for humans and the environment and their activity in the vapor phase, together with the lack of microbial resistance to plant essential oils are the benefits of essential oils with antifungal properties [25-58]. Therefore, essential oils are considered as a suitable alternative to chemical fungicides and often help to develop factors that inhibit the growth and proliferation of plant pathogenic fungi and reduce the production of mycotoxins by seed pathogens.

## Destructive effects of fungi on plant seeds after harvest

The most common symptoms and disorders in seeds often include several cases mentioned below: [40]

Seeds abortion: The host flower organs are replaced when the parasites fertilize. Fungal pathogens include *Claviceps purpurea* in cereals, *Fusarium* spp. In wheat, corn and rice, *Ascochyta rabiei* in chickpeas and *Drechslera verticillata* in cotton grass and wheat cause systemic contamination of cereals and grasses. Seeds necrosis: Such symptoms are usually caused by fungal pathogens affecting the protective layers of the seeds, such as *Colletotrichum* spp. and *Ascochyta* spp. In bean seeds, soybeans, chickpeas, kidney beans and other hosts occur and cause damage to fleshy cotyledons.

Shrunken seeds: Fungal pathogens such as *Alternaria brassicicola* and *Phoma lingam* in crosses, *Septoria linicola* in flax, *Drechslera teres* in barley, *Fusarium graminearum* and *Septoria nodorum* in wheat reduce seed size and germination rate. Seeds rot: Caused by many seed fungal pathogens include *Fusarium avenaceum*, *F. graminearum*, *F. moniliforme*, *Bipolaris sorokiniana*, *B. maydis* and *B. oryzae* in cereals, *Colletotrichum graminicola*, *Diaporthe Phaseolorum* and *Fusarium* spp. in soybeans, *Botrytis allii* in onions, *Verticillium dahliae* in spinach and *Botrytis cinerea* in the seeds of many host trees, germination leads to seed rot. Sclerotization and stromatization: In certain groups of fungi such as *Claviceps purpurea* and other *Claviceps* species in cereals and wheatgrass, *Phomopsis viterbensis* in chestnut, *Pleospora herbarum* in spinach and *Ciboria* spp. In the seeds of forest trees and weeds, the flower or seed organs turn into sclerotia or stromata.

Seeds discoloration: Seeds discoloration is a very important destructive factor and is considered as a general indicator of low quality for consumption (cereals) or for industrial use (flour, semolina, oil seeds). Many brown discoloration reactions in some fungal pathogens such as *Ascochyta pisi* in chickpeas, *Colletotrichum lindemuthianum* in beans, *B. sorokiniana* in wheat, *B. oryzae* in rice and *Cercorpora kikuchii* in soybeans are due to oxidation of phenolic compounds. Reduction of germination capacity: Deeper necrosis or decay of some hosts, such as wheat seed rot caused by *Drechslera verticillata*, reduces seed shelf life and germination. Production of toxic metabolites: Some seed fungal pathogens such as *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. due to the toxicity of mycotoxins, they cause harmful effects on seeds as well as toxicity to animals and humans.

## Origin and composition of plant essential oils

Some essential oils of secretory cavities and cell wall hairs as liquid droplets in leaves, flowers, roots, fruits, stems and bark of plants have been reported with antimicrobial activity [30], antibacterial, antifungal [28-35], antiviral [16-33], antiparasitic [43-44] and also with insecticidal activity [13-58]. Essential oils are a natural mixture of various terpenoid compounds and their oxygenated derivatives. Essential oils contain various terpenoids, mainly monoterpenes, sesquiterpenes, hydrocarbons with common formula (C5H8)n, sometimes excellent terpenes as oxygen compounds and oxygen compounds derived from these hydrocarbons, including alcohols, aldehydes, esters, ketones [30].

