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### INSECTICIDAL ACTIVITIES OF *CINNAMOMUM TAMALA* (LAURACEAE) ESSENTIAL OIL AGAINST *SITOPHILUS ORYZAE* L. (COLEOPTERA: CURCULIONIDAE)

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#### ABSTRACT

*Cinnamomum tamala* (Lauraceae) essential oil and its major constituent, eugenol were evaluated for repellent, insecticidal, feeding inhibitory, oviposition inhibitory and acetylcholinesterase enzyme inhibitory activities in rice weevil, *Sitophilus oryzae*. In repellency assay, *C. tamala* oil and eugenol repelled *S. oryzae* adults significantly at 0.2% concentration. Essential oil of *C. tamala* and eugenol caused fumigant and contact toxicity in *S. oryzae* adults. In fumigation toxicity assay, median lethal concentrations (LC<sub>50</sub>) were found 0.249 and 0.198  $\mu\text{l}/\text{cm}^3$ ; and 0.167 and 0.152  $\mu\text{l}/\text{cm}^3$  of *C. tamala* oil and eugenol after 24 and 48 h exposure of *S. oryzae* adults respectively. In contact toxicity assay, median lethal concentrations (LC<sub>50</sub>) were found 0.241 and 0.218  $\mu\text{l}/\text{cm}^2$ ; and 0.185 and 0.126  $\mu\text{l}/\text{cm}^2$  of *C. tamala* oil and eugenol after 24 and 48 h exposure of *S. oryzae* adults respectively. Essential oil of *C. tamala* and eugenol showed feeding deterrent activity and oviposition inhibition activity in *S. oryzae* adults when exposed to sub-lethal concentrations. Fumigation of *S. oryzae* with *C. tamala* oil and eugenol inhibited acetylcholinesterase enzyme (AChE) activity. This study concludes that *C. tamala* and its components can be used as alternative in management of stored-grain insects.

**Keywords:** *Cinnamomum tamala*, essential oil, *Sitophilus oryzae*.

#### INTRODUCTION

With the beginning of agricultural practices, storage of food grains started as a safeguard against poor harvests and famine. Since then, insects also started damaging stored grains both qualitatively and quantitatively. To protect stored grains from insect infestation, several synthetic pesticides have been used, but, these synthetic pesticides have increased the risk of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects among non-target species and cross-resistance and multi resistance in insects (WMO, 1991; Lu, 1995; UNEP, 2000; Beckel et al., 2002). This has led to increased public awareness on human safety and possible environmental damage diverting attention towards plant products especially volatile chemicals in stored-grain insect pest management. Essential oils are highly volatile and non-persistent. Some of these exhibit adulticidal, larvicidal and antifeedant activity, capacity to

delay development, adult emergence and fertility, and have deterrent effects on oviposition (Callero-Gallardo et al., 2011; Isman et al., 2011; Liu et al., 2011; Stefanazzi et al., 2011). There are 17,500 aromatic species among higher plants belonging to families Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Myrtaceae, Piperaceae, Poaceae, Rutaceae and Zingiberaceae (Bakkali et al., 2008). Of 3,000 essential oils known, 10% have commercial importance in cosmetic, food and pharmaceutical industries (Bakkali et al., 2008). These are natural, complex, secondary metabolites characterized by a strong odour and low density (Bruneton, 1999). These contain 20 to 60 compounds in different concentrations and characterized by two or three major components at fairly high concentrations (20 to 70%). The components include two groups of distinct biosynthetic origin. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight (Bakkali et al., 2008). Biological activities of essential oils depend on

