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Research Article
Study of Insecticidal Properties of Garlic, *Allium sativum* (Alliaceae) and Bel, *Aegle marmelos* (Rutaceae) Essential Oils Against *Sitophilus zeamais* L. (*Coleoptera*: Curculionidae)

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Abstract

Background and Objectives: The continuous use of synthetic pesticides has increased the risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects among non-target species and cross-resistance and multi-resistance in insects. These have created increased public awareness on human safety and possible environmental damage diverting attention towards other alternatives especially the use of plant products in stored-grain insect pest management. In the present study, essential oils of *Allium sativum* (*A. sativum*) and *Aegle marmelos* (*A. marmelos*) have been evaluated for their repellent, insecticidal, anti-ovipositional and acetylcholine esterase inhibitory activities against maize weevil, *Sitophilus zeamais* (*S. zeamais*). **Materials and Methods:** Garlic, *Allium sativum* and bel, *Aegle marmelos* essential oils have been isolated and evaluated for repellent, insecticidal, oviposition inhibitory and acetyl cholinesterase enzyme inhibitory activities in maize weevil, *Sitophilus zeamais*. One-way analysis of variance (ANOVA, $p<0.01$), correlation and linear regression analysis were used for data analysis. **Results:** In repellency assay, both essential oils showed repellent activity against *S. zeamais* adults. These essential oils caused toxicity in *S. zeamais* adults when applied by fumigation and contact methods. In fumigation toxicity assay, median lethal concentrations (LC_{50}) recorded were 0.297 and 0.22 μL cm^{-3} air, 0.312 and 0.184 μL cm^{-3} air of *A. sativum* and *A. marmelos* oils after 24 and 48 h exposure of *S. zeamais* adults, respectively. In contact toxicity assay, median lethal concentrations (LC_{50}) were found 0.208 and 0.116 μL cm^{-2} area and 0.227, 0.146 6,37 μL cm^{-2} area of *A. sativum* and *A. marmelos* oils after 24 and 48 h exposure of *S. zeamais* adults, respectively. Essential oils of *A. sativum* and *A. marmelos* oils were found to inhibit progeny production by inhibiting oviposition in *S. zeamais* adults when exposed to sub-lethal concentrations. Fumigation of *S. zeamais* with *A. sativum* and *A. marmelos* oils caused neurotoxicity by inhibiting acetylcholine esterase enzyme (AchE) activity. **Conclusion:** *A. sativum* and *A. marmelos* oils can be used as alternative in management of stored-grain insects.

Key words: *Allium sativum*, *Aegle marmelos*, *Sitophilus zeamais*, acetylcholine esterase, neurotoxicity

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects damage grains under storage both qualitatively and quantitatively since the time immemorial. Several synthetic insecticides have been developed and formulated to protect stored grains from insect infestation. But the continuous use of these insecticides has been developing global problems like ozone depletion, carcinogenicity, teratogenicity and mutagenic effects among non-target species as well as cross-resistance and multi resistance among insect populations[1-4]. These public issues of human health and environmental damage have diverted attention towards other alternatives especially the use of phytochemicals in stored-grain insect pest management. Plant derived essential oils are complex mixtures of 20-60 compounds of different chemical nature in different concentrations[5]. Each essential oil is characterized by a specific essence due to two or three major compounds. The essential oil composition depends on plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, genotype of the plant, soil nature and environmental conditions[6,7]. These oils possess adulticidal, larvicidal and antifeedant activity, oviposition inhibitory activities, capacity to delay development and adult emergence[8-11]. These essential oil producing aromatic plants belong to families like Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae and Zingiberaceae. These essential oils have commercial application in food, cosmetic and pharmaceutical industries[12].

