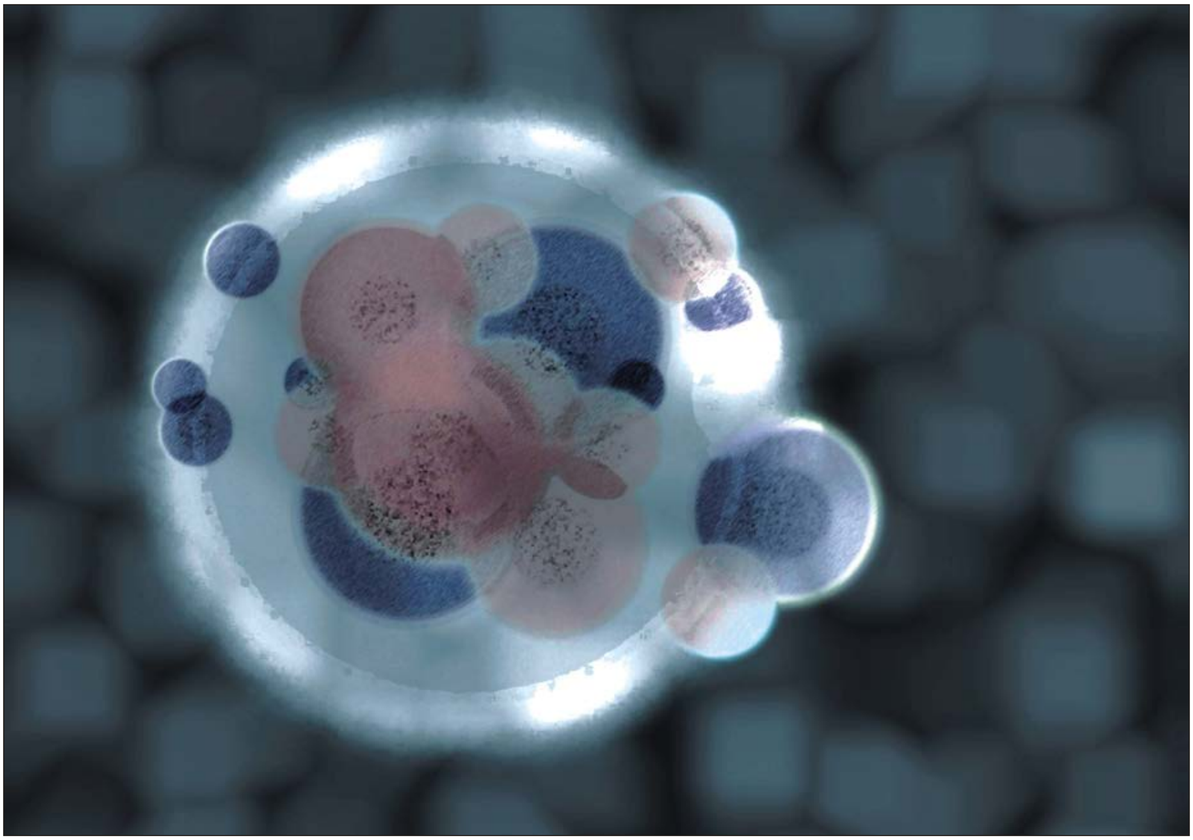

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Toxicity of Naturally Occurring Compounds of Plant Essential Oil Against *Tribolium castaneum* (Herbst)

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Abstract: Four compounds (trans-anethol, thymol, eugenol and cinnamaldehyde) occurring naturally in the plant essential oils were evaluated for contact and fumigant toxicity against adult, 10 day and 18 day old larvae of *Tribolium castaneum* (Herbst). The insecticidal activities varied with concentration, compound and the exposure time. The most sensitive stage was 10 day old larvae followed by adult *T. castaneum.* Eighteen day old larvae were highly tolerant of the tested compounds. Cinnamaldehyde and eugenol were highly effective against *T. castaneum* when applied for highest exposure time of 48 h at the lowest dose. At the highest dose level of 0.288 mg cm^{-2} and lowest exposure time of 6 h, trans-anethol achieved 100% mortality of 10 day old larvae as contact toxicity, whereas highest dose level of 115.38 and 6.153 mg^{-1}, thymol and eugenol achieved only 36.66 and 30% of 10 day old larvae and adult of *T. castaneum* as fumigant toxicity. Against 18 day old larvae, eugenol and cinnamaldehyde achieved 100% mortality after exposure for 48 h, even with the highest dose volume. These compounds may be suitable as contact and fumigants because of their high effectiveness and their safety.