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their chemical composition, which, in turn, varies with plant parts used for extraction, extraction method, plant phenological stage, harvesting season, plant age, soil nature and environmental conditions (Masotti et al., 2003; Angioni et al., 2006). *Cinnamomum tamala* belonging to family Lauraceae is a tree native to India, Bangladesh, Nepal, Bhutan and China. It has aromatic leaves called tejpatta which are used for culinary and medicinal purposes. Historically, it is one of the oldest known and used spices. It is mainly used for flavouring food and in pharmaceutical preparation because of its hypoglycaemic, stimulant and carminative properties (Hussain et al. 1980). Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhoea, nausea and vomiting. Ancient literature has revealed that in the first century A.D, dried leaves and bark of this plant were prescribed for fever, anaemia and body odour. Its seeds were crushed and mixed with honey or sugar and administered to children for dysentery or cough (Edwards, 1993). Essential oil of *C. tamala* leaves has excellent inhibitory effects on bacteria (Minakshi et al., 1999). Due to its aroma, the leaves are kept in clothes and also chewed to disguise bad mouth odour. The oil of *C. tamala* is used in fever, fungus disease of skin, fractures, eye disease, foul odour of body, diseases of oral cavity, herpes and in disorders of breast milk. The oil is extensively used as fragrance component in soaps, detergents, cosmetics, perfumes, toothpastes, and industrial fragrances (Jantan and Goh, 1990). Hydrodistillation of *C. tamala* leaves yields 1.2% colourless essential oil. The major components of the oil have been found to be Eugenol (60.2%),  $\alpha$ -Phellandrene (11.7%),  $\beta$ -Phellandrene (7.2%),  $\alpha$ - Pinene (2.8%), Elixene (1.8%), cis-Caryophyllene (1.6%), Myrcene (1.5%) and Limonene (1.4%) (Vishwam, 2015). In rural area, *C. tamala* leaves have been used to protect store grains from insect infestation since time immemorial. Eugenol (1-allyl-4-hydroxy-3-methoxybenzene) is a phenylpropene, an allyl chain-substituted guaiacol. It is a colourless to pale yellow oily liquid extracted from certain essential oils. Eugenol is used in perfumes, flavourings, and essential oils. It is also as a local antiseptic and anaesthetic (Jadhav et al., 2004). Attempts have been made to develop eugenol derivatives as intravenous anesthetics, as an alternative to propanidid which produces unacceptable side effects around the site of injection in many patients (Right and

Payne, 1962). It can be used to reduce the presence of *Listeria monocytogenes* and *Lactobacillus sakei* in food (Gill and Holley, 2004). In the present study, insecticidal activities of *C. tamala* leaves essential oil and its major component, eugenol has been investigated.

#### MATERIAL AND METHODS

***C. tamala* oil:** *C. tamala* leaves were purchased from Gorakhpur, U.P., India. Ground leaves were hydrodistilled in Clevenger apparatus continuously for 5 h at 100°C to yield essential oil. The oil extracted was collected and kept in Eppendorf tubes at 4°C until use.

**Pure compound:** Eugenol was purchased from Sigma Chemical Company, USA.

**Insects:** Rice weevil, *S. oryzae* was used to determine the insecticide nature of *C. tamala* essential oil. The insects were reared on whole wheat grain in the laboratory at 28±4°C, 75±5% RH, and photoperiod of 10:14 (L:D) hours.

**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. tamala* essential oil and eugenol 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. oryzae* 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:  $PR = (C-T)/(C+T) \times 100$ , C = number of insects in the untreated halves and T = number of insect in treated halves.

Preference index (PI) was calculated using the following formula:  $PI = (\text{percentage of insects in treated halves} - \text{percentage of insects in untreated halves}) / (\text{percentage of insects in treated halves} + \text{percentage of insects in untreated halves})$ . PI values between - 1.0 and - 0.1 indicate repellent essential oil, - 0.1 to + 0.1 neutral essential oil and + 0.1 to + 1.0 attractant essential oil.

**Fumigant toxicity:** Formulations of different dilutions of essential oil/eugenol (10, 15, 20 and 25  $\mu\text{l/ml}$  of solvent and 4, 8, 12 and 16  $\mu\text{l/ml}$  for eugenol) was made 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 *C. tamala* essential oil/eugenol formulations and left for two minutes 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/eugenol and control. After 24 and 48 h of fumigation, mortality in adults was recorded.

