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# Insecticidal and fungicidal efficacy of essential oils and nanoencapsulation approaches for the development of next generation ecofriendly green preservatives for management of stored food commodities: an overview

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## ABSTRACT

To date, several synthetic preservatives have been used for management of food losses caused by insect and fungal pathogens, however, development of resistant races and adverse effects on environment have limited their applicability. Currently, essential oils and their bioactive components are well acknowledged for control of insects. Insecticidal and antifungal efficacy of essential oils are reported to result from neurotoxic mechanisms like alteration in octopamine receptors,  $\gamma$ -aminobutyric acid (GABA) ion channels, inhibition of acetylcholine esterase (AChE) activities in insects and dysfunctioning of mitochondria as well as plasma membrane in fungal pathogens. Nanoencapsulation of essential oils is currently under practice to prevent environmental degradation, enhance insecticidal and fungicidal efficacy with wide commercial applications and recommend them for possible industrial utilization. The present review explores i) insecticidal and fungicidal efficacy of essential oils and bioactive components, ii) their possible mode of action, iii) the employment of modern nanoencapsulation approaches for the development of nontoxic and ecofriendly green insecticides/fungicides, and most importantly iv) patenting of developed plant based nanoformulation with desired application in management of stored products biodeterioration.

## KEYWORDS

Essential oil; insecticidal efficacy; nanoencapsulation; lipid peroxidation; acetylcholine esterase activity; neurotoxicity

## 1. Introduction

Agricultural commodities in storage conditions are major substrates for different insects and fungal pathogens (Ogendo et al. 2008). These storage insects cause qualitative and quantitative losses of agricultural commodities and the proportion of losses may depend on the seasonal variation, toxigenicity, sporulation, harvested periods and different processing parameters. In most of the under developed countries farmers generally store harvested commodities in the cow-dung ash and wooden cribs in which the commodities are easily infested by different insects such as *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae), *Callosobruchus*

*chinensis* L. (Coleoptera: Bruchidae), *Oryzaephilus suramensis* L. (Coleoptera: Silvanidae) and a number of saprophytic fungi such as *Aspergillus flavus* Link. (Eurotiales: Trichocomaceae), *Alternaria alternata* (Fries) Keissler (Hyphomycetes: Dematiaceae), *Penicillium italicum* Wehmer (Eurotiales: Trichocomaceae), *Cladosporium herbarum* (Pers.) Link. Ex S. F. Gray (Capnodiales: Davidiellaceae), *Curvularia lunata* (Wakker) Boed. (Pleosporales: Pleosporaceae) and *Fusarium oxysporum* Schltdl. (Hypocreales: Nectriaceae) (Abbasipour et al. 2011; Rajkumar et al. 2019; Das et al. 2019a). In addition to fungal pathogens, variety of mycotoxins viz. aflatoxins, trichothecenes, fumonisins, ochratoxins, zearalenone, deoxynivalenol, and patulin have been associated with food biodeterioration making more gravity to severe spoilage (Kalagatur et al. 2020). Different synthetic insecticides and fungicides such as phosphine, methyl bromide, organophosphate, pyrethroids, and carbamates have been utilized for management of fungal contamination and insect

infestation in the stored food commodities (Shaaya and Rafaeli 2007). The primary targets of these fungicides and insecticides are on the membrane ergosterol, cell wall porosity for fungal contaminants (Tian et al. 2012) and inhibitory action on acetylcholine esterase (neurotransmitter modulator), octopamine, cholinergic receptors, and  $\gamma$ -amino butyric acid (GABA) gated ion channels and oxidative impairment (Upadhyay et al. 2018a) for insects. However, excessive utilization of these synthetic insecticides and fungicides leads to the development of resistant insect and fungal races, damage to environmental sustainability, residual toxicity, toxicity to nontarget organisms and severe health disorders (Kumar 2012). Hence, researchers are currently focused towards insecticides/fungicides of natural origin offering an efficient biorational approach of plant based bioactive products for inhibition of insect infestation, fungal association, and mycotoxin contamination. Among varied pool of plant secondary metabolites, essential oils of aromatic plant species are gaining cumulative interest based on their varied phytochemical constituents and largely sound to the consumers due to their rapid volatility, efficient biodegradability, and effectiveness as fumigant, fungitoxicant, oviposition deterrant, antifeedant, and repellent activities (Jaya et al. 2014). Moreover, essential oils being complex mixture of different terpenoid, phenolic, and glycosidic components actively participate in inhibition of resistant races of insects and fungi after long term utilization for practical purposes. The essential oils and their bioactive components have been listed in Generally Recognized as Safe (GRAS) products (Dima and Dima 2015). Different mono- and sesquiterpenoids are common volatile ingredient of essential oil which inhibit the acetylcholine esterase activity, GABA gated ion channels and octopamine receptors leading to neuromuscular toxicity in insects (Cao et al. 2019). Essential oils and bioactive components have been reported to inhibit the cytotoxic intermediate product methylglyoxal which indirectly reduce the aflatoxin secretion in the stored food commodities (Das et al. 2020a, 2021a). In spite of several merits of essential oils, their practical applicability limits its direct application in the industrial scale due to volatile nature and most of the active components are oxidized and degraded in the presence of air. Therefore, efficient strategy to deploy the insecticidal and fungicidal efficacy of essential oil involves modern nanotechnological approach with great promise in targeted delivery system in order to manage the losses of food commodities under postharvest conditions. Nanotechnology include the encapsulation of essential oil within any carrier system eventually

developing into very small particles which critically protect themselves from variety of undesirable environmental effects and provides an efficient way for the development of nanoinsecticides/nanofungicides with controlled release properties (Rajkumar et al. 2020a).

In our opinion, a number of reports have been published focusing on insecticidal efficacy of essential oils, however, paper concerning the effective mechanism of action for two important groups of stored product organisms *viz.* insects and fungi, safety assessment for commercial exploitation and modern nanoencapsulation technology have not been fully clarified. Despite different shortcomings, on the basis of several toxicological studies we have presented simplified and better cooperations of essential oils and their nanoformulations as environmentally safe insecticides and fungicides with botanical prospects. Hence, the purpose of the review is to present an updated account of essential oils and bioactive components for their potential efficacy against infestation of insects and fungal pathogens in stored food commodities with special emphasis to their biochemical mechanism of actions. Moreover, the paper focuses on modern nanoencapsulation technologies with enhancement in insecticidal and fungicidal efficacy of essential oils and new insight for development of novel nontoxic, safe, and eco-friendly preservative demonstrating their practical application in food and agricultural industries.

## 2. Major insects and fungal pathogens contaminating food commodities in postharvest conditions

Different insects and fungal pathogens have been found to be associated with stored food commodities due to variable nutrients such as sugar contents, minerals, lipids, and proteins (Hashem et al. 2018). Insect infestation in stored food commodities act as vector for several food contaminating diseases and ultimately causes adverse impact on food quality and its esthetic values. Mostly, cereal grains, pulses, oil seeds, nuts, and spices are common source of carbohydrates, proteins, and fatty acids in the tropical regions of the world. Major insects *viz.* *Rhyzopertha dominica*, *Sitophilus zeamais*, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), *Sitophilus oryzae*, *Sitophilus granarius* L. (Coleoptera: Curculionidae), *Prostephanus truncatus* Horn. (Coleoptera: Bostrichidae), *Tribolium castaneum*, *Tribolium confusum*, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Lasioderma serricornis* Fabricius (Coleoptera: Anobiidae), and

*Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae), and *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) cause significant deterioration of cereal grains, pulses, and oil seeds in storage condition by forming holes, rupturing the outer seed coat resulting into loss of grain weight (Kim et al. 2003; Jayasekara et al. 2005; Tapondjou et al. 2005; Sharifian et al. 2013). In tropical regions, ambient temperature, and chemical constituents of the food products provide a suitable platform for buildup of different grain insects. The postharvest insects are characterized by primary and secondary feeding insects based on their pattern of infestation and feeding property in germ or cotyledons of seed (Throne et al. 2003). Different species of legume seeds or fruits such as *Cicer arietinum* L., *Glycine max* L., *Phaseolus vulgaris* L., *Vigna aconitifolia* (Jacq.) Maréchal, *V. radiata* (L.) Wilczek, *V. mungo* (L.) Hepper, *Cajanus cajan* (L.) Millspaugh, and *Lens culinaris* L. (Fabales: Fabaceae) are significantly damaged by *Callosobruchus maculatus* and *C. chinensis*, the most destructive pathogens of pulses in South East Asia and Africa (Maharjan et al. 2019). Gujar and Yadav (1978) reported the damage of chickpea grains by *Callosobruchus chinensis* causing 57–69% loss in weight and >65% loss in cellular protein. Chickpea (*Cicer arietinum* L.) and maize (*Zea mays* L.) (Poales: Poaceae) in the storage conditions are associated with *Tribolium castaneum*, causing maximum loss in weight as well as cause major changes in essential fatty acids and provide susceptibility towards the qualitative and quantitative deterioration (Kedia et al. 2014; Mehmood et al. 2018). Significant deterioration in terms of weight loss (~ 18.4%) in stored wheat and bean by infestation of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) has been reported by Padin et al. (2002). *Rhyzopertha dominica*, the lesser grain borer infested most of the storage grain or grain products leading to fragmentation of grain and produced powdery residues with characteristic pungent odor (Toews et al. 2006). Bushra and Aslam (2014) considered *Sitotroga cerealella* as top listed cereal grain destructive insect (>10–20% loss) by feeding the inner grain contents leading to ultimate deterioration of germ. Athanassiou et al. (2016) demonstrated *Trogoderma granarium* as major infesting insect for wheat, triticale, oats, barley, rye, and other millets causing 5–25% of overall loss because the diapausing period of the larvae can last for 4 years utilizing most of the nutritional contents of kernels and survive in wide environmental conditions. In the tropical countries, *Tribolium confusum* and *Sitophilus granarius* are common weevil insects of cereal grains and higher infestation of

these insects causes generation of heat resulting into proliferation of fungal growth and sporulation (Ziaee et al. 2014). *Lasioderma serricornis*, the cigarette beetle effectively damage the stored food products such as cereal grains, cocoa bean, spices, oil seeds, dry fruits and pulses due to suitability of the stored commodities as oviposition site and compatible for larval growth (Hori et al. 2011). Osipitan et al. (2011) described the infestation of *Prostephanus truncatus* causing 40–70% loss in stored maize and cassava roots by reducing the nutritional contents basically the amino acids (tryptophan and lysine) and carbohydrate content. *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) has been considered as primary insect for rice and reported to be imported with stored rice grains from Vietnam to Czech Republic. Another species viz. *Liposcelis corrodens* Heymons (Psocoptera: Liposcelididae) has been documented to be exported from European Union to China with seed grains (Stejskal et al. 2015). Kučerová (2012) reported effective deterioration of nutritional content in wheat grain after *Liposcelis bostrychophila* infestation leading to significant loss in grain weight. Kiran and Prakash (2015a) reported huge loss of barley, wheat, rye, and oats in world wide agricultural market due to postharvest infestation of two foremost coleopteran insects viz. *Sitophilus oryzae* and *Rhyzopertha dominica*. Riudavets et al. (2018) reported *Sitophilus zeamais* as an important deteriorating insect of stored rice and maize causing excessive damage (2–5%) to outer membrane of grain facilitating infestation of toxigenic fungi leading to loss in grain weight. Atta et al. (2020) reported damaging potential reached upto 10–30% by increasing the growth rate of *Tribolium castaneum*, alteration in feeding behavior and weight loss of postharvested wheat grains within 6–12 months. Table 1 represents major storage insects infesting stored foods.