Key words: Trans-anethol, thymol, eugenol, cinnamaldehyde, toxicity, *Tribolium castaneum*

INTRODUCTION

The widespread use of synthetic insecticides has led to many negative consequences (i.e., insecticide resistance, toxicity to mammals and other non-target animals, residue problems, environmental pollution) resulting in increasing attention being given to natural products (Isman, 2005). Plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Much effort has, therefore, been focused on plant derived materials for potentially useful products as commercial insect-control agents (Kim *et al*., 2003). Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts (Shaaya *et al*., 1991, 1997).

Plant essential oils in general have been recognized as an important natural resource and their major components, often various monoterpenoids, are among the best-known substances to have attracted attention in recent years as potential pest control agents due to their insecticidal, repellent and/or antifeedant properties (Tunç *et al*., 2000; Papachristos and Stamopoulos, 2002a, b; Lee *et al*., 2003, 2004; Trypathy, 2004; Ketoh *et al*., 2005; Isman, 2005). Most of these substances are volatile and can act as fumigants, thus offering the prospect of use against stored-product insects. In this context, many studies of the fumigant activity of such natural substances have been undertaken to establish new control practices with lower mammalian toxicity and low persistence in the environment (Isman, 2000; Tripathi *et al*., 2002; Erler, 2005; Isikber *et al*., 2006).

Focus on the vapour or fumigant toxicity of essential oils of plants and their constituents has sharpened since the 1980s. There are many reviews dealing with the use of plant products in general, against insect pests of stored products (Adler *et al*., 2000; Weaver and Subramanyam, 2000; Isman, 2005), specifically on essential oils and others only on monoterpenoids (Coats *et al*., 1991).

The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used (Lee *et al*., 2001). Numerous publications have presented data on the composition of the various essential oils. The major components of the economically interesting essential oils are summarized by Bauer *et al*. (2001). Essential oils can comprise more than sixty individual components (Senatore, 1996). Major components can constitute up to 85% of the essential oil, whereas other components are present only as a trace (Senatore, 1996; Bauer *et al*., 2001).

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The purpose of the present study was to investigate the contact and fumigant activity of naturally occurring compounds cinnamaldehyde, eugenol, thymol and trans-anethol against the red flour beetle, *T. castaneum* at different exposure hours after treatments.

MATERIALS AND METHODS

Compounds: The essential oil compounds used were trans-anethol [1-Methoxy-4-(1-proenyl) benzene], thymol (99.5% 5-methyl-2-isopropylphenol), eugenol [2-methoxy-4-(2-prophenyl) phenol] were purchased from Sigma, USA and cinnamaldehyde (trans-3-phenyl-2-propenal) was procured from Chem Industry, USA.

Test insect: Laboratory reared: Laboratory reared strain of *T. castaneum* (originally collected from the Slough Laboratory, UK and the culture are maintained in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi since, 1991) were used. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium. Adults used in the experiments were 3-7 day old and of mixed sex and larvae were 10 day and 18 day old used in contact and fumigant toxicity experiments. All stage were reared at 28±1°C without light and humidity control. The experiments were carried out during January to September 2008.