**Contact toxicity:** Formulations of different dilutions of essential oil/eugenol (10, 15, 20 and 25  $\mu\text{l/ml}$  solvent for *C. tamala*; 4, 6, 8, 10  $\mu\text{l/ml}$  solvent for eugenol) were made in acetone, applied on bottom surface of glass petri dish (diameter 8.5 cm, height 1.2 cm) and left for two minutes 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. Six replicates were set for each concentration of essential oil/eugenol. After 24 and 48 h of fumigation, mortality in adults was recorded.

**Antifeedant activity (AFA):** Antifeedant activity of *C. tamala* essential oil and eugenol was studied using flour disks. Flour disks were prepared by mixing 10 g wheat flour with 50 ml water until completely suspended. Wheat flour suspension was pipetted (200  $\mu\text{l}$ ) onto a plastic sheet, held for 24 h at room temperature and dried in oven at 60°C for 1 h. Flour disks were weighed between 70-76 mg each. Flour disk was treated with 2, 4, 6 and 8  $\mu\text{l}$  of *C. tamala* essential oil/eugenol, weighed, placed in glass petri dish and released twenty-five adults in each petri dish. Insects were allowed to feed and flour disks were reweighed after 4 days. Six replicates were set for each concentration of essential oil/eugenol and control. Antifeedant activity (AFA) was calculated using the following formula:  $\text{AFA} = \frac{[C-T]}{C} \times 100$ , C = consumption of flour disk in control group, and T = consumption of flour disc in treated group. The analysis of variance (ANOVA) was performed to test the significant antifeedant activity of *C. tamala* essential oil and eugenol in insect (Sokal and Rohlf, 1973).

**Oviposition inhibitory effect:** Ten *S. oryzae* adults of mixed sex were fumigated with two sublethal

concentrations; 40 % and 80 % of 24-h  $\text{LC}_{50}$  of *C. tamala* essential oil and eugenol for 24 h 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 oil/eugenol and control.

**Acetylcholinesterase enzyme (AChE) activity determination:** *S. oryzae* adult insects were fumigated with two sublethal concentrations viz. 40% and 80% of 24-h  $\text{LC}_{50}$  of *C. tamala* essential oil and eugenol as in toxicity assay. After 24 h of fumigation, adults were utilized for determination of acetylcholinesterase enzyme activity (Elman et al., 1961). Fumigated insects were homogenized in phosphate buffer saline (50 mM, pH 8) and centrifuged. Supernatant was used as the acetylcholinesterase 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-dithiobis 2-nitrobenzoic acid (DTNB) (0.33mM) and 1.45 ml phosphate buffer (50 mM, pH 8). Acetylcholinesterase 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  $\text{min}^{-1} \text{mg}^{-1}$  protein. Each enzymatic assay was replicated six times.

**Data analysis:** Median lethal concentration ( $\text{LC}_{50}$ ) was calculated using POLO programme (Russel et al., 1997). Analysis of variance (ANOVA) and correlation and linear regression analysis were conducted to define concentration-response relationship (Sokal and Rohlf, 1973).

## RESULTS

**Repellent activity:** Repellency was 48.33, 75.83, 88.33, 97.50 and 100% at 0.2, 0.4, 0.8, 1.6 and 3.2% concentrations of *C. tamala* essential oil respectively (Table 1). Preference Index (PI) was d -0.48, -0.75, -0.88, -0.97 and 1.0 at 0.2, 0.4, 0.8, 1.6, 3.2% concentrations of *C. tamala* essential oil respectively (Table 1). *C. tamala* essential oil and eugenol showed significant ( $F = 97.48$  for *C. tamala*; and  $F = 66.79$  for eugenol  $P < 0.01$ ) against *S. oryzae* adults based on negative values of Preference Index (Table 1).

**Fumigant toxicity:** Fumigation of *C. tamala* essential oil caused toxicity by vapour action. Median lethal concentrations ( $\text{LC}_{50}$ ) were 0.249 and 0.198  $\mu\text{l/cm}^3$  of *C. tamala* essential oil air after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. oryzae* adults

against *C. tamala* essential oil (F = 183.95 for 24h and 415.39 for 48h; P<0.01) (Table 3). Median lethal concentrations (LC<sub>50</sub>) were 0.167 and 0.152 µl/cm<sup>3</sup> air eugenol after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. oryzae* adults against eugenol (F = 187.46 for 24h and 431.5 for 48h; P<0.01) (Table 3).

