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Research Article
Evaluation of Insecticidal Properties of *Cuminum cyminum* and *Piper nigrum* Essential Oils against *Sitophilus zeamais*

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Abstract

Background and Objective: The increased and continuous use of synthetic insecticides develops risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenicity in non-target animal species and cross- and multi-resistance in insects. These possible consequences regarding human safety and possible environmental damage have diverted attention towards other alternatives especially the use of plant products in stored-grain insect pest management. In search of the environmentally safe green insecticides cumin (*Cuminum cyminum*) and black pepper (*Piper nigrum*) essential oils were evaluated for repellent, insecticidal, oviposition inhibitory and acetylcholine esterase enzyme inhibitory activities in maize weevil, (*Sitophilus zeamais*). **Materials and Methods:** Essential oils from cumin and black pepper were isolated by hydrodistillation method using Clevenger apparatus. *Sitophilus zeamais* (*S. zeamais*) adults were treated with both essential oils by fumigation and contact methods to determine the toxicity of essential oils. *S. zeamais* adults were treated with two sublethal concentrations of essential oils to determine its oviposition inhibitory and acetylcholine esterase enzyme (AChE) inhibitory activity (one-way ANOVA, $p<0.01$). **Results:** Both essential oils repelled *S. zeamais* adults significantly. In fumigation toxicity assay, median lethal concentrations (LC_{50}) were 0.346 and 0.253 $\mu L\ cm^{-3}$ air and 0.287 and 0.152 $\mu L\ cm^{-3}$ air for *C. cyminum* and *P. nigrum* oils after 24 and 48 h exposure period respectively. In contact toxicity assay, LC_{50} were 0.246 and 0. 185 $\mu L\ cm^{-2}$ area and 0.208 and 0.126 $\mu L\ cm^{-2}$ area for *C. cyminum* and *P. nigrum* oils after 24 and 48 h exposure period respectively. Both essential oils were found to inhibit oviposition in *S. zeamais* adults when exposed to sub-lethal concentrations. Fumigation of *S. zeamais* adults with *C. cyminum* and *P. nigrum* oils inhibited AChE activity. **Conclusion:** *C. cyminum* and *P. nigrum* oils can be used as alternative in management of stored-grain insects.

Key words: *Cuminum cyminum*, *Piper nigrum*, *Sitophilus zeamais*, acetylcholine esterase

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

INTRODUCTION

With the beginning of agricultural practices and storage of food grains, insects started damaging stored grains both qualitatively and quantitatively[1]. Several synthetic pesticides have been developed during second world war and used to protect stored grains from insect infestation. The continuous and extensive application of synthetic pesticides has developed the risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenicity in non-targets animal species and cross- and multi-resistance in insects[2-5]. This has created increased public concerns on human safety and possible environmental damage diverting attention towards other alternatives especially the use of plant products in stored-grain insect pest management. Essential oils, a class of plant origin product are highly volatile and non-persistent[6,7]. Some of these exhibit adulticidal, larvicidal and antifeedant activities, oviposition inhibitory activities, capacity to delay development and adult emergence[8-11]. About 17,500 aromatic species among higher plants belonging to families *Alliaceae*, *Apiaceae*, *Asteraceae*, *Cupressaceae*, *Lamiaceae*, *Lauraceae*, *Myrtaceae*, *Piperaceae*, *Poaceae*, *Rutaceae* and *Zingiberaceae*, only 3,000 essential oils are known[12]. These are complex mixtures of compounds of diverse chemical groups characterized by a strong odor and low density[13]. These oils are mixtures of 20-60 compounds of different chemical nature in different concentrations. Each essential oil is characterized by a specific essence due to two or three major components present in fairly high concentrations (20-70%). Biological activities of essential oils depend on their chemical composition, which, in turn, varies with plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, genotype of the plant, soil nature and environmental conditions[14,15]. Anethol, pulegone and carvacrol have been reported as the major component of cumin (*Cuminum cyminum*) oil and the insecticidal properties of cumin oil have been determined against *Callosobruchus chinensis Tetranychus cinnabarinus* and *Aphis gosoypii*[10,16,17]. All these components have been reported for their toxicity against Callosobruchus chinensis *Spodoptera litura*[18]. Insecticidal nature of black pepper, *Piper nigrum* has been determined in different insects[10,19]. Its different extracts inhibit oviposition in *Callosobruchus maculatus* and reduce the progeny emergence from the eggs[20]. The maize weevil, *Sitophilus zeamais* (*Coleptera*: *Curculionidae*) is a major pest of maize in humid tropical areas around the world where maize is grown[21,22].

