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Research Article

ROLE OF *CINNAMOMUM TAMALA* (*LAURACEAE*) AND *NIGELLA SATIVA* (*RANUNCULACEAE*) VOLATILE OILS IN MAIZE WEEVIL, *SITOPHILUS ZEAMAIS*, MOTSCHULSKY MANAGEMENT

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ABSTRACT. Synthetic insecticides used indiscriminately in insect pest management programme results in carcinogenicity, mutagenesis, neurotoxicity and teratogenicity in non-target animals and development of resistance in target animals. These issues have diverted the researches aiming insect pest management towards the use of plant volatiles. In this study, insecticidal properties of *Cinnamomum tamala* (Lauraceae) and *Nigella sativa* (*Ranunculaceae*) volatile oils have been evaluated against maize weevil, *Sitophilus zeamais* (Coleoptera: *Curculionidae*). Volatile oils were isolated and tested for repellent, toxic, oviposition inhibitory, developmental inhibitory and feeding inhibitory properties against *S. zeamais*. In toxicity assay, median lethal concentrations of *C. tamala* and *N. sativa* oils were 0.396 and 0.334 µlcm^{-3}; and 0.369 and 0.328 µlcm^{-3} air respectively when *S. zeamais* adults were fumigated for 24 and 48h. In contact toxicity assay, lethal concentrations of *C. tamala* and *N. sativa* oils were 0.287 and 0.205 µlcm^{-2}; and 0.246 and 0.195 µlcm^{-2} area for 24 and 48h respectively when *S. zeamais* adults were exposed. These two volatile oils used reduced acetylcholine esterase activity in adults when fumigated with sub-lethal concentrations. Both volatile oils significantly reduced oviposition, progeny production and feeding, but, increased developmental period in *S. zeamais*. Therefore, it can be concluded that these two oils viz. *C. tamala* and *N. sativa* oils can be used in preparation of volatile oil based formulations in insect pest management.

Keywords: *Volatile oil, insecticides, Sitophilus zeamais, oviposition inhibition, acetylcholine esterase.*

INTRODUCTION

Insect pest especially of grains under storage damage grains both qualitatively and quantitatively causing huge economic loss annually. To mitigate such losses, synthetic insecticides have been applied variously. But continuous and unlimited uses of these insecticides damage human health and environment greatly including depletion of ozone layer, neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals as well as cross- and multi resistance in target/non-target insects [1-3]. Synthetic insecticides are killing over thousands of people per year unintentionally [4]. Besides, insecticides of synthetic origin have entered in our ecosystems affecting food chain [5]. Among these insecticides, organochlorines are persistent and accumulate at population level causing mass killings of bees, fishes, amphibians, birds and mammals [6,7]. Thus,

scientific communities have diverted their researches towards the use of plant derived volatile oils as alternative of insect pest management. These volatile oils are secondary metabolites in plant families like *Alliaceae*, *Apiaceae*, *Asteraceae*, *Cupressaceae*, *Lamiaceae*, *Lauraceae*, *Myrtaceae*, *Piperaceae*, *Poaceae*, *Rutaceae* and *Zingiberaceae*. Volatile oils are complex mixtures of compounds in different proportions depending on plant parts used for extraction, method of extraction, phenological stage of plant, season of harvesting, age of plant, genotype of plant, nature of soil and conditions of environment [8-10]. Most of these oils possess larvicidal, adulticidal, antifeedant, oviposition, developmental and adult emergence inhibitory properties for a number on insects [11-15].

Cinnamomum tamala, Indian bay leaf also known as tejpat or tejapatta is a tree of *Lauraceae* family. It is native to India, Bangladesh, Bhutan, China and Nepal. Its leaves have a clove like smell. It is used mainly for culinary and medicines [16]. The volatile oil from fresh leaves contains eugenol as major constituent followed by spathulenol and eugenyl acetate while volatile oil from dried leaves contains eugenol as main constituents followed by spathulenol, methyl eugenol, β-humulene and caryophyllene [17]. *C. tamala* has known for antibacterial, antioxidant, antiulcer, anticancer and antimicrobial properties [18-24]. *C. tamala* leaves have several biological properties and act as diuretic, diaphoretic, lactagogue and hypoglycemic agents [25,26]. *C. tamala* oil is effective as hypoglycaemic and hypolipidemic agents [27].