**Contact toxicity:** *C. tamala* essential oil and eugenol caused contact toxicity in *S. oryzae* adults. Median lethal concentration (LC<sub>50</sub>) of *C. tamala* essential oil was 0.241, and

0.218 µl/cm<sup>2</sup> against *S. oryzae* adults after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. oryzae* adults against *C. tamala* essential oil (F = 645.84 for 24h and 541.41 for 48h; P<0.01) (Table 3). Median lethal concentration (LC<sub>50</sub>) of eugenol was 0.185, and 0.126 µl/cm<sup>2</sup> against *S. oryzae* adults after 24 and 48 h of exposure respectively (Table 2). Regression analysis showed concentration-dependent mortality in *S. oryzae* adults against eugenol (F = 186.72 for 24h and 421.21 for 48h; P<0.01) (Table 3).

Table 1. Percent repellency of *C. tamala* oil and eugenol against *S. oryzae* adults.

Oil/compound	Concentration %	Percent Repellency (PR) * Mean±SD	Preference Index** (PI)
<i>C. tamala</i>	0.2	48.53±1.23	- 0.48
	0.4	75.83±2.10	- 0.75
	0.8	88.33±1.36	- 0.88
	1.6	97.50±1.54	- 0.97
Eugenol	0.2	35.83±1.28	- 0.35
	0.4	67.5±1.06	- 0.67
	0.8	83.33±2.06	- 0.83
	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 repellent 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 *C. tamala* oil and eugenol against *S. oryzae* adults.

Oil/compound	Toxicity	Exposure period (h)	LC <sub>50</sub> <sup>a</sup>	LCL	UCL	g-value	Heterogeneity	t-ratio
<i>C. tamala</i>	Fumigant toxicity	24	0.249	0.231	0.267	0.17	0.34	3.97
		48	0.198	0.185	0.211	0.16	0.31	3.94
	Contact toxicity	24	0.241	0.227	0.255	0.19	0.33	4.16
		48	0.218	0.202	0.234	0.15	0.32	4.28
Eugenol	Fumigant toxicity	24	0.167	0.154	0.180	0.17	0.34	3.84
		48	0.152	0.141	0.163	0.15	0.31	3.56
	Contact toxicity	24	0.185	0.172	0.198	0.18	0.36	3.69
		48	0.126	0.117	0.133	0.16	0.33	3.96

a µl/cm<sup>3</sup> for fumigant and µl/cm<sup>2</sup> for contact toxicity.

Table 3. Regression analysis of fumigant and contact toxicity of *C. tamala* oil and eugenol against *S. oryzae* adults.

Oil/compound	Toxicity	Exposure period	Intercept	Slope	Regression Equation	Correlation coefficient	F- value*
<i>C. tamala</i>	Fumigant toxicity	24 h	- 4.958	3.473	Y = - 4.958+3.473X	0.983	183.95
		48 h	0.629	3.788	Y = 0.629+3.788X	0.983	415.39
	Contact toxicity	24 h	- 0.003	4.167	Y = - 0.003+4.167X	0.993	645.84
		48 h	5.404	4.257	Y = 5.404+4.257X	0.977	541.41
Eugenol	Fumigant toxicity	24 h	- 2.00	5.125	Y = - 2.00+5.125X	0.985	187.46
		48 h	3.666	5.750	Y = 3.666+5.750X	0.990	431.50
	Contact toxicity	24 h	0.332	5.042	Y = 0.332+5.042X	0.996	186.72
		48 h	6.0	5.791	Y = 6.0+5.791X	0.991	421.21

\*P<0.01

**Antifeedant activity (AFA):** *C. tamala* essential oil and eugenol significantly decreased consumption of flour disk by *S. oryzae* adults. Consumption of flour disk was reduced to 80.27, 46.59, 20.16 and 6.64% of control when treated with 3, 6, 9 and 12  $\mu\text{l}/\text{disk}$  of *C. tamala* essential oil ( $F = 541.19$ ,  $P < 0.01$ ) (Table 4). Eugenol also decreased consumption of flour disk by *S. oryzae* adults significantly. Consumption of flour disk was reduced to 77.26, 62.53, 37.40 and 12.41 % of control when treated with 3, 6, 9 and 12  $\mu\text{l}/\text{disk}$  of eugenol ( $F = 271.38$ ,  $P < 0.01$ ) (Table 4).