Sitophilus zeamais is an internal feeder of grains and attacks both standing crops and stored cereal commodities including wheat, rice, sorghum, oats, barley, rye, buckwheat, peas and cottonseed. Females cause damage by boring into the kernel and laying eggs. The larvae and pupae eat the inner parts of the kernel, resulting in a damaged kernel and reduced grain weight[23]. Apart from weight losses, the feeding damage caused by weevils leads to severe reductions in nutritive and economic values, reduced seed viability as well as contamination by chemical excretions (silk) and insect fragments[24]. The infestation also elevates temperature and moisture content in the stored grain mass, which can lead to mold growth, including toxigenic species such as *Aspergillus flavus*[25]. *S. zeamais* cause extensive losses in quality and quantity of the grain in the field as well as in storage[26]. In the present study, insecticidal activities of *C. cyminum* and *P. nigrum* essential oils have been investigated against maize weevil *S. zeamais*.

MATERIALS AND METHODS

Essential oils: Cumin (*Cuminum cyminum*) and black pepper (*Piper nigrum*) were purchased from Gorakhpur, U.P., India. Ground powdered materials were hydrodistilled in Clevenger apparatus continuously for 5 h at 100°C to yield essential oil. The oils extracted were collected and kept in eppendorf tubes at 4°C until use. This study was carried out from April-July, 2017.

Insects: Maize weevil (*S. zeamais*) was used to determine the insecticide nature of *C. cyminum* and *P. nigrum* essential oils. The insects were reared on whole maize grain in the laboratory at 30$\pm$4°C, 75$\pm$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 *C. cyminum* and *P. nigrum* 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. Treated and untreated halves both 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[27]:

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

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

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

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

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 *C. cyminum* and *P. nigrum* essential oils (10, 15, 20 and 25 µL mL^{-1}) were made by using acetone (Rankem, RFCL Ltd., India) 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 *C. cyminum* and *P. nigrum* essential oils (10, 15, 20 and 25 µL mL^{-1} solvent) were made in acetone (Rankem, RFCL Ltd., India), 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 *C. cyminum* and *P. nigrum* 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 *C. cyminum* and *P. nigrum* oils as in fumigant toxicity assay. After 24 h of fumigation, adults were used for determination of AChE enzyme activity[28]. 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 acetylthiocholine iodide (ATChI) (0.5 mM), 0.05 mL chromogenic reagent 5,5-Dithio-bis 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.

Statistical analysis: Median lethal concentration (LC_{50}) was calculated using POLO programme[29]. One-way analysis of variance (ANOVA, p<0.01) and correlation and linear regression analysis were conducted to define concentration-response relationship[30].

RESULTS

Repellent activity: In repellency assay, highest percent repellency (PR) and preference index (PI) was recorded at 0.8 and 1.6% concentration of *P. nigrum* and *C. cyminum* essential oils respectively (Table 1). *C. cyminum* and *P. nigrum* essential oils showed significant (F = 183.86 for *C. cyminum*, and F = 265.34 for *P. nigrum* p<0.01) repellency against *S. zeamais* adults. The values of preference index (PI) for both essential oils used indicate the insect repellent properties (Table 1).

Fumigant toxicity: Fumigation of *C. cyminum* and *P. nigrum* essential oils caused toxicity by vapour action. Median lethal concentrations (LC_{50}) were 0.346 and 0.253 µL cm^{-3} air for *C. cyminum* essential oil after 24 and 48 h of exposure respectively (Table 2). Median lethal concentrations (LC_{50})

were 0.287 and 0.152 µL cm^{-3} air for *P. nigrum* essential oil after 24 and 48 h of exposure respectively (Table 2). The index of significancy of potency estimation, g-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. Regression analysis showed concentration-dependent mortality in *S. zeamais* adults against *C. cyminum* and *P. nigrum* essential oils (Table 3).

