



Essential oils based formulations as safe preservatives for stored plant masticatories against fungal and mycotoxin contamination: A review

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ABSTRACT

Masticatories are prone to fungal and mycotoxin contamination during postharvest processing. The plant based preservatives may be recommended as safe alternatives of synthetic chemicals for their proper storage to protect them from fungal and mycotoxins contamination. This review presents an overview on mycoflora and mycotoxins associated with plant masticatories and prospectives of essential oils and their nanoencapsulated formulations as safe plant based preservatives.

1. Introduction

Plants are not only used for fulfilling the basic human needs but are also used as masticatories throughout the world. Different parts of plants like fruits, leaves, wood, seeds, nuts, roots, barks etc. have been used as masticatory since long (Borborah et al., 2014). Such plant parts, commonly used as stimulant and mouth freshener; also possess medicinal importance against some diseases. *Piper betle*, *Areca catechu*, *Elettaria cardamomum*, *Terminalia chebula*, *Terminalia bellirica*, *Phyllanthus emblica*, *Nicotiana tabacum* etc. are some commonly used plant masticatories, not only in India but around the world. Rooney (1995) reported some skeletons found in the Duyong cave in Philippines which showing the evidence of betel chewing and was dated to about 3000 BCE. Chinese chronicles of the second century BC have also been reported betel chewing. However, in India, betel quid chewing is at least 2000 years old (Dave et al., 1992). These findings indicate that betel leaf chewing was used by the people since ancient time. In India alone, about 15–20 million people and over 2 billion people around world are betel quid chewers on a regular basis (Jeng et al., 2002). Some tannin-rich masticatories have also been utilized in folk remedies practiced in Asiatic, African, Pacific, and Latin American countries since time immemorial. Masticatory plants are either cultivated or grow as wild. They may be either used singly or in combination with other masticatories.

Because of their high medicinal value and associated health benefits, masticatories have attracted the people world over and thus have always been a high demand commodity among consumers. India holds an important share in the global masticatory market. It holds second

place in the tobacco production & exports to other parts of the world (Garasiya et al., 2018). The Indian betel leaf market has expanded to about Rs 9 000 million per year. Betel leaves worth about Rs 40 million are exported to the other parts of world including USA, UK, Saudi Arabia, Bangladesh, Singapore, and Nepal (Das et al., 2016). In Bangladesh, 30% of adults are betel quid chewers and the rate is higher in rural areas (43%) than urban areas (19%). About 80% betel leaves chewed/used in Bangladesh are exported from India (Tani, 2018).

In the past two decades, consumptions and demand of cardamom (small and large) has increased throughout the world mainly in the Middle Eastern countries, South-East Asian countries, European countries, US, and Japan. India is the prime producer of large cardamom (*Amomum subulatum* Roxb) with 4000 MT of annual productions which has global market value of about Rs. 1.60 billion, followed by Nepal and Bhutan with 2500 MT and 1000 MT of annual production respectively. Moreover, India is the second largest producer of small cardamom (*Elettaria cardamomum* (L.) Maton) after Guatemala (Sengottuvelu, 2011). Small cardamom is one of the most costly spices in the world and commonly known as “Queen of Spices” (Rajathi et al., 2017).

However, there are reports on postharvest losses of the masticatories due to microbial contaminations especially in tropical and subtropical countries. During post harvest storage and transportation, in case of *Piper betle*, 35–70% losses have been reported because of fungal contamination (Guha, 2006). Fungal contaminations may cause deterioration both qualitatively and quantitatively including adverse effects on human health. Since, masticatories hold major share in global economy and have been the part of human daily life, their safe preservation must be in priority.

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Different methods viz. physical, chemical and biological have been developed in order to control toxigenic fungi. Among these, synthetic pesticides (grey chemicals) are the most commonly used from long time. But, continuous use of these synthetic pesticides had many adverse effects on human health, environment as well as on other non target organisms.

Recently, plant based natural pesticides (green chemicals) have gained attention as safer alternatives to synthetic pesticides. These botanical pesticides can be used in different forms viz. plant extract, powder and essential oil. Higher plant essential oils (EOs) are alternatives of other botanical pesticides, which have merits over them as they are volatile in nature. As EOs are complex mixtures of diverse volatile aromatic compounds thus, having wide spectrum of action sites unlike synthetic pesticides (Dima and Dima, 2015). The activity of essential oils as antifungal, antioxidant and antiaflatoxigenic against storage fungi is because of presence of different chemical constituents, mostly acting due to synergistic effect of overall constituents. As such, this review compiles the report of fungal and mycotoxin deterioration of masticatories as well as prospective of essential oils for their safe preservation.

