Univ. j. zool. Rajshahi Univ. Vol. 26, 2007. pp. 63-66 **ISSN 1023-6104**
http://journals.sfu.ca/bd/index.php/UJZRU

Contact and Fumigant Toxicity of Essential Oils Against *Callosobruchus maculatus*.

I. Mahfuz and M. Khalequzzaman
Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh

Abstract: Toxicity of five essential oils (EOs), viz. cardamom, cinnamon, clove, eucalyptus and neem oils were investigated against the cowpea weevil, *Callosobruchus maculatus* (Fab.) adults, through contact and fumigation bioassay. In the contact bioassay eucalyptus oil was found to be the most effective in inducing mortality both after 24 and 48 h of treatments. The toxicity of the oils followed in the order: eucalyptus > clove > cinnamon > cardamom > neem. In the fumigation bioassay, however, a reverse result was obtained with eucalyptus oil where it shows the last position for 24 h and fourth position for 48 h after treatments. The efficacy in respect of the toxicity followed in the order: clove > cinnamon > cardamom > neem > eucalyptus after 24 h after treatment, and clove > cinnamon > cardamom > eucalyptus > neem after 48 h after treatments.

Keywords: Essential oil, bioassay, LD_{50}, *C. maculatus*

Introduction

Callosobruchus maculatus has involved the great attention because it is widely distributed throughout the tropical and sub-tropical regions. It is an important pest of several pulses including cowpea [*Vigna unguiculata* (L.) (Walp.)], chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), soyabean (*Glycine max* Mer.) and haricot beans (*Phaseolus vulgaris* L.). The pulses are very important source of vegetable protein for millions of people of tropical and subtropical regions. Cowpea seeds *V. unguiculata* have seriously affected by *C. maculatus* and cause maximum damage of 2 to 5 kg seeds within 45 to 90 days in optimum temperature ($30^0 \pm 1^0$ C) and moisture conditions (75 ± 3 %).

In order to keep these stored grain products free from pest attack, various synthetic chemicals have been used. Synthetic pesticides are currently the method of choice to protect stored grain from insect damage. But, continuous or heavy uses of synthetic pesticides has created serious problems arising from factors such as direct toxicity to parasites, predators, pollinators, fish and man. It also develops pesticides resistance (Zettler, 1991; Mahmud *et al*., 2002), susceptibility of crop plant to insect pests (Pimentel, 1977) and increased environmental and social cost (Pimentel *et al*., 1980). Therefore, environment needs some other alternatives of chemical pesticides. One alternative to synthetic insecticides is the botanical pesticides i.e. insecticidal plants or plant compound and the use of natural compounds, such as essential oils that result from secondary metabolism in plants. Essential oil and their constituents have been shown to be a potent source of botanical pesticide. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of bruchid pests (Keita, *et al.,* 2000, 2001, Tripathi *et al*., 2002).

Plant essential oils and their constituents in relation to contact and fumigant insecticidal actions have been well demonstrated against stored product pests. Especially their main compounds monoterpenoids, offer promising alternatives to classical fumigants (Papachristos & Stamopoulos, 2003) and also have some effects on biological parameters such as growth rate, life span and reproduction (Pascual-Villalobos, 1996).

In the present investigation essential oils from cardamom, cinnamon, clove, eucalyptus and neem were studied for their contact and fumigant effects on the adult *C. maculatus*.

Materials and Methods

Insect: C. maculatus were collected from private store houses of Rajshahi and Chapai-Nawabganj district, and the cultures were maintained in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh.

Mass cultures were maintained in earthen pots (2 kg) and/or large plastic containers (1.5 kg) and sub-cultures in beakers (500 g) or small plastic containers (100 g) with the food medium, the seeds of cowpea. The beetles were allowed to grow in natural environment as in traditional storehouse and checked in regular intervals. A huge number of pulse beetles were thus reared to set a continuous supply of the newly formed adults. The foods were kept in an incubator for sterilization, about 24 hours at 60°C to disinfest them. Then the seeds were thoroughly washed with tap water to remove dusts and other insect present in the materials and carefully dried under sun-light, having 13-14% moisture content. The sterile foods were than preserved in airtight glass jars (1000 ml) in order to impede further infestation.

