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Recent advances and review on use of botanicals from medicinal and aromatic plants in stored grain pest management

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Abstract
Stored grain infestation is a very serious problem as various life stages of insects cause economic damage and deteriorates the quality of food grains and food products. There are number of stored grain insect pests that infest food grains in farmer stores and public warehouses and massively surge due to un-controlled environmental conditions and poor ware housing technology used. However, for suppression of multiplying insect population highly specific and more appropriate modern methods are to be used. Few important methods such as microwave and ionizing irradiation, pheromone baited traps, IGRs and use of entomopathogens are proved highly effective against stored grain insects. Botanicals have been used since time immemorial for protection of stored products against common pests. They acts as repellents, antifeedants, toxicants and behave as natural grain protectants by behaving as chemosterilants/reproduction inhibitors or insect growth and development inhibitors. Literature shows that some chemical constituents of these oils interfere with the nervous system in insects. Due to the recent surge in use of green pesticides, the use of plant based pesticides is gaining a lot of impetus. Considering the above points, the present review paper emphasises on the work done on use of botanicals to combat stored pest in food grains with special emphasis on the classification, the mode of action, recent advances in use of botanicals and the commercial and regulatory constrains.

Keywords: Botanicals, medicinal, aromatic plants, stored grain pest management

Introduction
Ever since the breakthrough discovery of DDT in 1939, the use of synthetic pesticides was exceedingly popular in pest management systems employed all over the world. The indiscriminate and un-controlled use of synthetic pesticides has led to problems like (1) pest resistance to pesticides, (2) resurgence of pests, (3) toxic residues on food, water, air and soil, (4) elimination of natural enemies and disruption of the ecosystem and (5) minor pests assuming major status. Presently, plant botanicals occupy a very small niche in the world of synthetic pesticides, but the increasing environmental concerns had led to a surge in use of environmentally sustainable and friendly “green “alternatives. Botanicals are advantageous in many ways as they are (1) naturally occurring, (2) they are highly specific to target pests, (3)Little or no adverse effect on beneficial insects, (4) Insect resistance is slow or less common, (5) they have no unknown environmental hazards, (6) have less residual activity and (7) are effective against insecticide resistance species of insects. Due to all these various reasons, botanicals are considered as commercially viable green pesticides and are gaining tremendous impetus. Although there are some problems faced during commercialization and application, new methods should be sought by which we can overcome these constraints.
The present review consists of extensive information on use and mode of action of different botanicals and their components against stored product pests. It also outlays the problems faced with respect to up scaling and commercialization of herbal based formulations and safety and regulatory issues involved.

Losses due to stored grain pests
Present estimates indicate that India suffers a loss of at 25% in rice and maize, 5% in wheat, and 15% in pulses due to insect pests [1]. More than 20,000 species of field and storage pests account for destruction of approximately one-third of the world’s food production which is valued annually at more than 100 billion dollars [2]. India being a tropical country suffers around 20–30% damage to stored grains and grain products due to insect pests which is only

around 5–10% in the temperate zone [3]. Food grain production in India was reported to be 250 million tons in the year 2010-2011, in which nearly 20–25% food grains are damaged by stored grain insect pests [2]. According to a study conducted by World Bank, 12-16 million tonnes of food grains are lost due to storage pests, which if prevented could feed one-third of the population [4]. Therefore, there is a dire need to develop effective, economical and environmentally sustainable methods for control and management of stored product pest.

Use of botanicals for stored product pest management

The list of primary and secondary pests of stored grains is depicted in Table 1.The concept of using natural sources for storage of various household items dates back to ages ago when no modern means of storage was available. There is evidence of ash, sand herbal and medicinal plants used in ancient civilization, which have been used for extending the storage life of many food products. Many of these practices find their credibility even in the modern era as these methods are cost effective and sustainable. The main activity is due to the presence of essential oils that are lipophilic volatile secondary metabolites. Many spices and herbs, and their extracts, are known to possess insecticidal properties that are frequently present in the essential oil fraction [5-7]. Presently, botanical insecticides presently constitute only 1% of the world insecticide market [8].