**Oviposition inhibition:** Fumigation of *S. oryzae* adults with *C. tamala* essential oil and eugenol significantly reduced oviposition potential. Reduction in oviposition was 45.33 and 18.86% of the control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h  $\text{LC}_{50}$  of *C. tamala* essential oil respectively ( $F = 209.26$ ,  $P < 0.01$ ) (Table 5). Reduction in oviposition was 36.97 and 20.47% of the control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h  $\text{LC}_{50}$  of eugenol

respectively ( $F = 149.17$ ,  $P < 0.01$ ) (Table 5).

**Acetylcholinesterase enzyme (AChE) activity:** Fumigation of *C. tamala* essential oil and eugenol against *S. oryzae* adults significantly reduced AChE activity. AChE activity was reduced to 63.34 and 48.61% of control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h  $\text{LC}_{50}$  of *C. tamala* essential oil respectively ( $F = 326.6$ ,  $P < 0.01$ ) (Table 6). Similar treatment of *S. oryzae* adults with eugenol significantly reduced AChE activity. AChE activity was reduced to 58.97 and 42.26% of control when *S. oryzae* adults were fumigated with 40 % and 80 % of 24-h  $\text{LC}_{50}$  of *C. tamala* essential oil respectively ( $F = 68.71$ ,  $P < 0.01$ ) (Table 6). The index of significance 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.

Table 4. Feeding inhibitory activities of *C. tamala* in *S. oryzae* adults.

Oil/Compound	Conc ( $\mu\text{l}/\text{disk}$ )	Consumption of flour disk in mg Mean $\pm$ SD	AFA*	F-value**
<i>C. tamala</i>	0	18.50 $\pm$ 0.39(100)	0.0	541.19
	2	14.85 $\pm$ 0.93(80.27)	19.73	
	4	8.62 $\pm$ 0.69(46.59)	53.40	
	6	3.73 $\pm$ 0.70(20.16)	79.84	
	8	1.23 $\pm$ 0.97(6.64)	93.35	
Eugenol	0	18.50 $\pm$ 0.39(100)	0.0	271.38
	2	14.29 $\pm$ 0.96(77.26)	22.75	
	4	11.56 $\pm$ 0.75(62.53)	37.51	
	6	6.92 $\pm$ 0.58(37.40)	62.59	
	8	2.29 $\pm$ 0.23(12.41)	87.62	

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

\*Antifeedant Activity (AFA) was calculated using formula:  $\text{AFA} = [(C-T)/C] \times 100$ ; where, C = consumption of flour disk in control group, and T = consumption of flour disc in treated group.

Table 5. Oviposition inhibitory activities of *C. tamala* oil and eugenol in *S. oryzae* adults. \*\*  $P < 0.01$

Oil/compound	Conc	No. of progeny emerged Mean $\pm$ SD	F-value*
<i>C. tamala</i>	Control	155.50 $\pm$ 14.33 (100%)	209.26
	40% of 48h- $\text{LC}_{50}$	70.50 $\pm$ 11.11 (45.33)	
	80% of 48h- $\text{LC}_{50}$	29.33 $\pm$ 5.20 (18.86)	
Eugenol	Control	155.50 $\pm$ 14.33 (100%)	149.17
	40% of 48h- $\text{LC}_{50}$	57.50 $\pm$ 9.86 (36.97)	
	80% of 48h- $\text{LC}_{50}$	31.83 $\pm$ 5.84 (20.47)	

Values in parentheses indicate per cent change with respect to control taken as 100%. \* $P < 0.01$

Table 6. Effect of 40 and 80% of 24h-LC<sub>50</sub> of *C. tamala* oil on Acetylcholinesterase enzyme (AChE) activity in *S. oryzae* adults.