Nigella sativa also known as black caraway, black cumin, kalonji or siyahdaneh is a herb of family *Ranunculaceae*. It is native to eastern European countries like Bulgaria and Romania and Asian countries like Cyprus, Iran, Iraq, Pakistan and Turkey. It is an important spice and drug in Indian traditional system of medicine [28]. *N. sativa* is used in lowering blood pressure, triglycerides cholesterol level [29]. It has anti-bacterial, anti-cancer, anti-dibetic, anti-convulsant activity and anti-oxidant properties [30-34]. The nigella seeds contain volatile oil (0.4-0.45 %) which has constituents like anethol, carvacrol, d-citronellol, *p*-cymene, d-limonene, nigellone, α-pinene, β-pinene, 4-terpineol, thymoquinone, thymohydroquinone, dithymoquinone and thymol and longifoline [35,36].

Sitophilus zeamais, Motschulsky (Coleoptera: *Curculionidae*) is a serious pest of maize, wheat, rice, sorghum, oats, barley, rye, buckwheat, peas and cottonseed [37]. It is commonly found in humid tropical areas of the world where maize is cultivated. Adults attack whole grains and larvae cryptically feed and develop inside grains [38]. The present study aims to evaluate repellent, insecticidal, acetylcholine esterase inhibitory, oviposition inhibitory, developmental inhibitory and antifeedant activities of *C. tamala* and *N. sativa* volatile oils against maize weevil, *S. zeamais*.

MATERIALS AND METHODS

Essential oils

Dried leaves of *C. tamala* and seeds of *N. sativa* were purchased from local market of Gorakhpur. These were grounded and hydrodistilled in Clevenger apparatus to isolate volatile oils. Oils were stored in Eppendorff tubes at 4 ℃ till further use.

Insects

Maize weevil, *S. zeamais* was cultured on whole maize grain at 28±4℃, 50±5% relative humidity (RH) and photoperiod of 10:14 (Light:Dark) hours to evaluate insecticidal properties of *C. tamala* and *N. sativa* volatile oils.

Repellent activity

Repellent activity of *C. tamala* and *N. sativa* volatile oils was assayed by filter paper disc method. Experimental solutions of *C. tamala* and *N. sativa* oils were prepared in acetone. Whatman filter papers were cut into two halves: one half was treated with test solution and the other half was treated with solvent only. Oil treated and solvent treated halves were dried to evaporate solvent, attached with cellophane tape and placed in each glass petri dish (diameter 8.5 cm, height 1.2 cm). Now, forty *S. zeamais* adults were released in petri dish, covered and kept in dark. In the experiment six replicates were set for each concentration of oil. After 4 hours of exposure, adults in treated and untreated halves were counted and percent repellency (PR) was calculated using formula: PR = [(C-T)/(C+T)] ×100, C = Number of insects in the untreated half of the filter paper and T = number of insect in treated half of the filter paper. Preference index (PI) was calculated using formula: 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).

Fumigant toxicity

Experimental solutions of *C. tamala* and *N. sativa* oils were made in acetone. Ten adults taken from laboratory culture were placed in glass petri dish and added 2 gm of maize grains. Experimental oil formulation was applied in a filter paper strip (2 cm diameter) and was left for few minutes to evaporate solvent. Now, treated paper was pasted on the inner surface of the petri dish cover, air tightened with parafilm and kept in rearing conditions in dark. For each concentration of oil and control, six replicates were set. Mortality in adults was recorded after 24 and 48h of exposure.

Contact toxicity

Experimental solutions of *C. tamala* and *N. sativa* oils were made in acetone. Experimental oil solution was applied on the inner surface of glass petri dish (diameter 8.5 cm, height 1.2 cm). Petri dish was left open for few minutes to evaporate solvent completely. Now, ten *S. zeamais* adults were released in petri dish, covered and kept in rear conditions in dark. For each concentration of oil and control, six replicates were set. Mortality in adults was recorded after 24 and 48h of exposure.

