Plant essential oils as natural fungicides against stored product fungi

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Stored product fungi cause severe degradation of food items during storage and also cause health hazards by secreting various mycotoxins. Due to negative concerns on health and environment, many synthetic fungicides have been removed through the Food Quality Protection Act in the United States which hinted the exploration of plant products as safe fungicides. Different *in vitro* studies have demonstrated antifungal activity of plant essential oils (EOs) against a wide range of food spoiling fungi. EOs are composed of a large number of components having several targets in the cell. Being hydrophobic in nature, they easily pass through plasma membrane, cause partition in the lipids of the cell membrane, and make it more permeable and subsequently leakage of cell contents occur. EOs also reported to retard biosynthesis of ergosterol, the fungal specific sterol, making EOs specific to fungal target. A few EO based fungicides viz. Sporan™, Promax™ etc. are already commercially available and many are in process. Most of the EOs are in the list of generally recognized as safe (GRAS) in the U.S.A. favouring their use as safe fungicide. In the present chapter, the potential of plant EOs as safe fungicide for future application is discussed. In addition, mode of antifungal action, safety concerns and challenges for future prospects are also discussed.

Keywords: essential oil; safe; fungicide; antifungal; mode of action

1. Introduction

The food items such as cereals, pulses, oilseeds, spices, dry fruits etc. must be stored properly after harvest as their production varies from year to year. The point of production is not always the point of consumption and the time of production is not always the time of consumption. Hence, the food items should be stored and transported properly for future needs. However, during storage and transportation, a number of fungal pathogen attacks the food items leading to their qualitative and quantitative loss. On an estimate, around 25% of agricultural food items become unsuitable for consumption annually due to the invasion by different food-borne molds and their toxic metabolites [1]. The condition is more serious in tropical and sub-tropical regions of the world due to the congenial factors like high temperature, relative humidity and moisture content of stored products which favor the development of fungal population. The major fungi found associated with stored food items include *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., *Curvularia* spp., members of Mucoralesetc. [2]. Fungal attack in food items leads to their biodeterioration through increase in free fatty acid content, change in colour and texture and decrement in nutritional value and germination ability [3]. In addition, various mycotoxins secreted by different food borne molds cause serious health hazards to consumers. The major mycotoxins detected in stored food items are aflatoxins, ochratoxins, patulin, fumonisin, zeralenone and deoxynivalenol [1]. Mycotoxins are highly stable compounds which cannot be destroyed even after cooking. Mycotoxins are well known carcinogens, teratogens, tremorogens, mutagens, nephrotoxicants and neurotoxicants to a wide range of organisms [4]. Consumption of the mycotoxins along with the affected food items unknowingly may cause cancer, liver damage, kidney failure, paralysis or even death [5].Consumption of mycotoxin contaminated food items particularly that with the aflatoxins have caused many deaths of human and livestock in the past history in Asia and Africa [6]. Hence, mycotoxin contamination to food items is a serious health concern which should not be overlooked.

Various strategies to get rid of these noxious fungal pathogens during storage have been developed from time to time. The methods include physical methods such as solar heating of grains, mixing of inert dust, low temperature maintenance, irradiation, and various cultural, biological and chemical methods [7]. Most of the physical treatments have their own limitations as solar heating practice is restricted to semi-arid tropics only, low temperature maintenance is costly, admixture of inert dust causes inhalation problems etc. Biological control has less practical application to fungi because of its dependence on environmental conditions. Hence, chemical control is applied mainly throughout the world and large numbers of synthetic pesticides such as organochlorines, organophosphorus, organomercurals, dithiocarbamic acids, systemic fungicides etc. are continuously used around the world against fungi during storage. However, the chemical pesticides have also negative concerns due to their post application side effects to non-target organisms, pest resistance and residual toxicity threatening food security [8]. Some of the well-known synthetic pesticides have been banned in the developed countries due to their post application side effects. Lindane, a well knownorganochlorine, has proved highly toxic when consumed along with the food; Malathion, Dichlorvos (organophosphates) have reported to cause residual toxicity; Permethrin, Deltamethrin (Pyrethroids) proved to have carcinogenic properties and most of the dithiocarbamates reported to have mutagenic, carcinogenic and teratogenic properties in animals including human [9-12]. These reports make the statement true that “Pests are problem but

pesticides are more problematic". Due to well established health hazards of synthetic fungicides, there is a quick need of some safer alternatives to protect the food items from fungal and associated mycotoxin contamination during storage conditions.