Oil/compound	Conc.	Enzyme activity* Mean±SD	F- value** (2,15)
<i>C. tamala</i>	Control	0.0862±0.0037 (100)	326.6
	40% of 24h-LC <sub>50</sub>	0.0546±0.0026(63.34)	
	80% of 24h-LC <sub>50</sub>	0.0419±0.0021(48.61)	
Eugenol	Control	0.0862±0.0037(100)	68.71
	40% of 24h-LC <sub>50</sub>	0.0508±0.0023(58.97)	
	80% of 24h-LC <sub>50</sub>	0.0364±0.0019(42.26)	

\*Enzyme activity was expressed as mol of 'SH' hydrolysed min<sup>-1</sup>mg<sup>-1</sup> protein. Values in parentheses indicate per cent change with respect to control taken as 100%. \*\* P<0.01

## DISCUSSION

Among plant-based insecticides, plant volatiles have received much attention in the scientific community in insect pest management programme (Isman et al., 2011; Liu et al., 2011; Stefanazzi et al., 2011). *Acorus calamus*, *Syzygium aromaticum*, *Hyptis spicigera*, *Ocimum canum* and *Vepris heterophylla* essential oils exhibited repellent activity, insecticidal effect and inhibition of progeny in *S. oryzae* (Sharma and Meshram, 2006; Ngassoum, 2007). Essential oil components also have been evaluated for their role in insect pest management programme. Linalool and linalyl acetate exhibited significant fumigant toxicity to rice weevils (Singh et al., 1989). Menthol, methonene, limonene, β-pipene, α-pipene, and linalool exhibited toxicity in *S. oryzae* and inhibited AChE activity (Lee et al., 2001). In present study, repellent, insecticidal, feeding, oviposition and AChE inhibitory activities of *C. tamala* essential oil and eugenol in *S. oryzae* was studied. This essential oil and eugenol showed significant repellent activity against *S. oryzae* adults. *C. tamala* essential oil and eugenol induced high mortality in *S. oryzae* adults when treated by fumigation or contact methods. *C. tamala* essential oil and eugenol reduced progeny production in *S. oryzae* which ultimately may be reduced damage caused by the insect. *C. tamala* essential oil and eugenol decreased consumption of flour disk by *S. oryzae* adults. Similar results have been shown by *Schinus molle*, *Alpinia conchigera*, *Zingiber zerumbet* and *Curcuma zedoaria* essential oils and eugenol in *T. castaneum* and *S. oryzae* (Chaubey, 2012a,b,c; Sithisut et al., 2011, Cardiet et al., 2012). Enan (2001) has reported insecticidal activity and mechanism of eugenol against American cockroaches, *Periplaneta americana*. Exposed American cockroaches showed hyperactivity followed by

hyperextension of the legs and abdomen, then fast knockdown or quick immobilization followed by death. Ants and German cockroaches showed fast immobilization/knockdown followed by mortality. It causes blockage of octopamine receptors binding sites at lower concentrations (Enan, 2001). Little is known about the mode of action of essential oils and their constituents in insects, but studies suggested their neurotoxicity (Isman et al., 2007; Rana et al., 2010). In present study, fumigation of *S. oryzae* adults with *C. tamala* significantly reduced AChE activity. Recent research has demonstrated the interference of monoterpenes with acetylcholinesterase activity in *S. oryzae* and *Callosobruchus chinensis* (Chaubey, 2014, 2016). 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 (Enan, 2001) or GABA-gated chloride channels (Priestley et al., 2006). 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 (Hollingworth et al., 1984). 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 (Veal, 1996). 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 (Hollingworth et al., 1984).

In conclusion, *C. tamala* essential oil can be used as an alternative of synthetic insecticides in the stored-grain insect pest management.