Acetylcholine esterase activity

S. zeamais adults were fumigated with 40 and 80% of 24h-LC_{50} of *C. tamala* and *N. sativa* oils for 24 hours. After fumigation, adult insects were collected and used for acetylcholine esterase enzyme activity estimation [39]. The experimental adult insects were homogenized in phosphate buffer saline (50mM, pH8), centrifuged and supernatant collected was used as enzyme source for estimation of acetylcholine esterase activity. To 0.1ml of enzyme source, 0.1ml acetylthiocholine iodide (0.5 mM), 0.05 ml, 5,5-Dithio-bis 2-nitrobenzoic acid (0.33 mM) and 1.45 ml phosphate

buffer (50 mM, pH 8) were added. The reaction mixture was incubated for 3 min at 25℃. Now, changes in absorbance of reaction mixture were measured at 412nm for the determination of acetylcholine esterase acticity. Acetylcholine esterase activity was estimated in terms of mmol of 'SH' hydrolysed $min^{-1}mg^{-1}$ protein.

Oviposition inhibition

In this assay**,** *S. zeamais* adults of mixed sex were taken from the laboratory culture and fumigated with 40 and 80% of 24h-LC_{50} and 48h-LC_{50} of *C. tamala* and *N. sativa* oils for 24 hours and 48 hours respectively. After fumigation, the treated adults were reared on maize grain. After 10 days, discarded adults and counted the number of F_1 progeny after 45 days. Six replicates were set for each concentration of oil as well as control. The percentage oviposition deterrence (POD) was calculated using the formula [40]: Percentage oviposition deterrence (POD) = $[(E_C-E_T)/E_C] \times 100$, where E_C = number of adults emerged in control and E_T = number of adults emerged in test.

Developmental inhibition

Ten *S. zeamais* adults of mixed sex were placed in a 250 ml plastic container with 20 gm of maize grains and allowed to mate and lay eggs for seven days in condition applied for rearing of insects. Now, adults were removed and a filter paper strip (2 cm diameter) impregnated with *C. tamala* and *N. sativa* oil was pasted on undercover of the container. The eggs and juveniles were fumigated with three concentrations (0.2, 0.4 and 0.6 μlcm^{-3}) of *C. tamala* and *N. sativa* oils. The duration from the end of fumigation till the emergence of F_1 adults and the number of adults emerged in control as well as in test was recorded. Six replicates were set for each concentration of oil and control. Inhibition rate (IR) was calculated using formula [41]: Inhibition rate (IR) = $[(Cn-Tn)/Cn] \times 100$, where Cn = number of adults emerged in control and Tn = number of adults emerged in test.

Antifeedant activity

For the determination of antifeedant activity of *C. tamala* and *N. sativa* oils, flour discs were prepared by mixing 10 gm of maize flour with 50 ml water until completely suspended. Maize flour suspension was pipetted out (200 µl) onto a plastic sheet, held for 24 hours at room temperature and then dried in an oven for one hour at 60^0 C. Each flour disc was treated with 40 and 80% of 96h-LC_{50} of *C. tamala* and *N. sativa* oil, weighed, placed in glass petri dish and twenty-five *S. zeamais* adults were introduced into it and allowed to feed. After four days of feeding, flour discs were taken and reweighed to calculate antifeedant activity (AFA) using formula [42]: AFA = $[C-T/C] \times 100$, where C = consumption of food in control group and T = consumption of food in treated group. Six replicates were set for each concentration of oil and control.

Statistical analysis

Median lethal concentration was estimated by POLO programme [43]. One-way analysis of variance and correlation and regression were conducted to study concentration-response relationship [44]. Tukey's test was performed to detect the significant difference between means [45].

RESULTS AND DISCUSSION

Repellent activity

Percent Repellency (PR) and Preference Index (PI) were increased with increase in the concentration of *C. tamala* and *N. sativa* oils and were recorded maximum at 0.8% concentrations (Table1).

Table 1. *Repellent activity of C. tamala and N. sativa volatile oils against S. zeamais* adults

Oil	Concentration (%)	Percent Repellency (PR)* Mean±SD	Preference Index (PI)**
C. tamala	0.1	22.50±1.04	-0.22
	0.2	48.52±0.93	-0.48
	0.4	72.50±1.09	-0.72
	0.8	98.35±0.08	-0.98
N. sativa	0.1	15.80±0.98	-0.15
	0.2	35.80±0.75	-0.35
	0.4	64.25±0.84	-0.64
	0.8	90.80±0.07	-0.90