The essential oils are usually liquid and can be easily converted from liquid to vapor at room temperature or slightly above that temperature without any decomposition. In the essential oils of different plants, a main element or a combination of terpenes are predominant. For example, the predominant composition of basil essential oil (*Ocimum basilicum*) is methyl chavicol at 75% and rhizomes of wedge essential oil (*Acorus calamus*) contain 70-80% B-asarone. The main components of coriander essential oil (*Coriandrum sativum*) include decanal and 2-decenol, as well as other compounds such as linalool in the amount of 50-60% and p-cymene, terpinene, camphor and limonene [34]. Very small compositions of plant essential oils significantly affect the taste, aroma and biological activity of the essential oils. Phenolic compounds of the essential oils, followed by alcohols, aldehydes, ketones, ethers and hydrocarbons have the highest antimicrobial properties [23]. The biological activities of essential oils depend on the qualitative and quantitative characteristics of their components, and such compounds are influenced by plant genotype, agronomic conditions, plant chemistry, geographical location, environmental factors, extraction methods and storage conditions of essential oils and even plant tissues [9-45].

## Investigating effect of medicinal plants in seed protection

### *Azadirachta indica*

The bioactive compounds of neem (*Azadirachta indica*) are terpenes and azadirachtin and they are of special importance due to the presence of azadirachtin. A variety of bioactive compounds such as nimbin, nimbidin, salannin, thionemon and meliantriol are found in the seeds, leaves and bark of neem trees at high concentrations. The most bioactive compounds are in the kernels of seeds and leaves, respectively [29].

Treatment with neem essential oil at concentration of 0.5% for 16 hours is known as an effective method to control 50% of *Macrophomina phaseolina* disease in cowpea and *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *F. nivale*, *F. semitectum*, *Alternaria alternate* and *Drechslera hawiinesis* in different plant species [37]. Neem leaf extract is very effective in reducing contamination of the seeds of african bean (*Sphenostylis stenocarpa*) caused by *F. moniliforme* and *Botryodiploida theobromae* and complete control of *A. niger* and *A. flavus* as well as increasing seed germination [42]. Treatment with neem extract (*A. indica*) significantly reduced the synthesis of aflatoxin B1 in maize [11] and also inhibited 83% of *Bipolaris oryzae* infection in 1:1 dilution [5] and also corn treatment using the neem seed extract for 1 to 24 hours reduces infection caused by *F. moniliforme*, *Acremonium strictum* and *B. theobromae*, which are pathogenic on maize [2].

## *Allium sativum* L.

Garlic (*Allium sativum*) contains at least 33 sulfur compounds such as alline, allicin, agon, diallyl sulfides, vinylditines, allyl cysteine, s-allyl mercaptocysteine as well as sulfur compounds including 17 amino acids and glycosides, minerals including selenium and germanium, together with enzymes such as alinase, peroxidase and myrosinase [14].

Treatment of sorghum seeds with garlic extract tablets (*A. sativum*) controls 90% of fungal seed borne pathogens such as *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. Also, application of garlic extract leads to 84.6% reduction in *F. moniliforme* infection [36] as well as control of mustard fungal pathogens including *Alternaria*, *Aspergillus*, *Chaetomium*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus* [32]. Treatment with alcoholic extracts of garlic (*A. sativum*) controlled the contamination of wheat seeds caused by *B. sorokiniana* [27] and also lead to inhibition of fungal pathogens including *A. alternata*, *Fusarium oxysporum*, *Aspergillus* sp., *Rhizopus* sp. and *C. graminicola* and increased germination of sorghum and peanut seeds (*Arachis hypogaea*) [56]. Investigating the inhibitory effect of garlic extract against mold infestation caused by *Alternaria*, *Curvularia*, *Drechslera*, *Fusarium* and *Phoma* species using different concentrations of the extract (including 25%, 12.5%, 6.25% and 3.12%) revealed that concentration of 12.5% was effective for controlling storage mold on the seeds for one to two months. Whereas, the concentration of 25% was effective for longer storage of the seeds [39].

## *Lantana camara* L.

*Lantana camara* L. extract contains volatile compounds including monoterpene hydrocarbons, oxygen monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and aliphatic components, as well as important compounds such as germacrene, phellandrene, β-caryophyllene and 1,8-cineole [57].

