J. bio-sci. 14: 43-48, 2006 ISSN 1023-8654

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

Mina Mondal and M Khalequzzaman*

Department of Zoology, Rajshahi University, Rajshahi, Bangladesh

Abstract

Contact and fumigant toxicity of the three essential oils, viz., cardamom (*Elletaria cardamomum* Maton), Cinnamon (*Cinnamomum aromaticum* Nees), and Clove (*Syzygium aromaticum* (L.) Merr. and Petry) were tested against the red flour beetle, *Tribolium castaneum* (Herbst) larvae and adults. Residual film bioassay was employed in Petri dish (5 cm dia.) for contact toxicity studies and 6cm × 1.8cm glass vials were used for testing fumigation actions. Three day old adults and 10- day old larvae were equally susceptible to the contact toxicity of cinnamon oil, with LD_{50} values of 0.074 and 0.196 mg cm^{-2} respectively. Cardamom oil provided higher toxicity to 14-day and 18- day old larvae having LD_{50} value of 0.10mg cm^{-2}. In fumigation bioassay cinnamon oil provided the highest toxicity to adult and 10-, 14-, and 18-day old larvae, with LD_{50} values of 0.03, 0.05, 0.088 and 0.09 mg cm^{-3} respectively. Furthermore, 10-day old larvae were more tolerant than the adults to the contact toxicity of the essential oils, but 14- day old larvae had the same susceptibility as the adults. In contact and fumigation toxicity adults and all stages of larvae were more resistant to clove oil.

Key words: Bioassay, cardamom, cinnamon, clove, toxicity, essential oil.

Introduction

The red flour beetle, *Tribolium castaneum* (Herbst) is one of the major pests of stored grains and grain products in the tropics. The control of this insect relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users. An alternative to synthetic pesticides is the use of natural compounds, such as essential oils that result from secondary metabolism in plants. The toxicity of a large number of essential oils and their constituents have been evaluated against a number of stored-product insects (Paranagama *et al*. 2003).

Essential oils are commercially used in four primary aspects: as aromas in fragrances and perfumes, as flavouring food additives, as pharmaceuticals, and as insecticides. They recently have received much attention due to their multi-functions as antimicrobial, antifungal, antitumor and insecticidal agents (de Souza *et al*. 2005). Essential oils and especially their main compounds monoterpenoids, offer promising alternatives to classical fumigants (Peterson and Ems-Wilson 2003, Aslan *et al.* 2004). Essential oils are volatile and can act like fumigants offering the prospect for use in stored-product protection (Lee *et al*. 2001, 2002a,b, 2004), contact insecticides (Tapondjou *et al*. 2002, Peterson and Ems-Wilson 2003), antifeedent or repellent effects (Kim *et al.* 2003a,b, Park *et al.* 2003a,b, García *et al*. 2005) and may also affect some biological parameters such as growth rate, life span and reproduction (Tunç *et al.* 2000, Kathuria and Kaushik 2005, Rahmat *et al*. 2006).

* Corresponding author. E-mail: kzaman@ru.ac.bd.

In the present experiment three essential oils, e.g. cardamom, *Elletaria cardamomum* (L.), cinnamon (*Cinnamomum aromaticum* Nees) and clove (*Syzygium aromaticum* L. Meer and Perry) were tested in a series of toxicological experiments in order to determine their contact and fumigant effects on *T. castaneum.*

Materials and Methods

Biological materials

T. castaneum were used in this study. All larvae and adults were obtained from laboratory cultures maintained in the incubators at 30 ± 1ºC and 70 ± 5% r.h at dark. The beetles were reared on wheat flour mixed with yeast (10:1 w:w). The larvae of *T. castaneum* used in contact and fumigant toxicity experiments were 10-, 14- and 18- days old and adults were three to four days post-eclosion. All 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.