Garlic, *Allium sativum* (Family: Alliaceae) is one of the most important ingredients of human food and Ayurvedic medicines since ancient time. Allicin, a key component of garlic reduces blood pressure by inhibiting angiotensin II and vasodilating effects[13]. Its various preparations have antidiabetic properties[14]. Its consumption protects human from cancer[9]. Garlic inhibits proliferation of atherosclerotic cells and other cell types as well as collagen synthesis and accumulation in the aorta[15]. Garlic preparations having allyl sulfides show antibacterial activity against both gram-negative and gram-positive bacteria like *Bacillus*, *Clostridium, Escherichia, Klebsiella*, *Proteus*, *Salmonella*, *Staphylococcus* and *Streptococcus* and antifungal activities against *Candida albicans*[16,17]. Diallyl sulfide and diallyl disulfide act as free radical scavangers by activating antioxidant enzymes like glutathione-s-transferase and catalase[18]. Alcoholic extract of garlic shows anthelmintic activity against *Ascaris lumbricoldes*.

Garlic bulbs contain a number of active compounds especially sulphur containing compounds which are responsible for the pharmacological activities. Steam distillation of garlic bulb produces essential oil containing diallyl, allyl methyl and dimethyl mono to hexa sulfide[19]. *A. sativum* essential oil extracted by steam distillation method have allyl methyl trisulfide (34.61%) and diallyl disulfide (31.65%) as major components[20]. Other components of low percentage are allyl methyl disulfide, diallyl sulfide, diallyl trisulfide and diallyl tetrasulfide. Douiri *et al.*[21] have reported that principal groups of components present in *A. sativum* essential oil are sulfur componunds represented mainly by trisulfides (57.4%) and disulfides (23.16). *A. sativum* essential oil contains 1,3-Dithiane, di-2-propenyl, 1-Propene,3,3'-thiobis, methyl 2-propenyl, 3-vinyl-1,3-dithiin, 2-vinyl-1,3-dithiin, di-2-propenyl, 3-vinyl-1,2 dithiin1-chloro-4-(1- ethoxy)-2-methylbut-2-ene, methyl 2-propenyl, diallyl disulfide, 3-vinyl-1,2 dithiin, methyl1-methyl-2-butenyl sulphide, octane 4-brom[21]. These components contribute to acaricidal[22], antibacterial[23], fungicidal[24], insecticidal[25], molluscicidal[26], nematicidal[27] and antiparasitic[28] properties of garlic. Bel, *Aegle marmelos* (Family: Rutaceae) is a tree native to Northern India but also found throughout Ceylon, Burma, Bangladesh, Thailand and Indo-China[29]. It is traditionally used for treatment of various diseases such as dysentery, fever, diabetes, asthma, heart problems, ophthalmia, haemorrhoids, urinary problems, ulcer[30,31]. Moreover, the alcoholic leave extracts are used as antibacterial and antifungal activities[32,33]. The leaf extracts significantly inhibit the dermatophytic fungi like *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum canis*, *M.gypseum* and *Epidermophyton floccosum*[34]. The oil isolated from *A. marmelos* leaves of Cuba sources contains δ-cadinene (12.1%) and β-caryophyllene (10%) as major compounds[35]. On the other hand, the oil isolated from *A. marmelos* leaves is composed mainly of α-phellandrene (39.2%) and limonene (26.8%)[36]. The maize weevil, *Sitophilus zeamais* (*Coleoptera*: Curculionidae) is a major pest of maize in humid tropical areas around the world where maize is grown[37]. This species attacks both standing crops and stored cereal products including wheat, rice, sorghum, oats, barley, rye, buckwheat, peas and cotton seed[38]. The maize weevil also infests other types of stored, processed cereal products such as pasta, cassava and various coarse milled grains. In the present study, essential oils of *A. sativum* and *A. marmelos* have been evaluated for their repellent, insecticidal, anti-ovipositional and acetylcholine esterase inhibitory activities against maize weevil, *S. zeamais*.