Traditional methods of pest management

Traditional age-old practises include storage of red gram (*Cajanus cajan*) grains with common salt, which are then packed in jute gunny bags and then stored. This method gave protection for 6-8 months from insects. Karthikeyan, C. *et al* [9] reported that farmers in Tamil Nadu believed that addition of ash with sorghum (*Sorghum bicolor*) help ward of insects, e.g. rice weevil (*Sitophilus oryzae*), rodents (*Tatera indica*) and mite (*Oligonychus indicus*) and help to reduce losses up to 80%. *Azadirachta indica* leaves are most commonly used in traditional storage practices all over India. Use of lime and camphor are also common in storage of paddy in gunny bags. The use of neem (*Azadirachta indica*) oil in the seed storage treatment employs mixing 20 ml oil of for 1 kg of pulse seeds and use of neem seed kernel extract for a dip treatment to the jute gunny bags with before storage is also employed. Storage of grains with sweet flag (*Acorus calamus*.) powder prevented insect infestation for 6 months as the strong odour emitted from sweet flag acted as a repellant against all the storage pests.There is extensive research being carried to determine the efficacy and practical use of locally available plants for controlling insect pests. However most studies are based on laboratory trials that have been carried out for small durations and data obtained from this literature does not simulate real farm applications.

Mode of action

Essential oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes. The mode of action against pests is by means of a neurotoxic mode of action as studies have showed that it inhibits the acetyl cholinesterase enzyme (AChE) activity [10] which interferes with the neuromodulator octopamine [11] or GABA-gated chloride channels [12] in the pests leading to their destruction. Lee *et al*. reported that there was no direct correlation between insect toxicity and AChE inhibition and inferred that, in addition to AChE inhibition, the monoterpenes may act on other vulnerable sites (e.g. cytochrome P450-dependent mono-oxygenases) [13]. The lethal concentration of essential oils against stored grain pests is given in Table 2.

Classification of Botanical Insecticides

Jacobson classified the plant components into 6 groups namely repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants, and attractants based on the effect they have on insects [14].

Repellents. Repellents are minimal impact substituents that avert away the insect pest from the treated materials by stimulating olfactory or other receptors. Ebenezer O Owusu reported that some traditionally useful Ghanaian plant materials namely *Ocimum viride* and *Chromolaena odorata* reduced survival of stored product insect pests to less than 25% after 10 days of treatment at concentrations of 0.1 mg ml−1 and above [15]. Essential oils obtained from members of the Lamiaceae (mint family), *Poaceae* (aromatic grasses) and *Pinaceae* (pine and cedar family) are commonly used as insect repellents throughout the globe [16] Saljoqi, A. U. R. *et al* reported the percent mortality or repellency data of the insects where in bakain drupes showed 61.2% mortality, followed by habulas (48.40%), mint (47.40%) and bakain leaves (46.80%), while harmal (16.80%) was found less effective followed by lemon grass (35.20%) against rice weevil, *Sitophilus oryzae* L [17].

Antifeedants/Feeding Deterrents

Antifeedants or "feeding deterrents" are defined as chemicals that inhibit feeding or disrupt insect feeding by rendering the treated materials unattractive or unpalatable [18]. Most natural occurring antifeedants include glycosides of steroidal alkaloids, aromatic steroids, hydroxylated steroid meliantriol, triterpenehemiacetal, and others [14]. Antifeedants are obtained from secondary metabolites– alkaloids, phenolics and terpenoids [19], the later being the most potent. Most prevalent anti feedants are limonoids from the neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) trees, exemplified by azadirachtin and toosendanin, and limonin from *Citrus* species. Among plant phenolics, the best known antifeedants are the furanocoumarins and the neolignans. Murray Isman reported that the limitations of anti-feedants are due to differences in response between pest species, potential desensitization of pests, rapid environmental degradation, and its efficacy [20].

Toxicity: The toxicity of essential oil varies and is dependent on various factors like the chemical composition of the oil, source obtained from, season and ecological conditions, method of extraction, time of extraction and plant part used [21, 13]. The essential oil of *C. sativum* exhibited volatile toxicity to stored product insects [22]. Nicotine, an active component of *Nicotiana tabacum*, was reported to be a strong organic poison which acts as a contact-stomach poison with insecticidal properties which also has toxicity in humans [23]. Many species of the genus *Ocimum* and its oils, extracts, and their bioactive compounds have been reported to have insecticidal activities against various insect species [24].