**Percent repellency (PR) was calculated using formula: PR = [(C-T)/(C+T)] ×100, C = number of insects in the untreated halves and T = number of insect in treated halves; **Preference index (PI) was calculated using formula: 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 oil, -0.1 to +0.1 neutral oil and +0.1 to +1.0 attractant oil.*

Fumigant toxicity

Median lethal concentrations were recorded 0.396 and 0.334 µlcm^{-3} air for *C. tamala* oil after 24 and 48 hours exposure period respectively (Table 2). On the other hand, LC_{50} values were 0.369 and 0.328 µlcm^{-3} air for *N. sativa* oil after 24 and 48 hours exposure period respectively (Table 2). The index of significancy of potency estimation, g-value indicates that the mean value is within the limits of all probability levels (P<0.1, 0.5 and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicated that regression was 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 as lethality was found to increase with increase in concentration of oils (Table 2).

Contact toxicity

Median lethal concentrations were 0.287 and 0.205 µlcm^{-2}; and 0.246 and 0.195 µlcm^{-2} area for *C. tamala* and *N. sativa* essential oils after 24 and 48 hours exposure period respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults by *C. tamala* and *N. sativa* oil (Table 2).

***Table 2**. Fumigant and contact toxicity of C. tamala and N. sativa volatile oils against S. zeamais adults*

Oil	Toxicity	Exposure period (h)	LC_{50}*	g-value	Heterogeneity	t-ratio	Regression Equation	Correlation Coefficient
C. tamala	Fumigant toxicity	24	0.396	0.20	0.33	3.78	Y = - 3.87+5.87X	0.98
		48	0.334	0.15	0.35	4.67	Y = 5.23+2.87X	0.99
	Contact toxicity	24	0.287	0.28	0.34	4.19	Y = - 4.05+2.86X	0.98
		48	0.205	0.23	0.33	3.87	Y = 9.08+5.31X	0.99
N. sativa	Fumigant toxicity	24	0.369	0.29	0.35	3.93	Y = - 7.46+7.91X	0.99
		48	0.328	0.27	0.34	4.18	Y = 6.84+3.54X	0.98
	Contact toxicity	24	0.246	0.24	0.32	4.79	Y = - 7.98+6.54X	0.99
		48	0.195	0.26	0.35	3.83	Y = 7.43+6.43X	0.99

**μlcm^{-3} for fumigant toxicity and μlcm^{-2} for contact toxicity*

Acetylcholine esterase enzyme activity

Fumigation of *S. zeamais* adults with 40 and 80% of 24h-LC_{50} of *C. tamala* oil and *N. sativa* oil reduced acetylcholine esterase activity to 82.38 and 55.97%; and 74.73 and 50.83% of control respectively (Table 3). Both *C. tamala* and *N. sativa* oils significantly inhibited activity of acetylcholine esterase enzyme in *S. zeamais* adults (For *C. tamala* oil F=106.43; For *N. sativa* oil F = 120.00; $P<0.05$; Table 3). Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant ($P<0.05$) difference between all the treated and control groups (Table 4).

***Table 3**. Effect of C. tamala and N. sativa volatile oils on acetylcholine esterase activity in S. zeamais*

Oil	Concentration	Enzyme activity* (Mean±SD)	F-value (df = 2,15)
C. tamala	Control	0.0954±0.0029(100)	106.43**
	40% of 24h-LC_{50}	0.0768±0.00024(82.38)	
	80% of 24h-LC_{50}	0.0534±0.0016(55.97)	
N. sativa	Control	0.0954±0.0029 (100)	120.00**
	40% of 24h-LC_{50}	0.0713±0.0022(74.73)	
	80% of 24h-LC_{50}	0.0485±0.0015(50.83)	

**mmol of 'SH' hydrolysed $min^{-1}mg^{-1}$ protein; Values in parentheses indicate per cent change with respect to control taken as 100%; **Significant at $P<0.05$ (df = 2,15)*

***Table 4**. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of effect of C. tamala and N. sativa volatile oils on acetylcholine esterase activity in S. zeamais*

Oil	Pair wise comparison		HSD0.05 = 0.0074 HSD0.01 = 0.0098	Q0.05 = 3.6734 Q0.01 = 4.8359
	T1:T2	M1 = 0.10 M2 = 0.07	0.02	Q = 11.37*
C. tamala	T1:T3	M1 = 0.10 M3 = 0.06	0.04	Q = 20.61*
	T2:T3	M2 = 0.07 M3 = 0.06	0.02	Q = 9.24*
	T1:T2	M1 = 0.10 M2 = 0.07	0.03	Q = 13.43*
N. sativa	T1:T3	M1 = 0.10 M3 = 0.07	0.05	Q = 21.70*
	T2:T3	M2 = 0.07 M3 = 0.05	0.02	Q = 8.27*