In addition to storage insects, a number of fungal pathogens viz. *Aspergillus flavus*, *Aspergillus niger* van Tieghem (Eurotiales: Trichocomaceae), *Aspergillus pseudotamarii* Yoko Ito, S.W. Peterson, Wicklow & T. Goto (Eurotiales: Trichocomaceae), *Aspergillus candidus* Link. (Eurotiales: Trichocomaceae), *Aspergillus repens* (Corda) Sacc. (Eurotiales: Trichocomaceae), *Aspergillus luchuensis* Inui (Eurotiales: Trichocomaceae), *Alternaria alternata*, *Curvularia lunata*, *Cladosporium herbarum*, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (Capnodiales: Cladosporiaceae), *Fusarium oxysporum* and *Mycelia sterilia* (Deuteromycetes) have been noted to be associated with different stored food commodities leading to considerable alteration

**Table 1.** Major storage insects infesting stored foods.

Food commodity	Botanical name	Infestation of storage insects	References
Rice	<i>Oryza sativa</i> L.	<i>Sitophilus granarius</i> L.	Sharaby (1988)
		<i>Sitophilus oryzae</i> L.	Carvalho et al. (2012) Chayengia et al. (2010) Edde (2012)
Wheat	<i>Triticum aestivum</i> L.	<i>Rhyzopertha dominica</i> Fabricius	Togola et al. (2010)
		<i>Sitotroga cerealella</i> Olivier	Boon and Ho (1988)
		<i>Tribolium castaneum</i> Herbst	Gałęcki et al. (2019)
		<i>Tribolium confusum</i> du Val	Trematerra et al. (2000) Edde (2012)
Maize	<i>Zea mays</i> L.	<i>Rhyzopertha dominica</i> Fabricius	Obeng-Ofori (1995) Mishra et al. (2019)
		<i>Sitotroga cerealella</i> Olivier	Togola et al. (2010)
		<i>Acanthoscelides obtectus</i> Say	Padin et al. (2002)
		<i>Sitophilus oryzae</i> L.	Mehta and Kumar (2021) Khanal et al. (2021)
Legumes	<i>Cajanus cajan</i> (L.) Millsp. <i>Vigna subterranean</i> (L.) Verdc. <i>Vigna unguiculata</i> (L.) Walp. <i>Cicer arietinum</i> L. <i>Vigna radiata</i> (L.) R.Wilczek <i>Vigna angularis</i> (Willd.) Ohwi and Ohashi <i>Cicer arietinum</i> L. <i>Phaseolus vulgaris</i> L.	<i>Sitophilus zeamais</i> Motschulsky	Noudegbessi et al. (1970) Moreno-Martinez et al. (2000) Chinaru Nwosu et al. (2015)
		<i>Sitotroga cerealella</i> Olivier	Togola et al. (2010)
		<i>Tribolium castaneum</i> Herbst	Li and Arbogast (1991)
		<i>Callosobruchus maculatus</i> Fabricius	Ekeh et al. (2013) Iturralde-García et al. (2016) Hamdi et al. (2017)
		<i>Callosobruchus chinensis</i> L.	Maharjan et al. (2019) Banga et al. (2019)
		<i>Acanthoscelides obtectus</i> Say	Gad et al. (2021)
Tobacco	<i>Nicotiana tabacum</i> L.	<i>Lasioderma serricornis</i> Fabricius	Gatehouse et al. (1989) Rumbos et al. (2018)
Millet	<i>Sorghum</i> spp. <i>Pennisetum glaucum</i> L.R.Br.	<i>Sitophilus oryzae</i> L.	Bhargude et al. (2021)
		<i>Rhyzopertha dominica</i> Fabricius	Edde (2012) Mishra et al. (2019)
		<i>Sitotroga cerealella</i> Olivier	Togola et al. (2010)

in chemical constituents and overall esthetic value (Kumari et al. 2019; Das et al. 2019a). Excessive fungal infestation leads to secretion of mycotoxins, hazardous secondary metabolites causing ill effects on human health. Several reports have been found showing effective influence of insect infestation on excessive mycotoxin contamination in different food grains. Gorman and Kang (1991) reported positive correlation between damage of maize grains by insects and level of aflatoxin in different storage conditions. In India, Sinha and Sinha (1992) suggested severe aflatoxin contamination of maize grains due to *Sitophilus oryzae* and *Tribolium castaneum* infestation in postharvest conditions. During feeding, insects efficiently break the outer pericarp of the food grain and expose the surface for excessive mycelial proliferation and sporulation. Moreover, the metabolic activity of insects causes increment in relative humidity, moisture content and pH, providing suitable conditions for *Aspergillus flavus* infestation and mycotoxin production (Aristil et al. 2020). Therefore, both the fungal pathogens and insects management at an extensive scale is a major requirement to control losses of storage food commodities with regular periodicity. Table 2 presents the major fungal species contaminating stored foods.

### 3. Overview of current postharvest management strategies

Different physical and chemical management practices have been employed for control of insects infestation as well as fungal and mycotoxin contamination. Radiation treatment, atmospheric packaging, refrigeration, and heat therapy are common physical management strategies for controlling fungal pathogens and insects infestation (Fields and White 2002; Gonçalves et al. 2019). However, these physical methods require technological experts, therefore quite expensive for practical applications. Synthetic preservatives such as chloropicrin, phosphine, methyl bromide, organophosphate, and benzimidazoles have great contribution towards inhibition of insects and fungi, however, the undesirable side effects of these chemicals on human health, environmental sustainability and induction of resistance against insects and fungal species restrict their utilization in commercial market (Shukla et al. 2013). Moreover, the synthetic chemicals induce mycotoxin production and lipid peroxidation in different stored food commodities (Singh et al. 2019). Hence, utilization of plant products is gaining cumulative attention for the formulation of eco-friendly and safer alternative of synthetic insecticides and fungicides. Botanical

**Table 2.** Major fungal species contaminating stored foods.

Food commodity	Botanical name	Infestation of storage fungi	References
Rice	<i>Oryza sativa</i> L.	<i>Aspergillus flavus</i> Link. and <i>Aspergillus niger</i> van Tieghem	Paranagama et al. (2003)
		<i>Aspergillus flavus</i> Link., <i>Alternaria alternata</i> (Fries) Keissler, <i>Aspergillus fumigatus</i> Fresenius and <i>Aspergillus luchuensis</i> Inui	Das et al. (2020b)
Maize	<i>Zea mays</i> L.	<i>Aspergillus flavus</i> Link., <i>Aspergillus candidus</i> Link., <i>Aspergillus fumigatus</i> Fresenius, <i>Penicillium islandicum</i> Sopp, <i>Penicillium fellutanum</i> Biourge	Mannaa and Kim (2018)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus parasiticus</i> Speare, <i>Aspergillus ochraceus</i> Wilhelm and <i>Aspergillus niger</i> van Tieghem	Reddy et al. (2008)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus candidus</i> Link., <i>Penicillium citrinum</i> Thom and <i>Penicillium verrucosum</i> Dierckx	Park et al. (2005)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus niger</i> van Tieghem, <i>Aspergillus ochraceus</i> Wilhelm	Dadzie et al. (2019)
Capsicum pepper	<i>Capsicum</i> spp.	<i>Aspergillus flavus</i> Link., <i>Fusarium verticilloides</i> (Sacc.) Nirenberg, <i>Fusarium graminearum</i> Schwabe and <i>Fusarium proliferatum</i> (Matsush.) Nirenberg	Chulze (2010)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus penicillioides</i> Speg, <i>Aspergillus wentii</i> Wehmer, <i>Fusarium verticilloides</i> (Sacc.) Nirenberg and <i>Rhizopus stolonifer</i> Vuillemin	Kaaya and Kyamuhangire (2006)
		<i>Fusarium verticilloides</i> (Sacc.) Nirenberg	Tran et al. (2021)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus niger</i> van Tieghem	Bauchet et al. (2021)
Legumes	<i>Lens culinaris</i> Medik.	<i>Penicillium spp.</i> and <i>Alternaria spp.</i>	Chaudhari et al. (2020a)
		<i>Rhizopus stolonifer</i> Vuillemin and <i>Alternaria alternata</i> (Fries) Keissler	Costa et al. (2019)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus niger</i> van Tieghem and <i>Rhizoctonia solani</i> Kühn	Shukla et al. (2009)
Oil seeds	<i>Vigna mungo</i> (L.) Hepper	<i>Aspergillus niger</i> van Tieghem and <i>Fusarium nivale</i> Ces. ex Berl. & Voglino	
		<i>Aspergillus versicolor</i> (Vuill.) Tirab. and <i>Aspergillus flavus</i> Link.	Upadhyay et al. (2018b)
		<i>Penicillium citrinum</i> Thom and <i>Aspergillus repens</i> (Corda) Sacc.	
Spices	<i>Sesamum indicum</i> L.	<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries and <i>Alternaria tenuis</i> Nees	
		<i>Aspergillus niger</i> van Tieghem, <i>Aspergillus fumigatus</i> Fresenius, <i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries and <i>Alternaria alternata</i> (Fries) Keissler	Prakash et al. (2010)
	<i>Brassica campestris</i> L.	<i>Aspergillus niger</i> van Tieghem, <i>Mycelia sterilia</i> , <i>Curvularia lunata</i> (Wakker) Boed. and <i>Aspergillus fumigatus</i> Fresenius	
		<i>Penicillium italicum</i> Wehmer, <i>Aspergillus candidus</i> Link., <i>Aspergillus niger</i> van Tieghem, <i>Aspergillus fumigatus</i> Fresenius and <i>Aspergillus sydowii</i> (Bainier & Sartory) Thom and Church	
	<i>Arachys hypogea</i> L.	<i>Aspergillus flavus</i> Link., <i>Aspergillus sydowii</i> (Bainier & Sartory) Thom and Church, <i>Alternaria alternata</i> (Fries) Keissler and <i>Aspergillus niger</i> van Tieghem	
		<i>Aspergillus flavus</i> Link., <i>Aspergillus niger</i> van Tieghem, <i>Aspergillus candidus</i> Link. and <i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	
Masticatories	<i>Prunus amygdalus</i> (Mill.) D. A. Webb	<i>Aspergillus glaucus</i> (L.) Link., <i>Aspergillus niger</i> van Tieghem, <i>Aspergillus terreus</i> Thom, <i>Cladosporium herbarum</i> (Pers.) Link. Ex S. F. Gray and <i>Penicillium italicum</i> Wehmer	Singh et al. (2019)
		<i>Aspergillus spp.</i> and <i>Penicillium spp.</i>	Aiko and Mehta (2016)
	<i>Glycyrrhiza glabra</i> L.	<i>Fusarium spp.</i> , <i>Alternaria spp.</i> , <i>Curvularia spp.</i> and <i>Fusarium spp.</i>	Prakash et al. (2011)
		<i>Aspergillus spp.</i> and <i>Aspergillus spp.</i>	Kedia et al. (2015)
Coffee bean	<i>Nicotiana tabacum</i> L.	<i>Aspergillus brasiliensis</i> Varga, Frisvad & Samson, <i>A. sclerotioniger</i> Samson & Frisvad and <i>A. uvarum</i> G. Perrone, Varga & Kozak.	Perrone et al. (2007)
		<i>Aspergillus spp.</i> and <i>Fusarium spp.</i>	
Millets	<i>Sorghum bicolor</i> (L.) Moench	<i>Aspergillus spp.</i> , <i>Penicillium spp.</i> , <i>Fusarium spp.</i> and <i>Rhizopus spp.</i>	Taye et al. (2018)
		<i>Aspergillus flavus</i> Link., <i>Aspergillus niger</i> van Tieghem, <i>Aspergillus terreus</i> Thom, <i>Cheatomium spirale</i> Zopf. and <i>Penicillium purpurogenum</i> Stoll	Taye et al. (2016)
	<i>Pennisetum glaucum</i> (L.) Morrone		Kumar et al. (2018)

(Continued)

**Table 2.** (Continued)

Food commodity	Botanical name	Infestation of storage fungi	References
Dry fruits	<i>Juglans regia</i> L.	<i>Aspergillus flavus</i> Link., <i>Aspergillus repens</i> (Corda) Sacc., <i>Aspergillus minutus</i> E.V. Abbott and <i>Aspergillus luchuensis</i> Inui	Dwivedy et al. (2017)
	<i>Phoenix dactylifera</i> L.	<i>Aspergillus flavus</i> Link., <i>Alternaria humicola</i> Oudem., and <i>Aspergillus sulphureus</i> Desm.	
	<i>Ficus carica</i> L.	<i>Alternaria humicola</i> Oudem. and <i>Aspergillus luchuensis</i> Inui	
	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai	<i>Aspergillus flavus</i> Link. and <i>Mucor</i> spp.	
	<i>Buchanania lanzan</i> Spreng.	<i>Aspergillus niger</i> van Tieghem and <i>Aspergillus flavus</i> Link.	

preservatives have been recognized as a sustainable measure in place of synthetic ones because of less residual toxicities, multi target mechanism of action and most notably biodegradable nature (Pang et al. 2020).

#### 4. Essential oils and bioactive components: a green approach for controlling insects and fungal pathogens

Among the varied pools of plant bioactive metabolites, essential oils from aromatic plant species act as a hub for complex phytochemical constituents, bioactive components and are one of the best known volatile substances for control of different storage fungi and insects. Essential oils and their bioactive components have been included under the Generally Recognized as Safe (GRAS) category and act as promising fumigant, repellent, antifeedant, oviposition deterrent and feeding deterrent against a number of storage insects (Chaudhari et al. 2021; Prakash et al. 2021). Essential oils, commonly isolated from different aromatic plant parts *viz.* seeds, fruits, leaves, flowers, rhizomes, bulbs through steam/hydro distillation possess a lot of advantages *viz.* easy availability, significant biodegradability, negligible residual toxicity, broad scale mammalian safety as well as long term utilization in food and pharmaceutical industries as herbal medicines, beverages, and ayurvedic formulations (Tang et al. 2018). In addition to essential oils, different bioactive components of essential oils such as carvone, limonene, apiol, geranyl acetate, linalyl acetate, 1,8 cineol, cuminaldehyde, geraniol, neral, phenyl ethyl alcohol,  $\alpha$ -pinene,  $\alpha$ -terpenol,  $\beta$ -pinene, myristicine, elemicine, linalool, cinnamaldehyde, eugenol, carvacrol, methyl cinnamate, methyl eugenol and fenchone have also been tested for efficient inhibition of insects infestation as well as fungal and mycotoxin secretion in stored food commodities (Prakash et al. 2015; Sun et al. 2016; Das et al. 2019b). The bioactive components of essential oils with potent insecticidal efficacy have been grouped into different

categories such as terpenoids, sulfur compounds (allyl sulphides), cyanates, alkaloids, and phenolics. As essential oils are composed of very complex mixture of variety of components (about 20–60) with different concentrations; among them components with high concentrations (20–70%) have been recognized as major constituents and may represent maximum bioefficacy of essential oils. Most importantly, the bioefficacy of essential oils is also dependent on chemical structures of components having different functional groups and catalytic sides. The structure-activity relationship of the components is mainly determined by the functional groups of essential oils, type of toxicity, target insects, and fungal pathogens. Components containing phenolic hydroxyl groups in their chemical structures such as carvacrol and thymol are reported to show maximum insecticidal activity (Seo et al. 2009; Pavela 2011). Moreover, the hydroxyl groups in benzene rings critically affect the antifungal and insecticidal activities (Lee et al. 2008; Park et al. 2008). Enan (2005) reported that the insecticidal activity of phenyl propanoid is determined by presence as well as location of hydroxy and spacing groups (methoxy) in benzene rings. Regnault-Roger et al. (2012) and Park et al. (2016) reported that the orthoposition of hydroxy groups in methylsalicylate exhibited better insecticidal activity with ability to disrupt the octopaminergic and GABA systems in insects. Lee et al. (2008) reported the role of double bonds in propenyl groups of phenyl propanoid in effective antifungal activities. Badawy et al. (2010) reported that the monoterpene components have the property to donate one hydrogen bond (geraniol, cuminaldehyde, linalool, thymol, and menthol), thus, are more toxic to spider mite as compared to the components without any hydrogen bond donating capacity. Similar observations based on the hydrogen bond donating capacity of different oxygenated monoterpenes *viz.* linalool, methone, isosafrol, eugenol, and 1,8 cineol for efficient insecticidal activity against *Sitophilus oryzae* has been demonstrated by Lee et al. (2001). Antifungal effectivity of essential oil components