MATERIALS AND METHODS

Essential oils: Dried *A. sativum* bulbs were purchased from the local market of Gorakhpur. Essential oil was isolated by

crushing the bulb and hydrodistillation of crushed bulbs for 4 h in clevenger apparatus. Young and green leaves of *A. marmelos* were taken from the campus of M.G.P.G. College Gorakhpur (U.P.) and hydrodistilled for 4 h in clevenger apparatus. Essential oil was kept in Eppendorf tubes at 4°C till further use.

Insects: Maize weevil, *S. zeamais* was used to determine the insecticide nature of *A. sativum* and *A. marmelos* essential oils. The insects were reared on whole maize grain in the laboratory at 30±4°C, 75±5% RH and photoperiod of 10:14 (L:D) h.

Repellent activity: Repellency assay was performed in glass petri dishes (diameter 8.5 cm, height 1.2 cm). Test solutions of different dilutions (0.2, 0.4, 0.8 and 1.6% vol:vol) of *A. sativum* and *A. marmelos* were prepared in acetone. Whatman filter papers were cut into two halves and each test solution was applied to filter paper half as uniform as possible using micropipette. The other half of the filter paper was treated with acetone only. Essential oil treated and acetone treated halves were dried to evaporate the acetone completely. Both treated and untreated halves were then attached with cellophane tape in a manner so that seepage of the test samples from one half to other half can be avoided and placed at the bottom in each petri dish. Forty *S. zeamais* adults were released at the centre of the filter paper disc and the petri dish was covered and kept in dark. Six replicates were set for each concentration of essential oil. After 4 h of treatment, number of adults in treated and untreated halves was counted. Percent repellency (PR) was calculated using formula:

$$PR = \frac{(C - T)}{(C + T)} \times 100$$

C = Number of insects in the untreated halves
T = Number of insect in treated halves

Preference index (PI) was calculated using the following formula:

$$PI = \frac{\text{Percentage of insects in treated halves} - \text{percentage of insects in untreated halves}}{\text{Percentage of insects in treated halves} + \text{percentage of insects in untreated halves}}$$

PI values between -1.0 and -0.1 indicate repellant essential oil, - 0.1 to +0.1 neutral essential oil and + 0.1 to + 1.0 attractant essential oil.

Fumigant toxicity: Formulations of *A. sativum* and *A. marmelos* essential oils (10, 15, 20 and 25 µL mL^{-1}) were made by using acetone as solvent. Ten adults taken from the laboratory culture were placed with 2 g of wheat grains in glass petri dish (diameter 8.5 cm, height 1.2 cm). Filter paper strip (2 cm diameter) was treated with essential oil formulations and left for 2 min for evaporation of acetone. Treated filter paper was pasted on the undercover of petri dish, air tightened with parafilm and kept in dark in conditions applied for rearing of insect. Six replicates were set for each concentration of essential oil and control. After 24 and 48 h of fumigation, mortality in adults was recorded.

Contact toxicity: Formulations of *A. sativum* and *A. marmelos* essential oils (10, 15, 20 and 25 µL mL^{-1} solvent) were made in acetone, applied on bottom surface of glass petri dish (diameter 8.5 cm, height 1.2 cm) and left for 2 min for evaporation of acetone. Ten adults taken from the laboratory culture were released at the centre of petri dish, covered and kept in dark in conditions applied for rearing of insect. After 24 and 48 h of fumigation, mortality was recorded.

Oviposition inhibitory effect: Ten *S. zeamais* adults of mixed sex were fumigated with sublethal concentrations viz. 40 and 80% of 24 h LC_{50} and 48 h LC_{50} of *A. sativum* and *A. marmelos* essential oils for 24 and 48 h, respectively and reared on wheat grain in a 250 mL plastic box for 10 days. After 45 days, adults were discarded and number of F_1 progeny was counted. Six replicates were set for each concentration of essential oils and control.

