J. bio-sci. 17: 57-62, 2009 ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index

OVICIDAL ACTIVITY OF ESSENTIAL OILS AGAINST RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

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Abstract

Context: The essential oils are being tried as potential candidates for pest and disease management. Several essential oils of botanical origin have been reported for their repellant, toxic and developmental inhibitory activities. The ovicidal effect of essential oil is probably the major factor in the suppression of the development of adults from treated eggs.

Objectives: To investigate the ovicidal effect of vapours of five essential oils viz., cardamom (*Elletaria cardamomum* L.), cinnamon (*Cinnamomum zeylanicum* Blume), clove (*Sygium aromaticum* L. Merrill. et. Perry), *Eucalyptus* spp. and neem (*Azadirectica indica* A. juss) against the eggs of red flour beetle, *Tribolium castaneum* (Herbst).

Materials and Methods: Ten eggs (24 h old) of *T. castaneum* were placed in each petridish with wheat flour as food medium and without flour medium and then the petridish were kept inside 650 ml jars with screwed lids. Aliquots of 0.5 ml of each dose of essential oils were applied on filter paper attached to the lower side of the lids. The exposure periods were 24, 48, 72 and 96 h, respectively. After treatment periods, petridishes were taken out of the jars and the final mortality counts were made after 11 days. Mortality data were subjected to probit analysis.

Results: The oils had high-fumigant activity against eggs and toxicity progressively increased with increase in exposure time and concentration. At the highest concentration of 5.769 mg/l air and exposure period of 24 h, cinnamon oil achieved 100% mortality in flour and without flour media. The vapours of essential oils from cardamom and clove resulted in 100% mortality of the eggs. Neem oil achieved mortalities as high as 51.66 and 50% mortality at the highest concentration and exposure period in with-flour and without flour medium respectively. At a concentration of 5.769 mg/ l air cardamom oil, the LT_{90} values were 50.80 and 62.78 h for with-flour and without flour medium respectively.

Conclusion: The essential oils of cinnamon and clove, proved to be promising as control agents against stored-product insects, especially *T. castaneum*.

Key words: Essential oil, Fumigant toxicity, Ovicidal activity, *Tribolium castaneum*

Introduction

World over, pests (especially weeds, pathogens and insects) are the largest competitor of agricultural crops and severely reduce the crop production in the range of 25–50% (Oerke 2006). To protect agricultural crops enormous amount of synthetic pesticides are used. As per Agrow (2007) report, the total value of world's agrochemical market was between US$ 31–35 billion and among the products herbicides accounted for 48 % followed by insecticides (25%) and fungicides (22%).

However, the excessive use of synthetic pesticides in the croplands, urban environment, and water bodies to get rid of noxious pests has resulted in an increased risk of pesticide resistance, enhanced pest resurgence and development of resistance/ cross-resistance, toxicological implications to human health and increased environmental pollution. In fact, combating of environmental pollution and its ill-effects on the life and life

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support systems is one of the most serious challenges before the present day world. Efforts are thus being made world over to replace these synthetic chemicals with alternatives, which are safer and do not cause any toxicological effects on the environment. The natural pest and disease control either directly or indirectly using natural plant products/botanicals, including essential oils, holds a good promise (Isman 2000, 2006, Bakkali *et al*. 2008).

Aromatic plants and their essential oils have been used since antiquity in flavor and fragrances, as condiment or spice, in medicines, as antimicrobial / insecticidal agents, and to repel insect or protect stored products (Dorman and Deans 2000, Isman and Machial 2006, Bakkali *et al*. 2008). These constitute effective alternatives to synthetic pesticides without producing adverse effects on the environment (Isman 2000, Isman and Machial 2006). However, the attempts to characterize their pest control activity under *in vitro* conditions started in 1900s (Dorman and Deans 2000). Moreover, the interest in essential oils has regained momentum during the last decade, primarily due to their fumigant and contact insecticidal activities and the less stringent regulatory approval mechanisms for their exploration due to long history of use (Isman 2006).