**Significant (P<0.05) difference between all the treated and control groups.*

Oviposition inhibition

Fumigation of *S. zeamais* adults with *C. tamala* and *N. sativa* oils reduced oviposition capacity of insects (Table 5). Oviposition was reduced to 63.47 and 58.56% of control respectively when *S. zeamais* adults were fumigated with 80% of 24h-LC_{50} of *C. tamala* and *N. sativa* oils (For *C. tamala* oil F = 186.95; For *N. sativa* oil F = 173.62; P<0.05; Table 5). Percent oviposition deterrence was recorded 40.33 and 41.14% when *S. zeamais* adults were fumigated with 80% of 24h-LC_{50} of *C. tamala* and *N. sativa* oils respectively (Table 5).

***Table 5.** Oviposition inhibitory activities of C. tamala and N. sativa volatile oils in S. zeamais*

Oil	Conc.	No. of progeny emerged (Mean±SD)	POD*	F-value	Conc.	No. of progeny emerged (Mean±SD)	POD*	F-value
	Control	94.00±3.52 (100%)	-		Control	94.00±3.52 (100%)	-	
C. tamala	40% of 24h-LC_{50}	72.67±2.34 (78.37)	22.63	186.95**	40% of 48h-LC_{50}	55.00±2.13 (58.51)	41.49	163.26**
	80% of 24h-LC_{50}	59.67±2.23 (63.47)	40.33		80% of 48h-LC_{50}	39.33±1.20 (41.84)	58.16	
	Control	94.00±3.52 (100%)	-		Control	94.00±3.52 (100%)	-	
N. sativa	40% of 24h-LC_{50}	69.33±2.12 (73.75)	26.25	173.62**	40% of 48h-LC_{50}	53.00±2.08 (56.38)	43.62	177.28**
	80% of 24h-LC_{50}	55.33±2.17 (58.86)	41.14		80% of 48h-LC_{50}	39.50±1.07 (42.02)	57.98	

*Values in parentheses indicate per cent change with respect to control taken as 100%; *Percentage of oviposition deterrence (POD) = [(EC-ET)/EC] ×100; EC = number of adults emerged in control and ET = number of adults emerged in test; **Significant at P<0.05 (df = 2,15)*

Similarly, reduction in oviposition was 41.84 and 42.02% of control when *S. zeamais* adults were fumigated with 80% of 48h-LC_{50} of *C. tamala* and *N. sativa* oils respectively (For *C. tamala* oil F = 163.26; For *N. sativa* F = 177.28) (Table 5). Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant ($P<0.05$) difference between all the treated and control groups (Table 6).

Table 6. *Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of oviposition inhibitory activities of C. tamala and N. sativa volatile oils in S. zeamais*

Oil	**Exposure period 24h**			**Exposure period 48h**				
	Pair wise comparison	$HSD_{0.05}$ = 6.6693 $HSD_{0.01}$ = 8.7799	$Q_{0.05}$ = 3.6734 $Q_{0.01}$ = 4.8359	Pair wise comparison		$HSD_{0.05}$ = 7.3195 $HSD_{0.01}$ = 9.6359	$Q_{0.05}$ = 3.6734 $Q_{0.01}$ = 4.8359	
C. tamala	T_1:T_2	M_1 = 94.00 M_2 = 72.67	21.33	Q = 11.75*	T_1:T_2	M_1 = 94.00 M_2 = 55.00	39.00	Q = 19.57*
	T_1:T_3	M_1 = 94.00 M_3 = 59.67	34.33	Q = 18.91*	T_1:T_3	M_1 = 94.00 M_3 = 39.33	54.67	Q = 27.44*
	T_2:T_3	M_2 = 72.67 M_3 =59.67	13.00	Q = 7.16*	T_2:T_3	M_2 = 55.00 M_3 = 39.33	15.67	Q = 7.86*
	Pair wise comparison	HSD0.05 = 8.1099 HSD0.01 = 10.6764	Q0.05 = 3.6734 Q0.01 = 4.8359		Pair wise comparison		HSD0.05 = 7.9684 HSD0.01 = 10.4902	Q0.05 = 3.6734 Q0.01 = 4.8359
N. sativa	T_1:T_2	M_1 = 94.00 M_2 = 69.33	24.67	Q = 11.17*	T_1:T_2	M_1 = 94.00 M_2 = 53.00	41.00	Q = 18.90*
	T_1:T_3	M_1 = 94.00 M_3 = 55.33	38.67	Q = 17.51*	T_1:T_3	M_1 = 94.00 M_3 = 39.50	54.50	Q = 25.12*
	T_2:T_3	M_2 = 69.33 M_3 = 55.33	14.00	Q = 6.34*	T_2:T_3	M_2 = 53.00 M_3 = 39.50	13.50	Q = 6.22*