2. Fungal and mycotoxin contamination of masticatories

Currently, the presence of different toxigenic fungi along with their secondary metabolites (as mycotoxins) on food and herbal products are of great concerns worldwide due to their serious effects on health of consumers. Mycotoxins have different acute and chronic effects on consumers like humans and animals. Majority of the mycotoxins reported till date, are potentially carcinogenic, teratogenic, tremorgenic, immunotoxic and nephrotoxic. Fungal contamination in plant materials is very common during storage period. Such microbial contaminations have harmful impacts on plant materials also as they are capable of altering their chemical composition either by transforming chemical profile or by producing toxic compounds. For example, *Melilotus officinalis* (sweet clover) plant is used as herbal medicine for its anticoagulant, antispasmodic and astringent properties and helpful in treatment of wounds, inflammation, swollen joints and stomach ulcers. But microbial contamination causes chemical transformation of its constituents and resulting chemical compound is found to be capable in hemorrhaging (Patwardhan, 2007).

Mycotoxins are highly stable compounds which are normally not destroyed even after cooking. Aflatoxins B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂), ochratoxins (OTA), deoxynivalenol (DON or vomitoxin), zearalenone (ZEA), fumonisin, T-2 toxin and HT-2 toxins, alternariol, beauvericin, bikaverin, fusaric acid, fusarin C, moniliformin and sambutoxin are the most common mycotoxins.

Mycotoxins contamination of foods and feeds greatly depends on environmental conditions that lead to fungal growth and toxins production. Moisture content of raw material is also an important factor in tropical and sub tropical countries. The presences of moisture content in stored plant samples provide adequate ground for the favorable impact of fungal growth (Halt, 1998).

According to International Agency for Research on Cancer (IARC 1993), it has been reported that among all known mycotoxins, AFB₁ is considered as the most toxic and are classified in group 1 carcinogen category for human. AFB₁ is produced by *Aspergillus* species namely *Aspergillus flavus*, *Aspergillus nomius* and *Aspergillus parasiticus*. Further, because of their thermostable and ubiquitous nature, contamination of AFB₁ is considered as an unavoidable food contaminant by US Food and Drug Administration (FDA) (Wagacha and Muthomi, 2008). In humans and animals, Aflatoxin M₁ (AFM₁) is a key metabolite of AFB₁, which may be present in milk from animals fed with AFB₁ contaminated feed. Further, it has been already reported that AFB₁ contamination enhances generation of free radicals and lipid peroxidation which negatively affects stored plant products quality by retarding nutritional value, increased toxicity, off-flavour, and induction of several chronic and

degenerative health ailments (Kiran et al., 2016). Due to potent toxicity of aflatoxins, their permissible limit in food has been decided to 20 µg/kg (Bhat et al., 2010).

Masticatories are used as raw and stored in unscientific manner which accelerates fungal association and ultimately mycotoxins production. These are mostly procured from the forests of tropical and subtropical regions where the environmental conditions particularly temperature and relative humidity are prone to fungal infestation and mycotoxin elaboration. In addition, their moisture content remains high because of the frequent occurrence of unseasonal rains in these regions.

Fungal infestations have been reported from different masticatories which are commonly used by people. Roy and Kumari (1991) reported *Alternaria alternata*, some *Aspergillus* sp. like *A. flavus*, *A. niger*, *A. ochraceus*, *Penicillium citrinum*, *Curvularia* sp. and *Fusarium moniliforme* from *Piper betle* leaf. Chourasia (1995) reported *Alternaria alternata*, *Aspergillus* sp., *Curvularia* sp., *Fusarium* sp. and *Penicillium* sp. from *Ellettaria cardamomum*. Besides Chourasia (1995), Mandeel (2005) and Prakash et al. (2011) also reported *Aspergillus* sp. from *Ellettaria cardamomum*. Singh et al. (2008) reported *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium italicum*, *Curvularia* sp. and *Fusarium* sp. from *Terminalia chebula*. Prakash et al. (2010) reported different species of *Aspergillus*, *Cladosporium* and *Penicillium* from *Areca catechu*. Kedia et al. (2015) reported *Aspergillus* sp. and *Alternaria* sp. from *Nicotiana tabacum*. *Aspergillus* sp. and *Penicillium* sp. have been reported from *Terminalia bellerica* by Kumar and Roy (1993), Gautam et al. (2009), Mishra et al. (2012), Aiko and Mehta (2016). Different *Aspergillus* sps. like *A. flavus*, *A. glaucus*, *A. ochraceus*, *A. niger*, *A. terreus* and other fungi like *Cladosporium herbarum*, *Fusarium* sps. *Penicillium italicum* have been reported from *Glycyrrhiza glabra* by Hitokoto et al. (1978), Roy and Chourasia (1990), Arino et al. (2007), Singh et al. (2008) and Aiko and Mehta (2016). AFB₁ contaminations have been also reported from *Acacia catechu* by Roy et al. (1988). Fungi contaminating the masticatories and the mycotoxins secreted by them is listed in Table 1.