Contact toxicity: To examine the contact toxicity of the tested compounds to different days of the insect, 6 cm petridishes were used. A serial dilution of each compound of essential oils was prepared using acetone as a solvent. Aliquots of 0.5 mL of the dilutions were applied into the petridish. After evaporated the solvent for 5 min the adults (3-7 days old) and larvae (10 and 18 day old) were released in group of 10 for each replication of doses and control. Then the petridishes were kept at 28±1°C in the incubator. Each concentration and control was replicated three times. Mortality was determined after 6, 12, 18, 24 and 48 h from commencement of exposure.

Fumigant toxicity: Fumigant toxicity was done in 650 mL gastight jars with screwed caps as exposure chamber. A filter paper (Whatman No. 1), cut into 6 cm diameter was attached to the under surface of the cap to serve as a diffuser, on which varying doses of the tested compounds were applied while the control diffuser was left untreated. Insects were exposed to the 5 cm Petri dish that were placed at the bottom of each jar, ten individuals for each replication was placed and exposed to the various concentrations of vapour. The insects had no contact with the diffuser and stayed at the bottom of the chamber throughout the experiment. A 6, 12, 18, 24 and 48 h exposure time was considered adequate to assess mortality. After exposure, the insects were taken out of the jar and subsequently the dead insects were counted. Five replications were performed for each dose and control.

Statistical analysis: Data on percentage mortality were corrected for heterogeneity of treatment variances using arcsine-transformation (Papachristos and Stamopoulos, 2002a; Leatemia and Isman, 2004) before being subjected to ANOVA. Differences between treatment means were determined using Least Significant Difference (LSD) test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In contact toxicity for the tested monoterpene compounds, it can be seen from Table 1 and 2 that, at the lowest concentration of 0.036 mg cm^{-2} and highest exposure time of 48 h, trans-anethol caused 70% mortality for adult, 76.66% mortality for 10 day old larvae and no mortality were observed for 18 day old larvae. Whereas, at the lowest concentration of 0.053 mg cm^{-2} and highest exposure time of 48 h, thymol showed 43.33% mortality for adult, 76.66% mortality for 10 day old larvae and 23.33% mortality for 18 day old larvae. In case of eugenol, at the lowest concentration of 0.013 mg cm^{-2} and highest exposure time of 48 h, 100% mortality were observed for adult, 96.66% mortality for 10 day old larvae and 46.66% mortality for 18 day old larvae. At the lowest concentration of 0.057 mg cm^{-2} and highest exposure time of 48 h, cinnamaldehyde showed 93.33% mortality for adult, 100% mortality for 10 day old larvae and 30.0% mortality for 18 day old larvae. Complete mortality was obtained after 48 h at highest dose level above with all compounds for adult and 10 day old larvae, but not for 18 day old larvae. Here, the toxic order is Eugenol> cinnamaldehyde> trans-anethol> thymol in comparing the percent mortality at the lowest dose level and highest exposure time of 48 h.

In fumigation toxicity, at the lowest concentration of 1.08 mg^{-1} and highest exposure time of 48 h, trans-anethol caused 3.33% mortality for adult, 60.0% mortality for 10 day old larvae and no mortality were observed for 18 day old larvae. Whereas, at the lowest concentration of 14.43 mg^{-1} and highest exposure time of 48 h, thymol showed 56.66% mortality for adult, 90.0% mortality for 10 day old larvae and 6.66% mortality for 18 day old larvae. In case of eugenol, at the lowest concentration of 0.769 mg^{-1} and highest exposure time of 48 h, 50.0%

Table 1: Percentage mortality of different stages of *Tribolium castaneum* in contact toxicity to trans-anethol and thymol