Natural Grain Protectants: Extracts of some plant species *viz. Lantana camara* [25] (*Illicium verum* [26], *Tithonia diversifolia* [27] have been reported to possess strong insecticidal activity against different storage insects. The usage of (*Azadirachta indica*) leaves in storage of ragi to deter storage pests like lesser grain borers (*Rhyzopertha dominica*), saw toothed beetle (*Oryzae philussurina mensis*) and flat grain beetle (*Cryptolestes minutus*) is a common practice in Indian villages, also fresh pungam (*Pongamia glabra*) leaves are placed in layers in between the gunny bags arranged one above other in storerooms which helps in repelling the Angoumois grain moth (*Sitotroga cerealella*) and rice weevils (*Sitophilus oryzae*) [9]. Neem based pesticides are marketed in India in different trade names containing 300, 1500, 3000, 5000, 10000 and 50000 ppm of azadirachtin. Some of them are Ozoneem Trishul, Margocide OK, Godrej Achook, Nimbicidine, Bioneem, Neemark, Neem gold, Neemax, Rakshak, Econeem, Limnool and Repelin containing 300ppm of azadirachtin [28].

Chemosterilants/Reproduction Inhibitors

Plants and their various parts when mixed with grains have shown to have an effect on the insect oviposition, egg hatchability, postembryonic development, and progeny production [29]. Botanicals cause malfunctioning of the ovariole in female insects [30]. Oviposition inhibition occurs either due to death of female before laying their eggs in contact with botanical products or due to failure during egg laying of live females [31].

Insect Growth and Development Inhibitors

Insect eggs were found to be more sensitive than at other developmental stages which is probably due to the effect of the botanicals on the physiological and biochemical processes associated with the embryonic development [32]. Essential oils and their constituent mono-terpenoids behaves neurotoxins, imparting ovicidal activity on the development of the nervous system [33], whereas the non-volatile constituents prevent the exchange of gases by blocking the funnel leading to suffocation and consequent death of the embryo [34]. *Prosophis* sp., *Nerium* sp., *Ocimum* sp., *Acalypha* sp., *Catharanthus* sp., *and Vitex* sp. leaf extracts showed caused significant ovipositional deterrent effect against pulse beetle. Leaf extract of *Vitex* sp. caused maximum reduction in egg viability (61.7%) followed by *Catheranthus* sp. leaf extract (56.7%). *Vitex* sp. treated seeds at 5% level caused maximum reduction in adult emergence (85.0%) followed by *Catheranthus* sp. (83.7%), *Acalypha* sp. (73.3%), *Nerium* sp. (70.0%), *Ocium* sp. (68.7%) and minimum reduction was recorded in case of *Prosphis* sp. (68.0%) [35].

Insecticidal activity

The insecticidal activity of plant extracts and essential oils is determined by means of contact or fumigant toxicity.

Contact toxicity

The extracts from *Cinnamomum cassia* bark, cinnamon oil, horseradish oil and mustard oil acted rapidly, causing 100% mortality after 1 day of treatment against *L. serricorne* adults exposed to direct contact. Also, the extracts from *Agastache rugosa* whole plant and *Acorus calamus* var. *angustatus* rhizome produced 100% mortality by 2 and 4 days for the same *L. serricorne* adults [36]. Mahfuz and Khalequzzaman [37] observed that in the contact bioassay against *C. Maculates* eucalyptus oil was found to be the most effective in inducing mortality both after 24 and 48 h of treatments. The toxicity of the oils followed in the order: eucalyptus > clove > cinnamon > cardamom > neem.

Fumigant toxicity

Kim *et al*. [36] reported insecticidal activity of horseradish oil, mustard oil and *Foeniculum* fruit extract by fumigation technique with 100% mortality in sealed containers and 2-4% mortality in open containers. Mahfuz and Khalequzzaman [37] reported that in the fumigation bioassay, treatment with eucalyptus showed the highest toxicity after 24 h of treatment followed by neem, cardamom, cinnamon and clove, and after 48 h of treatment the toxicity followed in the order of clove > cinnamon > cardamom > eucalyptus > neem against *C. maculates*.