**Significant ($P<0.05$) difference between all the treated and control groups*

Developmental inhibition

Progeny production was reduced to 51.27 and 48.43% as compared to the control when fumigated with 0.6 µlcm^{-3} of *C. tamala* and *N. sativa* oils respectively (Table 7). Both *C. tamala* and *N. sativa* oils slow down the developmental progression of *S. zeamais* larva and pupa (Table 7). The duration of larval phase to adult emergence was increased significantly to 120.25% and 128.27% as compared to the control when fumigated with 0.6 µlcm^{-3} of *C. tamala* and *N. sativa* essential oils respectively (For *C. tamala* oil F = 34.65; For *N. sativa* 48.79; P<0.01; Table 7). Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (P<0.05) difference between all the treated and control groups (Table 8).

Table 7. *Effect of C. tamala and N. sativa volatile oils on development of S. zeamais*

Oil	Conc.	No. of progeny emerged (Mean±SD)	PR*	F-value	Duration of developmental period in days (Mean±SD)	F-value
C. tamala	Control	85.17±6.01 (100)	-	59.11**	24.83±0.54 (100)	34.65**
	0.2 µlcm^{-3}	69.83±3.18 (81.98)	18.02		26.50±0.96 106.59)	
	0.4 µlcm^{-3}	56.67±2.68 (66.53)	33.47		28.33±0.76 (114.09)	
	0.6 µlcm^{-3}	43.67±1.47 (51.27)	48.73		29.86±0.47 (120.25)	
N. sativa	Control	85.17±6.01 (100)	-	65.82**	24.83±0.54 (100)	48.79**
	0.2 µlcm^{-3}	67.00±3.03 (78.66)	21.34		27.00±0.69 (108.73)	
	0.4 µlcm^{-3}	53.88±2.26 (63.26)	36.74		29.00±0.57 (116.65)	
	0.6 µlcm^{-3}	41.00±2.05 (48.13)	51.87		32.00±0.34 (128.87)	

*Values in parentheses indicate per cent change with respect to control taken as 100%; *Inhibition rate (IR) = [(Cn-Tn)/Cn] ×100, Cn = number of adults emerged in control and Tn = number of adults emerged in test; **Significant at P<0.05 (df = 3,20)*

Table 8*. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of effect of C. tamala and N. sativa volatile oils on development of S. zeamais*

Oil	Progeny emergence				Developmental period			
	Pair wise comparison		$HSD_{0.05}$ = 9.1580 $HSD_{0.01}$ = 11.6097	$Q_{0.05}$ = 3.9583 $Q_{0.01}$ = 5.0180	Pair wise comparison		$HSD_{0.05}$ = 1.4842 $HSD_{0.01}$ = 1.8773	$Q_{0.05}$ = 3.9419 $Q_{0.01}$ = 4.9859
C. tamala	T_1:T_2	M_1 = 85.17 M_2 = 69.83	15.33	Q = 6.63*	T_1:T_2	M_1 = 24.83 M_2 = 26.50	1.67	Q = 4.43*
	T_1:T_3	M_1 = 85.17 M_3 = 56.67	28.50	Q = 12.32*	T_1:T_3	M_1 = 24.83 M_3 = 28.33	3.50	Q = 9.30*
	T_1:T_4	M_1 = 85.17 M_4 = 43.67	41.50	Q = 17.94*	T_1:T_4	M_1 = 24.83 M_4 = 29.86	5.02	Q = 13.34*
	T_2:T_3	M_2 = 69.83 M_3 = 56.67	13.17	Q = 5.69*	T_2:T_3	M_2 = 26.50 M_3 = 28.33	1.83	Q = 4.87*
	T_2:T_4	M_2 = 69.83 M_4 = 43.67	26.17	Q = 11.31*	T_2:T_4	M_2 = 26.50 M_4 = 29.86	3.36	Q = 8.92*
	T_3:T_4	M_3 = 56.67 M_4 = 43.67	13.00	Q = 5.62*	T_3:T_4	M_3 = 28.33 M_4 = 29.86	1.52	Q = 4.05*