			Mortality (%) after (Mean±SE)				
Compound	Life stage	Dose (mg cm^{-2})	6 h	12 h	18 h	24 h	48 h
Trans-anethol	Adult	0.288	66.66±6.66a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
		0.144	56.66±3.33ab	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
		0.072	43.33±3.33bc	60.00±5.77b	66.66±8.81b	100.0±0.00a	100.0±0.00a
		0.036	23.33±3.33c	40.00±5.77c	50.00±5.7 b	56.66±3.33b	70.00±5.77b
		Control	0	0	0	0	0
Statistical analysis							
F-value			16.94**	120.46**	54.73**	457.82**	81.48**
LSD			13.01	11.419	15.03	4.562	8.673
	10 day larvae	0.288	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a	100.0±0.00a
		0.144	90.00±5.77a	86.66±3.33b	96.66±3.33a	100.0±0.00a	100.0±0.00a
		0.072	56.66±3.33b	70.00±5.77b	63.33±3.33b	80.00±5.77bc	83.33±8.81ab
		0.036	53.33±6.66b	70.00±0.00b	66.66±3.33b	70.00±0.00c	76.66±3.33b
		Control	0	10.00±5.77c	3.330±3.33c	6.660±6.66d	16.66±3.33c
Statistical analysis							
F-value			21.80**	45.33**	66.11**	57.17**	32.14**
LSD			18.20**	18.31**	19.70**	21.51**	21.254**
	18 day larvae	0.288				20.0±5.77	23.33±3.33
		0.144				3.33±3.33	6.66±3.33
		0.072				0	3.33±3.33
		0.036				0	0
		Control				0	0
Statistical analysis							
F-value						7.089 NS	6.38 NS
LSD							
Thymol	Adult	0.43	86.66±6.66a	93.33±6.66a	90.0±5.77a	100.0±0.00a	100.0±0.00a
		0.215	60.0±10.00ab	53.33±3.33b	63.33±8.81ab	70.0±15.27ab	93.33±6.66a
		0.107	33.33±8.81bc	30.0±5.77b	60.0±10.00ab	63.33±8.81b	60.0±10.00b
		0.053	6.66±6.66c	20.0±0.00b	36.66±8.81bc	36.66±12.01bc	43.33±3.33b
		Control	0	0	10.0±5.77c	6.66±6.66c	0
Statistical analysis							
F-value			12.81**	24.83**	11.11**	12.55**	18.27**
LSD			35.478	23.184	29.76	29.75	25.974
	10 day larvae	0.43	93.33±6.66a	83.33±8.81a	93.33±6.66a	100.0±0.00a	100.0±0.00a
		0.215	73.33±6.66ab	53.33±8.81ab	56.66±8.81b	90.00±10.00a	93.33±6.66a
		0.107	43.33±3.33bc	46.66±3.33ab	43.33±6.66b	53.33±6.66b	93.33±6.66a
		0.053	13.33±6.66c	13.33±6.66b	26.66±6.66bc	36.66±8.81bc	76.66±14.52a
		Control	0	0	3.33±3.33c	6.660±3.33c	6.660±6.66b
Statistical analysis							
F-value			16.25**	8.68**	21.25**	24.38**	13.23**
LSD			31.685	34.524	26.58	28.64	40.37
	18 day larvae	0.43	56.66±6.66a	70.00±5.77a	70.0±5.77a	86.66±8.81a	83.33±8.81a
		0.215	36.66±8.81a	40.00±5.77b	36.66±8.81b	53.33±8.81ab	56.66±12.01ab
		0.107	30.00±5.77ab	46.66±8.81a	43.33±3.33ab	46.66±3.33b	30.00±5.77bc
		0.053	6.660±6.66b	23.33±3.33b	23.33±3.33b	26.66±6.66b	23.33±3.33bc
		Control	0	0	3.330±3.33c	0	6.660±6.66c
Statistical analysis							
F-value			8.25**	9.63**	19.47**	8.87**	10.44**
LSD			27.754	17.84	18.91	28.118	31.84

Means in each column and row followed by the same letters do not differ significantly using Least Significant Difference (LSD) test at **p<0.01

mortality were observed for adult, 46.66% mortality for 10 day old larvae and no mortality were observed for 18 day old larvae. At the lowest concentration of 10.14 mg^{-1} and highest exposure time of 48 h, cinnamaldehyde showed 100% mortality for adult, 83.33% mortality for 10 day old larvae and 56.66% mortality for 18 day old larvae. Here the toxic order is Eugenol> trans-anethol > cinnamaldehyde > thymol in comparing the percent mortality at the lowest dose and concentration level and highest exposure time of 48 h (Table 2-4).