Table 1: Repellency of *C. cyminum* and *P. nigrum* essential oils against *S. zeamais* adults

Oils	Concentration (%)	Percent repellency (PR) Mean±SD	Preference index (PI)
C. cyminum	0.2	49.75±2.81	-0.49
	0.4	73.69±2.76	-0.73
	0.8	95.40±1.84	-0.95
	1.6	100.00±0.00	-1.00
P. nigrum	0.2	53.80±2.47	-0.53
	0.4	77.36±2.08	-0.71
	0.8	100.00±0.00	-1.00
	1.6	100.00±0.00	-1.00

Contact toxicity: *C. cyminum* and *P. nigrum* essential oils caused contact toxicity in *S. zeamais* adults. Median lethal concentration (LC_{50}) of *C. cyminum* essential oil was 0.246 and 0.185 µL cm^{-2} area against *S. zeamais* adults after 24 and 48 h of exposure respectively (Table 2). Median lethal concentration (LC_{50}) of *P. nigrum* essential oil was 0.208 and 0.126 µL cm^{-2} area against *S. zeamais* adults after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults against *C. cyminum* and *P. nigrum* essential oils (Table 3).

Oviposition inhibition: Fumigation of *S. zeamais* adults with *C. cyminum* and *P. nigrum* essential oils significantly reduced oviposition potential. Maximum reduction in oviposition was 50.49 and 38.99% of the control when *S. zeamais* adults were fumigated with 80% of 24 h LC_{50} of *C. cyminum* (F = 203.74, p<0.01) and *P. nigrum* (F = 246.84, p<0.01) essential oil respectively (Table 4). The same treatment with 80% of 48 h LC_{50} of *C. cyminum* (F = 254.66, p<0.01) and *P. nigrum* (F = 307.08, p<0.01) essential oils reduced oviposition to 31.50 and 22.23% of the control (Table 4).

Table 2: Fumigant and contact toxicity of *C. cyminum* and *P. nigrum* essential oils against *S. zeamais* adults

Oils	Toxicity	Exposure period (h)	LC_{50}^{a}	g-value	Heterogeneity	t-ratio
C. cyminum	Fumigant toxicity	24	0.346	0.17	0.31	3.67
		48	0.253	0.15	0.35	3.84
	Contact toxicity	24	0.246	0.19	0.32	3.66
		48	0.185	0.16	0.29	3.32
P. nigrum	Fumigant toxicity	24	0.287	0.17	0.36	4.16
		48	0.152	0.19	0.31	3.59
	Contact toxicity	24	0.208	0.20	0.32	3.73
		48	0.126	0.19	0.29	3.84

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

Table 3: Regression analysis of fumigant and contact toxicity of *C. cyminum* and *P. nigrum* essential oils against *S. zeamais* adults

Oils	Toxicity	Exposure period (h)	Intercept	Slope	Regression equation	Correlation coefficient
C. cyminum	Fumigant toxicity	24	-4.57	3.36	Y = -4.57+3.36X	0.99
		48	0.84	3.59	Y = 0.84+3.59X	0.98
	Contact toxicity	24	-0.89	4.27	Y = -0.89+4.27X	0.99
		48	4.10	4.95	Y = 4.10+4.95X	0.98
P. nigrum	Fumigant toxicity	24	-2.94	4.42	Y = -2.94+4.42X	0.99
		48	3.80	4.93	Y = 3.80+4.93X	0.99
	Contact toxicity	24	0.54	4.02	Y = 0.54+4.02X	0.98
		48	5.32	4.62	Y = 5.32+4.62X	0.98

Table 4: Oviposition inhibitory activities of *C. cyminum* and *P. nigrum* essential oils in *S. zeamais*

Oils	Concentration	Number of progeny emerged Mean±SD	F-value** (2,15)	Concentration emerged Mean±SD	Number of progeny (2,15)	F-value** (2,15)
C. cyminum	Control	86.80±7.54 (100%)	203.74	Control	86.80±7.54 (100%)	254.66
	40% of 24h-LC_{50}	73.66±5.81 (84.86)		40% of 48 h-LC_{50}	52.80±5.47 (49.07)	
	80% of 24h-LC_{50}	43.83±4.72 (50.49)		80% of 48 h-LC_{50}	27.35±4.33 (31.50)	
P. nigrum	Control	86.80±7.54 (100%)	246.84	Control	86.80±7.54 (100%)	307.08
	40% of 24h-LC_{50}	54.60±4.46 (62.90)		40% of 48 h-LC_{50}	41.70±4.11 (40.78)	
	80% of 24h-LC_{50}	29.73±3.54 (38.99)		80% of 48 h-LC_{50}	19.23±2.43 (22.23)	

Values in parentheses indicate percent change with respect to control taken as 100%

Table 5: Effect of *C. cyminum* and *P. nigrum* essential oils on acetylcholine esterase enzyme (AChE) activity in *S. zeamais* adults

