Short Communication

Repellent activity of common spices against the rice weevil, *Sitophilus zeamais* Motsch (Coleoptera, Curculionidae)

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ABSTRACT. Five essential oils and eight extracts of common spices were screened and evaluated for repellent activity against adults of the rice weevil, *Sitophilus zeamais* (Motsch.), using the modified filter paper impregnation method. Both extracts of cinnamon (*Cinnamomum zeylanicum*) and essential oils of gingers, *Etlingera elatior*, *E. pyramidosphaera* and *Zingiber officinale*, showed strong repellent activity against *S. zeamis* at 20 mg/ml. Cinnamon methanol extract exhibited potent repellent activity at the concentration of 10 mg/ml. Extracts of turmeric (*Curcuma longa*) and black pepper (*Piper nigrum*) showed moderate repellent activity at 20 or 50 mg/ml. However, extracts of lemongrass (*Cymbopogon citratus*) and dry chili (*Capsicum annuum*) exhibited weak attractant activity. The findings from this research demonstrate the possible use of these spices as repellent of rice weevil in stored products.

Keywords: bioassay, essential oil, repellency, rice weevil, *Sitophilus zeamais*

INTRODUCTION

Worldwide, rice is an important food and is continuously attacked by several insect pests during storage. *Sitophilus* species are major stored product pests throughout the world, and widely infests rice, other grains and their products (Asmanizar *et al.*, 2008). The protection of stored products against attack by such pests is essential in many countries, particularly those that do not have adequate storage facilities. Control of these pests relies on the widespread use of various synthetic chemical insecticides and fumigants. It has led to a number of serious problems such as environmental pollution, pesticide residue in food grains, pesticide resistance and toxicity to non-target organisms (Yusof & Ho, 1992; Cosimi *et al.*, 2009). Therefore, the development of safer alternatives to conventional synthetic insecticides and fumigants is highly desirable.

In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides. Terrestrial plants are known to contain a rich source of bioactive metabolites which show antifeedant, repellent and toxic effects in a wide range of insects (Rajendran & Sriranjini, 2008; Ukeh *et al.*, 2009). Tripathi *et al*. (2002) reported that the leaf essential oil of turmeric, *Curcuma longa*, exhibited contact and fumigant toxicity against three stored-product beetles. Ukeh *et al.*, (2009) found that *Zingiber officinale* extract were repellent toward adult *S. zeamais* Motschulsky. In addition, a methylene chloride extract of cinnamon, and acetone extracts of black pepper seeds have been reported to be toxic to grain storage insects

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(Ho *et al.*, 1996; Huang & Ho, 1998). Ho *et al*. (2003) screened more than 60 Malaysian plants against two species of insects and found that *Melicope subunifoliolata* showed strong feeding deterrent activity against *S. zeamais*. Thus, a wide variety of vascular plants may provide new sources of natural pesticides and repellents, and their extracts and essential oils have potential for use in stored product protection.

The use of locally available common spices to reduce pest damage for stored food is common practice in traditional farm storage. Research on the evaluation of local materials for stored product protection is vital in Sabah as rice farming is carried out traditionally in villages. This study was undertaken to examine repellent activities of several common spices against adult *S. zeamais* using the slightly modified filter paper impregnation assay.

MATERIALS AND METHODS

Sample Collection

Specimens such as black pepper (*Piper nigrum*), fresh chili (*Capsicum annuum*), dry chili powder (*Capsicum annuum*), dried bark of cinnamon (*Cinnamomum zeylanicum)*, and lemongrass (*Cymbopogon citratus*) were purchased from a market in Kota Kinabalu, Sabah. Ginger, *Zingiber officinale* was bought from a market in Keningau, Sabah. *Etlingera brevilabrum* and *E. pyramidosphaera* were collected from Ulu Kimanis, Sabah, while *E. elatior* was collected from the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. Fresh rhizome of *Curcuma longa* (turmeric) was collected from Tambunan, Sabah.

Sample Preparation

Essential oils of five plant samples (*E. brevilabrum*, *E. elatior*, *E. pyramidosphaera*, lemongrass and *Z. officinale*) were extracted by the hydrodistillation method using a Clevenger apparatus. Resulting oil was dehydrated over Na_2SO_4 anhydrous, prior to use. Black pepper and dried bark of cinnamon were ground to powder before extraction. Crude extracts were prepared by immersing samples in ethanol. Crude extracts of black pepper and cinnamon were further partitioned between hexane and 90% methanol.

Insect

Sitophilus zeamais was collected from infested rice obtained from a local market and reared on rice grain in glass jars covered with nylon mesh, held with a rubber band for the passage of air at 28±2^{o}C and 70-80% relative humidity (r.h.).