Contact Bioassay

Series of dilutions of essential oils were prepared using acetone as a solvent. Aliquots of 1 ml of the dilutions were applied into 6 cm dia. petridishes for surface-film bioassay (Busvine 1971). The solvent was allowed to evaporate for 1 hour and the treated insects were transferred to petridishes. Controls were treated with acetone alone. Fifteen adults or larvae of the species were used for each concentration and 20 adults or larvae were used for control. The petridishes were kept in the incubator and mortality was observed after 24 h.

Fumigant bioassay

Series of dilutions of essential oils were prepared using acetone as a solvent. Glass vials (6 cm long, 1.8 cm dia.) capped with polypropylene stoppers were used for the bioassays. Adult or larvae were transferred to the vials in groups of ten individuals and the vials were covered with fine nylon cloth secured with adhesive tape. Aliquots of 0.05 ml of the dilution were placed into similar vials. After evaporating the solvent, the vials containing the insects were turned upside down over the vials containing the oil so that the oil vapours saturated the atmosphere of the vials containing the beetles. Four replications of each treatment were set up. Controls were maintained in the similar way with the solvent only. The vials were kept in the incubator and mortality was observed after 24 h pose-exposure.

Data analysis

Mortality data were corrected using Abbott's formula (Abbott 1925). The observed data were subjected to probit analysis according to Finney (1947) and Busvine (1971) using a software developed at the Department of Agricultural and Environmental Science, University of Newcastle Upon Tyne, U K.

Results

In surface-film bioassay cinnamon oil offered the highest toxicity to adults and 10- day old larvae, whereas cardamom oil provided the maximum toxicity to 14- and 18- day old larvae. Cinnamon oil vapour (by fumigation) caused the highest mortality of various life stages of *T. castaneum* at LD_{50} level (Table 1). The adult were more susceptible than larvae to contact and fumigant actions. As the larvae grew older, they became less susceptible. Among the three essential oils clove oil was less toxic to *T. castaneum* to the contact and fumigation actions. In contrast to contact toxicity, adults were susceptible to the fumigant toxicity of all the three essential oils, and they were more susceptible than larvae.

Table 1. Contact and fumigant toxicity of some essential oils against *Tribolium castaneum* larvae and adults.

Oil	Life stage	Dose LD_{50} (mg cm^{-2})	95% confidence limits		Regression equation	χ^2 (2df)
			Lower (mg cm^{-2})	Upper (mg cm^{-2})		
Surface-film bioassay						
	Adult	0.122	0.092	0.160	Y=3.465192 + 1.414348X	0.151
	Larval age					
Cardamom	10 day	0.294	0.193	0.450	Y=3.690108 + 0.891879X	0.202
	14 day	0.101	0.072	0.142	Y=3.775373 + 1.218838X	0.446
	18 day	0.092	0.062	0.136	Y=3.924968 + 1.116663X	0.039
	Adult	0.074	0.051	0.106	Y=3.938027 + 1.22351X	0.538
	Larval age					
Clnnamon	10 day	0.196	0.138	0.280	Y=3.574011 + 1.102561X	0.140
	14 day	0.108	0.075	0.154	Y=3.902944 + 1.06257X	0.287
	18 day	0.235	0.133	0.417	Y=3.861711+ 0.82974X	0.888
	Adult	0.341	0.269	0.433	Y=4.137235 + 1.618698X	0.434
	Larval age					
		1.134	0.496	2.592	Y=4.075463 + 0.87668X	0.139
Clove	10 day	0.922	0.386	2.199	Y=4.296858 + 0.728981X	0.808
		0.779	0.392	1.549	Y=4.261798 + 0.82794X	2.238
	14 day					
	18 day					
Fumigation bioassay						
	Adult	0.223	0.150	0.332	Y=3.376679 + 1.203566X	1.853
	Larval age					
Cardamom	10 day	0.346	0.206	0.582	Y=3.598921 + 0.910077X	0.189
	14 day	0.355	0.252	0.499	Y=2.80085 + 1.419142X	0.662
	18 day	0.348	0.243	0.497	Y=2.933418 + 1.340766X	0.198
	Adult	0.030	0.017	0.052	Y=4.428527 + 1.204901X	0.928
	Larval age					
Clnnamon	10 day	0.050	0.030	0.084	Y=4.325145 + 0.960671X	0.090
	14 day	0.088	0.048	0.160	Y=4.262812 + 0.781603X	0.065
	18 day	0.090	0.060	0.133	Y=3.844475 + 1.213423X	0.660
	Adult	0.120	0.080	0.180	Y=3.762674 + 1.145967X	0.246
	Larval age					
Clove	10 day	0.214	0.101	0.452	Y=3.986094 + 0.76256X	0.333
	14 day	0.163	0.091	0.289	Y=3.963585 + 0.85587X	0.258
	18 day	0.131	0.082	0.207	Y=3.88088 + 1.002968X	0.251