Oils	Concentration	Enzyme activity* Mean±SD	F- value** (2,15)
C. cyminum	Control	0.0947±0.0029 (100)	276.84
	40% of 24 h-LC_{50}	0.0627±0.0022 (66.90)	
	80% of 24 h-LC_{50}	0.0296±0.0012 (31.59)	
P. nigrum	Control	0.0937±0.0029 (100)	301.76
	40% of 24 h-LC_{50}	0.0497±0.0019 (53.04)	
	80% of 24 h-LC_{50}	0.0366±0.0014 (39.06)	

*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%

Acetylcholine esterase enzyme (AChE) activity: *C. cyminum* and *P. nigrum* essential oils reduced AChE activity in *S. zeamais* adults significantly. Fumigation of *S. zeamais* adults with 40 and 80% of 24 h LC_{50} of *C. cyminum* essential oil reduced AChE activity to 66.9 and 31.59% of control respectively (F = 276.84, p<0.01) (Table 5). Similar treatment of *S. zeamais* adults with *P. nigrum* essential oil significantly reduced AChE activity to 53.04 and 39.06% of control (F = 301.26, p<0.01) (Table 5).

DISCUSSION

Plant derived volatiles have received much attention in the scientific community in stored grain insect pest management programme[8-11,27-33]. *Acorus calamus*, *Syzygium aromaticum*, *Hyptis spicigera*, *Ocimum canum* and *Vepris heterophyla* essential oils exhibited repellent, insecticidal activities and inhibition of progeny production in *S. oryzae*[34,35]. Essential oil components have been evaluated for their role in insect pest management programme. Linalool and linalyl acetate exhibited significant fumigant toxicity to rice weevils[36]. Menthol, methonene, limonene, α-pipene, β-pipene and linalool exhibited toxicity in *S. oryzae* and inhibited AChE activity[37]. In present study, repellant, insecticidal, oviposition and AChE inhibitory activities of *C. cyminum* and *P. nigrum* essential oils in *S. zeamais* were studied. Both essential oils showed significant repellant activity against *S. zeamais* adults. *C. cyminum* and *P. nigrum* essential oils induced high mortality in *S. zeamais* adults when treated by fumigation or contact methods. *C. cyminum* and *P. nigrum* essential oils reduced progeny production in *S. zeamais* which ultimately may reduce damage caused by the insect. Similar results have been shown in *T. castaneum* and *S. oryzae*[31-33]. Betancur *et al.*[38] have reported insecticidal property of *Peumus boldus* leaf oil against *S. zeamais*. *P. boldus* oil has shown repellent and lethal actions against *S. zeamais* adults. This oil reduced F_1 emergence in *S. zeamais* adults when fumigated. *Mentha longifolia* leaf oil has also shown similar results[39]. *C. cyminum* and *P. nigrum* essential oils have been reported for their fumigant toxicity against *T. castaneum*[17]. Both oils reduced oviposition potency of adults, pupation and adult emergence. These oils also increased developmental period of the insect[17]. In present study, fumigation of *S. zeamais* adults with *C. cyminum* and *P. nigrum* essential oils significantly reduced AChE activity. Recent researches have demonstrated the interference of monoterpenes with acetylcholinesterase activity in *S. oryzae* and *T. castaneum*[32,33]. Essential oils are lipophilic in nature and can be inhaled or ingested. The rapid action against insect pests is indicative of a neurotoxic mode of action and interference with the neuromodulator octopamine[40] or GABA-gated chloride channels[41]. Scott *et al.*[42] have reported isobutyl amide as a constituent which acts as neurotoxins in insects[42]. Several essential oil 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[43]. 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[44]. 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[43]. In conclusion, *C. cyminum* and *P. nigrum* essential oils can be used as an alternative of synthetic insecticides in the stored-grain insect pest management.

CONCLUSION

Piper nigrum and *C. cyminum* essential oils caused repellent, toxic, oviposition inhibitory and developmental inhibitory activities against *S. zeamais* adults. Thus, *P. nigrum* and *C. cyminum* essential oils can be used as an alternative of synthetic insecticides in the stored-grain insect pest management as they can be obtained from nature and are biodegradable.

SIGNIFICANCE STATEMENTS

This study established the insecticidal nature and acetylcholine esterase inhibitory activity of *C. cyminum* and *P. nigrum* essential oils in maize weevil, *S. zeamais*. The outcomes of this study help in the preparation of essential oil based formulations for stored grain insect pest management.

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