The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility they have fumigant activity that might be of importance for controlling stored-product insects (Regnault-Roger and Hamraoui, 1995; Ahn *et al*., 1998). The insecticidal activity varied with insect species, oil concentrations and exposure time. In the space fumigation studies the oil and its aromatic constituents showed strong species-specific toxicity that was highly dependent upon the dosage and time after treatment (Ogendo *et al*., 2008). Essential oil products are generally broad-spectrum,

Table 2: Percentage mortality of different stages of *Tribolium castaneum* in contact toxicity to eugenol and cinnamaldehyde

Compound	Life stage	Dose (mg cm^{-2})	Mortality (%) after (Mean±SE) 6 h	12 h	18 h	24 h	48 h
Eugenol	Adult	0.101	30.0±5.77a	50.0±5.77	63.33±12.01	100.0±0.00a	100.0±0.00
		0.05	23.33±3.3ab	30.0±5.77	70.0±5.77	100.0±0.00a	100.0±0.00
		0.025	10.0±5.77bc	43.33±8.81	50.0±5.77	93.33±6.66ab	100.0±0.00
		0.013	3.33±3.33c	30.0±5.77	50.0±10.00	76.66±3.33b	100.0±0.00
		Control	0	0	0	0	0
Statistical analysis							
F-value			5.21*	2.24NS	1.31NS	8.84**	
LSD			17.68			21.657	
	10 day larvae	0.101	26.66±6.66	70.0±5.77a	76.66±3.33a	100.0±0.00	100.0±0.00
		0.05	20.0±5.77	50.0±11.54ab	76.66±12.01a	100.0±0.00	96.66±3.33
		0.025	23.33±3.33	36.66±3.33b	60.0±5.77ab	86.66±6.66	100.0±0.00
		0.013	0	20.0±0.00b	33.33±3.33b	80.0±11.54	96.66±3.33
		Control	0	0	0	0	0
Statistical analysis							
F-value			0.41	10.32**	4.35*	2.52NS	0.66NS
LSD			NS	18.938	21.21		
	18 day larvae	0.101		30.0±5.77a	43.33±3.33	66.66±3.33	100.0±0.00a
		0.05		23.33±8.81a	40.0±5.77	60.0±11.54	70.0±17.32ab
		0.025		20.0±5.77a	26.66±12.01	46.66±8.81	63.33±3.33b
		0.013		3.33±3.33b	20.0±11.54	43.33±3.33	46.66±17.6b
		Control		0	0	0	0
Statistical analysis							
F-value				5.27*	1.49NS	2.03NS	4.77*
LSD				16.79			30.18
Cinnam-aldehyde	Adult	0.458		93.33+6.66a	100.0±0.00a	100.0±0.00a	96.66±3.33
100.0±0.00							
		0.229	43.33+3.33b	86.66±6.66a	100.0±0.00a	96.66±3.33	96.66±3.33
		0.114	43.33+8.81b	56.66±3.33b	56.66±3.33b	83.33±8.81	100.0±0.00
		0.057	20.0+5.77b	50.0±5.7b	43.33±3.33c	93.33±6.66	93.33±6.66
		Control	0	3.33±3.33c	0	0	0
Statistical analysis							
F-value			17.56**	38.32**	370.27**	0.671NS	0.687NS
LSD			26.729	22.93	6.452		
	10 day larvae	0.458	63.33±6.66a	93.33±6.66a	100.0±0.00a	100.0±0.00	100.0±0.00a
		0.229	46.66±3.33ab	93.33±6.66a	96.66±3.33a	96.66±3.33	93.33±3.33b
		0.114	26.66±6.66b	46.66±3.33b	93.33±6.66a	100.0±0.00	100.0±0.00a
		0.057	23.33±6.66b	30.0±5.77bc	53.33±3.33b	86.66±6.66	100.0±0.00a
		Control	0	6.66±6.66 c	6.66±6.66c	0	0
Statistical analysis							
F-value			8.509**	19.65**	29.49**	2.4NS	4*
LSD			18.562	31.84	28.22		10.01
	18 day larvae	0.458	10.0±5.77	66.66±6.66a	66.66±6.66a	80.0±5.77a	86.66±6.66a
		0.229	3.33±3.33	40.0±5.77ab	43.33±3.33ab	53.33±3.33b	76.66±12.01ab
		0.114	0	30.0±5.77ab	30.0±5.77ab	43.33±6.66bc	43.33±6.66bc
		0.057	0	10.0±5.77b	16.66±8.81b	20.0±0.00c	30.0±10.00c
		Control	0	0	0	0	0
Statistical analysis							
F-value			0.788NS	10.45**	6.26**	25.53	5.18*
LSD				24.255	27.85	14.51	27.30