with basic chemical structures follow the general order of phenols > alcohol > aldehydes > ketone > esters > hydrocarbons. High oxygenated sesquiterpene components in *Tagetes riojana* M. Ferraro essential oil showed prominent antifungal activity with steric, electrostatic, and lipophilic effects in cell membrane. Reactive peroxide functional groups in bioactive components such as ascaridol extracted from *Chenopodium ambrosioides* L. essential oil have potential ability to destroy plasma membrane and inhibit conidial germination (Li et al. 2019). Carvacrol and thymol have been recognized as most effective phenolic compound for inhibition of wide range of microorganisms based on the molecular structure having nonpolar part that may develop passage for membrane interaction and hydroxyl groups with delocalized electrons conferring the acidic character to the compounds facilitating greater interactions (Pizzolitto et al. 2015). Dambolena et al. (2012) reported the antifungal activity of essential oil components viz. thymol, cresol, carvacrol, eugenol, and isoeugenol based on the hydrophobic properties and lipophilicity that induce changes in physico-chemical properties in plasma membrane, cell wall, and major cellular organelles. Moreover, synergism, additive, and complementary factors are prominent characteristics of essential oils and their major and minor components for inhibition of different insects and fungal pathogens against qualitative and quantitative biodeterioration of different stored food products. Yang et al. (2010) demonstrated insecticidal activity of *Allium sativum* L. essential oil and the major components diallyl trisulfide and methyl allyl disulfide against *Sitophilus zeamais* and *Tribolium castaneum* in stored rice samples. Akami et al. (2019) reported induced toxicity in *C. maculatus* through fumigation of *Lippia adoensis* Hochst essential oil leading to modulation in cytochrome P<sub>450</sub> based monooxygenase and glutathione-S-transferase genes. The essential oil of *Perovskia abrotanoides* Kar. exhibited significant fumigant toxicity and morbidity effect (32 µL/L air caused 100% mortality) on *Sitophilus oryzae* and *Tribolium castaneum* at LC<sub>50</sub> doses (Arabi et al. 2008). Upadhyay et al. (2019) reported the effect of *Melissa officinalis* L. essential oil fumigation in *Tribolium castaneum* leading to modulation in antioxidative defense enzyme systems such as glutathione, superoxide dismutase, catalase, and reactive oxygen species and increased fumigant toxicity, oviposition deterrent and larvicidal activities.

In addition, essential oils have also been documented to inhibit fungal infestation and mycotoxin secretion in storage conditions. Nguefack et al. (2004) investigated fungitoxic and mycotoxin inhibitory efficacy of five different essential oils such as

*Thymus vulgaris* L. (Lamiales: Lamiaceae), *Zingiber officinalis* Roscoe (Zingiberales: Zingiberaceae), *Monodora myristica* (Gaertn.) Dunal (Magnoliales: Annonaceae), *Cymbopogon citratus* (DC.) Stapf. (Poales: Poaceae) and *Ocimum gratissimum* L. (Lamiales: Lamiaceae) against *Aspergillus flavus*, *Aspergillus fumigatus* Fresenius (Eurotiales: Trichocomaceae) and *Fusarium moniliformae* J. Sheld. (Hypocreales: Nectriaceae). Zabka et al. (2014) tested antifungal efficacy of 20 different essential oils extracted from medicinal plants against toxigenic fungi such as *Penicillium expansum* Link. (Eurotiales: Trichocomaceae), *Penicillium brevicompactum* Dierckx (Eurotiales: Trichocomaceae) *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium verticilloides* (Sacc.) Nirenberg (Hypocreales: Nectriaceae) and *Fusarium oxysporum* having high dose acute toxicity. Tian et al. (2012) described the antifungal and aflatoxin inhibitory efficacy of *Anethum graveolens* L. (Apiales: Apiaceae) essential oil against *Aspergillus flavus* through dose dependent reduction in ergosterol and mitochondrial dehydrogenase activities. Ferreira et al. (2018) reported the inhibition of *Fusarium graminearum* Schwabe (Hypocreales: Nectriaceae) infestation and deoxynivalenol contamination in maize grains by *Zingiber officinale* essential oil. Inhibitory efficacy of *Curcuma longa* L. (Zingiberales: Zingiberaceae), *Cinnamomum zeylanicum* Blume (Laurales: Lauraceae) *Ocimum basilicum* L. (Lamiales: Lamiaceae), *Cymbopogon martini* (Roxb.) Wats (Poales: Poaceae) and *Zingiber officinale* against infestation of *Penicillium verrucosum* Dierckx (Eurotiales: Trichocomaceae) and *Aspergillus ochraceus* Wilhelm (Eurotiales: Trichocomaceae) and ochratoxin A contamination in maize grains has been recently reported (Kalagatur et al. 2020).

## 5. Insecticidal activity of essential oils and bioactive components

Essential oils and their bioactive components exhibit multi-target mechanism of toxicity for different storage insects. Several literatures have reported insecticidal actions of essential oils via fumigant toxicity, repellent toxicity, antifeedant activity, oviposition deterrent nature and ovicidal activity and have reported significant toxicity against different insects causing stored food biodeterioration. Moreover, the neurotoxic activity through inhibition of acetyl choline esterase enzymatic activity, blocking of octopamine receptors and impairment in different oxidative enzymes such as superoxide dismutase, catalase, and glutathione peroxidase may also lead to mortality of insects by application of different essential oils and their bioactive ingredients (Kiran et al. 2017;



Nattudurai et al. 2017). However, in case of fungal pathogens, essential oils mainly act on cell wall causing dissolution, disintegration of membrane integrity through ergosterol inhibition and alteration in membrane permeability through leakage of vital cellular ions and eventually culminating into dissolution of osmotic homeostasis of cell (Tian et al. 2018; Ma et al. 2019).

### 5.1. Fumigant toxicity

Strong volatile nature of essential oil components such as terpenes, sesquiterpenes and phenolics offers the fumigant efficacy against different insects. Current research on essential oils and their active ingredients have exhibited fumigant toxicity of several essential oils leading to cumulative interest in the industrial markets for preparation of plant based insecticidal formulation. Lee et al. (2004a) described fumigant toxicity of six different essential oils viz. *Eucalyptus blakelyi* Maiden (Myrtales: Myrtaceae), *Eucalyptus nicholii* Maiden & Blakely (Myrtales: Myrtaceae), *Eucalyptus codonocarpa* Blakely & McKie (Myrtales: Myrtaceae), *Callistemon sieberi* DC. (Myrtales: Myrtaceae), *Melaleuca fulgens* R.Br. (Myrtales: Myrtaceae) and *Melaleuca armillaris* (Sol. ex Gaertn.) Sm. (Myrtales: Myrtaceae) against *Rhyzopertha dominica* and *Tribolium castaneum* in terms of lethality expressed as LD<sub>50</sub> and LD<sub>95</sub>. The fumigant natures of essential oils indicate mere absorption of active constituents on the surface of the stored grain and are usually accepted for food flavoring. Fumigant toxicity of eight different essential oil components viz. eugenol, 1,8 cineol, (-) methone, (-)-β-pinene, (-)-limonene, (+)-α-pinene, linalool and carvacrol at different concentrations (0.25–60 μL/L) have been tested against major insect weevil, *Callosobruchus maculatus*. Among different components, fumigant toxicity of carvacrol, eugenol, and 1,8 cineol exhibited 100% mortality at the adult stage, while least toxicity was reported for α- and β-pinene suggesting the negative correlation of vapor pressure and toxicity of bioactive components for potential insect mortality (Ajayi et al. 2014). Sahaf et al. (2008) suggested increased susceptibility to fumigant toxicity shown by *Vitex pseudo-negundo* (Haukskn.) Hand.-Mzt. (Lamiales: Lamiaceae) essential oil against eggs, larvae and adults of *Callosobruchus maculatus* at LC<sub>50</sub> values 2.20, 8.42, 9.39 recorded for concentrations 1.01, 2.50, 0.91 μL/L air, respectively during 24h treatments. Suthisut et al. (2011) investigated fumigant toxicity of essential oils isolated from *Zingiber zerumbet* (L.) Smith (Zingiberales: Zingiberaceae), *Alpinia conchigera* Griff. (Zingiberales: Zingiberaceae) and *Curcuma*

*zedoaria* (Christm.) Roscoe (Zingiberales: Zingiberaceae) and major bioactive components viz. terpene 4-ol, α-humuline, β-pinene, camphene, isborneol and camphor against *Tribolium castaneum* and *Sitophilus zeamais* after 14, 24, and 24h of exposure duration and exhibited better activity of *Alpinia conchigera* essential oil towards the adults of *Tribolium castaneum* (LC<sub>50</sub> = 73 μL/L) and *Sitophilus oryzae* (LC<sub>50</sub> = 85 μL/L) than eggs, larva, and pupa stages. Papachristos and Stamopoulos (2004) tested the fumigant toxicity of *Eucalyptus globulus* Labill. (Myrtales: Myrtaceae), *Lavandula hybrida* Reverchon (Lamiales: Lamiaceae) and *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) essential oil against eggs of *Acanthoscelides obtectus* and determined LC<sub>50</sub> values in between 1.30–35.0 μL/L air at different egg ages and observed subsequent larval mortality. Pourya et al. (2018) reported fumigant toxicity of *Pistacia atlantica* Desf. (Sapindales: Anacardiaceae) and *Pistacia khinjuk* Stocks (Sapindales: Anacardiaceae) essential oil against *Callosobruchus maculatus* and displayed strong mortality effect after 24h of treatment (LC<sub>50</sub> = 22 μL/L air). In an another study, Rajkumar et al. (2019) suggested 100% mortality of *Sitophilus oryzae* and *Tribolium castaneum* at 75 and 100 μL/L air of *Mentha piperita* L. essential oil after 24h of exposure. Significant fumigant toxicity of pinene rich *Haplophyllum dauricum* (L.) G.Don (Rutales: Rutaceae) essential oil has been explained by active contact of major components as promising fumigant (LC<sub>50</sub> of 12.09 mg/L) against *Tribolium castaneum* and *Lasioderma serricorne* (Cao et al. 2019).

### 5.2. Repellent toxicity

Repellent activity of essential oils and bioactive components against different storage insects depend on chemical profile, synergism, additive effects and insect susceptibility. Tapondjou et al. (2005) demonstrated the average repellent effects of *Eucalyptus saligna* Sm. (Myrtales: Myrtaceae), *Cupressus sempervirens* L. (Pinales: Cupressaceae) essential oils and cymol against *Sitophilus zeamais* and *Tribolium confusum* and categorized into different classes of repellency as II (32 ± 8%), III (55 ± 11%), IV (73 ± 15%) and V (90–100%) based on the % mean repellency values (%PR). In a filter paper arena test, Wang et al. (2006) reported the higher repellent behavior of *Artemisia vulgaris* L. essential oil against *T. castaneum* at 0.6 μL/mL. Liu et al. (2006) performed the repellent toxicity assay of *Cinnamomum camphora* (L.) J.Presl. (Laurales: Lauraceae) and *Artemisia princeps* Pamp. (Asterales: Asteraceae) essential oils against *Bruchus rugimanus* L. (Coleoptera: Bruchinae) and *Sitophilus oryzae* and

exhibited significant repellency of these essential oils, while much better efficacy was observed for 1:1 mixture of these essential oils at a varying concentration of 250–1000 µg/g. Jemâa et al. (2012) performed repellent bioassay of *Laurus nobilis* L. (Laurales: Lauraceae) essential oil against *Rhyzopertha dominica* and *Tribolium castaneum* and described the results by median repellent dose (RD<sub>50</sub>). They exhibited RD<sub>50</sub> value of essential oil against *Rhyzopertha dominica* and *Tribolium castaneum* as 0.013, 0.036, 0.033 and 0.045, 0.139, 0.096 µL/cm<sup>2</sup>, respectively during 24 h of exposure. Liu et al. (2014) tested the repellent activity of *Kaempferia galanga* L. (Zingiberales: Zingiberaceae) essential oil and its major components viz. trans-cinnamaldehyde, ethyl cinnamate, 1,8-cineol, dimethyl phthalate and ethyl p-methoxy cinnamate against *Liposcelis bostrychophila* and dimethyl phthalate with potent repellent activity at 0.8, 1.6 and 3.6 µL/cm<sup>2</sup> after 2 and 4 h of exposure. Abdel-Sattar et al. (2010) evaluated the repellent toxicity of *Schinus molle* L. (Sapindales: Anacardiaceae) essential oil extracted from leaves and fruits against *Trogoderma granarium* and *Tribolium castaneum* showing 84.94 and 85.11% toxicity against *Trogoderma granarium* and *Tribolium castaneum*, at 1000 µL dose of essential oil extracted from leaf and fruit, respectively. Chen et al. (2019) demonstrated the repellent activity of *Alpinia katsumadai* Hayata (Zingiberales: Zingiberaceae) seed essential oil through area preference method against *Liposcelis bostrychophila*, *Tribolium castaneum* and *Lasioderma serricornis* and showed percent repellent activity (%PR) higher than 90% at 3.15, 15.73, and 78.63 µL/cm<sup>2</sup> after 2 to 4 h of exposure. Repellent nature of *Zanthoxylum planispinum* L. var. *dintanesis* (Sapindales: Rutaceae) essential oil extracted from leaves and fruit pericarp against adult insects of *Lasioderma serricornis* and *Lasioderma bostrychophila* exhibited potential toxicity at 15.73 and 12.63 µL/cm<sup>2</sup>. Moreover, the oxygenated monoterpenes of the *Zanthoxylum planispinum* L. (Sapindales: Rutaceae) essential oil such as linalool, 2-dodecanone, and terpinen-4-ol exhibited greater repellency than the synthetic insecticide DEET at 4 h post-exposure (Wang et al. 2019). Toxicity of *Ferula asa-foetida* L. and *Ferula gummosa* Boiss. (Apiales: Apiaceae) essential oils against invasive stored product insect *Prostephanus truncatus* and *Trogoderma granarium* in stored maize upto 6.7–40 and 81.1–85.6% after 7 days of exposure has been recently demonstrated by Pavela et al. (2020). Positive allosteric modulation of chloride gated GABA and acetyl choline esterase activity rendered by conjugated double bonds of monoterpenoids determine the higher repellent

action of essential oils and their bioactive components against common storage insects.