#### REFERENCES

- Angioni, A., A. Barra, V. Coroneo, S. Dessi and P. Cabras. 2006. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *Stoechas* essential oils from stem/ leaves and flowers. *J. Agric. Food Chem.* 54, 4364-4370.
- Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils. A review. *Food Chem. Tox.* 46, 446-475.
- Beckel, H., Lorini, S.M.N., 2002. Resistencia de *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) a inseticidas piretro'ides e organofosforados usados em trigo armazenado [Resistance of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) to insecticides pyrethroids and organophosphates used in stored wheat]. In: Resumos e Atas do III Seminário Técnico do Trigo/XVII Reunião da Comissão Centro-sul Brasileira de Pesquisa de Trigo [Summaries and Minutes of III Technical Seminar of Trigo/XVII Reunião of the Commission I south-Center Brazilian of Inquiry of Wheat]. 44.
- Bruneton, J. 1999. Pharmacognosy, Phytochemistry, Medicinal Plants: Essential oils. 2nd ed. Lavoisier Publishing, New York, 461-780.
- Caballero-Gallardo, K., Olivero-Verbel, J., Stashenko, E.E. 2011. Repellent activity of essential oils and some of their individual constituents against *Tribolium castaneum* Herbst. *J. Agric. Food Chem.* 59, 1690-1696.
- Cardiet, G., Fuzeau, B., Barreau, C., Fleurat-Lessard, F. 2012. Contact and fumigant toxicity of some essential oil constituents against a grain insect pest *Sitophilus oryzae* and two fungi, *Aspergillus westerdijkiae* and *Fusarium graminearum*. *J. Pest Sci.* 85,351-358
- Chaubey, M.K., 2012a. Fumigant toxicity of essential oils and pure compounds against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Biol. Agri. Horti.* 28(2), 111-119 (2012a).
- Chaubey, M.K., 2012b. Responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) against essential oils and pure compounds. *Herba Polonica* 58(3), 33-45.
- Chaubey, M.K., 2012c. Biological effects of essential oils against Rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *J. Essential Oil Bearing Plants* 15(5), 809-815.
- Chaubey, M.K., 2014. Biological activities of *Allium sativum* essential oil against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Herba Polonica* 60(2), 41-55.
- Chaubey, M.K., 2016. Fumigant and contact toxicity of *Allium sativum* (Alliaceae) essential oil against *Sitophilus oryzae* L. (Coleoptera: Dryophthoridae). *Appl. Sci. Let.* 3(2), 43-48.
- Edwards, D.M., 1993. Rural Development Forestry Network (Overseas Development Institute, London), 15b, 1-21.
- Elman, G.L., Courtney, K.D., Andrews, V.Jr., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88-95.
- Enan, E., 2001. Molecular and pharmacological analysis of an octopamine receptor from American cockroach and fruit fly in response to plant essential oils. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 130(3), 325-37.
- Gill, A.O., Holley, R.A., 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *Appl. Environ. Microbiol.* 70(10), 5750.
- Hollingworth, R.M., Johnstone, E.M., Wright, N. 1984. Pesticide Synthesis through Rational Approaches, ACS Symposium Series No. 255, American Chemical Society, Washington, DC, 103-125.
- Hussain, A., Virmani, O.P., Popil, S.P., Mishra, L.N., Gupta, A.K., 1980. Dictionary of Indian Medicinal Plants, CIMAP Lucknow.
- Isman, M., Machial, C., Miresmailli, S., Bainard, L., 2007. Essential oil based pesticides: New insight from old chemistry. *Pesticide Chemistry.* Wiley-VCH, Weinheim, Germany. 201-209.
- Isman, M.B., Miresmailli, S., Machial, C., 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem. Rev.* 10,197-204.
- Jadhav, B.K., Khandelwal, K.R., Ketkar, A.R., Pisal, S.S., 2004. Formulation and evaluation of mucoadhesive tablets containing eugenol for the treatment of