Acetylcholine esterase enzyme (AChE) activity determination: *S. zeamais* adults were fumigated with two sublethal concentrations viz. 40 and 80% of 24 h LC_{50} of *A. sativum* and *A. marmelos* oils as in fumigant toxicity assay. After 24 h of fumigation, adults were used for determination of acetylcholine esterase enzyme activity[39]. Fumigated insects were homogenized in phosphate buffer saline (50 mM, pH 8) and centrifuged. Supernatant was used as the acetylcholine esterase source. To 0.1 mL of enzyme source, added 0.1 mL substrate acetyl thiocholine iodide (ATChI) (0.5 mM), 0.05 mL chromogenic reagent 5,5-Dithiobis 2-Nitrobenzoic acid (DTNB) (0.33 mM) and 1.45 mL phosphate buffer (50 mM, pH 8). Acetylcholine esterase enzyme activity was determined by measuring changes in the optical density at 412 nm by incubating the reaction mixture for 3 min at 25°C. Enzyme activity was expressed as mmol of 'SH' hydrolysed min^{-1} mg^{-1} protein.

Data analysis: Median lethal concentration (LC_{50}) was calculated using POLO programme[40]. One-way analysis of

variance (ANOVA, p<0.01), correlation and linear regression analysis were conducted to define concentration-response relationship[41].

RESULTS

Repellent activity: Maximum repellency was observed at 0.8% concentrations of *A. sativum* and *A. marmelos* essential oils (Table 1). Maximum preference index (PI) was found at 0.8% concentrations of *A. sativum* and *A. marmelos* essential oils (Table 1). *A. sativum* and *A. marmelos* essential oils showed significant (F = 198.58 for *A. sativum* and F = 201.64 for *A. marmelos* p<0.01) repellency against *S. zeamais* adults.

Fumigant toxicity: Fumigation of *A. sativum* and *A. marmelos* essential oils caused toxicity by vapour action. Median lethal concentrations (LC_{50}) were 0.297, 0.22 μL cm^{-3} and 0.312, 0.184 μL cm^{-3} air for *A. sativum* and *A. marmelos* essential oils after 24 and 48 h of exposure, respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults against *A. sativum* and *A. marmelos* essential oils (F = 257.33 for 24 h and 314.67 for 48 h for *A. sativum* essential oil and F = 213.64 for 24 h and 257.84 for 48 h for *A. marmelos* essential oil, p<0.01) (Table 3). The index of significancy of potency estimation, p-value indicates that the mean value is within the limits of all probabilities (p<0.1, 0.5 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicate that the regression is significant. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate.

Contact toxicity: *A. sativum* and *A. marmelos* essential oils caused contact toxicity in *S. zeamais* adults. Median lethal concentrations (LC_{50}) were 0.208 and 0.116 μL cm^{-2}, and 0.227 and 0.146 μL cm^{-2} area for *A. sativum* and *A. marmelos* essential oils after 24 and 48 h of exposure, respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults (F = 249.34 for 24 h and 279.87 for 48 h for *A. sativum* essential oil and F = 216.39 for 24 h and 226.51 for 48 h for *A. marmelos* essential oil, p<0.01) (Table 3).

Oviposition inhibition: Fumigation of *S. zeamais* adults with *A. sativum* and *A. marmelos* essential oils significantly reduced oviposition potential. Maximum reduction in oviposition was 43.9 and 49.63% and 22.6 and 33.27% of the control when *S. zeamais* adults were fumigated with 80% of 24 h LC_{50} and 48 h LC_{50} of *A. sativum* and *A. marmelos* essential oils, respectively (p<0.01, Table 4).