2. Essential oils (EOs) as green fungicide

Plants contain a number of secondary metabolites viz. terpenoids, phenolics, alkaloids etc. being used by them since antiquity as defence weapons for their protection against herbivores and pathogens. This valuable defensive chemistry can be exploited for the management of harvested plant materials from fungal contamination. Plant based formulations would be a better alternative to synthetic fungicides as they are chiefly biodegradable and eco-friendly. The fungicidal property of plant products against a wide range of fungi is well reported [2, 13]. Among these products, plant-based essential oils (EOs)are emerging as better alternative of synthetic fungicides all over the world due to their high efficacy, volatile nature and hence can be used as fumigant, and having many active compounds which provide less chance to development of resistance in fungi.

EOs are aromatic oily liquids obtained from different plant parts viz. flowers, seeds, leaves, twigs, barks, fruits and roots and have been used in traditional medicine and pharmaceutical preparations since many years ago [14]. EOs can be extracted by hydro-distillation, steam-distillation, cold pressing, expression, fermentation, effleurage or extraction. However, the most commonly used method for commercial production is steam or hydro-distillation. The antifungal properties of some EOs against a wide range of stored-product fungi have shown potential in formulation of plant-based fungicide. Table 1 represents some of the EOs studied for a wide range of storage fungi.

Even though most of the EOs inhibit postharvest fungi in *in vitro* conditions, their *in vivo* practical efficacy, however, is less researched. The most common method used for observing the antifungal activity of EOs is poisoned food method or micro-dilution method (Table 1). The method provides direct contact to the test pathogen with the test EO and is suitable for *in vitro* studies. Most of the authors have calculated MIC (minimum inhibitory concentration) value from this study which is the concentration required to cause 100% growth inhibition of test fungi. The calculation of MIC is beneficial as it provides least wastage of applied chemical and it can be tested for further detailed study. Most of the *in vivo* studies have used fumigation as say [15-16], however, the method of spraying or dipping to control postharvest decay of fruit and vegetables has also been tried [17-18].

Table 1 Essential oils (EOs) as antifungal agents against a wide range of post-harvest fungi.

Sr. no.	Plant for EO isolation. ( ) indicates plant part	Family	Against storage fungi	Method used	Observation	Reference
1.	*Brassica nigra* (S)	Brassicaceae	A.n., A.o., P.c.	Direct contact and vapor phase	100% growth inhibition at 4 µl ml^{-1}concentration in direct contact method and at47 µl l^{-1}air concentration in vapor phase	[15]
2.	*Chenopodiumambrosioides* (L)	Amaranthaceae	A.f., A.g., A.n., A.o., C.g., C.m., F.o., F.s.	Poison food assay	100% growth inhibition at 0.3% concentration	[19]
3.	*Cicutavirosa* (F)	Apiaceae	A.f., A.o., A.n., A.a.	Poisoned food method	100% growth inhibition at 5 µl ml^{-1}concentration	[20]
4.	*Cinnamomumjensenianum*(Ba)	Lauraceae	A.f.	Poisoned food method	MIC at 8 µl ml^{-1}concentration	[21]
5.	*Cuminum cyminum* (S)	Apiaceae	A.f., A.n., C.l., A.g., A.a., P.c., A.u., A.t., R.s., A.nid., *Mucor* sp., M.s., P.i., C.c., P.p., P.i., F.o., A.r., S.a.	Poisoned food method	100% growth inhibition at 0.6 µl ml^{-1}concentration except R.s.	[22]