**Significant (P<0.05) difference between all the treated and control groups.*

***Table 8**. Continues*

Oil	Progeny emergence				Developmental period			
	Pair wise comparison	$HSD_{0.05}$ = 9.9424 $HSD_{0.01}$ = 12.6359	$Q_{0.05}$ = 3.9766 $Q_{0.01}$ = 5.0539		Pair wise comparison	$HSD_{0.05}$ = 1.7266 $HSD_{0.01}$ = 2.1889	$Q_{0.05}$ = 3.9583 $Q_{0.01}$ = 5.0180	
N. sativa	T_1:T_2	M_1 = 85.17 M_2 = 68.33	16.80	Q = 6.73*	T_1:T_2	M_1 = 24.83 M_2 = 27.00	2.17	Q = 4.97*
	T_1:T_3	M_1 = 85.17 M_3 = 54.33	30.83	Q = 12.33*	T_1:T_3	M_1 = 24.83 M_3 = 29.00	4.17	Q = 9.55*
	T_1:T_4	M_1 = 85.17 M_4 = 42.60	42.57	Q = 17.03*	T_1:T_4	M_1 = 24.83 M_4 = 32.00	7.17	Q = 16.43*
	T_2:T_3	M_2 = 68.33 M_3 = 54.33	14.00	Q = 5.60*	T_2:T_3	M_2 = 27.00 M_3 = 29.00	2.00	Q = 4.58*
	T_2:T_4	M_2 = 68.33 M_4 = 42.60	25.73	Q =10.29*	T_2:T_4	M_2 = 27.00 M_4 = 32.00	5.00	Q = 11.46*
	T_3:T_4	M_3 = 54.33 M_4 = 42.60	11.73	Q = 4.69*	T_3:T_4	M_3 = 29.00 M_4 = 32.00	3.00	Q = 6.88*

**Significant (P<0.05) difference between all the treated and control groups.*

Antifeedant activity

Both *C. tamala* and *N. sativa* oils inhibited feeding in *S. zeamais* adults (Table 9). Both oils decreased consumption of flour disc by *S. zeamais* adults. Antifeedant activity was reduced to 75.47 and 79.36% with respect to control at 80% of 96h-LC_{50} of *C. tamala* and *N. sativa* oil respectively (For *C. tamala* oil F = 261.48; For *N. sativa* F = 703.46; P<0.01; Table 9) Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (P<0.05) difference between all the treated and control groups (Table 10).

***Table 9**. Antifeedant activity of C. tamala and N. sativa volatile oils against S. zeamais*

Concentration	***C. tamala***		***N. sativa***	
	Consumption of flour disc (mg) (Mean±SD)	**AFA***	**Consumption of flour disc (mg) (Mean±SD)**	**AFA***
Control	11.29±0.15 (100)	-	11.29±0.15 (100)	-
40% of 96h-LC_{50}	6.49±0.24 (57.48)	42.51	5.60±0.23 (49.61)	50.39
80% of 96h-LC_{50}	2.77±0.12 (24.53)	75.46	2.33±0.12 (20.64)	79.36
	F = 261.48**		F = 703.46**	

*Values in parentheses indicate per cent change with respect to control taken as 100%; *Antifeedant activity was calculated using AFA = [C-T/C] × 100; Where, C = consumption of flour disc in control group, and T = consumption of flour disc in treated group. Six replicates were set for each concentration of oil and control; **Significant at P<0.05 (df = 2, 15)*

Table 10. *Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of antifeedant activity of C. tamala and N. sativa volatile oils against S. zeamais*