King-like shrub methanolic extracts (*L. camara*) have effective antifungal activity against soybean seed contamination caused by *Colletotrichum truncatum* and *M. phaseolina* [8]. Shrub-like leaf extracts (*L. camara*) effectively inhibit seed borne fungal pathogens, including *Alternaria*, *Rhizopus*, *Aspergillus* and *Penicillium* in mustard and chickpeas and reduce the germination rate of crops [3-59] as well as environmentally friendly essential oils of shrub (*L. camara*) based on antifungal and anti-aflatoxigenic potentials suppress fungal pathogens such as *A. flavus* and *A. parasiticus* on various plant species [3].

## *Cymbopogon citratus*

The chemical compounds of lemongrass (*Cymbopogon citratus*) mainly contain terpenes, alcohols, ketones, aldehydes and esters and some essential oils containing citral α and citral β as the main compounds of nerol geraniol, citronellal, terpinolene, geranyl acetate, myrcene and terp flavonoids and phenolic compounds, including luteolin, isoorientin 2′-O-rhamnoside, quercetin, kaempferol and apiginin [50].

Lemongrass essential oils and extracts (*C. citratus*) control the production of aflatoxins and *A. flavus* growth in rice and inhibit *A. flavus* and *A. fumigatus* in corn [1] lemongrass essential oil (*C. citratus*) with concentrations of 0.6 mg/ml and 1 mg/ml has fungistatic and fungicidal effects against *A. flavus*, as well as aqueous extracts of lemongrass (*C. citratus*), which has inhibitory effect against *C. graminicola*, *Phoma sorghina*, *F. moniliforme*, *C. graminicola* and *P. sorghina* [53].

Essential oils of *C. giganteus*, *C. nardus* and *C. schoenanthus* significantly reduced seed contamination caused by *Phoma* sp., *Fusarium moniliforme*, *Sphacelotheca sorghi* and *Tolyposporium ehrenbergii* in sorghum [60] as well as essential oil of lemongrass (*C. citratus*) in concentrations of 0.1 ml/100g and 0.25 ml/100g, which control cantaloupe deterioration caused by *A. flavus* [12].

### *Mentha piperita* L.

Peppermint essential oils (*Mentha piperita* L.) contain several chemical compounds, such as menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, B-pinene, α-pinene, germacrene-D trans-sabinene hydrate and pulegone. The essential oil of peppermint controls the infection caused by *B. oryzae* in rice, increases the germination of seeds and root growth of rice seedlings [6]. Also, treatment with steam of this essential oil reduces contamination of the most common fungal pathogens of cucumber, including *Fusarium*. spp., *Alternaria*, *Rhizoctonia*, *Helminthosporium* and *Penicillium* and reduce the transfer of fungi from seeds to seedlings [22].

### *Thymus vulgaris*

Thyme essential oil (*Thymus vulgaris*) contains phenolic compounds, thymol and carvacrol [18] with anti-aflatoxigenic effects, especially on production of aflatoxin B1 at the rate of 0.6 µl/ml, which causes complete inhibition of fungal pathogens such as *A. flavus*. Application of thyme essential oil at 0.7 µl/ml concentration leads to reduction of rice contamination by 48-100% and control of rice fungal pathogens including *B. oryzae*, *A. padwickii* and *F. moniliforme*, together with inhibitory effect on transmission of the above mentioned pathogens at 76-95% [41]. Also, toxicity to food fungal pathogens, such as *Cladosporium herbarum*, *Curvularia lunata*, *F. oxysporum*, *Aspergilus terreus*, *A. niger*, *A. fumigatus*, *Alternaria alternata* and *B. theobromae* is a characteristic of thyme essential oil [31].

### *Ocimum gratissimum* L.

The chemical composition of clove essential oil (*Ocimum gratissimum* L.) includes eugenol, thymol, citral, ethyl cinnamate, geraniol and linalool [55], which increases the germination capacity of seeds and reduces the severity of *A. padwickii* in rice. Aqueous extracts of clove (*O. gratissimum*) are well known to have control effects against *A. padwickii* fungal pathogens in rice [41]. Application of the essential oil obtained from clove can be considered as a suitable method for controlling *A. flavus*, *A. parasiticus* and *A. ochraceus* in peanuts as well as *F. oxysporum* and inhibition of aflatoxin production by such fungal pathogens [4]. The essential oils of basil (*O. basilicum*) and clove (*O. gratissimum*) have significant inhibitory effect on *F. verticilloides* and FB1 (Fumonizin B1) contamination in maize [21].