6.	*Cympopogoncitratus* (AP)	Poaceae	B.c., C.h., R.s., A.n.	Dilution method	100% growth inhibition at 500 ppm	[23]
7.	*Cymbopogon martini* (L)	Poaceae	A.f., A.n., A.t., A.fu.,A.w., A.l., A.c., A.t., C.l., *Fusarium* spp., *Penicillium* spp.	Broth dilution method	100% growth inhibition at 0.5 µl ml^{-1}concentration	[24]
8.	*Foeniculumvulgare* (S)	Apiaceae	A.f.	Broth dilution method	MIC at 10 µg l^{-1} concentration	[25]
9.	*Laurusnobilis* (L)	Lauraceae	B.c., P.d., M.l.	Poisoned food technique	At 1000 µg ml^{-1} conc. 100% growth inhibition of B.c. and M.l. but 71% inhibition to P.d.	[26]
10.	*Lippiarugosa* (L)	Lamiaceae	A.f.	Agar medium assay	MIC at 1000 ppm	[27]
11.	*Menthaarvensis* (AP)	Lamiaceae	A.f., A.n., A.fu., *Rhizopus* spp., *Mucor* spp., *Curvularia* spp., P. o.	*In vivo* fumigation assay on wheat seeds	100% protection at 600 ppm except A.f.	[16]
12.	*Mentha spicata* (AP)	Lamiaceae	A.f., A.n., C.l., A.g., A.a., A.lu., P.c., A.u., A.t., R.s., A.nid., Mucor sp., M.s., P.i., C.c., P.p., P.i., F.o., A.r., S.a.	Poisoned food method	100% growth inhibition at 1.0 µl ml^{-1}conc. except A.lu. and A.t.	[28]
13.	*Ocimum sanctum* (L)	Lamiaceae	A.a., A.c, A. fu., A.t., A.v., C.l., F.n., Penicillium sp., A.f.	Poisoned food technique	100% growth inhibition at 0.3 µl ml^{-1}concentration	[29]
14.	*Rosmarinusofficinalis* (L)	Lamiaceae	A.f., A.n., A.t., A.c., A.s., A.fu., A.a., C.c., A.l., F.o., P.i., Mucor sp.	Contact assay	100% growth inhibition at 1.5 µl ml^{-1}conc. except A.a and C.c.	[30]
15.	*Saturejahortensis*(AP)	Lamiaceae	A.f.	Agar dilution method	MIC at 500 ppm	[31]

16.	*Syzygiumaromaticum*(B)	Myrtaceae	A. f., A. fu., A.n.	Broth dilution method	100% inhibition at 0.64 µl ml^{-1}concentration	[32]
17.	*Tagetespatula* (Fl)	Asteraceae	P.d., B.c.	Poisoned food method	MIC for B.c. at 10 µl $ml^{-1}$and for P.d. at 1.85 µl ml^{-1}concentration	[33]
18.	*Thymus pulegioides* (AP)	Lamiaceae	A. f., A. fu., A.n.	Broth dilution method	100% inhibition at 0.32 µl ml^{-1}concentration	[34]
19.	*Trachyspermum ammi* (F)	Apiaceae	A.f., A.n., C.l., A.g., A.a., A.lu., P.c., A.u., A.t., R.s., A.nid., *Mucor*sp., M.s., P.i., C.c., P.p., P.i., F.o., A.r., S.a.	Poisoned food method	100% growth inhibition at 0.8 µl ml^{-1}concentration	[35]
20.	*Zataria multiflora* (AP)	Lamiaceae	A.f.	Agar dilution method	MIC at 400 ppm	[36]

- Plant part: AP= aerial part; B= bud; Ba= Bark; F= Fruit; Fl= flower; L= leaf; S= seed.
- Fungal species: A.a. = *Alternariaalternata*; A.c., *Aspergillus conicus*; A.f. = *Aspergillus flavus*; A.fu. = *Aspergillus fumigatus*; A.g. = *Aspergillus glaucus*; A.l. = *Aspergillus luchuensis*; A.n. = *Aspergillus niger*; A.nid. = *Aspergillus nidulans*; A.o. = *Aspergillus ochraceous*; A.r. = *Absidiaramosa*; A.s. = *Aspergillus sydowi*; A.t.=*Aspergillus terreus*; A.ta. = *Aspergillus tamari*; A.u. = *Aspergillus unguis*; A.v. = *Aspergillus versicolor*; A.w. = *Aspergillus wentii*; B.c. = *Botrytis cinerea*; C.c. = *Cladosporiumcladosporioides*; C.g. = *Colletotrichumgloesporioides*; C.h. = *Cladosporiumherbarum*; C.l. = *Curvularialunata*; C.m. = *Colletotrichummusae*; F.n. = *Fusariumnivale*; F.o. = *Fusariumoxysporum*; F.s. = *Fusariumsemitectum*; M.l. = *Monilinialaxa*; M.s. = Mycelia sterilia; P.c. = *Penicilliumcitrinum*; P.d. = *Penicilliumdigitatum*; P.i. = *Penicilliumitalicum*; P.l. = *Penicillium luteum*; P.o. = *Penicilliumoxalicum*; P.p. = *Penicilliumpurpurogenum*; R.s. = *Rhizopusstolonifer*; S.a. = *Spondylocladium austral*.
- MIC= Minimum inhibitory concentration.