### 5.3. Oviposition deterrent and ovicidal activity

Essential oils are active inhibitors of egg deposition and maturity in different insect present in stored food commodities. Varieties of bioactive components participate in alteration of fecundity, egg laying, and adult emergence. It causes premature death of the insects without the production of F<sub>1</sub> adult generation. Fumigation of stored insects with essential oil hampers the synthesis of vitellogenin, active glycolipoprotein precursor for egg yolk development. Moreover, the fumigation also affects the hatchability of eggs by changing permeability of egg vitelline membrane. Abbasipour et al. (2011) reported the oviposition deterrence activity of *Elettaria cardamomum* (L.) Maton essential oil against *Callosobruchus maculatus* at concentrations 57.12 and 64.26 µL/mL within 48 h of treatments. Shukla et al. (2016) reported dose dependent increase in efficacy expressed as percent oviposition deterrence in *Callosobruchus maculatus* by applying *Acorus calamus* L. (Acorales: Acoraceae) essential oil. In fumigated sets (0.1 µL/mL of *Callistemon lanceolatus* (Sm.) Sweet essential oil) of stored legumes, 12 eggs of *Callosobruchus maculatus* were reported as compared to 302 eggs in the non fumigated sets, proving *Callistemon lanceolatus* (Sm.) Sweet essential oil effectiveness for food preservation against storage insects. Toxicity and repellent activity of *Mentha piperita* L. essential oil and its major components viz. menthol and L-menthone by increasing oviposition deterrent and ovicidal activity against three important storage insects viz. *Tribolium castaneum*, *Liposcelis bostrychophila* and *Lasioderma serricornis* in stored food commodities has been recently investigated (Pang et al. 2020). Matos et al. (2020) demonstrated the insecticidal efficacy of *Eugenia caryophyllata* Thunb. (Myrtales: Myrtaceae) and *Illicium verum* Hook.f. (Austrobaileyales: Schisandraceae) essential oils by greater efficiency in oviposition inhibition at 20 µL/20g and 40 µL/20g in *Callosobruchus maculatus*.

### 5.4. Pupicidal and larvicidal activity

Storage insects complete their reproductive cycles and their development from LI-LIV stage of larva and pupa generation generally occurs inside the food commodities. Treatment of storage commodities with essential oils and their bioactive components check the embryonic maturity and egg lying capacity of different insects during storage periods. Shukla et al. (2011) demonstrated 21.87, 22.81, and 50.93%

inhibition of *Callosobruchus chinensis* larvae at 16, 10, and 6 days interval, respectively with *Lippia alba* (Mill.) N.E. Br. ex Britton & P. Wilson (Lamiales: Verbenaceae) essential oil. Kiran et al. (2017) observed LI/LII (64.34–71.33%), LIII/LIV (55.03–55.60%) larvicidal activity and pupicidal activity (31.13–42.63%) at LC<sub>50</sub> doses of *Boswellia carterii* essential oil against *Callosobruchus maculatus* and *Callosobruchus chinensis*. Shukla et al. (2016) suggested the significant efficacy of *Acorus calamus* L. essential oil and  $\beta$ -asarone against LI/LII, LIII/LIV *Callosobruchus chinensis* larval development and found 75–33.43, 79.06–38.43, and 56.25–38.43%. The essential oil also exhibited 35.31 and 38.43% pupicidal activity at 0.05 and 0.1  $\mu$ L/mL concentrations, respectively. Sahaf and Moharrampour (2008) performed the larvicidal and pupicidal toxicity of *Carum copticum* L. and *Vitex pseudo-negundo* essential oil at LC<sub>50</sub> doses 2.50 and 8.42  $\mu$ L/mL, respectively against the neonate larvae of *Callosobruchus maculatus*. Recent investigation of Ammar et al. (2020) suggested toxicity of *Pulicaria arabica* (L.) Cass. (Asterales: Asteraceae), *Saccocalyx satureioides* Cosson & Durieu (Lamiales: Lamiaceae) and *Artemisia campestris* L. (Asterales: Asteraceae) essential oils against 3rd instar larvae of *Spodoptera littoralis* Boisduval with LD<sub>50</sub> value 61.2, 68.9, and > 200  $\mu$ g/larva.

The types of insecticidal toxicity and mechanism of action with respect to different essential oils and their bioactive components are represented in Table 3.

## 6. Insecticidal mechanism of action of essential oils and their components

### 6.1. Neurotoxic modulation of essential oils and components

Several reports have been published on considerable fumigant, repellent, antifeedant, pupicidal, larvicidal and ovicidal activity of essential oils, however, very fragmentary reports are available dealing with the neurotoxic action of essential oils on storage insects. Acetylcholinesterase (AChE) is one of the crucial enzymes responsible for neurotransmission through catalysis of cholinergic synaptic impulses. Monoterpenoid components of essential oils act as competitive inhibitor of AChE and modulate the enzyme kinetics through binding with serine hydroxyl group at the active site of the enzyme. Abdelgaleil et al. (2009) reported strong inhibition of AChE enzyme in *Sitophilus oryzae* and *Tribolium castaneum* by L-fenchone, 1,8-cineol, cuminaldehyde and limonene. In a primary inhibitory assay of

AChE, Kim et al. (2013) demonstrated  $\alpha$ -pinene (97.36%),  $\beta$ -pinene (54.96%) and limonene (51.23%) as significant inhibitor of AChE at 1 mg/mL. Nattudurai et al. (2017) reported significant effect of *Atalantia monophylla* (L.) Corr. Serr. (Sapindales: Rutaceae) essential oil on reduced biosynthesis of AChE enzyme upto 10.96–45.21% in the *C. maculatus* at the LC<sub>10</sub> and LC<sub>30</sub> doses. Moreover, the *Atalantia monophylla* essential oil exhibited better efficacy in AChE inhibition for *Sitophilus oryzae* at LC<sub>10</sub> and LC<sub>20</sub> doses. It has also been demonstrated that the mixture of monoterpenoids viz. linalyl acetate, limonene and linalool for better inhibition of AChE than single application of bergamot essential oil. Chaubey (2017) reported active inhibition of AChE activity in adult *Sitophilus zeamais* upto 40–80 and 31.59–66.9% after 24 h of treatment at LC<sub>50</sub> doses of *Cuminum cyminum* L. (Apiales: Apiaceae) and *Piper nigrum* L. (Piperales: Piperaceae) essential oils, respectively. In addition to AChE, octopamine, a circulating neurohormone and neurotransmitter has significant neuromodulatory effects in insects regulated through octopamine-1 and octopamine-2. Interruption in functional activity of octopamine breaks down the transfer of neuronal impulses. Therefore, octopaminergic site of action of essential oils and bioactive components has been represented as a biorational approach for control of storage insects. Kostyukovsky et al. (2002) demonstrated the modulation in octopamine receptor based on intracellular cAMP stimulation by fumigation with (+)-limonene as a possible target site of action for controlling *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) a model insect.  $\gamma$ -aminobutyric acid is a secondary target site for different insecticide by recognizing the picrotoxinin site with noncompetitive inhibition of ionotropic GABA gated chloride channels. Several reports on insecticidal activity of essential oils by modulating the GABA receptors have been published. Park et al. (2016) suggested the role of phenolic hydroxyl groups of essential oils and bioactive components such as *Gaultheria fragrantissima* Wall. (Ericales: Ericaceae), *Illicium verum* Hook.f. and methyl salicylate in blocking GABA receptors of *Callosobruchus chinensis*. Bloomquist et al. (2008) demonstrated the inhibition kinetics of chloride ion uptake through GABA channel at varying concentrations of >100  $\mu$ M silphinenes (tricyclic sesquiterpene). Pajaro-Castro et al. (2017) described the superior insecticidal activity of bioactive components of essential oils such as linalool and  $\beta$ -pinene against *Tribolium castaneum* infestation via interfering the neuromodulator of GABA gated ions channels. Thymol is reported to

**Table 3.** Some essential oils and bioactive components for inhibition of insects with type and mechanism of toxicity.

Type/mechanism of toxicity	Targeted insects	Essential oils/components applied	References	
Fumigant toxicity	<i>Sitophilus zeamais</i> Motschulsky	<i>Mosla chinensis</i> Maxim. <i>Carum curvi</i> L.	Lu et al. (2020) Fang et al. (2010)	
	<i>Sitophilus granarius</i> L.	<i>Cymbopogon citratus</i> (DC.) Stapf <i>Acorus calamus</i> L.	Plata-Rueda et al. (2020) El-Nahal et al. (1989)	
	<i>Sitophilus oryzae</i> L.	<i>Melaleuca fulgens</i> R.Br. and <i>Eucalyptus blakelyi</i> Maiden <i>Artemisia sieberi</i> Besser and <i>A. tridentata</i> Nutt. <i>Artemisia scoparia</i> Waldst. & Kit. and <i>A. sieberi</i> Besser <i>Thymus persicus</i> Ronniger ex Rech. f. <i>Mentha microphylla</i> K.Koch <i>Perovskia abrotanoides</i> Kar. <i>Acorus calamus</i> L.	Negahban et al. (2007) Negahban et al. (2006, 2007) Saroukolai et al. (2010) Mohamed and Abdelgaleil (2008) Arabi et al. (2008) El-Nahal et al. (1989)	
	<i>Callosobruchus maculatus</i> Fabricius	<i>Artemisia scoparia</i> Waldst. & Kit. and <i>A. sieberi</i> Besser <i>Artemisia sieberi</i> Besser and <i>A. tridentata</i> Nutt.	Negahban et al. (2006, 2007) Negahban et al. (2007)	
	<i>Callosobruchus chinensis</i> L.	<i>Cuminum cyminum</i> L. and <i>Anethum graveolens</i> L. <i>Acorus calamus</i> L.	Chaubey (2008) El-Nahal et al. (1989)	
	<i>Tribolium castaneum</i> Herbst	<i>Artemisia scoparia</i> Waldst. & Kit. and <i>A. sieberi</i> Besser <i>Chenopodium ambrosoides</i> L., <i>Cinnamomum camphora</i> (L.) J.Presl. and <i>Ocimum basilicum</i> L. <i>Allium sativum</i> L.	Negahban et al. (2006, 2007) Nenaah and Ibrahim (2011) Yang et al. (2010)	
	<i>Tribolium confusum</i> du Val	<i>Artemisia sieberi</i> L. and <i>A. tridentata</i> Nutt. <i>Artemisia vulgaris</i> L.	Negahban et al. (2007) Wang et al. (2006)	
	<i>Acanthoscelides obtectus</i> Say	<i>Acorus calamus</i> L. <i>Rosmarinus officinalis</i> L., <i>Lavendula hybrida</i> L. and <i>Eucalyptus globulus</i> Labill.	El-Nahal et al. (1989) Papachristos and Stamopoulos (2004)	
	<i>Trogoderma granarium</i> Everts	<i>Chenopodium ambrosoides</i> L., <i>Cinnamomum camphora</i> (L.) J.Presl. and <i>Ocimum basilicum</i> L.	Nenaah and Ibrahim (2011)	
	Repellent toxicity	<i>Lycoriella ingénue</i> Dufour	<i>Schizonepeta tenuifolia</i> Briq.	Park et al. (2006)
		<i>Sitophilus oryzae</i> L.	<i>Artemisia scoparia</i> Waldst. & Kit. and <i>A. sieberi</i> Besser <i>Cinnamomum camphora</i> (L.) J.Presl. and <i>Artemisia princeps</i> Pamp.	Negahban et al. (2006, 2007) Liu et al. (2006)
		<i>Sitophilus granarius</i> L.	Geranyl acetate and citral <i>Cymbopogon citratus</i> (DC.) Stapf <i>Hyptis spicigera</i> Lam. and <i>H. suaveolens</i> (L.) Poit.	Plata-Rueda et al. (2020) Plata-Rueda et al. (2020) Conti et al. (2011)
		<i>Sitophilus zeamais</i> Motschulsky	<i>Eucalyptus saligna</i> Sm. and <i>Cupressus sempervirens</i> L.	Tapondjou et al. (2005)
		<i>Tribolium castaneum</i> Herbst	<i>Laurus nobilis</i> L. <i>Artemisia scoparia</i> Waldst. & Kit. and <i>Artemisia sieberi</i> Besser	Jemâa et al. (2012) Negahban et al. (2006, 2007)
		<i>Tribolium confusum</i> du Val	<i>Eucalyptus saligna</i> Sm. and <i>Cupressus sempervirens</i> L.	Tapondjou et al. (2005)
		<i>Callosobruchus maculatus</i> Fabricius	<i>Artemisia scoparia</i> Waldst. & Kit. and <i>A. sieberi</i> Besser	Negahban et al. (2006, 2007)
		<i>Bruchus rugimanus</i> Bohem	<i>Cinnamomum camphora</i> (L.) J.Presl. and <i>Artemisia princeps</i> Pamp.	Liu et al. (2006)
		<i>Rhyzopertha dominica</i> Fabricius	<i>Laurus nobilis</i> L.	Jemâa et al. (2012)
		<i>Lasioderma serricorne</i> Fabricius	<i>Zanthoxylum planispinum</i> var. <i>dintanesis</i> Siebold & Zucc.	Wang et al. (2019)
<i>Lasioderma bostrychophila</i> Badonnel				
Contact toxicity		<i>Tribolium castaneum</i> Herbst	<i>Lavendula angustifolia</i> Mill., <i>Allium cepa</i> L., <i>Carum carvi</i> L. and <i>Cyperus fuscus</i> L. Eugenol, $\alpha$ -terpeniol, carvacrol, eugenol, thymol, 1,8-cineol, trans-anethole, verbenone and fenchone	Gharsan et al. (2018) Kanda et al. (2017)
		<i>Sitophilus oryzae</i> L.	Allylisothyanate, eugenol, carvacrol and ethyl formate	Cardiet et al. (2012)
	<i>Tribolium confusum</i> du Val	<i>Chrysanthemum</i> spp.	Haouas et al. (2012)	
	<i>Sitophilus zeamais</i> Motschulsky	<i>Eucalyptus saligna</i> Sm. and <i>Cupressus sempervirens</i> L.	Tapondjou et al. (2005)	
	<i>Callosobruchus maculatus</i> Fabricius	<i>Citrus sinensis</i> (L.) Osbeck	Oboh et al. (2017)	