Discussion

Cardamom oil was generally a more effective contact poison and fumigant against the adults of *T. castaneum*. These findings are similar to those of nutmeg oil (Huang *et al*. 1997). The essential oil of garlic was more toxic to *T. castaneum* than to *S. zeamais* by contact (Ho *et al*. 1996), while cinnamaldehyde, the

main constituent of cinnamon oil, exerted equal contact toxicity to both *T. castaneum* and *Sitophilus zeamais* (Huang and Ho 1998). *T. castaneum* adults showed similar susceptibility to the contact of cinnamaldehyde, having an LC_{50} of 0.7 mg cm^{-2}. However, it had higher fumigant toxicity with an LC_{50} of 0.28 mg cm^{-2}. The larvae became less susceptible to both contact and fumigant toxicity of cinnamaldehyde with age (Huang and Ho 1998). Contact toxicities of cardamom oil were tested by Huang *et al*. (2000). They observed that adults of *S. zeamais* and *T. castaneum* were equally susceptible to the contact toxicity of the cardamom oil at the LD_{50} values of 56 and 52 mg mg^{-1} insect respectively. Furthermore, 12-day old larvae of *T. castaneum* were more tolerant than the adults to the contact toxicity of the oil, but 14- and 16-day old larvae had the same susceptibility as the adults.

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.* 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 *T. castaneum* and *S. zeamais.* Cinnamaldehyde is a more potent insecticide against *T. castaneum* than anethole and eugenol. Therefore, cinnamaldehyde is more advantageous as a contact poison as it is equally effective against both species of insects. The larvae of *T. castaneum* were progressively more tolerant to cinnamaldehyde with age, a trend similarly observed with the 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). As in the case of contact toxicity, a similar trend was observed in the fumigant toxicity test to *T. castaneum* larvae, and the larvae became less susceptible to cinnamaldehyde with age.

A direct comparison of the potency of contact toxicities of the essential oils could not be made because different experimental methods were employed. These studies suggest that cardamom oil may be a potential grain protectant by killing various life stages of *T. castaneum* through contact and fumigant actions. This study suggests that cinnamon, cardamom and clove could be potent grain protectants due to their contact and fumigant activities against the *T. castanium.* The advantages of using essential oils as a grain protectants are: (1) they can be easily extracted by steam distillation; (2) they have very low toxicity to mammals since the popular spices consumed by people in various parts of the world; and (3) the essential oils are volatile and this can be potentially used fumigants.

References

Abbott W S (1925) A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**: 265–267.

Aslan İ, Özbek H, Çalmaşur Ö and Şahİn F (2004) Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Indust. Crops Prod*. **19**: 167–173.

Busvine J R (1971) A *Critical Review of the Techniques for Testing Insecticides.* Commonwealth Agricultural Bureau, London. 345 pp.

de Souza E L, Lima E O, de Luna K R, Freire K R L and de Sousa C P (2005) Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. *Brazilian Arch. Biol. Technol.* **48(2)**: 245–250.

Finney D J (1947) *Probit Analysis.* Cambridge University Press, London. 333 pp.

García M, Donadel O J, Ardanaz C E, Tonn C E and Sosa M E (2005) Toxic and repellent effects of *Baccharis salicifolia* essential oil on *Tribolium castaneum. Pest Manage. Sci.* **61**: 612–618.