Means in each column and row followed by the same letters do not differ significantly using Least Significant Difference (LSD) test at *p<0.05 and **p<0.01, NS: Not significant

due to the presence of several active ingredients that operate via several modes of action (Chiasson *et al*., 2004).

Positive results for contact and fumigant activity of monoterpenes were obtained against *T. castaneum*. Monoterpenoids are typically volatile and rather lipophilic compounds, which can rapidly penetrate into insects and interfere with their physiological functions (Lee *et al*., 2002). Eugenol a monoterpenoid had fumigant toxicity to *T. castaneum* (Rozman *et al*., 2007). The essential oil from *Vitex pseudonegundo*, 1, 8-cineol was determined to be the major constituents has fumigant toxicity to *T. castaneum* (Sahaf *et al*., 2008). Of all the oils tested thus far, cinnamaldehyde is the only essential oil-derived compound that exerts contact toxicity to *T. castaneum*. The essential oil of garlic (Ho *et al*., 1996) as well as the n-hexane extract of star anise (Ho *et al*., 1995) and its main constituent, anethole (Ho *et al*., 1997) were more toxic to *T. castaneum* than to *Sitophilus zeamais*. On the other hand, the n-hexane extract of clove flower buds (Ho *et al*., 1994) and its main constituent, eugenol were more toxic to *S. zeamais* than to *T. castaneum*. Therefore,

Table 3: Percentage mortality of different stages of *Tribolium castaneum* in fumigant toxicity to trans-anethol and thymol

			Mortality (%) after (Mean±SE)				
Compound	Life stage	Dose (mg L^{-1})	6 h	12 h	18 h	24 h	48 h
Trans-anethol	Adult	8.7				60.00±5.77a	83.33±8.81a
		4.35				33.33±8.81a	70.00±11.54a
		2.175				3.33±3.33b	6.66±3.33b
		1.087				3.33±3.33b	3.33±3.33b
		Control				0	0
Statistical analysis							
F-value						16.96**	17.33**
LSD						25.562	36.56
	10 day larvae	8.7		33.33±8.81	56.66±3.33 a	70.00±10.0a	100.0±0.00a
		4.35		33.33±18.55	53.33±8.81 a	66.66±8.81a	100.0±0.00a
		2.175		6.66±3.33	26.66±6.66 b	53.33±3.33ab	90.0±10.0ab
		1.087		3.33±3.33	30.00±5.77 b	23.33±8.81b	60.0±10.0b
		Control		0	0	0	0
Statistical analysis							
F-value				3.628 NS	5.65*	6.58**	**8.39
LSD					12.8	24.551	30.03
	18 day larvae	8.7				13.33±6.66	36.66±6.66
		4.35				10.0±5.77	20.0±5.77
		2.175				3.33±3.33	0
		1.087				0	0
		Control				0	0
Statistical analysis							
F-value						0.617NS	3.63NS
Thymol	Adult	115.38		13.33±6.66	53.33±8.81	56.66±8.81	100.0±0.00a
		57.69		10.0±5.7	33.33±6.66	46.66±6.66	90.0±10.00ab
		28.85		3.33±3.33	30.00±5.77	33.33±6.66	60.0±5.77b
		14.43		0	0	0	56.66±8.81b
		Control		0	0	0	0
Statistical analysis							
F-value				0.617NS	2.97NS	2.47NS	10.44**
LSD							30.07
	10 day larvae	115.38	36.66±6.66	50.0±11.54	80.0±15.27a	86.66±8.81a	100.0±0.00a
		57.69	30.0±5.77	36.66±8.81	70.0±11.54ab	73.33±17.63a	100.0±0.00a
		28.85	26.66±6.66	36.66±6.66	63.33±3.33ab	73.33±8.81ab	100.0±0.00a
		14.43	26.66±3.33	33.33±8.81	30.0±5.77b	63.33±3.33ab	90.0±10.0a
		Control	0	20.0±5.77	0	30.00±5.77b	30.0±5.77b
Statistical analysis							
F-value			0.673NS	1.64NS	3.638*	3.104*	22.48**
LSD					25.629	26.83	23.365
	18 day larvae	115.38			16.66±3.33	20.0±0.00	30.0±5.77
		57.69			0	10.0±5.77	16.66±8.81
		28.85			0	0	10.0±5.77
		14.43			0	0	6.66±6.66
		Control			0	0	0
Statistical analysis							
F-value					2.16NS	1.64NS	