Acetylcholine esterase enzyme (AChE) activity: Fumigation of *A. sativum* and *A. marmelos* essential oils against

Table 1: Repellency of *A. sativum* and *A. marmelos* essential oils against *S. zeamais* adults

Oils	Concentration (%)	Percent repellency (PR)* Mean±SD	Preference Index** (PI)
A. sativum	0.2	79.5±2.67	-0.79
	0.4	97.68±1.34	-0.97
	0.8	100±1.84	-1.0
	1.6	100±0.0	-1.0
A. marmelos	0.2	73.8±2.38	-0.73
	0.4	93.16±0.96	-0.93
	0.8	100±0.0	-1.0
	1.6	100±0.0	1.0

*Percent repellency (PR) was calculated as: PR = (C-T)/(C+T) ×100, Where C = Number of insects in the untreated halves and T = Number of insect in treated halves, **Preference index (PI) was calculated as: PI = (percentage of insects in treated halves - percentage of insects in untreated halves)/(percentage of insects in treated halves+percentage of insects in untreated halves). PI value between -1.0 to -0.1 indicates repellant essential oil, -0.1 to +0.1 neutral essential oil and +0.1 to +1.0 attractant essential oil

Table 2: Fumigant and contact toxicity of *A. sativum* and *A. marmelos* essential oils against *S. zeamais* adults

Oils	Toxicity	Exposure period (h)	LC_{50a}	g-value	Heterogeneity	t-ratio
A. sativum	Fumigant toxicity	24	0.297	0.16	0.34	3.84
		48	0.220	0.17	0.31	3.10
	Contact toxicity	24	0.208	0.16	0.33	3.54
		48	0.116	0.17	0.32	3.97
A. marmelos	Fumigant toxicity	24	0.312	0.18	0.31	4.21
		48	0.184	0.20	0.35	3.35
	Contact toxicity	24	0.227	0.19	0.34	3.95
		48	0.146	0.18	0.30	3.34

[a]μL cm^{-3} for fumigant and μL cm^{-2} for contact toxicity

Table 3: Regression analysis of fumigant and contact toxicity of *A. sativum* and *A. marmelos* essential oils against *S. zeamais* adults

Oils	Toxicity	Exposure period (h)	Intercept	Slope	Regression equation	Correlation coefficient
A. sativum	Fumigant toxicity	24	- 4.98	3.98	Y = - 4.98+3.98X	0.98
		48	2.56	3.55	Y = 2.56+3.55X	0.99
	Contact toxicity	24	- 3.86	5.67	Y = - 3.86+5.67X	0.98
		48	5.76	4.07	Y = 5.76+4.07X	0.99
A. marmelos	Fumigant toxicity	24	- 4.96	3.98	Y = - 4.96+3.98X	0.99
		48	6.13	4.09	Y = 6.13+4.09X	0.99
	Contact toxicity	24	2.45	5.68	Y = 2.45+5.68X	0.99
		48	1.68	3.97	Y = 1.68+3.97X	0.98

Table 4: Oviposition inhibitory activities of *A. sativum* and *A. marmelos* essential oils in *S. zeamais*

Oils	Concentration	No. of progeny emerged Mean±SD	F-value** (2,15)	Concentration	No. of progeny emerged Mean±SD	F- value** (2,15)
A. sativum	Control	87.84±6.84(100%)	212.66	Control	87.84±6.84(100%)	263.9
	40% of 24h-LC_{50}	65.36±4.99(74.40)		40% of 48h-LC_{50}	42.66±5.68(48.56)	
	80% of 24h-LC_{50}	38.57±3.95(43.90)		80% of 48h-LC_{50}	19.56±2.62(22.26)	
A. marmelos	Control	87.84±6.84(100%)	198.43	Control	87.84±6.84(100%)	223.66
	40% of 24h-LC_{50}	60.84±3.61(69.26)		40% of 48h-LC_{50}	42.98±4.09(48.92)	
	80% of 24h-LC_{50}	43.60±2.34(49.63)		80% of 48h-LC_{50}	29.23±2.02(33.27)	

**F-values significant (p<0.01), Values in parentheses indicate per cent change with respect to control taken as 100%

Table 5: Effect of *A. sativum* and *A. marmelos* essential oils on acetylcholine esterase enzyme (AChE) activity in *S. zeamais* adults