Of late, the essential oils are being tried as potential candidates for pest and disease management (Isman 2000, Pawar and Thaker 2006, Abad *et al*. 2007). Essential oils may act as fumigants (Negahban *et al*. 2006, 2007, Ogendo *et al*. 2008, Sahaf *et al*. 2008), contact insecticides (García *et al*. 2007), repellents (Prajapati *et al*. 2005, García *et al*. 2007, Ogendo *et al*. 2008, Cosimi *et al*. 2009, Nerio *et al*. 2009), antifeedants (Harwood *et al*. 1990, Han *et al*. 2006) and may affect some biological parameters such as ovicidal activity, growth rate, life span and reproduction inhibition (Isman 2000, Weaver and Subramanyam 2000, Prajapati *et al*. 2005).

Therefore, in order to better understand the response of the egg stage to essential oils and especially their vapours or their constituents, there is a need for systematic investigations of the effect of different essential oil concentrations and exposure periods on stored-product insects. The present study was undertaken to investigate the effect of vapours of five essential oils against the eggs of red flour beetle, *Tribolium castaneum* (Herbst)

Materials and Methods

Collection of eggs: The wheat flour was used as the food medium for *T. castaueum* culture. A standard mixture of whole-wheat flour with powdered dry yeast in a ratio of 19:1 (Khalequzzaman *et al*. 1994) was used as food medium throughout the experimental period. Form culture of the red flour beetle, about 500 beetles was placed in 500 ml beaker containing food medium of whole-wheat mill flour. The beaker was covered with a piece of cloth and kept in an incubator at 28 ±1ºC. After 24 h interval the eggs were collected by sieving the food medium by two sieves of 500 and 250 µm aperture separating the adults and eggs respectively.

Essential oils: Five essential oils; cardamom (*Elletaria cardamomum* L.), cinnamon (*Cinnamomum zeylanicum* Blume), clove (*Sygium aromaticum* L. Merrill et. Perry), *Eucalyptus* spp. and neem (*Azadirectica indica* A. Juss) were used. Those essential oils were procured from the pharmacy as of 90 % purity and were further purified in the rotary evaporator in the Crop Protection and Toxicology Laboratory, Department of Zoology, Rajshahi University.

Ovicidal activity of essential oils: The eggs were exposed to essential oils on 6 cm petridish. Ten eggs (24 h old) were placed in each petridish with flour as food medium and without flour as no food medium and then the petridish were kept in 650 ml jars with screwed lids. Aliquots of 0.5 ml of each dose of essential oil was applied on filter paper (Whatman No. 1), cut into 6 cm diameter, and were attached to the lower side of the lid of the jar. After evaporation of the solvent in about two minutes, the lids were closed tightly with the jars. The exposure periods were 24, 48, 72 and 96 h, respectively. After exposure, petridishes were taken out of the jars and kept in the incubator at at 28 ±1ºC. The final mortality counts were made after 11 days with the help of a stereomicroscope. Unhatched eggs were counted as dead.

Analysis of the data: Percentage mortality was calculated and data were corrected for natural mortality in controls using the Abbott (1925) formula. The corrected mortality were then subjected to probit analysis to estimate LT_{50} and LT_{99} values (Sokal and Rohlf 1973). To demonstrate the difference between flour and without-flour media t-test were done. Analysis of variance (ANOVA) was used to determine the effect of essential oil concentrations on ovicidal activity. Following a significant ANOVA, differences amongst means were established using Least Significant Difference (LSD) test at 1% level.

Results

Essential oil vapours showed variable toxicity to egg of *T. castaneum*. There is no significant difference between flour and without flour media (t-values were insignificant) in all oils except neem oil ($P < 0.05$) at 24 h treatment.. However, only the cinnamon oil achieved a mortality of 100% to all exposure periods against the eggs of *T. castaneum* both in flour and without flour media. Cardamom oil achieved 100% mortality at the highest concentration and exposure period of 96 h in both media. Clove oil showed 100% mortality at the highest concentration in without flour medium to 24 h exposure period, whereas, 48.33% mortality in flour medium. Eucalyptus oil showed 25.00 and 10.00% egg mortality in without flour and flour media respectively after 24 h exposure. Neem oil was less toxic and achieved 51.66 and 50.00% mortality at the highest concentration at 96 h exposure period in flour and without flour media respectively (Table 1).