Means in each column and row followed by the same letters do not differ significantly using Least Significant Difference (LSD) test at *p<0.05. and **p<0.01, NS: Not significant

cinnamaldehyde is more advantageous as a contact poison as it is equally effective against stored product of insects. The larvae of *T. castaneum* were progressively more tolerant to cinnamaldehyde with age, a trend similarly observed by other authors with hexane extracts of clove (Ho *et al*., 1994) and star anise (Ho *et al*., 1995) as well as the essential oil of garlic (Ho *et al*., 1996). Clemente *et al*. (2000) obtained positive results by treating *Tribolium castaneum* with the essential oil of *Lavandula spica* L. that contained 40% of 1,8-cineole. The laboratory studies of Lee *et al*. (2003) proved that *T. castaneum* could be controlled by 1,8-cineole, 1-fechone and pulegone, but only at the highest doses. The results of present study are mostly in the agreement with the results of other investigators.

Erler (2005) reported the fumigant activity of thymol against adults and eggs of *T. confusum* and larvae and eggs of *Ephestia kuehniella* Zeller. Concerning the larval stage, Stamopoulos *et al*. (2007) observed that susceptibility to vapours varied with age. In fact, as the

Table 4: Percentage mortality of different stages of *Tribolium castaneum* in fumigant toxicity to eugenol and cinnamaldehyde