(Continued)

**Table 3.** (Continued)

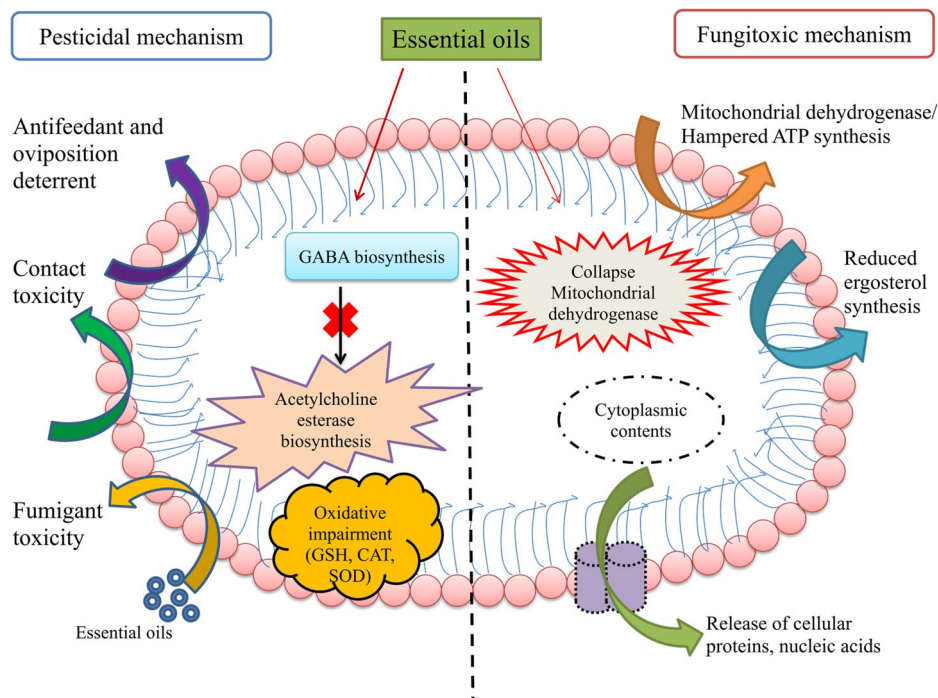
Type/mechanism of toxicity	Targeted insects	Essential oils/components applied	References
Antifeedant activity	<i>Tribolium castaneum</i> Herbst	<i>Eucalyptus globulus</i> Labill. and <i>Lavandula stoechas</i> L. Cinnamaldehyde	Ebadollahi (2011) Huang and Ho (1998)
	<i>Tribolium confusum</i> du Val	<i>Callistemon viminalis</i> (Sol. ex Gaertn.) Byrnes and <i>Eucalyptus camaldulensis</i> Dehnh. S.G.Harrison and <i>Illicium verum</i> Hook.f.	Hamzavi and Moharrampour (2017)
Oviposition deterrent and ovicidal activity	<i>Callosobruchus maculatus</i> Fabricius	<i>Acorus calamus</i> L. <i>Elettaria cardamom</i> (L.) Maton <i>Eugenia caryophyllus</i> (Spreng.) Bullock & S.G.Harrison and <i>Illicium verum</i> Hook.f.	Shukla et al. (2016) Abbasipour et al. (2011) Matos et al. (2020)
	Larvicidal and pupicidal activity	<i>Callosobruchus chinensis</i> L.	<i>Anethum sowa</i> Roxb. ex Fleming <i>Lippia alba</i> (Mill.) N.E. Br. ex Britton & P. Wilson <i>Acorus calamus</i> L. <i>Mentha spicata</i> L.
<i>Callosobruchus maculatus</i> Fabricius		<i>Carum copticum</i> L. and <i>Vitex pseudo-negundo</i> (Hauuskn.) Hand.-Mzt.	Sahaf and Moharrampour (2008)
<i>Tribolium confusum</i> du Val <i>Oryzaephilus surinamensis</i> L.		<i>Azadirachta indica</i> A. Juss and cannabidiol	Mantzoukas et al. (2020)
<b>Mechanism of toxicity</b>			
Imbalance in acetylcholine esterase and glutathione-S-transferase activity	<i>Sitophilus oryzae</i> L.	<i>Atalantia monophylla</i> (L.) Corr. Serr. <i>Rosmarinus officinalis</i> L.	Nattudurai et al. (2017) Kiran and Prakash (2015b)
	<i>Oryzaephilus surinamensis</i> L. <i>Callosobruchus maculatus</i> Fabricius	<i>Rosmarinus officinalis</i> L. <i>Atalantia monophylla</i> (L.) Corr. Serr.	Kiran and Prakash (2015b) Nattudurai et al. (2017)
	Impairment in enzymatic (SOD, CAT) and nonenzymatic antioxidative defense	<i>Callosobruchus chinensis</i> L. <i>Callosobruchus maculatus</i> Fabricius	<i>Boswellia carterii</i> Bird.
<i>Sitophilus zeamais</i> Motschulsky <i>Sitophilus oryzae</i> L. <i>Tribolium confusum</i> du Val		Allylisoithiocyanate <i>Mentha piperita</i> L. <i>Carum carvi</i> L.	Wu et al. (2014) Rajkumar et al. (2019) Petrović et al. (2019)
Cytochrome P-450 dependent monooxygenase inhibitor	<i>Callosobruchus maculatus</i> Fabricius	<i>Petroselinum sativum</i> Hoffm.	Massango et al. (2017)

potentially inhibit GABA<sub>A</sub> receptors by competitively binding at 3H[EBOB] site of the GABA gated chloride channels (Priestley et al. 2003). Casida and Tomizawa (2008) performed an experiment dealing with site directed mutagenesis of insect GABA receptor by ligand docking simulation and exhibited alteration in pores and channels of the chloride receptors leading to alteration in feeding deterrence activity of insect suggesting a novel method for development of insecticidal formulation.

### 6.2. Insecticidal efficacy through impairment in cellular enzymatic and nonenzymatic antioxidative defense system

Different cellular antioxidative enzymes viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPS) and nonenzymatic biomolecules (ratio of oxidized and reduced glutathione) catalyze different physiological processes in insect growth and development. Any type of impairment in oxidative enzymes elucidates the biochemical mechanism underlying the insect mortality as well as changes in feeding behavior. Kiran and Prakash (2015a) described profound stimulatory effect on SOD activity and decrease in CAT activity in

*Rhyzopertha dominica* and *Sitophilus oryzae* after fumigation with *Gaultheria procumbens* L. (Ericaceae) essential oil at LC<sub>50</sub> doses. Both, CAT and SOD are marked as first line of defensive enzymes modulated in insects. Rajkumar et al. (2019) conducted an experiment on the effect of *Mentha piperita* L. essential oil as well as major components menthone and menthol on *Tribolium castaneum* and *Sitophilus oryzae* revealing increased production of SOD and decrement in CAT activity at the LC<sub>50</sub> doses. Moreover, the ratio of cellular oxidized and reduced glutathione was decreased upon fumigation with *Mentha piperita* L. essential oil to mitigate the oxidative stress. Cytotoxicity and modulation in mitochondrial dehydrogenase activity by intracellular enzyme systems viz. lactate dehydrogenase and phenol oxidase in *Trogoderma granarium* by fumigation of *Cinnamomum camphora* (L.) J.Presl. and *Cymbopogon citratus* (DC.) Stapf essential oil and their synergistic combinations has been recently reported as prime insecticidal mechanism of action (Feroz 2020). Oni et al. (2019) reported dose dependent increment of SOD and CAT in *Callosobruchus maculatus* by application of *Acalypha wilkesiana* Müll.Arg. (Malpighiales: Euphorbiaceae) essential oil, however, the



**Figure 1.** Insecticidal and fungitoxic mechanism of action of essential oils and bioactive components.

glutathione peroxidase activity was decreased with respect to higher doses of essential oil. Alteration in common antioxidative enzymes suggests the toxic effects of *Acalypha wilkeshiana* essential oil on enzyme kinetics through generation of reactive oxygen species. Upadhyay et al. (2019) showed *Melissa officinalis* L. (Lamiaceae) essential oil induced oxidative stress in *Tribolium castaneum* causing dose dependent increment in endogenous ROS production. Moreover, at  $LC_{20}$  and  $LC_{50}$  doses, SOD and CAT activity was increased to 6.88, 22.96 and 22.03, 38.67%, respectively. Kiran and Prakash (2015b) observed time and concentration dependent variation in SOD, CAT, and GSH in *Oryzaephilus surinamensis* and *Sitophilus oryzae* after fumigation with *Rosmarinus officinalis* essential oil as compared to control set. SOD activity in *Sitophilus oryzae* elevated up to 118.88% at 0.15  $\mu\text{L}/\text{mL}$ , while in *Oryzaephilus surinamensis* the increment reached upto 78.46% after 6 h of exposure. CAT (87.84–54.55%) and GSH (30.06–24.76%) activity was also observed to be reduced after 6 and 9 h of treatment by *Rosmarinus officinalis* essential oil. SOD and CAT antioxidant system act as first line of defense in insect oxidative stress while glutathione are identified as nonenzymatic second line of defense system. Therefore, modulation in the antioxidative enzymes changes the biochemical and physiological behavior of insects with the resultant of insect mortality. The neurotoxic as well as enzymatic and nonenzymatic antioxidative impairment for insecticidal efficacy of essential oils and bioactive components is represented in Figure 1.

## 7. Antifungal and antimycotoxigenic activity of essential oils and their components

Many literature about antifungal efficacy of essential oil have been published but the exact mechanism of fungitoxic efficacy has not been clearly demonstrated. Most of the workers have described the antifungal efficacy of essential oil due to its cytotoxic nature on fungal cell wall and plasma membrane by disintegration of osmotic homeostasis of cell and efflux of several small ions or molecules (Figure 1).

### 7.1. Antifungal mechanism of action

#### 7.1.1. Effect on cell wall and plasma membrane

A number of studies have been conducted on effect of essential oil on cell wall and plasma membrane of food contaminating fungi enumerating the possible target site of action. Suppression of fungal growth may also be associated with shrinkage of plasma membrane, deformation in hyphal wall, cytoplasmic coagulation and lomasome formation. Tolouee et al. (2010) described the antifungal effect of *Matricaria chamomilla* L. (Asterales: Asteraceae) essential oil against food contaminating *Aspergillus flavus* at highest doses (1000  $\mu\text{g}/\text{mL}$ ) due to disorganization of plasma membrane and cell wall and depletion of cytoplasmic constituents. Tian et al. (2012) observed the effect of *Anethum graveolens* L. (Apiaceae) essential oil on aflatoxigenic strain of *Aspergillus flavus* and exhibited noticeable decrement in ergosterol production and spore germination, suggesting plasma membrane as potential site of action of essential oil. Yamamoto-Ribeiro et al.

(2013) noticed an interesting finding for correlation of *Zingiber officinale* Roscoe essential oil induced membrane ergosterol depletion in *Fusarium verticillioides* and inhibition of fumonisin B<sub>1</sub> secretion suggesting impairment in cell wall and membrane integrity leading to leakage of fatty acids, polysaccharides and phospholipids, the active constituents for fumonisin biosynthesis. Imbalance in permeability of plasma membrane and leakage of vital intracellular metal ions with notable increment in electrical conductivity by fumigation of Tea tree essential oils and its major components  $\alpha$ -terpeniol and terpene-4-ol suggested the antifungal effectiveness (against *Aspergillus niger*) due to quick loss of membrane integrity with prominent fractures (An et al. 2019). Recent investigation of Das et al. (2020c) demonstrated excessive release of cellular cations (Ca<sup>2+</sup>, K<sup>+</sup>, and Mg<sup>2+</sup>) from *Aspergillus flavus* after fumigation with *Pimpinella anisum* L. essential oil suggesting imbalance in cellular homeostasis and disintegration of fungal plasma membrane (Figure 1).