Table I. Mean percent of egg mortality of *Tribolium castaneum* in different essential oils treated in different hours of time.

Essential oil (mg^{-l} air)	% mortality after 24h		% mortality after 48h		% mortality after 72h		% mortality after 96h	
	without flour	with flour	without flour	with flour	without flour	with flour	without flour	with flour
				Cinnamon				
5.769	100	100	100	100	100	100	100	100
2.884	100	100	100	100	100	100	100	100
1.442	100	100	100	100	100	100	100	100
0.721	60.00	35.00	100	100	100	100	100	100
Control	00	00	00	00	00	00	00	00
	df=5, t=0.327, NS							
				Cardamom				
5.769	56.66	55.00	83.33	88.33	91.66	96.66	100	100
2.884	40.00	30.00	68.33	41.66	70.00	66.66	100	70.00
1.442	16.66	11.66	30.00	20.00	46.66	35.00	46.66	58.33
0.721	11.66	3.33	18.33	16.66	28.33	18.33	31.66	18.33
Control	00	00	00	00	00	00	00	00
	df=6, t=0.40, NS		df=6, t=0.369, NS		df=6, t=0.225, NS		df=6, t=0.322, NS	
				Clove				
5.769	100	48.33	100	70.00	100	83.33	100	93.33
2.884	41.66	28.33	91.66	33.33	91.66	46.66	96.66	53.33
1.442	28.33	25.00	60.00	26.66	73.33	33.33	80.00	43.33
0.721	10.00	16.66	26.66	25.00	48.33	28.33	76.66	35.00
Control	00	00	00	00	00	00	00	00
	df= 4, t=0.749, NS		df=5, t=1.559, NS		df=6, t=1.80, NS		df=4, t=2.262, NS	
				Eucalyptus				
5.769	25.00	10.00	70.00	65.00	85.00	80.00	96.66	90.00
2.884	13.33	26.66	55.00	53.33	71.66	58.33	83.33	68.33
1.442	8.33	5.00	33.33	21.66	41.66	28.33	51.66	46.66
0.721	3.33	3.33	13.33	6.66	23.33	18.33	31.66	20.00
Control	00	00	00	00	00	00	00	00
	df=6, t=0.176, NS		df=6, t=0.340, NS		df=6, t=0.460, NS		df=6, t=0.455, NS	
				Neem				
5.769	23.33	00	31.66	20.00	38.33	40.00	50.00	51.66
2.884	18.33	00	25.00	15.00	31.66	21.66	38.33	46.66
1.442	11.66	00	18.33	6.66	25.00	13.33	23.33	18.33
0.721	5.00	00	6.66	3.33	11.66	5.00	13.33	5.00
Control	00	00	00	00	00	00	00	00
	df=3, t=3.655, p<0.05		df=5, t=1.398, NS		df=6, t=0.708, NS		df=5, t=0.06, NS	

All the doses caused 100% mortality of eggs of *T. castaneum* in cinnamon oil, so the lethal time could not be calculated. At the highest concentration level of cardamom oil, the time required for 50% (LT_{50}) and 90% (LT_{90}) kill of eggs were 22.49 and 50.80 h in flour medium and 20.73 and 62.78 h in without flour medium, respectively. For clove oil the LT_{50} was 26.00 h and LT_{90} was 96.02 h at the highest concentration level in flour medium, but without flour medium, 100% mortality was occurred. The LT_{50} and LT_{90} were estimated as 44.42 and 90.61 h in flour medium and 35.90 and 76.73 h in without flour medium at the highest concentration level of eucalyptus oil. However, the LT_{50} and LT_{90} of neem oil at the same dose were 87.93 and 191.98 h for flour medium and 114.41 and 1425.43 h for without flour medium, respectively (Table 2).

Table 2. LT_{50} (h) and LT_{90} (h) of different concentrations of essential oils against the mortality of eggs of *T. castaneum*.