Commercialization of botanical pesticides

In a recent review paper on neem and other botanical insecticides, three barriers to the commercialization of new products of this type were identified: (i) the scarcity of the natural resource; (ii) the need for chemical standardization and quality control; and (iii) difficulties in registration [20]. Other studies have been reported where controlled release mechanisms are employed. Botanocap a green pesticide technology made using oil in-water micro emulsions as a nano-pesticide delivery system to replace the traditional emulsifiable concentrates (oil), to reduce the use of organic solvent and increase the dispersity, wettability and penetration properties of the droplets is being developed [38]. Nisar [39] reported use of polymer- and clay-based coats containing azadirachtin-A and were evaluated for quality maintenance of soybean seed during storage. The coats behaved as a barrier to moisture thus reducing azadirachtin-A degradation and prevented proliferation of storage fungi. In commercial products such as Eco SMART these products are exempt from Environmental Protection Agency registration and are approved as direct food additives or classifies as GRAS (generally recognized as safe) by the Food and Drug Administration. Several smaller companies in the U.S. and the U.K. have developed garlic oil-based pest control products and in the U.S. there are consumer insecticides for home and garden use containing mint oil as the active ingredient.

In India, as Sarkar and Kshirsagar [40] reports that plant based research in India is under the purview of Biodiversity legislation, 2002. For plant based research permission from the State Biodiversity Board (SBB) has to be sought. The SBB organizes meetings with local management committees and experts from the field of plant taxonomy, social forestry, zoology, geology, ornithology, etc and then permission is granted. After which the actual collection, processing and screening samples for proposed bioactivity is initiated. One bioactivity is determined, commercialization of such plant based product remains to be great challenge. The Central Insecticides Board & Registration Committee under the Ministry of Agriculture & Farmers Welfare has set up guidelines for registration for botanical pesticides which covers the following. 1) Neem based products. 2) Neem based products containing azadirachtin for house hold uses. 3) Formulation based on cymbopogom plant extract. 4) Eucalyptus extract containing eucalyptol. The present commercial botanical products are enlisted in Table 3.

Advances in botanical based pesticides for stored grain pest: Indian scenario

A number of patents and formulations have been developed in India in the context of use of botanicals for stored grain pests. Most of them include botanicals from underutilized or by-products of waste processing industries. Indian patent 221311 [41] describes use of a herbal ant repellent composition for the control of ants containing 35% Turmeric powder extract containing 10% of Curcuminoid, 50% Neem powder extract containing 15% of liminoid, 10% Clove oil containing 90% of Eugenol and 5% Eucalyptus oil containing 70% of Cineol, sorbed on particulate sorptive substrate to obtain a controlled release system for controlling ants in stored food grains. Patent No 222111 [42] enlists the use of potato peels obtained from potato processing industries as an agro-based stored grain protectant. A novel synergistic formulation useful as pest repellent for stored grains was developed which comprises of powdered menthol, solid preservative powder and a liquid preservative along with a suitable binder [43]. Patent 188378 [44] enlists a synergistic composition useful as a fumigant against stored grain insect-pests comprising of a mixture of 1, 8 cineole, obtained from the plant *Eucalpytus globulus* and one or more constituents of essential oil. Pant, M., *et al* [45] reported use of aqueous filtrate of de-oiled karanja (*Pongamia glabra*) and jatropha (*Jatropha curcas*) cakes left after extracting oil for preparing biodiesel to enhance the activity of eucalyptus oil (*Eucalyptus globulus*) as a pesticide by making a nano-emulsion for the control of *Tribolium castaneum*. Nisar *et al.* [39] reported use of pesticidal seed coats based on azadirachtin-A to improve the storage of soybean seed.

Constraints

The main problem with essential oils is their volatile nature which hinders their use and further scale-up. Although botanicals have a lot of advantages they also have certain constrains such as 1) higher application rates (as high as 1% active ingredient) and frequent reapplication when used out-of-doors. 2) Availability and variability of sufficient quantities of plant material, 3) standardization of products 4) protection of technology (patents) and regulatory approval.