Ho S H, Cheng L P L, Sim K Y and Tan H T W (1994) Potential of cloves *(Syzygium aromaticum* (L.) Merr. and Perry as a grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Postharvest Biol. Technol.* **4**: 179–183.

Ho S H, Koh L, Ma Y, Huang Y and Sim K Y (1996) The oil of garlic, *Allium satiuum* L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *Postharvest Biol. Technol*. **9**: 41–48.

Ho S H, Ma Y and Huang Y (1997) Anethole, a potential insecticide from *Illicium verum* Hook F. against two stored product insects. *Int. Pest Contr.* **39** (2): 50–51.

Ho S H, Ma Y, Goh P M and Sim K Y (1995) Star anise, *Illicium uerum* Hook f. as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus reamais* Motsch. *Postharvest Biol. Technol.* **6**: 341–347

Huang Y and Ho S H (1998) Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. stored Prod. Res.* **34(1)**: 11–17.

Huang Y, Lam S L and Ho S H (2000) Bioactivities of essential oils from *Elletaria cardamomum* (L.) to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *J. stored Prod. Res.* **36**: 107–117.

Huang Y, Tan J M W L, Kini R M and Ho S H (1997) Toxic and antifeedant action of nutmeg oil against monoterpenes on *Acanthoscelides obtectus* (Say) (Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.*). J. stored Prod. Res.* **31**: 291–299

Kathuria V and Kaushik T (2005) Feeding inhibition of *Helicoverpa armigera* (Hübner) by *Eucalyptus camaldulensis* and *Tylophora indica* extracts. *Insect Sci.* **12**: 249–254.

Kim S I, Park C, Ohh M H, Cho H C and Ahn Y J (2003a) Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). *J. stored Prod. Res.* 39(1): 11–19.

Kim S I, Roh J Y, Kim D H, Lee H S and Ahn Y J (2003b) Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* (L.) and *Callosobruchus chinensis* (L.). *J. stored Prod. Res.* **39(3)**: 293–303.

Lee B H, Annis P C, Tumaalii F and Choi W S (2004) Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects. *J. stored Prod. Res.* **40**: 553–564.

Lee B H, Choi W S, Lee S E and Park B S (2001) Fumigant toxicity of essential oil and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). *Crop Prot*. **20**: 317–320.

Lee B H, Lee S E, Annis P C, Pratt S J, Part B S and Tumaalii F (2002b) Fumigant toxicity of essential oils and monoterpenes against the red flour beetle, *Tribolium castaneum* Herbst. *J. Asia-Pacific Entomol*. **5(2)**: 237-240.

Lee Y H, Park E K and Lee S E (2002a) Adverse effect of essential oil fumigation on *Proisotoma minuta* (Collembola: Entomobryoidae). *J. Asia-Pacific Entomol*. **5(1)**: 131-133.

Paranagama P A, Abeysekera K H T, Abeywickrama K P and Nugaliyadde L (2003) Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. *Let. App. Microbiol.* **36**: 1–5.

Park C, Kin S and Ahn Y J (2003a) Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. *J. stored Prod. Res.* **39(3):** 333–342.

Park K, Lee S G, Hoi D H, Park J D and Ahn Y J (2003b) Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtuse* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J. stored Prod. Res.* **39(4)**: 375–384.

Peterson C J and Ems-Wilson J (2003) Catnip essential oil as a barrier to subterranean termites (Isoptera: Rhinotermitidae) in the laboratory. *J. Econ. Entomol*. **96(4)**: 1275–1282.

Rahmat A, Edrini S, Ismail P, Hin T Y Y and Bakar M F A B (2006) Chemical constituents, antioxidant and cytotoxic effects of essential oil from *Strobilanthes crispus* and *Lawsonia inermis*. *J. Biol. Sci.* **6(6)**: 1005-1010.

Tapondjou L A, Adler C, Bouda, H and Fontem D A (2002) Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. *J. stored Prod. Res.* **38**: 395–402.

Tunç I, Berger B M, Erler F and Dağli F (2000) Ovicidal activity of essential oils from five plants against two stored-product insects. *J. stored Prod. Res.* **36**: 161-168.