Dose (mg^{-l})	Cardamom		Clove		Eucalyptus		Neem	
	LT_{50}	LT_{90}	LT_{50}	LT_{90}	LT_{50}	LT_{90}	LT_{50}	LT_{90}
With flour								
5.769	22.49	50.80	26.00	96.02	44.42	90.61	87.93	191.98
2.884	50.18	249.70	88.47	1174.56	50.58	278.65	102.88	225.05
1.442	93.42	341.15	219.22	9546.90	108.69	363.02	202.51	631.35
0.721	377.47	3444.6	286.38	7777.68	248.61	1180.1	771.33	4430.67
Without flour								
5.769	20.73	62.78	*	*	35.90	76.73	114.41	1425.4
2.884	31.19	179.23	25.60	58.32	48.49	116.68	209.80	3969.3
1.442	97.42	629.45	40.80	145.73	86.44	317.19	643.16	24414.9
0.721	239.45	2805.97	67.60	166.44	154.21	561.13	1486.9	34838.2

* It was not possible to estimate LT50 or LT90 values due to 100% mortality at all exposure periods used.

Discussion

Ovicidal activity is only apparent when the target system (the nervous system in this case), begins to develop (Michaelides and Wright 1997). Alternatively, changes in the permeability of the chorion and/ or vitelline membrane may occur during embryogenesis and may facilitate the diffusion of vapours into older eggs so that vital physiological and biochemical processes are affected (Gurusubramanian and Krishna 1996). These factors have been considered to play a dominant role in the susceptibility of eggs of many insect species to different ovicides including organophosphates, carbamates and dinitrophenols (Smith and Salkeld 1966, Michaelides and Wright 1997).

Previously for the management of economic loss caused by *T. castaneum*, several essential oils of botanical origin have been reported for their repellant, toxic and developmental inhibitory activities. Essential oils of *Anethum sowa* (Tripathi *et al*. 2000a), *Artemisia annua* (Tripathi *et al*. 2000b), *Lippia alba* (Verma *et al*. 2000) and *Elletaria cardomum* (Huang *et al*. 2000) have been reported for their repellant and toxic behavior against *T. castaneum*. Although cardamom oil did not prevent the oviposition but it was ovicidal to the eggs of *T. castaneum*. In addition, it also prevented the eggs treated with the oil from developing to the adult stage. Hence, the ovicidal effect of cardamom oil was probably the major factor in the suppression of the development of adults from treated eggs (Huang *et al.* 2000). An ovicidal effect on *T. castaneum* was also found in the essential oils of garlic (Ho *et al*. 1996) and nutmeg (Huang *et al*. 1997). Ho *et al*. (1995) investigated that hexane extracts of star anise were ovicidal to eggs of *T. castaneum.* Moreover, it is well known that rosemary oil has ovicidal activity against other insects such as *T. confusum* du Val and *Ephestia kuehniella* Zeller (Tunç *et al.* 2000).

Comparison of the results with earlier investigations (Saraç and Tunç 1995) demonstrates that responses of the egg stage and active stages of stored-product insects to essential oils are different. Eggs of *T. confusum* were more tolerant towards anise oil than the adults (Tunç *et al.* 2000). However, the eggs *of E. kuehniella* were less tolerant towards the essential oil than the last larval instars at comparable concentrations and exposure periods (Saraç and Tunç 1995). Compared with the investigation of Shaaya *et al.* (1993), the results demonstrate a much lower toxicity of the essential oil of oregano against eggs of *T. confusum* and *E. kuehniella* (Tunç *et al*. 2000). The exposure to vapours of essential oils from anise and cumin resulted in 100% mortality of the eggs of *T. confusum*. Oregano achieved mortalities as high as 77% and 8% in *T. confusum* and *E. kuehniella*, respectively. At a concentration of 98.5 ml anise essential oil/l air, the LT_{99} values were 60.9 h and 253.0 h for *E. kuehniella* and *T. confusum*, respectively. For the same concentration of the essential oil of cumin, the LT_{99} value for *E. kuehniella* was 127.0 h. As the essential oils from other plants investigated were less active their estimated LT_{99} values were too far beyond the tested exposure range to be reliable (Tunç *et al*. 2000).

Conclusion

Our results and those reported earlier clearly indicate variations in the activity of essential oils regarding the stage of the insect, the species of the insect and the plant origin of the essential oil. Not all the essential oils tested showed satisfactory activity, but the essential oils of cinnamon, and also clove, proved to be promising as control agents against stored-product insects, especially *T. castaneum*.

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