Oils: The essential oils were purchased as pure oil (Branded in India) from a shop at Dhaka, Bangladesh.

The five essential oils were Cardamom [*Elettaria cardamomum* (L.) Maton.], Cinnamon (*Cinnamomum aromaticum* Nees), Clove [*Syzygium aromaticum* (L.) Merr. & Perry], Eucalyptus [*Eucalyptus* spp.] and Neem [*Azadirachta indica* A. Juss]. The oils were dehydrated in a vacuum rotary evaporator and then collected in sealed glass containers and refrigerated in the dark at 4°C.

Residual film bioassay: The adult *C. maculatus* were used for the residual film bioassay which is treated with the oils separately. Fifteen pulse beetles were used in each of the three replications. Oil was weighed and diluted in acetone and the actual doses were calculated from the amount of oil present in 1 ml of the solution. The doses of cardamom, cinnamon, clove and neem oils were 62.85, 31.42, 15.71 and 7.85 µg cm^{-2}, whereas the doses of eucalyptus oil were 62.85, 31.42, 15.71 and 13.04 µg cm^{-2}. For treatment 1 ml of each of the doses was poured down on to each petri dish (9 cm dia.) and air dried. The adults were than put in treated petri dishes and kept at the room temperature. A control experiment was maintained in which treatment was made with acetone. Only the mortality of the beetles was recorded after 24 and 48 h of treatment.

Fumigation bioassay: Glass vials (5.5 cm long by 2 cm in diameter), capped with polypropylene stoppers were used for the bioassay. Pulse beetles were transferred to the vials in groups of 15 adults. The vials were covered with fine nylon cloth secured with adhesive tape. The doses used for cardamom, cinnamon and clove oils were 95.45, 63.63, 47.72 and 31.81 µg cm^{-2}, whereas the doses used for eucalyptus and neem oils were 190.90, 127.26, 95.45 and 63.63 µg cm^{-2}. The vials containing the insects were then turned upside down over the vials containing the oil such that the oil vapours saturated the atmosphere of the containers containing the pulse beetles. The control consisted of a similar setup but without essential oil. This procedure was replicated three times. The vials were placed at room temperature with a photoperiod of 14 h light and 10 h dark. Mortality counts were made 24 and 48 h after treatment.

Results

The results of toxic effect of different essential oils applied as residual film and fumigation method are presented in Table 1.

In the residual film bioassay eucalyptus oil was found to be the most effective in inducing mortality both after 24 and 48 h of treatment. The efficacy of the oils followed in the order: eucalyptus > clove > cinnamon > cardamom > neem. The LD_{50} value in case of 24 h after treatment were 31.26, 26.64, 21.85, 12.22 and 488.63 µg cm^{-2}, and after 48 hours of treatment were 24.72, 22.18, 13.84, 8.86 and 57.99 µgcm^{-2} respectively for cardamom, cinnamon, clove, eucalyptus and neem oils.

In the fumigation bioassay, however, a reverse result was obtained with eucalyptus oil where it is placed in the last position for 24 h after treatment and fourth position for 48 h after treatment. The efficacy in respect of the toxicity followed in the order: clove > cinnamon > cardamom > neem > eucalyptus after 24 h of treatment, and clove > cinnamon > cardamom > eucalyptus > neem after 48 h oftreatment. The LD_{50} values recorded for these oils were 304.12, 279.18, 92.81, 652.64 and 507.69 µg cm^{-2} (for 24 h of treatment); and 205.00, 118.53, 69.62, 403.10 and 420.34 µg cm^{-2} (for 48 h of treatment) for cardamom, cinnamon, clove, eucalyptus and neem oils, respectively.

Discussion

Several oils were tested against *C. maculatus* attacking *Vigna* spp. There were differences in oil efficacy at the doses tested under different experimental conditions, as noted by Pierrard (1986). The present results corroborates the findings of Jilani & Malik (1973) and Ali *et al.* (1983) who also reported the toxic effect of neem oil, coconut oil, rapeseed oil, mustard oil, sesame oil, dalda and palm oil on *C. chinensis*. More current research illustrated that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Huang *et al*., 2000; Tunc *et al*., 2000; Lee *et al*., 2001a,b).