## Seed fungi produce mycotoxins

Post-harvest economic losses due to fungal contamination, complexity of mycotoxins and contamination of more than 25% of world cereals with mycotoxins [31-48] are considered as critical concerns worldwide. Biosynthesis of fumonizines as the most common maize mycotoxins by some soil borne and seed borne fungal pathogens, such as *Fusarium verticillioides* and *F. proliferatum* is the cause of contamination of a wide range of crops [17]. Some molds or parasitic fungi such as *A. flavus* and *A. parasiticus* have attracted considerable attention from research communities due to their abundance, ubiquity, and ability to synthesize mycotoxins as well as colonization of various food products [15-47].

Treatment with 0.05% concentration of citrus lemon essential oil and 0.2% of orange essential oil (*Citrus aurantium*) reduces patulin production and inhibits *Penicillium expansum* in apples, as well as controlling *Aspergillus* strains [26]. Lemongrass essential oil (*Lippia alba*) contains the main geranial and neral compounds with the effect of increasing seed germination and inhibiting the production of aflatoxin B1 induced by *A. flavus* in legume seeds as an effective preservative for edible legumes against fungal and mycotoxin contamination [51]. Treatment with essential oils of clove (*O. gratissimum*), potassium (*C. citratus*), *Xyloppia aethiopica* and *cinnamon silane* (*Cinnamomum verum*) can be effective for preventing formation of non-sorbic acid and aflatoxin synthesis precursors [10] as well as treatment with essential oil of garcinia (*Garcinia indica*), which containing mainly garcinol and inhibits *A. flavus* and aflatoxin B1 synthesis [49]. One of the methods to control diseases caused by *Aspergillus* sp. is treatment with marjoram essential oil (*Origanum vulgare* L.), which mainly contains carvacrol and thymol with antioxidant effects and also contains γ-terpinene, p-cymene, terpinen-4-ol and sabinene hydrate at concentration of 20 μl/l [7-52].

Treatment with essential oils of *Pimpinella anisum* and boldo (*Peumus boldus*) increases seed germination and controls the synthesis of aflatoxin B1 in maize and finally inhibits *Aspergillus* section *Flavi* (*A. parasiticus* and *A. flavus*) [17]. Combination of 1:1 equine tail extracts (*Equisetum arvense*) with licorice (*Stevia rebaudiana*) contains antioxidant compounds with antifungal activity to inhibit *A. flavus* and aflatoxin production in maize. Thus, such natural compounds obtained from plants are proposed as alternative treatments for controlling mycotoxin-producing fungi in corn. Essential oils of cinnamon (*Cinnamomum zeylanicum*), peppermint (*M. piperita*), basil (*O. basilicum*), marjoram (*O. vulgare*), apazot (*Teloxys ambrosioides*), clove (*Syzygium aromaticum*) and thyme (*T. vulgaris*) have protective effects on corn and are effective in controlling *A. flavus* [24].

## Conclusion

Identification of alternative disease control methods to be replaced with application of chemical fungicides in order to prevent biodegradation of plant products during long-term storage without any toxicity to consumers is a necessary and practical matter for plant pathology researchers. The multicomponent properties of essential oils make it more difficult to develop resistance in fungal pathogen populations, as well as the selection of such natural plant products over synthetic fungicides in the control of destructive plant pathogens. In recent years, the use of extracts and essential oils of medicinal plants as a safe and environmentally friendly alternative to fungicides and synthetic pesticides to control fungal pathogens of plant products have been proposed. A close look at the native plants containing abundant antimicrobial compounds as natural protectors of agricultural products against fungal diseases is essential and can be an interesting subject for future researches in this field.

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