The most studied mycotoxins present in masticatories are aflatoxins. Other mycotoxins viz. ochratoxin A, citrinin, fumonisin and ZEA have also been reported from common plant masticatories.

Roy et al. (1988) reported AFB₁ from *Acacia catechu*, *Glycyrrhiza glabra*, *Terminalia bellerica* and *Terminalia chebula* which were found at the level of 90 µg/kg, 590 µg/kg, 620 µg/kg and 180 µg/kg respectively. Roy and Chourasia (1990) reported 170 µg/kg AFB₁ and 50 µg/kg OTA from *Glycyrrhiza glabra*. Roy and Kumari (1991) reported 20–1000 µg/kg AFB₁ and 10–720 µg/kg citrinin from *Piper betle* leaf. Roy and Kumar (1993) reported 930–1740 µg/kg OTA from *Emblia officinalis* and they also reported 90–1230 µg/kg OTA from *Terminalia chebula*. Kumar and Roy (1993) reported 280–1070 µg/kg AFB₁ and 130–670 µg/kg AFB₂ from *Emblia officinalis* and also 1,430–2190 µg/kg AFB₁, 570–1340 µg/kg AFB₂ and 230–670 µg/kg AFG₁ from *Terminalia chebula*. 7.0 µg/kg ZEA and 1.2 µg/kg total aflatoxin have been reported from *Foeniculum vulgare* by Patel et al. (1996). 216.5 µg/kg and 1.4–252.8 µg/kg OTA have been reported by Bresch et al. (2000) and Arino et al. (2007), respectively from *Glycyrrhiza glabra*. Santos et al. (2009) reported 647 µg/kg fumonisin from *Glycyrrhiza glabra*. Amounts of AFB₁ reported from stored masticatories have been found to be higher than the permissible limit.

3. Prospectives of botanical preservatives in protection of masticatories during storage from fungal and mycotoxin contaminations

Synthetic pesticides and fumigants have been conventionally used as common chemical methods for pest control. Several synthetic fungicides/insecticides have contributed significantly in the management of post harvest losses but the use of synthetic chemicals (grey chemicals) as preservatives of various foodstuffs is not desirable because of

Table 1
Storage fungi and mycotoxins associated with stored masticatories.

S.No.	Masticatories	Contaminating fungi and mycotoxins produced	Investigators
1	<i>Acacia catechu</i>	AFB ₁ contamination	Roy et al. (1988)
2	<i>Areca catechu</i>	The species of <i>Aspergillus</i> , <i>Cladosporium</i> and <i>Penicillium</i> were predominantly found. AFB ₁ produced by <i>Aspergillus</i> spp.	Prakash et al. (2010)
3	<i>Elettaria cardamomum</i>	<i>Alternaria alternata</i> , <i>Aspergillus</i> spp., <i>Curvularia</i> spp., <i>Fusarium</i> spp., <i>Penicillium</i> spp., and other moulds. Aflatoxins also reported.	Chourasia (1995), Mandeel (2005), Prakash et al. (2011)
4	<i>Glycyrrhiza glabra</i>	<i>Aspergillus</i> spp. like <i>A. flavus</i> , <i>A. glaucus</i> , <i>A. ochraceus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>Cladosporium herbarum</i> , <i>Fusarium</i> spp., <i>Penicillium italicum</i> and other moulds	Hitokoto et al. (1978), Roy and Chourasia (1990), Arino et al. (2007), Singh et al. (2008), Aiko and Mehta (2016)
5	<i>Nicotiana tabacum</i>	Species of <i>Aspergillus</i> and <i>Alternaria</i> were found predominantly with AFB ₁ contaminations.	Kedia et al. (2015)
6	<i>Piper betle</i>	<i>Alternaria alternata</i> , <i>Aspergillus</i> spp. like <i>A. flavus</i> , <i>A. niger</i> , <i>A. ochraceus</i> , <i>Penicillium citrinum</i> , <i>Curvularia</i> spp., <i>Fusarium moniliforme</i> , and other moulds have been reported.	Roy and Kumari (1991)
7	<i>Terminalia chebula</i>	<i>Alternaria alternata</i> , <i>Aspergillus</i> spp. like <i>A. flavus</i> , <i>A. niger</i> , <i>A. terreus</i> , <i>Curvularia</i> spp., <i>Fusarium</i> spp., <i>Penicillium italicum</i> have been reported.	Singh et al. (2008)
8	<i>Terminalia bellirica</i>	The species of <i>Aspergillus</i> and <i>Penicillium</i> were predominantly found along with AFB ₁ contaminations.	Kumar and Roy (1993), Gautam et al. (2009), Mishra et al. (2012), Aiko and Mehta (2016)