Cinnamaldehyde, the main constituent of cinnamon oil, exerted equal contact toxicity to both *T. castaneum* and *S. zeamais* (Huang & Ho, 1998). Oil of clove was toxic to *S. oryzae* and *Rhyzopertha dominica* (Sighamony *et al*., 1986). Non-polar extracts of the flower buds of clove, *Syzygium aromaticum* and star anise (*Illicium uvrum* Hook f.) are insecticidal to *T. castaneum* and *S. zeamais* Motsch., and suppress progeny production (Ho *et al*., 1994). It was found that against C. *maculatus*, *C. chinensis* and *C. analis* attack in *V. radiata*, neem oil *(Azadirachta indica* A. Juss) allowed no adult emergence, reduced oviposition, and prevented insect development (Babu *et al.*, 1989).

In the present residual film bioassay eucalyptus oil was found to be highly effective in inducing mortality of adult *C. maculatus*. Clove, cinnamon and cardamom oils were also found to be effective as the LD_{50} values are very close to the eucalyptus oil. Neem oil exhibited very high LD_{50} value in comparison to other oils after 24 h of treatment indicating its ineffectiveness in this regard. However, the difference in the results of neem oil with that of other four oils was decreased to a large extent after 48 h of treatment. The fumigation bioassay indicated that the treatment of eucalyptus gave the highest toxicity after 24 h of treatment followed by neem, cardamom, cinnamon and clove, and after 48 h of treatment the toxicity followed in the order of clove > cinnamon > cardamom > eucalyptus > neem. The present study demonstrated that the essential oils of

clove, cinnamon and cardamom, exhibited toxicity to C. *maculatus* adults in both the bioassay experiments.

Table 1. LD_{50}, 95% confidence limits and regression equations of essential oils to adult*Callosobruchus maculatus* after 24 and 48 h of residual film and fumigation treatment.

Treatment	Duration	Oils	LD_{50} (µg cm^{-2})	95% confidence limits		Regression equations	χ^2 (at 2 df)
				Lower (µg cm^{-2})	Upper (µg cm^{-2})		
Residual film bioassay	24 h	Cardamom	31.26	25.35	38.56	Y = 1.474 + 2.358 X	4.69
		Cinnamon	26.64	22.17	32.01	Y = 1.202 + 2.664 X	2.00
		Clove	21.86	18.17	26.29	Y = 1.496 + 2.616 X	1.60
		Eucalyptus	12.23	4.39	34.04	Y = 3.708 + 1.180 X	6.93*
		Neem	488.63	24.35	9806.74	Y = 3.199 + 0.670 X	2.15
	48 h	Cardamom	24.72	17.41	35.11	Y = 1.574 + 2.459 X	6.45*
		Cinnamon	22.19	18.66	26.39	Y = 1.162 + 2.851 X	3.57
		Clove	13.84	9.02	21.25	Y = 2.583 + 2.118 X	6.11*
		Eucalyptus	8.87	2.79	28.21	Y = 3.551 + 1.529 X	8.00*
		Neem	57.99	32.69	102.88	Y = 2.899 + 1.191 X	2.66
Fumigation bioassay	24 h	Cardamom	304.12	66.02	1400.92	Y = 1.177 + 1.540 X	0.248
		Cinnamon	279.19	56.84	1371.35	Y = 1.822 + 1.299 X	0.002
		Clove	92.81	62.64	137.52	Y = 1.497 + 1.780 X	0.399
		Eucalyptus	652.64	110.99	3837.80	Y = 1.266 + 1.327 X	0.015
		Neem	507.69	164.07	1570.95	Y = –0.397 + 1.995 X	0.046
	48 h	Cardamom	205.00	67.68	620.98	Y = 1.591 + 1.475 X	0.056
		Cinnamon	118.53	75.09	187.11	Y = 0.620 + 2.112 X	0.303
		Clove	69.63	53.47	90.62	Y = 1.557 + 1.869 X	0.405
		Eucalyptus	403.11	144.77	1122.41	Y = 0.768 + 1.624 X	0.379
		Neem	420.35	158.90	1111.94	Y = 0.131 + 1.856 X	0.115

* Variance has been adjusted for heterogenity

References:

Ali, S. I., Singh, O. P. & Misra, U. S. 1983. Effectiveness of plant oils against pulse beetle *Callosobruchus chinensis* Linn. *Indian J. Entomol.* **45:** 6-9.