3. Mechanism of antifungal activity

EOs are composed of a number of different components such as terpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones in different composition or combinations [37]. Some of the components remain present in very high concentration while some in very low concentration. The antifungal efficacy of EO is mainly either attributed to the overall synergistic effects of all the major and minor compounds or to the bioactivity of the major compounds [38]. The chemical composition can vary according to method of EO isolation, age of plant, time of harvest, ecological and geographical variations. Hence, before large scale application the chemical standardization of EO must be endorsed.

One of the important mechanisms of action of EO is the reduction in ergosterol synthesis in fungal cells. Some studies have shown that EOs have the potency to cause a considerable reduction in the quantity of ergosterol in fungal cell membrane [32,34]. Ergosterol is specific to fungal cell membrane and is its major sterolcomponent responsible for maintaining the cell function and integrity [39].Kelly *et al*. [40] stated that the primary action mechanism of azole antifungal drugs, is the interruption of sterol biosynthetic pathways resulting in reduced ergosterol biosynthesis. Further, the studies of Kedia *et al*. [22] with *Cuminum cyminum* EO, Kedia *et al*. [28] with *Mentha spicata* EO, Kedia *et al*. [35] with *Trachyspermum ammi* EO and Tian *et al*. [21] with *Cinnamomumjensenianum* EO have a clear evidence of reduced ergosterol biosynthesis due to EO treatment on *Aspergillus flavus* cells. Being lipophilic in nature, the EOs target the plasma membrane and the membranous organelles of the fungal cell by either crossing or accumulating in the cell membrane. This results in interaction with the enzymes and proteins therein, disturb cell permeability by producing a flux of protons towards the cellexterior which ultimately disrupt the fungal cell organization and cause cell death as supported by the transmission electron microscopic results of Nogueira *et al*. [41], Tian *et al*. [21] and Kedia *et al*. [35].These findings are also supported by leakages of Ca^{+2}, K^{+} and Mg^{+2} ions from EO treated cells of *Aspergillus*

flavus [42, 35]. The effects included membrane swelling, change in fluidity and increased passive flux of protons. Helal*et al*. [42] suggested that the release of ions were not based on their size and/or formation of holes in the membrane. However, the EO accumulates in the plasma membrane, causes increased membrane bilayer disorder and ion leakage. These effects cause disturbance in the osmotic balance of the cell, making its membrane associated proteins inefficient leading to inhibition of cell growth. Some of the well-known EO components viz. thymol, carvacrol, eugenol and other phenolic components have been reported to disrupt cell membrane by dissipating H^+ and K^+ ion gradients causing leakage of vital cellular constituents which results in water imbalance, depletion of intracellular ATP concentration and finally cell death [2]. Fig. 1 represents the diagrammatic outline of antifungal mode of action of EO.