### 7.1.2. Effect on mitochondrial system

In addition to cell wall and plasma membrane disorganizations, the hydro-phobicity of essential oil components modulates the permeability of small cations causing alteration in proton motive force and cellular synthesis of ATP. Tian et al. (2012) reported disruption of mitochondrial membrane permeability leading to dysfunction of chemiosmotic mechanism of ATP synthesis and further lethality of cell. Shao et al. (2013) demonstrated the antifungal activity of tea tree essential oil against *Botrytis cineria* Pers. (Helotiales: Sclerotiniaceae) due to increase in saturated fatty acids and decreased level of unsaturated fatty acids in mitochondrial membrane. Moreover, the potential of bioactive components of tea tree essential oil against postharvest infestation in strawberry through oxidative impairment in several enzymes such as peroxidase, superoxide dismutase, phenyl alanine lyase and  $\beta$ -1,3 glucanase activities has also been reported. Essential oils also caused membrane depolarization and vacuole formation with disintegration of membrane fluidity. Hu et al. (2017) described the antifungal and antiaflatoxigenic effect of *Curcuma longa* L. essential oil by effective disintegration of fungal endo-membrane system through inhibition of malate dehydrogenase, succinate dehydrogenase and mitochondrial ATPase activity. Recent investigation of Ju et al. (2020a) suggested the inhibition of *Penicillium roqueforti* Thom. (Eurotiales: Trichocomaceae) by combined effect of major components of *Litsea cubeba* (Lour.) Pers. (Laurales: Lauraceae) essential oil viz. eugenol and

citral with significant changes in mitochondrial TCA cycle by down-regulating the isocitrate dehydrogenase, succinate dehydrogenase and fumarate hydratase enzymes. Chen et al. (2014) demonstrated the fungitoxic activity of *Anethum graveolens* L. essential oil on *Candida albicans* (C.-P. Robin) Berkhout (Saccharomycetales: Saccharomycetaceae) by induced apoptosis through cytochrome c promoted meta-caspase activation, chromosomal fragmentation, decondensation and phosphatidylcholine externalization. Li et al. (2016) reported the effect of *Litsea cubeba* (Lour.) Pers. essential oil fumigation on hyphal and conidiophores causing *in vitro* fungal growth inhibition in culture media. Li et al. (2017) investigated the antifungal activity of tea tree essential oil against *Botrytis cineria* Pers. by affecting membrane permeability with increasing level of ROS and decreased the activities of mitochondrial isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, citrate synthetase and ATPase. Malformation in mitochondria with sunken surface causing intracellular ATP leakage and decreased the rate of ATP synthesis in *Penicillium digitatum* (Pers.) Sacc. (Eurotiales: Trichocomaceae) by citral has been reported as vital point for antifungal activity (Zheng et al. 2015).

### 7.2. Antimycotoxigenic mechanism of action

In addition to antifungal activity essential oils have prompt inhibitory activity against mycotoxin secretion by broad range toxigenic fungal species. Manso et al. (2014) reported aflatoxin B<sub>1</sub> inhibitory activity of cinnamon essential oil by inhibition of two virulence factor extracellular enzymes viz. elastase and keratinase with potent diminution of oxygenase activity in *Aspergillus fumigatus*. Reduction in cellular ROS by dose dependent treatment of cinnamaldehyde has been investigated as key inhibitory mechanism of aflatoxin B<sub>1</sub> secretion (Sun et al. 2016). Accumulation of free radicals and down-regulation of mitochondrial enzymes in *Fusarium graminearum* by *Curcuma longa* L. essential oil significantly reduced the zearalenone secretion (Kumar et al. 2016). Interruption of ergosterol biosynthesis, disruption of membrane integrity and osmotic disturbances in membrane bound proteins by *Rosmarinus officinalis* essential oil has been demonstrated for mitigation of fumonisin biosynthesis in *Fusarium verticillioides* (da Silva Bomfim et al. 2015). Dambolena et al. (2010) reported inhibitory effect of fumonisin secretion by *Ocimum basilicum* L. and *Ocimum gratissimum* L. essential oil with more specific interaction of phenolic components mediated -SH groups and

nonspecific proteins damaging pH homeostasis and leakage of inorganic ions. Kalagatur et al. (2015) described dose dependent reduction of zearalenone secretion in *Fusarium graminearum* contaminating maize grains with increasing concentrations of *Ocimum sanctum* L. essential oil by significant down-regulation of PKS4 and PKS13 gene expression. Inhibition of lipid peroxidation with significant restraining of ergosterol biosynthesis by *Zingiber officinale* Roscoe essential oil impaired the biosynthesis of fumonisin has been reported by Yamamoto-Ribeiro et al. (2013). Das et al. (2019a) recently reported the inhibition of cellular methylglyoxal (cytotoxic component act as aflatoxin B<sub>1</sub> inducer) by *Coriandrum sativum* L. essential oil facilitating the down-regulation of aflatoxin B<sub>1</sub> in *Aspergillus flavus*. Antifungal and antiaflatoxigenic activity of thyme essential oil in *A. flavus* has been associated with reduced expression of genes specific to secondary metabolism (*lae A*) and virulence factors viz. *me T* and *lip A* (Oliveira et al. 2020). *Cinnamomum zeylanicum* Blume, *Ocimum basilicum* L., *Curcuma longa* L., *Cymbopogon martini* (Roxb.) Stapf and *Zingiber officinale* Roscoe essential oil mediated down-regulation of *acOTAnrps*, *acOTApks*, *acpks*, *afl-T*, *afl-R*, *afl-M*, *afl-P* and *afl-D* gene expression for effective inhibition of fungal infestation, ochratoxin A and aflatoxin B<sub>1</sub> biosynthesis has been recently investigated (Kalagatur et al. 2020). The antifungal and mycotoxin inhibitory activity of essential oil and bioactive components with their mechanism of toxicity is presented in Table 4.

## 8. Nanoencapsulation of essential oils and bioactive components: boon to control food contaminating fungal pathogens and insects infestation

Currently, the increasing global population has led to optimize the agricultural production and minimize the losses resulting from fungal pathogens and insects infestation in food commodities. Essential oils and bioactive components play active role in inhibition of fungi and insect infestation and fungal contamination, but, ephemeral and volatile nature of essential oils cause easy oxidation and makes them more sensitive to environmental conditions reducing the overall bio-efficacy (Basak and Guha 2017; Rajkumar et al. 2020a). Nanotechnology involving the micro/nanoencapsulation of bioactive components within polymeric wall materials improves the natural effectiveness and offers a foundation for development of novel botanical formulation having significant contribution in development of green

preservatives (Campos et al. 2018; Hasheminejad et al. 2019). Tramon (2014) observed the utilization of nanoencapsulated green preservative as a counterpart of synthetic ones; however, standardization for maximum effectiveness is required for target specificity and controlled release. Nanoencapsulation offers the sustained release of volatility and minimizes the toxicity to nontarget organisms as well as modify the physico-chemical stability. There are different nanocarrier systems viz. chitosan, poly ethylene glycol, cashew gum, alginate and cyclodextrin to customize the nanostructure development such as nanoemulsion, nanogel, nanocomposite and nanoparticles representing reproducible interest for development of environmentally safe preservatives having promising future in sustainable agro-ecosystem (Jemaa et al. 2018; Zhang et al. 2019; Rajkumar et al. 2020b). Different encapsulation techniques such as nanoprecipitation, nanocomplexation, film hydration, ionic gelation, spray drying, coacervation and emulsification have been used for synthesis of essential oil nanoparticles (matrix through dispersion), nanocapsules (matrix surrounding the core), nanocomplexes (spatial disposition with nanosphere) and nanobubbles (essential oil bubbles dispersed within the wall material) (Mohammadi et al. 2015; Gonçalves et al. 2017; Ribes et al. 2018; Herrera et al. 2019). In case of essential oil based insecticides/fungicides, nanoencapsulation leads to optimum release of active bio-components to maintain the threshold limit in the environment.

### 8.1. Nanoencapsulated essential oils and their components for inhibition of insects infestation in stored foods

Encapsulation of essential oils and their components into suitable matrix polymers facilitate the controlled delivery of active constituents with greater bioefficacy for inhibition of insects in real food system without altering the organoleptic properties. Ziaee et al. (2014) reported the insecticidal activity of nanoencapsulated *Cuminum cyminum* L. essential oil against *Tribolium confusum* and *Sitophilus granarius* with altered fumigant and persistence bioassay. Upadhyay et al. (2019) observed the enhanced insecticidal, antifeedant, repellent and fumigant toxicity after encapsulation of *Melissa officinalis* L. essential oil within chitosan biopolymer with emerging and effective means for target site of delivery. Hashem et al. (2018) demonstrated potential of *Pimpinella anisum* L. essential oil nanoemulsion affecting midgut of *Tribolium castaneum* with reduced progeny and concentration dependent



**Table 4.** Some essential oils and bioactive components for inhibition of food contaminating fungal pathogens and mycotoxins with mechanism of toxicity.

Infesting fungi/ mycotoxins	Mechanism of toxicity	Essential oils/components used	References	
<i>Aspergillus flavus</i> Link.	Inhibition of enzymatic energy production, H <sup>+</sup> -ATPase proton motive force and biosynthesis of structural components	<i>Thymus vulgaris</i> L., <i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry and <i>Satureja hortensis</i> L.	Omidbeygi et al. (2007)	
	Leakage of cellular ions	<i>Cymbopogon citratus</i> (DC.) Stapf	Helal et al. (2007)	
	Inhibition of ergosterol synthesis	<i>Perilla frutescens</i> (L.) Britton	Hu et al. (2020)	
		<i>Anethum graveolens</i> L.	Tian et al. (2012)	
	Plasma membrane disintegration	<i>Curcuma longa</i> L.	Hu et al. (2017)	
		<i>Salvia sclarea</i> L. and <i>linalyl acetate</i>	Singh et al. (2021)	
	Disruption in membrane	P-cymene	Tian et al. (2018)	
mitochondrial potential and inhibition of spore germination	<i>Thymus vulgaris</i> L.	Oliveira et al. (2020)		
	E-(2)-hexenal	Ma et al. (2019)		
Modulation of cellular SOD, CAT and GSH/GSSG activity	<i>Anethum graveolens</i> L.	Tian et al. (2012)		
Imbalance in reactive oxygen species production and membrane damage	<i>Pimpinella anisum</i> L.	Das et al. (2020c)		
	Geranial and citral	Tang et al. (2018)		
<i>Aspergillus niger</i> van Tieghem	Cinnamaldehyde	Sun et al. (2016)		
	Disintegration of plasma membrane integrity, reduced synthesis of ergosterol and induced oxidative stress	Cinnamaldehyde	Sun et al. (2020)	
	Disorganization of membrane permeability	α-terpeniol and terpene-4-ol	An et al. (2019)	
	Increase in cellular malondialdehyde content and leakage of vital cellular components	Citral and eugenol	Ju et al. (2020b)	
	Inhibition of mycelial growth and spore germination	<i>Satureja khuzistanica</i> Jamzad, <i>Satureja hortensis</i> L. and <i>Satureja spicigera</i> (K.Koch) Boiss.	Farzaneh et al. (2015)	
<i>Fusarium graminearum</i> Schwabe	Inhibition of cellular ergosterol	<i>Zingiber officinale</i> Roscoe	Ferreira et al. (2018)	
<i>Fusarium verticilloides</i> (Sacc.) Nirenberg	Inhibition in cellular ergosterol and reduced production of conidia	<i>Curcuma longa</i> L.	Avanço et al. (2017)	
<i>Fusarium oxysporum</i> Schldt.	Inhibition through dissolution of cell wall integrity in biofilm	<i>Lippia rhemania</i> H.Pearson, <i>Helichrysum splendidum</i> (Thunb.) Less., <i>Cinnamomum zeylanicum</i> Blume, <i>Cymbopogon citratus</i> (DC.) Stapf and <i>Cinnamomum camphora</i> (L.) J.Presl.	Manganyi et al. (2015)	
<i>Penicillium cyclopium</i> Westling	Efflux of cytoplasmic content and vital ions	Nonanal and α-phellandrene	Zhang et al. (2017)	
<i>Penicillium expansum</i> Link.	Modulation in transcriptome profile by genes involved in patulin synthesis	Decanal	Zhou et al. (2018)	
<i>Alternaria alternata</i> (Fries) Keissler	Inhibition of mycelia growth and conidial germination	<i>Thymus vulgaris</i> L.	Perina et al. (2015)	
<i>Rhizopus stolonifer</i> Vuillemin	Increase in mitochondrial membrane potentiality and reduction in ergosterol content	Thymol and salicylic acid	Kong et al. (2019)	
<b>Mycotoxins</b>	Aflatoxin B <sub>1</sub>	Inhibition of mycelial growth and spore germination	<i>Satureja khuzistanica</i> Jamzad, <i>S. hortensis</i> L. and <i>S. spicigera</i> (K.Koch) Boiss.	Farzaneh et al. (2015)
		Modulation of cellular SOD, CAT and GSH/GSSG activity	<i>Pimpinella anisum</i> L.	Das et al. (2020c)
	Patulin	Apoptotic cell death by plasma membrane disintegration	<i>Thymus vulgaris</i> L.	Oliveira et al. (2020)
Modulation in transcriptome profile by genes involved in patulin synthesis		Decanal	Zhou et al. (2018)	
Fumonisin B <sub>1</sub> and B <sub>2</sub>	Inhibition in cellular ergosterol and reduced production of conidia	<i>Curcuma longa</i> L.	Avanço et al. (2017)	

mortality response. Nanoencapsulated peppermint and *Piper nigrum* L. essential oil encompassed into chitosan nanoparticle with effective biological efficacy against different storage insects such as *Sitophilus oryzae* and *Tribolium castaneum* by inhibition of acetylcholine esterase activity has been

recently reported (Rajkumar et al. 2020a, 2020b). Yang et al. (2009) synthesized polyethylene glycol coated nanoparticle loaded with garlic essential oil and evaluated the insecticidal activity against *Tribolium castaneum*. The nanoparticle showed prominent control efficacy against *Tribolium*