Oils	Concentration	Enzyme activity* Mean±SD	F- value** (2,15)
A. sativum	Control	0.0984±0.0027 (100)	253.66
	40% of 24 h-LC_{50}	0.0646±0.0023 (65.65)	
	80% of 24 h-LC_{50}	0.0278±0.0011 (28.25)	
A. marmelos	Control	0.0984±0.0027 (100)	207.7
	40% of 24 h-LC_{50}	0.0493±0.0017 (70.42)	
	80% of 24 h-LC_{50}	0.0384±0.0012 (39.02)	

*Enzyme activity was expressed as mol of 'SH' hydrolysed $min^{-1}mg^{-1}$ protein, **F-values significant (p<0.01), Values in parentheses indicate percent change with respect to control taken as 100%

S. zeamais adults significantly reduced AChE activity. Maximum reduction in AChE activity was 28.25 and 39.02% of control when *S. zeamais* adults were fumigated with 80% of 24 h LC_{50} of *A. sativum* and *A. marmelos* essential oils, respectively (F = 253.66 for *A. sativum* and F = 207.7 for *A. marmelos* essential oils, p<0.01) (Table 5).

DISCUSSION

Essential oils of plant origin have found its wide applicability in stored grain insect pest management programmes[9-11,42-46]. Besides essential oils, its individual components have also been known for its effectiveness against insect pests. Linalool and linalyl acetate show toxicity in rice weevils[47]. Menthol, methonene, limonene, α-pipene, β-pipene, β-caryophyllene and linalool show lethality and AChE inhibitory activities[44,48]. In present study, toxic, oviposition and AChE inhibitory activities of *A. sativum* and *A. marmelos* essential oils were studied in *S. zeamais*. Both essential oils repelled *S. zeamais* adults. *A. sativum* and *A. marmelos* essential oils caused lethality in *S. zeamais* adults. Reduction in progeny production in *S. zeamais* was observed when treated with *A. sativum* and *A. marmelos* oils which may reduce damage by the insect. Similar results have been observed in *Callosobruchus chinensis* and *Tribolium castaneum*[49-51]. Fumigation with *A. sativum* and *A. marmelos* essential oils significantly reduced AChE activity in *S. zeamais* adults. Essential oil monoterpenes have been reported to interfere with AChE activity in *S. oryzae* and *T. castaneum*[50,52]. The rapid action of essential oils in insects indicates is neurotoxic mode of action. These interference with the neuromodulator octopamine[53] or GABA-gated chloride channels[54]. Several essential oil and its components act on the octopaminergic system of insects. Octopamine is a neurotransmitter, neurohormone and circulating neurohormone-neuromodulator and its disruption results in total breakdown of the nervous system[55]. Thus, the octopaminergic system of insects represents a target for insect control. Low molecular weight terpenoids are too lipophilic to be soluble in the haemolymph after crossing the cuticle and the proposed route of entry is tracheae[56]. Most insecticides bind to receptor proteins in the insect and interrupt normal neurotransmission leading to paralysis and death. Recent evidence suggests that low molecular weight terpenoids with different structures may also bind to target sites on receptors that modulate nervous activity[55].

CONCLUSION

Use of essential oils as an alternative in insect pest management programmes is a sustainable alternative as they can be obtained from nature. Essential oils can be used as contact toxicity, fumigant toxicity, repellent, oviposition inhibitory and developmental inhibitory agents. These act on various levels in the insects so possibility of generating resistance is low. Thus, *A. sativum* and *A. marmelos* essential oils can be used as an alternative of synthetic insecticides in the stored-grain insect pest management.

SIGNIFICANCE STATEMENTS

This study determined the insecticidal and acetylcholine esterase inhibitory properties of *A. sativum* and *A. marmelos* essential oils in maize weevil, *Sitophilus zeamais*. The findings of this study helps in the preparation of essential oil based formulations for the management of stored grain insect pests.

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