			Mortality (%) after (Mean±SE)				
Compound	Life stage	Dose (mg L^{-1})	6 h	12 h	18 h	24 h	48 h
Eugenol	Adult	6.153	30.0±5.77	43.33±8.81a	53.33±6.66a	100.0±0.00a	100.0±0.00a
		3.076	16.66±3.33	23.33±3.33ab	30.0±5.77ab	96.66±3.33a	100.0±0.00a
		1.538	0	16.66±3.33b	20.0±0.00b	76.66±12.01ab	96.66±3.33a
		0.769	0	10.0±5.77b	20.0±5.77b	b	50.0±11.54b
		Control	0	0	0	43.33±+8.81b	0
Statistical analysis							
F-value			4.03NS	4.7*	8.13**	9.04**	22.34**
LSD				16.35	16.16	34.628	21.752
	10 day larvae	6.153	26.66±6.66	30.0±5.77a	36.66±12.01	80.0±5.77a	96.66±3.33a
		3.076	10.0±5.77	36.66±6.66a	43.33±8.8	66.66±3.33ab	93.33±6.66a
		1.538	3.33±3.33	26.66±6.66ab	36.66±3.33	53.33±6.66bc	86.66±8.81ab
		0.769	20.0±11.54	10.0±5.77ab	26.66±12.01	33.33±3.33c	46.66±6.66b
		Control	0	3.33±3.33b	10.0±5.77	0	0
Statistical analysis							
F-value			1.74NS	5.97**	2.58NS	14.42**	6.26**
LSD				24.22		15.211	35.44
	18 day larvae	6.153			16.66±6.66	26.66±6.66	33.33±3.33a
		3.076			10.0±5.77	13.33±3.33	20.0±5.77ab
		1.538			0	6.66±3.33	3.33±3.33b
		0.769			0	0	0
		Control			0	0	0
Statistical analysis							
F-value					0.811NS	4.08NS	11.04**
LSD							23.451
Cinnam-aldehyde	Adult	81.15	13.33±6.66	63.33±6.66a	93.33±6.66a	100.0±0.00a	9100.0±0.00a
		40.58	3.33±3.33	23.33±3.33b	63.33±18.55ab	100.0±0.00a	96.66±3.33a
		20.29	0	0	66.66±8.81ab	93.33±6.66a	100.0±0.00a
		10.14	0	0	23.33±3.33b	50.0±5.77b	100.0±0.00a
		Control	0	0	0	0	6.66±3.33b
Statistical analysis							
F-value			1.15NS	28.49**	4.96*	20.52**	77.34**
LSD				20.765	31.36	22.448	17.41
	10 day larvae	81.15	10.0±5.77	46.66±3.33a	86.66±8.81a	100.0±0.00a	100.0±0.00a
		40.58	3.33±3.33	43.33±8.81a	60.0±10.0ab	93.33±6.66a	100.0±0.00a
		20.29	0	10.0±5.77b	46.66±3.33abc	76.66±12.01ab	93.33±6.66a
		10.14	0	6.66±6.66b	33.33±8.81bc	46.66±8.81b	83.33±16.66a
		Control	0	13.33±6.66b	10.0±5.77c	0	20.0±5.77b
Statistical analysis							
F-value			0.788NS	5.015*	10.17**	6.67 **	11.07**
LSD				22.23	29.79	37.80	35.94
	18 day larvae	81.15	13.33±6.66		30.0±5.77	50.0±5.77a	100.0±0.00a
		40.58	13.33±3.33		33.33±8.81	30.0±5.77ab	70.0±17.32ab
		20.29	3.33±3.33		16.66±3.33	13.33±8.81ab	56.66±3.33b
		10.14	0		0	10.0±5.77b	56.66±6.66b
		Control	0		0	3.33±3.33b	6.66±3.33c
Statistical analysis							
F-value			1.5NS		2.108NS	5.57**	14.09**
LSD						29.47	33.44

Means in each column and row followed by the same letters do not differ significantly using Least Significant Difference (LSD) test at *$p<0.05$. and **$p<0.01$, NS: Not significant

larva develops it becomes less susceptible. The adult of *T. castaneum* to all tested compounds shows that adults were much more susceptible than larvae. As the larvae grew older, they became less susceptible, except for the 16 day old larvae. There was a tendency for the larvae to become more tolerant to the tested compounds as they grew older provided they did not moult during exposure. As in the case of contact toxicity, *T. castaneum* larvae became progressively more tolerant with age (Huang and Ho, 1998). Furthermore, the essential oil evaluated in this study is used as a pharmaceutical agent and is thus considered to be less harmful to humans and the environment than the majority of conventional insecticides (Isman, 2005). Consequently, the possibility of employing these natural fumigants to control insects in stored products may warrant further investigation.

We extended the range of active oil substances of aromatic plants tested by four more compounds (trans-anethol, thymol, eugenol and cinnamaldehyde) in the control of *T. castaneum* all of which proved capable of achieving complete control within 48 h at the lowest doses. In the present study, cinnamaldehyde, eugenol, thymol and trans-anethol were demonstrated both contact and fumigant toxicity to *T. castaneum* adults and larvae. The observed contact and fumigant activity demonstrates that essential oils are a source of biologically active compounds which may potentially prove to be efficient insecticides.

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