Bioassay

The repellent activities of the test materials against *S. zeamais* were evaluated using the filter paper impregnation method (Huang *et al.*, 1997; Liu & Ho, 1999) with some modification. Filter paper (Whatman No.1, 12 cm diameter) was cut in a square shape (4 cm^2) and folded in wave shape. 100 µl of appropriate concentration of each test sample was applied to prepared square filter papers. Ethyl acetate (EtOAc) or EtOH was used for the control in all experiments. The treated and control papers were air dried for 20 minutes to evaporate the solvent completely. After drying, pairs of the treated and control paper were placed in a glass Petri dish (90 mm diameter) and then 20 unsexed adult beetles were released at the centre of the Petri dish. Vaseline was applied to the inner vertical side of the Petri dish to prevent the beetles from climbing onto the side and lid of the Petri dish. The Petri dish was covered with a lid and kept in darkness at 28±2^{o}C and 70-80% r.h. Each treatment was replicated three times and the numbers of beetle settled on each filter paper were counted and recorded at hourly intervals for 6 hours. No significant difference was detected between the repellency of organic solvents impregnated and untreated filter papers in tests designed to check for any possible influence of organic solvents (EtOAc and EtOH). A χ^2-test was performed to compare the total number of beetles from three replicates on each filter paper.

RESULTS AND DISCUSSION

In this study, five essential oils and eight extracts of common spices were evaluated by a modified repellency test against adults of *S. zeamais*. Data from three repetitions of repellent test for these 13 samples are given in Table 1. The cinnamon methanol extract showed the highest repellent activity for *S. zeamais* adults at all time intervals. It had repellency at the concentration of 10 mg/ml (62.5 µg/cm^2), but its effect was not clearly demonstrated at 1 mg/ml. In addition, cinnamon hexane extract also highly repelled *S. zeamais* adults on filter papers at all time intervals with maximum activity after 2 hours of exposure. Among essential oils, *E. elatior*, *E. pyramidosphaera* and *Z. officinale* strongly repelled at all tested exposure time, while *E. brevilabrum* and lemongrass as well as its crude extract did not show any significant effect. Extracts of black pepper and turmeric gave moderate levels of repellency. Piperine, a pungent substance in black pepper and cinnamaldehyde, a principal component of cinnamon flavour, are reported to possess insecticidal activities (Huang & Ho, 1998; de Paula *et al.*, 2000). These findings indicate that such active compounds may play a role in the repellent activity against *S. zeamais* and show their potency at much lower concentrations. Chili and lemongrass are well known to demonstrate insect repellent effects (Oyedele *et al.*, 2002; Chomchalow 2003; Parugrug & Roxas, 2008). Nevertheless, repellent activity of chili and lemongrass against *S. zeamais* adults were unverified in our experiment and extracts of lemongrass and dry chili had weak attractant activity.

In conclusion, the present study performed screening and evaluation of 13 samples and revealed that eight of them exhibited repellent activity against *S. zeamais* adults. This result suggests that these active materials have potential to provide grain protectant and may be exploited for rice weevil control in grain storage in an environment-friendly way. This is a preliminary study conducted to find out suitable targets and further studies will be needed. Our next approach will concentrate on the isolation and evaluation of effective compounds from these active materials.

Table 1. Table 1. Repellent activity of 13 test samples on *Sitophilus zeamais* adults after different exposure times using the filter paper test.