AFB₁ = Aflatoxin B₁.

the residual toxicities and their non biodegradable nature. They can trigger allergic reactions, risk for leukemia, residual toxicity, hormonal imbalance and other types of cancer. The repeated use of the synthetic pesticides has also led to the development of resistant races of many pests and most of these pesticides are gradually becoming ineffective. In Nigeria, hundreds of people died because of consumption of food commodities treated with unbalanced amount of synthetic pesticides (Bankole and Adebajo, 2003). Thus, there is an urgent need to recommend some safer plant based pesticides (botanical pesticides) as fourth generation pesticides during storage of masticatories.

The plant world comprises a rich store house of different phytochemicals that could be tapped for use as pesticides. Some of these plant based bioactive compounds like azadirachtin (from *Azadirachta indica*), pyrethroids (from *Chrysanthemum cinerariifolium*) and carvone (from *Carum carvi*) are currently being used as botanical pesticides. Plant based pesticides (green chemicals) will be renewal in nature. EOs and other plant based natural products are important source of therapeutics since ancient times (Raut and Karuppaiyil, 2014). Higher plant secondary metabolites such as flavanoids, phenols, tannins, quinones, essential oils, alkaloids, saponins and sterols show variety of biological activities.

EOs are a complex mixture of low molecular weight compounds (usually < 500 Da), containing phenylpropanoids and terpenoids as major constituents. Some formulations of EO which are currently in use for food preservation have been kept in “GRAS” category because of their favorable safety profile. As EOs are volatile in nature, they may also be used as plant-based fumigants during storage of masticatories. Therefore, EOs may play a major role in overcoming problems related to storage losses, quality and shelf-life (Prakash et al., 2015). Due to their high efficacy and volatile nature, they can be used as fumigant. They have been reported to show diverse biological effects, thus, there is less chance of resistance development. Also, they have number of biological activities including, antiviral, anti bacterial, antifungal, antimutagenic, antioxidant, anti inflammatory, immunomodulatory, etc. In addition, they are ephemeral, biodegradable (environmental safety), systemic in nature, indigenous renewable and volatile. Such properties make essential oils highly suitable for their use as plant based pesticides. Many of them have been standardized and commercialized for plant protection. It has been reported that cardamom oil (*Elettaria cardamom*) possesses inhibitory activity against *Aspergillus niger* and *Aspergillus fumigatus* (Bansod et al., 2008).

Recently, different EOs and their bioactive components have been used against pest in the agricultural sector. Food and Drug Administration (FDA) and Environment Protection Agency (EPA) have kept some EOs viz. Cumin, Fennel, Oregano, Thyme, Anethum, Cinnamon, Lemongrass, nutmeg, basil, and their components viz.

carvone, eugenol, cinnamaldehyde, citral, limonene, menthol and linalool, etc. in generally recognized as safe (GRAS) category and are used as preservatives in USA with wide coverage. Some EO based formulations have been commercialized on large scale as safe food additives (Dwivedy et al., 2016a). For example, ‘DMC Base Natural’ which is a mixture of 50% EO from rosemary, sage and citrus and 50% glycerol is being widely used as food preservative. ‘Carvone’, a monoterpene of *Carum carvi* EO, introduced in the Netherlands under the trade name ‘TALENT’, is being commercially used to protect vegetables from microbial rotting. Similarly, ‘EcoPCOR’ based on eugenol has been introduced by EcoSMART technologies for control of crawling and flying insects without non target toxicity. ‘EcoTrol’, another plant based pesticide used for post harvest protection of horticultural fruits is produced from Rosemary EO (Dwivedy et al., 2016b). The antioxidant potential of EOs are also widely studied. Central Food Technological Research Institute (CFTRI) Mysore, India has reported a brown color natural anti-oxidant fraction from *Cinnamomum zeylanicum* fruits to be used as shelf-life enhancer of food items by controlling their spoilage from lipid per oxidation (Jayaprakasha et al., 2008). Moreover, biorational mode of action of EOs potentializes their application in agriculture sector for achieving green consumerism.