Oil	Pair wise comparison		$HSD_{0.05}$ = 0.9700 $HSD_{0.01}$ = 1.2769	$Q_{0.05}$ = 3.6734 $Q_{0.01}$ = 4.8359
C. tamala	T_1:T_2	M_1 = 11.29 M_2 = 6.49	4.80	Q = 18.18*
	T_1:T_3	M_1 = 11.29 M_3 = 2.77	8.52	Q = 32.25*
	T_2:T_3	M_2 = 6.49 M_3 = 2.77	3.72	Q = 14.07*
N. sativa	T_1:T_2	M_1 = 11.29 M_2 = 5.60	5.69	Q = 33.29*
	T_1:T_3	M_1 = 11.29 M_3 = 2.33	8.96	Q = 52.41*
	T_2:T_3	M_2 = 5.60 M_3 = 2.33	3.27	Q = 19.12*

**Significant (P<0.05) difference between all the treated and control groups.*

Volatile oils of plant origin have been well known for their insecticidal properties against several insect pests including those of stored grains [46-52]. These volatile oils show repellent, contact toxicity, fumigant toxicity, oviposition inhibitory and developmental inhibitory activities against a variety of coleopteran insect of stored grains including rice weevil, *S. zeamais*. These volatile oils cause mortality in insects as these have been reported to inhibit activity of acetylcholine esterase [53,54]. Besides crude and complete oils, its individual constituents also cause repellency, contact toxicity, fumigant toxicity, oviposition inhibition and developmental inhibition in insects. Linalool, linalyl acetate, menthol, methonene, limonene, α-pipene, β-pipene, β-caryophyllene and linalool have been shown to cause toxicity in *S. zeamais* [46,55].

In this study, repellent, toxic, oviposition inhibitory, developmental inhibitory and feeding inhibitory activities of *C. tamala* and *N. sativa* oils were studied in *S. zeamais*. Both *C. tamala* and *N. sativa* oils repel and cause mortality in *S. zeamais* adults. The rapid mortality caused shows neurotoxic mode of action of the two oils under investigation Similar results have also been obtained with *Piper cubeba*, *Zingiber officinale* and other oils in *S. zeamais* adults [56]. Several other volatile oils and its pure compounds have also been reported to inhibit acetylcholine esterase activity causing paralysis and death in insects [46,53,54]. These oils interfere with neuromodulator octopamine or GABA-gated chloride channels [57,58]. The octopaminergic system of insects uses octopamine as neurotransmitter, neurohormone and neurohormone-neuromodulator. Disruption in its activity breaks down the nervous system in insects [59].

Oviposition and progeny production was reduced *S. zeamais* when fumigated with *C. tamala* and *N. sativa*. Volatile oils reduce oviposition potency of insects probably by disrupting mating and sexual communication in *S. zeamais* adults. These two volatile oils inhibit development of juvenile phases and increase developmental period in *S. zeamais*. Reduced adult emergence may be due to the death of egg and larval phases while delay in development may be due to inhibition of metabolic processes or disturbances in hormonal networking and tissue responsiveness. Both *C. tamala* and *N. sativa* oils reduce feeding potency in *S. zeamais*. This reduction may be due to repellent behaviour of these oils. Similar results have been obtained with *Allium sativum*, *Anthum graveolense*, *P. cubeb* and *Z. officinale* oils in *S. zeamais* [56,60]. The rapid mode of action of the two

oils under investigation shows their low persistence in the environment. The persistence of the insecticidal activity depends on the chemical composition of volatile oils and nature and position of the functional groups in oil constituents [61]. Volatile oils possessing high content of hydrogenated compound loss their activity as compared to those containing high content of oxygenated compounds [62,63].

Further studies have also been carried out to explore insecticidal activity of oil's constituents and its role in antagonistic and synergistic relationship [64,65]. It must be kept in mind that oils/constituents should be effective against targets only but not against non-targets including humans. Issues like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity should also be considered and evaluated for compatible use of volatile oils for insect pest management.

CONCLUSION

The present study reports that *C. tamala* and *N. sativa* oils possess repellant, toxic as well as oviposition, developmental and feeding inhibitory activities against *S. zeamais*. These oils act at multiple target sites which minimize the possibility of resistance development. Since *C. tamala* and *N. sativa* are the parts of our food, these oils are safe for humans. For these reasons, *C. tamala* and *N. sativa* oils can be considered as a natural alternative in the management of stored grains insects.

Conflict of Interest. The authors declared that there is no conflict of interest.

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