Test sample	Conc. (mg/mL)	Trial	Number of insects on each paper after each exposure																				
			1 h				2 h				3 h				4 h				5 h				6 h
			Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr	Un	χ^2	χ^2r	Tr
ESSENTIAL OIL																							
Etlingera brevilabrum	20	1	10	1	7.36	1.06	15	4	6.37	0.56	15	5	5	0.3	14	3	7.12	1.2	11	6	1.47	4.08*	13
		2	6	6	0		6	8	0.29		13	6	2.58		3	9	3		5	13	3.56		4
		3	4	7	0.82		4	8	1.33		1	14	11.27		7	5	0.33		1	12	9.31		3
Etlingera elatior	20	1	1	15	12.25	22.09**	0	17	17	48**	0	16	16	44**	0	15	15	50**	0	13	13	41.09**	0
		2	0	9	9		0	17	17		0	13	13		0	17	17		0	15	15		1
		3	2	6	2		0	14	14		0	15	15		0	18	18		1	16	13.24		2
Etlingera pyramidosphaera	20	1	1	12	9.31	38.1**	1	11	8.33	34.38**	0	15	15	45.08**	0	9	9	36.1**	0	14	14	35.77**	0
		2	0	13	13		0	13	13		0	15	15		0	17	17		2	16	10.89		1
		3	0	16	16		1	16	13.24		1	18	15.21		1	13	10.29		1	16	13.24		1
Zingiber officinale	20	1	1	13	10.29	35.1**	0	13	13	34.38**	1	11	8.33	24.64**	0	19	19	45.08**	0	13	13	41**	2
		2	0	13	13		1	14	11.27		1	13	10.29		1	14	11.27		0	14	14		0
		3	0	12	12		1	13	10.29		2	11	6.23		0	15	15		0	14	14		0
Cymbopogon citratus (lemongrass)	20	1	13	5	3.56	2.08	7	11	0.89	0.72	10	7	0.53	1.65	6	9	0.6	0.82	4	6	0.4	4.08*	12
		2	3	7	1.6		6	9	0.6		10	9	0.05		12	5	2.88		10	9	0.05		13
		3	8	3	2.27		15	2	9.94		9	4	1.92		7	5	0.33		17	2	11.84		14
EXTRACT																							
Cymbopogon citratus (lemongrass)	50	1	4	6	0.4	0.64	5	9	1.14	0	11	1	8.33	5.77*	17	0	17	12.25**	16	0	16	8.02**	16
		2	11	2	6.23		14	0	14		16	0	16		20	0	20		20	0	20		20
		3	2	14	9		2	12	7.14		0	11	11		1	13	10.29		2	17	11.84		0
Capsicum annuum (dry chili)	50	1	7	2	2.78	2.13	19	0	19	8.4**	12	1	9.31	10.76**	14	0	14	3	12	2	7.14	8.1**	14
		2	7	4	0.82		12	2	7.14		16	2	10.89		16	3	8.89		15	1	12.25		10
		3	1	2	0.33		0	10	10		3	7	1.6		0	15	15		2	8	3.6		2
Capsicum annuum (fresh chili)	50	1	5	8	0.69	9.26**	4	12	4	8.65**	5	7	0.33	7.04**	14	5	4.26	5.79*	12	5	2.88	2.81	12
		2	2	9	4.45		9	8	0.06		9	9	0		5	13	3.56		4	7	0.82		4
		3	3	12	5.4		2	16	10.89		0	16	16		0	19	19		0	15	15		2
Curcuma longa (turmeric)	50	1	3	11	4.57	22.22**	5	10	1.67	19.57**	2	14	9	28.17**	2	11	6.23	24.08**	3	13	6.25	34.31**	4
		2	1	16	13.24		2	14	9		3	15	8		5	15	5		2	13	8.07		4
		3	4	14	5.56		1	14	11.27		0	12	12		0	15	15		0	19	19		0

Piper nigrum (black pepper hexane)	20	1	0	15	15	18.69**	2	13	8.07	18.69**	2	8	3.6	26.56**	1	16	13.24	31.39**	1	17	14.22	30.08**	0
		2	1	9	6.4		3	13	6.25		1	13	10.29		1	13	10.29		1	14	11.27		2
		3	5	9	1.14		3	11	4.57		1	16	13.24		2	13	8.07		3	12	5.4		3
Piper nigrum (black pepper methanol)	20	1	0	11	11	10.53**	2	4	0.67	4.83*	3	3	0	9.32**	3	15	8	24.92**	4	11	3.27	14.4**	0
		2	2	8	3.6		3	12	5.4		1	10	7.36		4	13	4.76		1	12	9.31		3
		3	7	10	0.53		6	8	0.29		3	11	4.57		1	16	13.24		3	9	3		5
Cinnamomum zeylanicum (cinnamon hexane)	20	1	0	13	13	29.88**	0	18	18	47**	1	13	10.29	39.09**	0	16	16	40.33**	1	15	12.25	40.33**	0
		2	3	12	5.4		0	14	14		0	14	14		0	18	18		1	15	12.25		0
		3	0	13	13		0	15	15		0	15	15		2	12	7.14		0	16	16		1
Cinnamomum zeylanicum (cinnamon methanol)	20	1	0	16	16	48**	0	18	18	50**	0	14	14	49**	1	15	12.25	45.08**	0	17	17	48**	1
		2	0	17	17		0	17	17		0	16	16		0	19	19		0	17	17		0
		3	0	15	15		0	15	15		0	19	19		0	14	14		0	14	14		0
	10	1	0	14	14	35.1**	0	17	17	38.35**	0	12	12	28.9**	2	16	10.89	31.39**	0	19	19	45.3**	0
		2	0	16	16		2	14	9		2	10	5.33		1	16	13.24		1	18	15.21		1
		3	1	8	5.44		0	13	13		1	15	12.25		1	13	10.29		1	14	11.27		1
	1	1	10	2	5.33	14.29**	0	16	16	10.08**	16	1	13.24	1.52	12	0	12	0.1	16	0	16	0.1	6
		2	12	1	9.31		13	4	4.76		7	7	0		8	7	0.07		5	10	1.67		0
		3	2	1	0.33		0	15	15		2	9	4.45		2	13	8.07		0	9	9		0
	0.1	1	5	6	0.09	0.03	10	2	5.33	2.19	13	1	10.29	7.71**	16	2	10.89	2.67	10	0	10	1.6	10
		2	8	3	2.27		4	11	3.27		2	10	5.33		3	16	8.89		4	12	4		2
		3	2	5	1.29		9	1	6.4		15	1	12.25		14	3	7.12		10	4	2.57		9

** Total number of three replicates for treated (Tr) and untreated (Un) papers significantly different at $P < 1\%$. * Total number of three replicates significantly different at $P < 5\%$.

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