- periodontal diseases. *Drug Dev. Ind. Pharm.* 30(2), 195-203.
- Jantan, I., Goh, S.H., 1990. The essential oils of *Cinnamomum mollissimum* as natural sources of safrole and benzyl benzoate. *J. Trop. Forest Sci.* 2(3), 252-259.
- Lee, B.H., Choi, W.S., Lee, S.E., Park, B.S. 2001. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). *Crop Prot.* 20,317-320.
- Liu, Z.L., Chu, S.S., Jiang, G.H., 2011 Insecticidal activity and composition of essential oil of *Ostericum sieboldii* (Apiaceae) against *Sitophilus zeamais* and *Tribolium castaneum*. *Rec. Nat. Prod.* 5, 74-81.
- Lu, F.C. 1995. A review of the acceptable daily intakes of pesticides assessed by the World Health Organization. *Reg. Toxicol. Pharmacol.* 21, 351-364.
- Masotti, V., F. Juteau, J.M. Bessiere and J. Viano, 2003. Seasonal and phonological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *J. Agric. Food Chem.* 51, 7115-7121.
- Minakshi, D., Krishna, D.A., Benerjee, A.B., 1999. Antimicrobial screening of some Indian spices. *Phytother. Res.* 13(7), 616-618.
- Ngassoum, M.B., Tinkeu, L.S.N., Ngatanko, L., Tapondjou, L.A., Lognay, G., Malaisse, F., Hance, T., 2007. Chemical composition, insecticidal effect and repellent activity of essential oils of three aromatic plants, alone and in combination, towards *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Nat. Prod. Comm.*, 2(12), 1229-1232.
- Priestley, C.M., Burgess, I.F., Williamson, E.M. 2006. Lethality of essential oils constituents towards the human louse *Pediculus humanus*, and its eggs. *Fitoterapia* 77, 303-309.
- Rana, T., Kashmiri, M.A., Ahmed, M., 2010. Studies of antioxidant activity of essential oils of Umbelliferae family. *Pakistan J. Sci.* 62, 67-70.
- Right, D.A., Payne, J.P. 1962. A clinical study of intravenous anaesthesia with a eugenol derivative, G.29.505 *British J. Anaesth.* 34(6), 379-385.
- Russel, R.M., Robertson, J.L., Savin, S.A., 1997. POLO: A new computer programme for probit analysis. *Bull. Entomol. Res.* 23: 209-213.
- Sharma, K., Meshram, N.M., 2006. Bioactive of essential oils from *Acorum calamus* Linnaeus and *Syzygium aromaticum* Linnaeus against *Sitophilus oryzae* (Linnaeus) in stored wheat. *Biopest. Intern.*, 2(2), 144-152.
- Singh, D., Siddiqui, M.S., Sharma, S., 1989. Reproductive retardant and fumigant properties in essential oils against rice weevil in stored wheat. *J. Econ. Entomol.* 82,727-733.
- Sithisut, D., Fields, P.G., Chandrapathya, A., 2011. Contact toxicity, feeding reduction and repellency of essential oils from three plants from the ginger family (Zingiberaceae) and their major components against *Sitophilus zeamais* and *Tribolium castaneum*. *J. Stored Prod.* 104(4), 1445-1454.
- Sokal, R.R., Rohlf, F.J. 1973. Introduction to biostatistics. W.H. Freeman and Co, San Francisco, CA, USA, 185-207.
- Stefanazzi, N., Stadler, T.A., Ferrero, A., 2011. Composition and toxic, repellent and feeding deterrent activity of essential oils against the stored-grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pest Mang. Sci.* 67, 639-646.
- United Nations Environment Programme [UNEP]. 2000. The Montreal Protocol on substances that deplete the ozone layer. Nairobi (Kenya).
- Veal, L., 1996. The potential effectiveness of essential oils as a treatment for headlice. *Complement Ther. Nurs. Midwifery*, 2, 97-101.
- Vishwam, S., Chakraborty, A., Karnan, J., Murugan, R., David, R.C., 2015. Chemical analysis of leaf essential oil of *Cinnamomum tamala* from Arunachal Pradesh, India. *J. Chem. Pharm. Sci.* 8(2), 246-248.
- World Meteorological Organization [WMO]. 1991. Scientific assessment of ozone depletion. Geneva (Switzerland), Report No. 25.