*castaneum* infestation in stored rice over 80% after five months that may be due to the persistent and controlled release of active constituents along with the targeted delivery. Insecticidal activity of *Mentha longifolia* essential oil nanoemulsion and its major components such as 1,8-cineol and  $\beta$ -pinene against *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) has been demonstrated by Louni et al. (2018). Malaikozhundan and Vinodhini (2018) developed the nano-insecticidal formulations containing *Pongamia pinnata* (L.) Pierre (Fabales: Fabaceae) leaf extract and its bioactive components into zinc oxide nanoparticle and tested against pulse beetle *Callosobruchus maculatus* which significantly affected the midgut digestive enzyme leading to reduction of hatchability and fecundity. *Rosmarinus officinalis* L. essential oil loaded polycaprolactone nanocapsule exhibited potent insecticidal activity against *Tribolium castaneum* with effective management of stored food products (Khoobdel et al. 2017). Giunti et al. (2019) demonstrated repellence and acute toxicity of sweet orange essential oil nanoemulsion against *Cryptolestes ferrugineus* Stephens (Coleoptera: Laemophloeidae) and *Tribolium confusum*. Insecticidal efficacy of polycaprolactone nanoparticle impregnated with *Zataria multiflora* and *Rosmarinus officinalis* essential oils against *Tribolium confusum* in stored food commodities ( $LC_{50} = 112.64$  and  $206.66 \mu\text{L/L}$  air) has been recently demonstrated (Ahsaei et al. 2020). Yadav et al. (2021) investigated the insecticidal efficacy of *Myristica fragrans* Houtt. (Magnoliales: Myristicaceae), *Zanthoxylum alatum* and *Bunium persicum* (Boiss.) B.Fedtsch. (Apiales: Apiaceae) essential oil nanoformulation against the infestation of *Callosobruchus chinensis* in stored *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae) seeds. Significant damage (~74%) was observed for control seeds, while the chitosan entrapped essential oil nanoformulation exhibited 100% feeding deterrence activity. At  $0.2 \mu\text{L/mL}$  the nanoformulation could reduce the rate of vitality, fecundity and fertility with consistent maintaining of seed weight.

## 8.2. Antifungal and antimycotoxigenic activity of nanoencapsulated essential oils and their components in stored foods

In addition to storage insects, nanoencapsulated essential oils also exhibit potent fungitoxicity and mycotoxin inhibitory activities as compared to synthetic fungicides. Moreover, nanoencapsulation enhances the bio-efficacy through greater surface to volume ratio in the surfaces and interfaces where microorganisms are preferably located. Zhang et al.

(2019) reported chitosan/sodium alginate loaded Cinnamon essential oil (developed through ultrasonication based degasser technology) against *Penicillium expansum* Link. through multilayer film development and coating on wounded apples in order to harness the controlled release properties. Nanoemulsion of selected essential oils viz. thyme, cinnamon, lemongrass, peppermint and clove oil in medium chain triglycerides effectively inhibited the two chemotypes of *Fusarium graminearum* at  $EC_{50}$ ,  $EC_{70}$  and  $EC_{90}$  doses with significant antifungal effect on ergosterol disorganization, alteration in pH homeostasis and unequilibrium of cellular ions (Wan et al. 2019a). Encapsulation of *Thymus vulgaris* L. essential oil through complex coacervation exhibited dose dependent toxicity against *Aspergillus niger* and *Candida albicans* with increment in shelf life of bakery products (Gonçalves et al. 2017). Impairment in mitochondrial dependent reactive oxygen species generation and SOD, CAT, and glutathione activity by *Myristica fragrans* essential oil nanoemulsion effectively reduced the fungal growth and aflatoxin secretion in food commodities (Das et al. 2020d). Nanocapsules containing polylactic acid and lemongrass essential oil against the pathogenic fungal infestation viz. *Colletotrichum acutatum* Simmonds (Glomerellales: Glomerellaceae) has been recently investigated by Antonioli et al. (2020). Chaudhari et al. (2020b) investigated antifungal and antiaflatoxigenic bioefficacy of *Origanum majorana* L. (Lamiales: Lamiaceae) essential oil loaded chitosan nanoparticle against toxigenic *Aspergillus flavus* and tested *in situ* efficacy in stored food system (maize). Recent report of García-Díaz et al. (2019) suggested noisome encapsulated *Satureja montana* L. (Lamiales: Lamiaceae) and *Origanum virens* (Hoffmannsegg & Link) Ietswaart (Lamiales: Lamiaceae) essential oils with effective inhibition of *A. flavus* growth and aflatoxin  $B_1$  secretion in maize grains during storage. Encapsulation of *Anethum graveolens* essential oil into chitosan nanomatrix and evaluation of antifungal and aflatoxin  $B_1$  inhibitory efficacy in stored rice has been reported by Das et al. (2021b). The nano-encapsulated essential oil showed superior fungitoxic activity (especially toxigenic *Aspergillus flavus*) as compared to the free form due to their nanometric size with greater surface area to volume ratio and controlled volatilization. Prominent inhibition of cellular methylglyoxal synthesis by nanoencapsulated essential oil suggested the novel antiaflatoxigenic mechanism of action. Complete inhibition of aflatoxin  $B_1$  contamination and 57.65–82.11% inhibition of *Aspergillus flavus* infestation without any negative impact in organoleptic properties after fumigation

**Table 5.** Encapsulated essential oils and bioactive components for inhibition of insects infestation in stored foods.

Matrix polymer	Encapsulation technique	Essential oil/components	Target insects	References
Chitosan	lonotropic gelation	<i>Piper nigrum</i> L.	<i>Sitophilus oryzae</i> L. and <i>Tribolium castaneum</i> Herbst	Rajkumar et al. (2020a)
		Peppermint oil	<i>Sitophilus oryzae</i> L. and <i>Tribolium castaneum</i> Herbst	Rajkumar et al. (2020b)
		<i>Melissa officinalis</i> L. <i>Myristica fragrans</i> Houtt., <i>Zanthoxylum alatum</i> Roxb. and <i>Bunium persicum</i> (Boiss.) B.Fedtsch. essential oil formulation	<i>Tribolium castaneum</i> Herbst <i>Callosobruchus chinensis</i> L.	Upadhyay et al. (2019) Yadav et al. (2021)
$\beta$ -cyclodextrin	Surface plasmon resonance method	Carvacrol and linalool	<i>Helicoverpa armigera</i> Hübner and <i>Tetranychus urticae</i> Koch.	Campos et al. (2018)
Poly caprolactone	Solvent displacement and interfacial deposition	<i>Rosmarinus officinalis</i> L.	<i>Tribolium castaneum</i> Herbst	Khoobdel et al. (2017)
Polysorbate	Sonication based nanoemulsion	<i>Eucalyptus globulus</i> Labill.	<i>Sitophilus granarius</i> L.	Mossa et al. (2017)
Polyethylene glycol	Melting dispersion	<i>Allium sativum</i> L.	<i>Tribolium castaneum</i> Herbst	Yang et al. (2009)
Myristic acid, linoleic acid and chitosan	Nanogel	<i>Cuminum cyminum</i> L.	<i>Sitophilus granarius</i> L. and <i>Tribolium confusum</i> du Val	Ziaee et al. (2014)

of rice seeds by essential oil nanoformulation were observed. An overview of different methods used for encapsulation of essential oils and their fungitoxic and inhibition to insect infestation are represented in Tables 5 and 6.

### 9. Safety assessment of essential oils and nano-formulations

Preservative potential of any essential oil, bioactive components or nanoformulation requires assessment of safety limit before practical recommendation at the commercial level. Hoffmanns. et Link essential oil treatment sets without any alteration in behavior of mice convulsions, abdominal contortions, muscle tones and piloerection described the safe application of essential oils/ or nanoformulation (Jemaa et al. 2018). Moreover, the LD<sub>50</sub> value was much higher than 2000 mg/kg of body weight, which was reportedly nontoxic according to the OECD guidelines. Das et al. (2019b) reported the formulation of *Apium graveolens* L. (Apiales: Apiaceae) essential oil and its mixture with linalyl acetate and geranyl acetate as novel safe preservative based on the LD<sub>50</sub> value on male mice that was found to be 12,568.36  $\mu$ L/kg body weight. High dose (2 g/kg) of *Piper vicosanum* Yunck. (Piperiales: Piperaceae) essential oil exhibited negligible toxicity in female rats (Brait et al. 2015). Liu et al. (2015) demonstrated long term oral ingestion of *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtales: Myrtaceae) essential oil at 400 mg/kg without any toxic effect on histopathological significance and

organ weight. de Lima et al. (2013) conducted oral toxicity assay of *Croton sonderianus* Mull. Arg. and *Croton argyrophyloides* Mull. Arg. (Euphorbiales: Euphorbiaceae) essential oils exhibiting LD<sub>50</sub> value greater than 6000 mg/kg body weight. Acute oral toxicity of *Nepeta cataria* L. (Lamiales: Lamiaceae) essential oil through LD<sub>50</sub> assessment for male and female rats was found to be 2710 and 3160 mg/kg of body weight. Luo et al. (2005) observed the inhalation, dermal and oral toxicity of *Litsea cubeba* essential oil and associated toxicity dose was found to be 12,500, 4000, and 5000 mg/kg, respectively in rats. Regnault-Roger et al. (2012) reported acute oral toxicity of *Satureja hortensis*, *Hyssopus officinalis* L. (Lamiales: Lamiaceae) *Origanum vulgare*, *Lavendula spicaclove*, *Sassafras albidum* (Nutt.) Nees (Laurales: Lauraceae) and *Artemisia dracuncululus* L. (Asterales: Asteraceae) essential oils ranging between 1000 and 2000 mg/kg body weight in rats. Sometimes, high level of exposure of essential oils or components such as pulegone in pennyroyal oil have been associated with reasonable toxicity, but their low level of exposure would help minimize the associated risks. Koul et al. (2008) reported 2-acetonaphthone and thujone as toxic to mammals with LD<sub>50</sub> 0.59 and 0.87 g/kg, respectively. The toxicity of specific essential oil compounds could be reduced when applied in combination with nontoxic components based on different congeneric groups. Exposure of variety of congeneric groups over a broad concentration range may reduce the toxicity issues in mammalian system (Baser and Buchbauer 2015). Therefore, some sort of congeneric standards of

**Table 6.** Encapsulated essential oils and bioactive components against infestation of fungal pathogens in stored foods.

Matrix polymer	Encapsulation technique/delivery strategy	Essential oil/components	Target fungi and mycotoxins	References
Chitosan	Ionotropic gelation	<i>Coriandrum sativum</i> L. <i>Zataria multiflora</i> Boiss.	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub> <i>Botrytis cinerea</i> Pers.	Das et al. (2019a) Mohammadi et al. (2015)
		<i>Eugenia caryophyllata</i> (L.) Merr. et Perry	<i>Aspergillus flavus</i> Link.	Hasheminejad et al. (2019)
		Eugenol	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Das et al. (2020b)
		<i>Myristica fragrans</i> Houtt.	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Das et al. (2020d)
		<i>Zingiber zerumbet</i> (L.) Roscoe ex Sm.	<i>Aspergillus flavus</i> Link., <i>Alternaria grisea</i> Svlv., <i>Aspergillus fumigatus</i> Fresenius, <i>Aspergillus repens</i> (Corda) Sacc., <i>Aspergillus sydowii</i> (Bainier & Sartory) Thom and Church, <i>Fusarium oxysporum</i> Schldtl. and aflatoxin B <sub>1</sub>	Deepika et al. (2021)
		Anethole	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Chaudhari et al. (2020c)
		<i>Pimpinella anisum</i> L.	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Das et al. (2020c)
		<i>Monarda citriodora</i> Cerv. ex Lag.	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Deepika et al. (2020)
		<i>Bunium persicum</i> (Boiss.) Pimenov & Kljuykov	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub>	Singh et al. (2020)
		<i>Origanum majorana</i> L. <i>Schinus molle</i> L.	<i>Aspergillus flavus</i> Link. and aflatoxin B <sub>1</sub> <i>Aspergillus parasiticus</i> Speare	Chaudhari et al. (2020b) Luque-Alcaraz et al. (2016)
β-cyclodextrin	Ultrasonication based degassing film layer method	Cinnamon essential oil	<i>Penicillium</i> spp.	Zhang et al. (2019)
	Inclusion complex	Eugenol Cinnamaldehyde and eugenol	<i>Peronophythora litchi</i> C.C. Chen <i>Botrytis cinerea</i> Pers.	Gong et al. (2016) Herrera et al. (2019)
Xanthum gum	Homogenization based stirring	Cinnamon bark oil	<i>Aspergillus niger</i> van Tieghem, <i>Penicillium expansum</i> Link., <i>Aspergillus flavus</i> Link., <i>Zygosaccharomyces rouxii</i> (Boutroux) Yarrow and <i>Zygosaccharomyces bailii</i> Barnett	Ribes et al. (2018)
Type B gelatin	Complex coacervation	Thyme essential oil	<i>Candida albicans</i> (C.-P. Robin) Berkhout and <i>Aspergillus niger</i> van Tieghem	Gonçalves et al. (2017)
<b>Mixed polymer</b>				
Tween 80, β-lactoglobulin and α-lactalbumin	High pressure homogenization	Cinnamon leaf oil, Bergamot oil and Lemon oil	<i>Aspergillus niger</i> van Tieghem	Ribes et al. (2017)
Gellan gum and cassava starch	Liposome dispersion method	<i>Thymus zygis</i> Loefl. ex L.	<i>Botryotinia fuckeliana</i> (de Bary) Whetzel and <i>Alternaria alternata</i> (Fries) Keissler	Sapper et al. (2018)
Chitosan and sodium alginate	Ultrasonication based degassing film layer method	Cinnamon essential oil	<i>Penicillium</i> spp.	Zhang et al. (2019)
Tween 80 and phosphate buffer solution	Coarse nanoemulsion	Pippermint oil, Lemongrass oil, Thyme oil, Cinnamon oil and Clove oil	<i>Fusarium graminearum</i> Schwabe and deoxynivalenol	Wan et al. (2019b)
PEG-40 hydroxy castor oil and polyoxyethylene 4-lauryl ether	Phase inversion temperature	<i>Origanum vulgare</i> L.	<i>Cladosporium</i> spp., <i>Fusarium</i> spp. and <i>Penicillium</i> spp.	Bedoya-Serna et al. (2018)
Chitosan and quinoa protein	Spontaneous method	Thymol	<i>Botrytis cinerea</i> Pers.	Robledo et al. (2018)