Babu, T. R., Reddy, V. S. & Hussaini, S. H. 1989. Effect of edible and non edible oils on the development of the pulse beetle *Callosobruchus chinensis* (L) and on viability and yield of mungbean (*Vigna radiata* (L) Wilczek). *Tropical Sci.* **29:** 215-220.

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

Huang, Y. & 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. & Ho, S. H. 2000. Bio-activities of essential oil from *Elletaria cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst). *J. stored Prod. Res*. **36 (2)**: 107-117.

Jilani, G. & Malik, M.M. 1973. Studies on neem plant as repellent against stored grain insects. *Pakistan. J. Sci. Ind. Res.* **16(6):** 251-254.

Keita, S. M., Vincent, C., Belanger, A. & Schmit, J. P. 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) [Coleoptera: Bruchidae]. *J. stored Prod. Res*. **36:** 355–364.

Keita, S. M., Vincent, C., Schmit, J. P. Arnason, J. T. & Bélanger, A. 2001. Efficacy of essential oil of *Ocimum basilicum* L. and *O. gratissimum* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). *J. stored Prod. Res*. **37:** 339-349.

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

Lee, S. E., Lee, B. H., Choi, W. S., Park, B. S., Kim, J. G. & Campbell, B. C. 2001b. Fumigant toxicityof volatile natural products from Korean spices and medicinal plants towards the rice weevil, *Sitophilus oryzae* (L.). *Pest Manag. Sci*. **57:** 548–553.

Mahmud, M. K., Khan, M. M. H., Husain, M., Alam, M. I. & Afrad, M. S. I. 2002. Toxic effects of different plant oils on pulse beetle *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae). *J. Asiat. Soc. Bangladesh, Sci*. **28(1)**: 11-18.

Papachristos, D.P. & Stamopoulos, D. C. 2003. Selection of *Acanthoscelides obtectus* (Say) for resistance to lavender essential oil vapour. *J. stored Prod. Res.* 39: 433-441.

Pascual-Villalobos, J. M. 1996. Evaluation of the insecticidal activity of *Chrysanthemum coronarium* L. plant extracts. *Bol. Sanidad Veg. Plagas*. **22:** 411–420.

Pierrard, G. l986. Control of the cowpea weevil, *Callosobruchus maculatus*, at the farmer level in Senegal. *Trop. Pest Management* **32:** 197-200.

Pimentel, D. 1977. Ecological basis of insect pest, pathogen and weed problems. In: *Origin of pest, parasites, disease and weed problems.* (Cherrett, S.M. & Sagar, G.R. eds.), p. 3. Blackwell's scientific publications, Oxford, UK.

Pimentel, D., Andow, D., Dyson-Hudson, R., Gallahan, D., Jacobson, S., Irish, M., Croop, S., Moss, A., Schreiner, I., Shepard, M., Thompson, T. & Vinzant, B. 1980. Environmental and social costs of pesticides: a preliminary assessment. *Oikos* **34:** 125-140.

Sighamony, S., Anees, I., Chandrakala, T. S. & Osmani, Z. 1986. Efficacy of certain indigenous plant products as grain protectants against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). *J. stored Prod. Res.* **22:** 21-23.

Singh, D. & Singh, A. K. 1991. Repellent and insecticide properties of essential oils against housefly, *Musca domestica* L. *Insect Sci. Applic*. **12 (4)**: 487-491.

Tripathi, A. K., Prajapati, V., Verma, N., Bhal, J. R., Bansal, R. P., Khanuja, S.P.S. & Kumar, S. 2002. Bioactivities of the leaf essential oil of *Curcuma longa* (Var. Ch-66) on three species of stored-product beetles (Coleoptera). *J. Econ. Entomol*. **95 (1)**: 183-189.

Tunc, I., Berger, B. M., Erler, F. & Dagli, F. 2000. Ovicidal activity of essential oils from five plants against two stored-product insects. *J. stored Prod. Res.* **36**: 161-168.

Zettler, J. L.1991. Pesticide resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. *J. Econ. Entomol*. 84: 763-767.

Manuscript received on 13.01.07, accepted on 07.12.07