Some EOs have been reported to protect stored masticatories from fungal infestation and toxin production during long term storage. Kedia et al. (2015) performed *in situ* experiments for the confirmation of preservative potential of *Foeniculum vulgare* seed EO in stored *Nicotiana tabacum*, and demonstrated absolute protection against AFB₁ for 6 months. Likewise, other EOs viz. *Cinnamomum camphora* EO for *Terminalia chebula* and *Glycyrrhiza glabra* (Singh et al., 2008), *Piper betle* leaf EO for *Areca catechu* (Prakash et al., 2010) and *Ocimum gratissimum* EO for *Elettaria cardamomum* (Prakash et al., 2011) have been reported for their considerable protection against fungal deterioration of raw plant masticatories during storage. The EOs are secondary metabolites of plants which are a complex of several components playing important roles in the defense of the host plant. However, chemotypic variations in the chemical profile of the EOs due to alteration in the ecological and geographical conditions including age of the plant used for extraction of oil and time of harvesting may influence their antimicrobial activity. Hence, it is necessary to standardize the essential oils chemically before recommending as additive (Prakash et al., 2010). A list of EOs which are efficacious against fungi and aflatoxins contaminating masticatories is given in Table 2.

Some EOs and their components such as eugenol, thymol, linalool, carvone, cinnamaldehyde, vanillin, carvacrol, limonene and citral have been accepted for use as additives and flavorings in food system by the European Commission But large scale use in food preservation is sometimes limited because a high concentration is needed for practical

Table 2
Plant products efficacy against fungi and aflatoxins contaminating masticatories during storage.

S.No.	Masticatory plants	Botanical preservatives used	Plant part	Efficacy against <i>A. flavus</i> growth & AFB ₁ production	Investigators
1	<i>Terminalia chebula</i>	<i>Cinnamomum camphora</i> EO	Leaf	MIC = 1.0 µL/mL MAIC = 0.75 µL/mL	Singh et al. (2008)
2	<i>Glycyrrhiza glabra</i>	<i>Cinnamomum camphora</i> EO	Leaf	MIC = 1.0 µL/mL MAIC = 0.75 µL/mL	Singh et al. (2008)
3	<i>Areca catechu</i>	<i>Piper betle</i> EO	Leaf	MIC = (0.3–0.73 µL/mL) MAIC = 0.1 µL/mL	Prakash et al. (2010)
4	<i>Elettaria cardamomum</i>	<i>Ocimum gratissimum</i> EO	Leaf	MIC = 0.7 µL/mL MAIC = 0.6 µL/mL	Prakash et al. (2010)
5	<i>Terminalia bellirica</i>	<i>Caesulia axillaris</i> EO	Aerial part	MIC = 1.0 µL/mL MAIC = 0.8 µL/mL	Mishra et al. (2012)
6	<i>Nicotiana tabacum</i>	<i>Foeniculum vulgare</i> EO	Seed	MIC = 1.25 µL/mL MAIC = 1.0 µL/mL	Kedia et al. (2015)

EO = Essential oil, MIC = Minimum inhibitory concentration, MAIC = Minimum antiaflatoxic concentration.

application (Hyldgaard et al., 2012). Since, EOs are complex mixture of liquid volatile aromatic compounds (Sultanbawa, 2015), they get evaporated easily from the system. Thus, recently, in order to boost the efficacy of EOs, different kinds of carriers are being utilized as delivery agents for the encapsulation of EOs. The encapsulation of EOs into materials or nano-carriers not only helps in controlled release (Gouin, 2004), but also enhances their efficacy. So far, different nanoencapsulated EOs have been used as food preservatives (Burt, 2004).

4. Conclusions

From the current study it can be concluded that, plant raw masticatories are prone to contamination by different toxigenic fungi and mycotoxins during postharvest processing. Further, the plant based preservatives i.e. EOs and their nanoencapsulated formulations may be recommended as safer alternatives of synthetic chemicals for their proper storage and protection from fungal and mycotoxin contaminations.

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