essential oils could be maintained for lowered toxicity and wide scale practical applications in food and agricultural industries. Ribeiro et al. (2014) reported very slight change in LD<sub>10</sub> and LD<sub>50</sub> values after nanoencapsulation of *Eucalyptus citriodora* Hook. essential oil within chitosan biopolymer. LD<sub>50</sub> and LD<sub>10</sub> values of *Eucalyptus citriodora* essential oil and its nanoencapsulated formulation was found to be 1365.5–2286.1, 2337.9–2967.5 and 551.7–1355.5,

1404.0–2269.4 mg/kg, respectively. However, after nanoencapsulation there were no vital toxicity effects on behavior of mice. The mucoadhesivity and better residential periods of chitosan within the abomasum of mice cause negligible change in LD<sub>50</sub> whether higher concentration of essential oil are incorporated during preparation of nanoformulation. Sosnik (2014) suggested that increasing surface area after nanoencapsulation promotes intramolecular

**Table 7.** Safety assessment of essential oils, bioactive components and nanoformulations.

Test animals	Administration	Essential oils/components/nanoemulsion	Safety assessment (LD <sub>50</sub> ) (g/kg)	References
Mice	Oral	<i>Eucalyptus citriodora</i> L.	2.7	Ribeiro et al. (2014)
		<i>Hofmeisteria schaffneri</i> (A.Gray) R.M.King & H.Rob.	5	Angeles-López et al. (2010)
		<i>Apium graveolens</i> L. essential oil mixed with linalyl and geranyl acetate	12.56	Das et al. (2019b)
		<i>Illicium verum</i> Hook.f.	11.25	Dwivedy et al. (2018)
		<i>Cymbopogon flexuosus</i> (Nees ex Steud.) W. Watson	>2	Chandrashekar and Prasanna (2010)
		<i>Ocimum sanctum</i> L.	3.75–5.67	Orafidiya et al. (2004)
		Limonene	2.77	Yılmaz and Özbek (2018)
		1,8 cineol	3.84	Dougnon and Ito (2019)
		Carvacrol	0.91	Moazeni et al. (2019)
		<i>Mentha spicata</i> L.	8.34	Kedia et al. (2014)
		<i>Trachyspermum ammi</i> (L.) Sprague ex Turrill	6.62	Kedia et al. (2015)
		<i>Cinnamomum glaucescens</i> (Nees) Hand.-Mazz.	3.97	Prakash et al. (2013)
		<i>Aegle marmelos</i> (L.) Corrêa	23.65	Singh et al. (2009)
		<i>Angelica archangelica</i> L. essential oil mixed with phenyl ethyl alcohol and α-terpeniol	9.56	Prakash et al. (2015)
		<i>Lippia alba</i> (Mill.) N.E.Br. ex Britton & P.Wilson and <i>Callistemon lanceolatus</i> (Sm.) Sweet	11.04 and 14.62	Shukla et al. (2011)
		<i>Pimpinella anisum</i> L.	19.87	Das et al. (2020c)
		<i>Anethum graveolens</i> L.	18.71	Das et al. (2021b)
		<i>Acorus calamus</i> L.	4.87	Shukla et al. (2013)
		<i>Dorema ammoniacum</i> D. Don	>5.0	Raeesdana et al. (2018)
		<i>Thymus capitatus</i> (L.) Hoffmanns. & Link	>2	Jemaa et al. (2018)
		<i>Thymus broussonetti</i> Boiss.	4.47	Elhabazi et al. (2012)
		<i>Origanum majorana</i> L. essential oil nanoemulsion	11.88	Chaudhari et al. (2020b)
		<i>Pimpinella anisum</i> L. essential oil nanoemulsion	13.64	Das et al. (2020c)
		2-acetonaphthone	0.59	Koul et al. (2008)
		<i>Anethum graveolens</i> L. essential oil nanoemulsion	15.98	Das et al. (2021b)
		<i>Myristica fragrans</i> Houtt. essential oil nanoemulsion	9.23	Das et al. (2020e)
		Rat	Intraperitoneal	Pulegone
Subcutaneous	Thujone		0.87	
Wistar rat	Oral	<i>Cuminum cyminum</i> L.	>2.0	Allahghadri et al. (2010)
	Intraperitoneal	<i>Artemisia dracunculus</i> L.	1.25	Maham et al. (2014)
	Oral	Garlic essential oil nanoemulsion	2.80	Ragavan et al. (2017)

interaction in gastric mucosa with adhesive interactions. Ragavan et al. (2017) reported garlic essential oil nanoemulsion developed through ultrasonic emulsification with LD<sub>50</sub> value higher than 2.80 mL/kg of body weight and subacute toxicity at 0.46 mL/kg without any toxic effects in rats rather improved efficacy for treatment of dyslipidemia. Al-Abodi et al. (2019) reported negligible toxicity of *Zataria multiflora* essential oil nanoemulsion after combined administration with albendazole into mice. The pharmacokinetics i.e. blood circulation and liver enzymes of mice on long term consumption of *Zataria multiflora* essential oil nanoemulsion showed therapeutic property against cyst and parasitic infection. The safety assessment of essential oils, bioactive components and their nanoformulations in model animal system are represented in Table 7.

## 10. Patenting essential oils and bioactive components: new insight for marketed insecticide and fungicide formulations

Being consolidated, the essential oil can effectively inhibit the infestation of food contaminating insects

including a number of toxigenic species of fungi. The current food and agricultural industries have paid more attention towards designing of new formulations based on essential oil and their bioactive components. Patenting the developed methodology for practical application at very low doses together with the development of fungi and insect resistant food packaging system could facilitate the general acceptance of food regulatory authorities and common consumers. Thyme essential oil and some of its major monoterpenoids such as anethol, citronellal, eugenol and thymol have been patented (file number U.S. Patent No. 6,841,577 and 7,320,966) for protection of stored food commodities against infestation of green peach aphid (Bessette and Beigler 2005, 2008). Khanuja et al. (2006) developed a novel formulation (file number US PP16,747 P3) of essential oil extracted from *Mentha spicata* L. var. *viridis* against stored grain insects *viz.* *Callosobruchus maculatus*, *Sitophilus oryzae*, and *Tribolium castaneum* and fungal pathogens such as *Aspergillus niger*, *Microsporum gypseum* (E. Bodin) Guiart & Grigoraki and *Aspergillus flavus*. Gomez and Coen (2013) have developed insect attractant

bioformulation by using methyleugenol (bioactive component of *Cinnamomum cassia* (L.) J.Presl essential oil) against *Bactrocera cucurbitae* Coquillett and *Bactrocera dorsalis* Hendel and patented the novel formulation with file number US 2013/0302269 A1. Application of Wintergreen and Rosemary essential oils to control the fungal species such as *Gibberella ziae* (Schwein.) Petch, *Aspergillus parasiticus* Speare, *Fusarium moniliformae*, *Sphaeria maydis* (Berkeley) Sutton, *Peronosclerospora sorghii* (Weston & Uppal) Shaw, *Sclerospora graminicola* (Sacc.) Schroet. and *Microdochium nivale* (Schaffnit) E. Müll has been investigated and patented (file number US 2013/0142893 A1) by Besette et al. (2013). Recent report of Suranyi (2019) suggested the novel formulation comprising of sabadilla alkaloids and at least one essential oil (geranium oil, rosemary oil, peppermint oil and citronella oil) for controlling insects, mites and nematodes and filed a patent with application number US 2019/0191715 A1. Synergistic and residual insecticidal efficacy of essential oils and bioactive components mixture such as citronellal, p-cymene, eugenol, geraniol, guaiacol, D-pulegon, perillaldehyde, menthol, isoeugenol and thymol against stored grain insects have been demonstrated by Besette (2007) and filed a patent with application number US 7.241.806 B2. As discussed above, there is tremendous potential in application of essential oil and bioactive component based green botanical insecticidal/fungicidal formulation, however, major factors pertaining to availability of essential oils and/or active ingredients, production cost, method standardization and extraction procedure need to be considered for large scale innovative applications in food and agricultural industries with consumer preferences and additional benefits for commercialization, with an objective to facilitate new insights in protection of stored food commodities.

### 11. Commercial challenges associated with essential oils based insecticidal and fungicidal product development

Apart from extreme competitive agrochemical business, the obvious effects of cost and availability for long term storage and stability of essential oils based insecticidal and fungicidal formulation are the burning challenge (Benelli et al. 2017). Typically, the nanoformulations containing essential oil with effective insecticidal property have been tested with short term stability trials, therefore, long term field trials and their environmental impact should be addressed in outdoor context for providing avenues to solve the associated problem (Kah et al. 2018; Vurro et al. 2019). In addition, the key factor i.e. the ultimate

“barrier to entry” in commercialization of essential oils based insecticidal and fungicidal formulation is the Government based regularity approval (Isman 2016). Currently, the USA government has listed some essential oil and bioactive components as “FIFRA’s list 25B” which has been exempted from federal approval system ensuring their commercialization for developing essential oils based insecticides. Modern EcoSMART technologies having governmental regulatory exemption developed Sporam™ fungicide based on clove and thyme essential oil, Ecotrol™, TetraCURB™ and Thyme Bomb™ by mixing rosemary and thyme essential oils with effective insecticidal properties (Isman 2020). Basically, the availability of natural resources and likelihood for selection of effective essential oils against fungal pathogens and insects are included under the regulatory regimes (Marrone 2019). It has also been reported that several promising essential oils extracted from aromatic plants whose cultivation is more expensive with specific climatic requirement have very low yield, thereby restrict their commercial utilization as insecticidal/fungicidal formulation. A common question germane to wide scale application of essential oil is the relationship between their key constituents and bioactivity in management of fungal and insects infestation during practical application. To explore this question a number of major retailers have followed the Wal-Mart Stores Inc. and Prentiss Inc. with Brandt consolidation and large scale agricultural applications and consumer use. Furthermore, the monoterpene active component proportions in essential oil depend on the circadian rhythm, temperature, moisture, photoperiods, and relative humidity facilitating the alteration in plant phenological phases (Hansted et al. 1994; Pavela and Benelli 2016). Finally, the nanoencapsulation of essential oils and active components into any biodegradable and biocompatible matrix polymer forming powders or films may establish the prospect of essential oils making new inroads in commercial market place by replacing the adverse effects of synthetic preservatives.

### 12. Conclusion

Essential oils and bioactive components exhibit prominent insecticidal, fungitoxic, and mycotoxin inhibitory activity suggesting their application as green alternative of synthetic preservatives having chemical origin. Moreover, the mechanism of insecticidal and fungitoxic action could be employed to develop a biorational preservative with multiple target site of action. To avoid the negative impacts of essential oils and their bioactive ingredients during

direct applications in food and agroecosystem, the newly emerging nanoencapsulation approach as an effective and innovative technology with great promises in bioefficacy improvement as well as shelf life enhancement can be recommended at industrial scale after safety assessment in order to manage the postharvest losses of food commodities caused by fungal as well as insects infestation. Additionally, the patenting of developed formulation could be helpful in industrial scale commercialization of plant based products.

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### Disclosure statement

The authors declare that they have no competing interests.

### Data deposition

Not applicable.

### Consent to publish

I confirm that any participants, who may be identifiable through the manuscript, have been given an opportunity to review the final manuscript.

### Ethics declaration

This is an observational study, no ethical approval is required.

### Geolocation information

Covers worldwide insects and fungal pathogens control

### Authors' contribution

The idea and concept of the review article was given by Somenath Das and wrote the original manuscript. Vipin Kumar Singh and Abhishek Kumar Dwivedy edited the written manuscript. Anand Kumar Chaudhari performed the review of literature. Nawal Kishore Dubey critically reviewed and edited the manuscript. All authors read and approved the manuscript.

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### Data availability statement

The data that support the findings of this study are cited in manuscript.

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