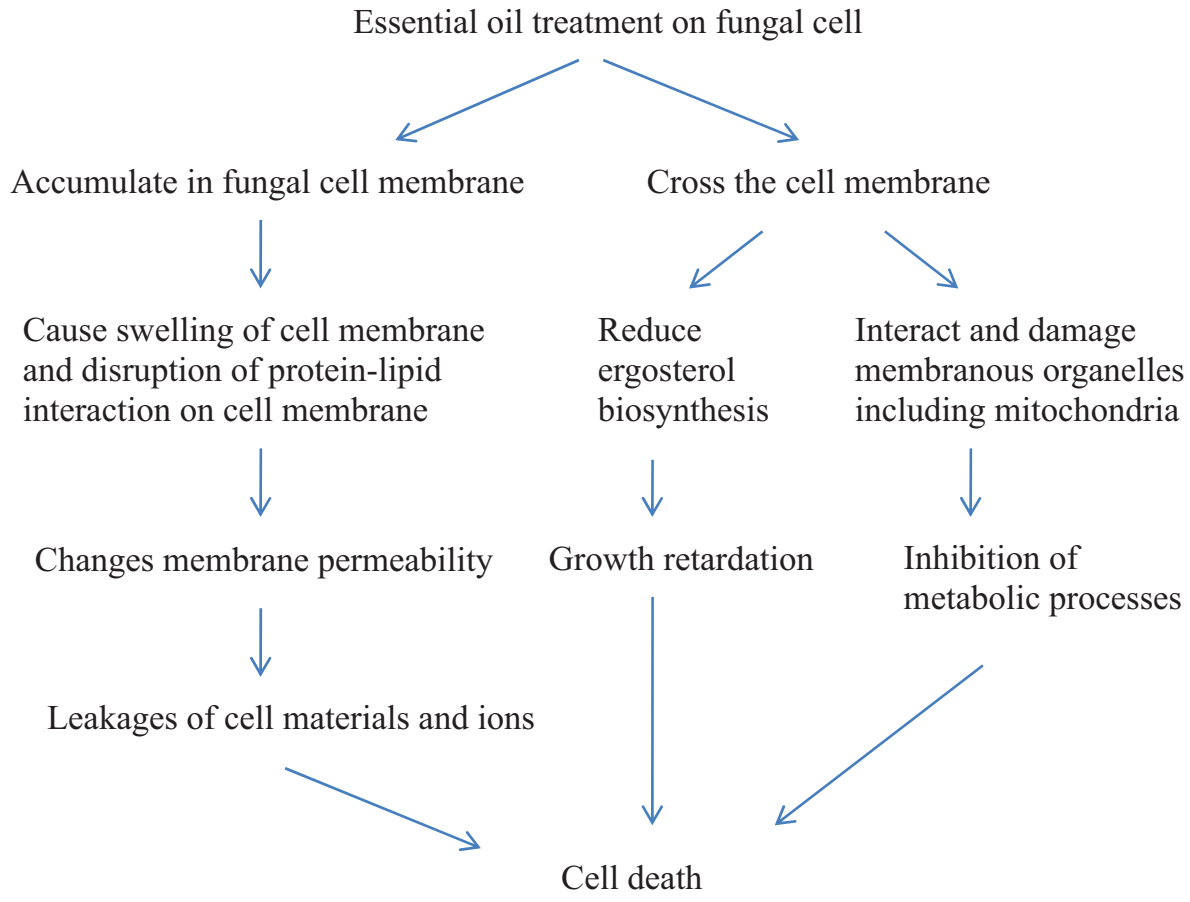

Fig. 1 Diagrammatic representation of antifungal mode of action of essential oil.

4. Potential and Challenges

Based on the results of a number of *in vitro* and *in vivo* studies and their multiple modes of action, EOs showed a great potential for the formulation of plant based green fungicide against wide range of food spoiling moulds. Further, most of the EOs are on the 'Generally Recognised as Safe' (GRAS) list fully approved by the Food and Drug Administration (FDA) and Environment Protection Agency (EPA) in USA, strengthening their applications on food items without any ill effects [43-44]. Some essential-oil-based insecticides, miticides, weedicides and fungicides formulated by different agricultural industries are already commercially available and are gaining popularity among farmers without any side effects. Some of the commercially available EO based fungicide developed for organic farmers include E-Rase™ (*Simmondsiacalifornica* EO), Sporan™ (*Rosemarinusofficianalis*EO), Promax™ (*Thymus vulgaris* EO) etc. [45].Further, EO treated seeds can be used for sowing purpose as the EOs have not shown any ill effect on seed viability [46].

The major drawback of using such products during storage conditions is that a closed system is required for long term storage as EOs are generally volatile and they most likely volatilize relatively quickly. However, Ilboudo *et al*. [47] observed the loss of activity of EO even in the airtight jars suggesting that the loss of activity may also occur due to degradation of the active compounds of the oil. Kim *et al*. [48] suggested that such degradation occur according to the chemical composition as the EO as EO having more hydrogenated compounds are more susceptible to degradation by oxidation. Temperature and light are two other abiotic factors enhancing oxidation process [49]. In the recent times, microencapsulation of EOs is being developed as the alternative formulations by a number of researchers throughout

the globe. The method will solve most of the application problems related to EOs during storage conditions. Firstly, it will reduce the amounts applied i.e. only little amount of EO would be needed. Secondly, it will increase the duration of effectiveness by reducing the volatilization i.e. controlled volatilization would occur at varying storage conditions. Thirdly, it will increase the activity of EOs at same concentration due to increase in surface area. Finally, it will slow down the rate of degradation in the environment as it would be entrapped within an inert material [45].

The variation in the chemical composition of EOs due to age of plant, plant parts, season, method of extraction, ecological and geographical variations also affects the bioactivity of EOs [43]. Hence it is strongly recommended to standardize the plant EOs before its application and commercialization.

5. Conclusion

In spite of a plethora of literature for EOs against storage fungi, only handful of EO formulations is commercially used by farmers and food industries. Most of the EOs are already in use as a flavoring agent in foods, as perfumes (fragrances and aftershave), in pharmaceuticals, aromatherapy etc. from a long time without any side effects. Theeco-chemical nature of EO and its biorational mode of action would be helpful in achieving “green consumerism”. In near future, many EO based formulations can be developed as safe fungicide for eco-friendly efficacious management of post-harvest losses of food items.

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