Table 1: List of primary and secondary pests of stored grains (Ahmed) [46]

Common name	Pest	Host
Primary pests		
Rice weevil	*Sitophilus Oryzae, Sitophilus zeamais, Sitophilus granarius*	Rice, wheat sorghum, barley, maize
Khapra beetle	*Trogoderma granarium*	Cereals, groundnut and pulses
Angoumois grain moth	*Rhyzopertha dominica*	Paddy, maize and wheat
Grain moth	*Sitotroga cerealelia*	Rice, wheat and maize
Rice moth	*Coroyra cephalonica*	
Lessergain borer	*R. dominica*	
Pulse beetle	*Callosobruchus chinensis Callosobruchus maculatus*	Pulses, bean and grain
Tamarind/ groundnut bruchid	*Caryedon serratus*	Groundnut, tamarind and other legumes
Cigarette beetle	*Lasioderma serricorne*	Wheat flour, cereal bran, groundnuts, cocoa beans, spices, turmeric,chillies, ginger, stored tobacco
Drug store beetle	*Stegobium paniceum*	Turmeric, coriander, ginger dry vegetables
Secondary pests		
Red flour beetle	*Tribolium castaneum, Tribolium confusum*	Broken grains, damaged grains, milled products
Saw toothe grain beetle	*Cryptolestus minutas Laemophloeus pusilus*	Maize, cereals and oilseeds

Table 2: Lethal concentrations of chemical constituents of commonly used oils against common stored grain pest.

Sl No	Stored grain pest	Essential oil used	Chemical constituent,	Lethal concentration (LC50, LD50, KC50, KD50, KT 50, LT90) mg/L	Action
1.	*Tribolium casteanum*	*Mustard oil*	Allyl isothiocynite	LC_{50} – 3.74 to 4.66 LC_{90}-4.89 to 7.87	Fumigant
2.	*Callosobruchus maculatus*	*Artemesia sieberi*	Camphor Camphene 1,8 Cineole Beta Thujone Alpha Pinene	1.45 ppm	Fumigant
3.	*Sitophilous oryzae*			3.86 ppm	Fumigant
4.	*Tribolium casteanum*			16.76 ppm	Fumigant
5.	*Tribolium casteanum*	*Eucalyptus*	Eucalyptus oil	LD 50- 28.9µL/L	Fumigant
6.	*Tribolium casteanum*	*Eucalyptus*	1,8 Cineole	LD 50-23.8 µL/L	Fumigant
7.	*Sitophilous oryzae*		1, 8 cineole	0.1 µL/720ml(100% mortality)	Fumigant
8.	*Rhizopertha dominica*		Camphor, Linalool, 1, 8 cineole	1 µL/720ml(100% mortality)	Fumigant
9.	*Rhizopertha dominica*	*Eucalyptus*	Cineole	59mg/L(100% mortality)	fumigant
10.	*Rhizopertha dominica*	*Eucalyptus codonophora*		10.4µL/L	Fumigant
11.	*Callosobruchus chinensis*	*Ocimum grattissimum*	Beta ocimene Eugenol	1 µL/L(100% mortality) 1 µL/L(100% mortality)(Eugenol)	Fumigant
12.	*Sitophilous oryzae*	*Vitex pseudo negundo Vitex pseudo negundo*		LC50=31.96 µL/L air	Fumigant

Table 3: List of different commercial botanical based pest management technologies

Product	Active ingredients	Used against
Aphidicide /miticide /fungicide for greenhouse and horticultural use (Mycotech Corporation)	Cinnamon oil with Cinnamaldehyde	Bush and tree fruits
EcoPCO (EcoSMART Technologies)	Eugenol and 2 -phenethyl propionate	Crawling and flying insects,
EcoTrolTM	Rosemary oil	Insecticide /miticide
SporanTM	Rosemary oil	Fungicide
Apilife VARTM (from Chemicals LAIF, Italy)	containing thymol and lesser amounts of cineole, menthol and camphor	Varroa mites in honeybees,
Ponneem	Neem	Field crop insect pest management

Conclusion
Presently, the use of plant based bio pesticides are gaining popularity due to recent commercialization techniques and initiatives taken by the Government. Extensive work needs to be carried out on developing the efficacy of botanicals for large scale and long term use, also farmers and extension workers need to be educated about its use and importance. Government should provide subsidies and schemes and take efforts to encourage the use of such products.

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