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Fumigant Effect of Essential Oils of Several Species of Plants on *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

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Abstract: Fumigant effects of essential oil vapours from 14 species were studied on adults of *Sitophilus zeamais* (Motschulsky). The oils came from *Mentha haplocalyx*, *M. spicata*, *Illicium verum*, *Myristica fragrans*, *Alpinia officinarum*, *Cinnamomum parthenoxylon*, *Acorus tatarinowii*, *Brassica juncea*, *Capsicum annuum*, *Litsea cubeb*, *Curcuma longa*, *Artemisia princeps*, *Pogostemon cablin* and *Cymbopogon citratus* was tested. Eight essential oils had fumigant activity on *S. zeamais* adults and oils from *M. haplocalyx* and *M. spicata* were the strongest. The LC_{50} values for oil from *M. haplocalyx* under the exposure periods of 24, 48 and 72 h were respectively 11.53, 9.49 and 7.93 μL/L. For *M. spicata* oil, LC_{50} values for 24, 48 and 72 h were respectively 13.43, 11.36 and 9.20 μL/L.

Key words: plant essential oils, *Sitophilus zeamais* (Motschulsky), fumigant effect, *Mentha haplocalyx*, *Mentha spicata*l

Introduction

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) is an important primary pest of stored products around world. *Sitophilus zeamais* seriously affects stored products, including rice, wheat, corn, potatoes and their process products, as well as some special local products and Chinese medicinal materials[1]. The three-months loss rate of infested foodstuff can be 11.25%, increasing to 35.12% after six months. Fumigants and chemical repellents are mainly used to control *S. zeamais* in the national and local grain depots in China[2]. But because of the misuse of chemicals, *S. zeamais* has developed resistance to some insecticides and phosphine[3,4,5]. The resistance factor of *S. zeamais* to phosphine can reach 116 times[6].

Essential oils are volatile secondary metabolites produced by plants for their own needs other than nutrition. In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. Studies found that they have various activities against insects, such as fumigant toxicity, contact toxicity, stomach toxicity, repellency and developmental retardation. Furthermore, there is no evidence of pests having resistance to essential oils[7,8,9,10,11].

In this report, we present results of a study on the fumigant effect of 14 species of plant essential oils on *S. zeamais*, aiming at providing a theoretical basis of developing insecticides using economical and safe plant materials against storage pests.

Materials and Methods

Insects and Rearing Conditions

Sitophilus zeamais were reared in our laboratory at the Institute of Urban Pest Control in Huazhong Agricultural University (China). The temperature in rearing room was kept at 27 ± 1℃, while the relative humidity was maintained at 70% ± 5%. Glass jars of 500 mL capacity, covered with calico, were used to contain whole wheat with a moisture content of 13 ± 1%. Wheat was washed in tap water, dried and heated at 80℃ for 2 h to prevent pre-infestation and then stored at the above laboratory conditions. When the second generation adults were 2 – 3 weeks old, they were used in the bioassays.

Essential oil Species

14 species of essential oils were tested. 8 essential oils were distilled in the laboratory and 6 were purchased in Jiangxi (Table 1).

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Table 1. List of 14 species of essential oils

Scientific name	Family	Chinese name	Extract from	Place
Mentha haplocalyx	Labiatae	Bohe	Leaf and stem	HZAU
Illicium verum	Illiciaceae	Bajiaohuixiang	Seed	HZAU
Myristica fragrans	Myristicaceae	Roudoukou	Seed	HZAU
Alpinia officinarum	Zingiberaceae	Gaoliangjiang	Rhizome	HZAU
Curcuma longa	Zingiberaceae	Jianghuang	Root	HZAU
Acorus gramineus	Araceae	Shichangpu	Rhizome	HZAU
Brassic juncea	Brassicaceae	Jiecai	Seed	HZAU
Capsicum annuum	Solanaceae	Lajiao	Fruit	HZAU
Mentha spicata	Labiatae	Liulanxiang	Leaf	Jiangxi
Cinnamomum parthenoxylon	Lauraceae	Huangzhang	Root	Jiangxi
Litsea cubeba	Lauraceae	Shancangzi	Fruit	Jiangxi
Artemisi princeps	Compositae	Aihao	Leaf	Jiangxi
Cymbopogon citratus	Gramineae	Xiangmao	Leaf	Jiangxi
Pogostemon cablin	Labiatae	Huoxiang	Leaf	Jiangxi

Extraction of Essential Oils

The plant materials were dried in the oven at 40℃, crushed using a vegetation disintegrator, and then they were filtered through a 40 mesh screen. Dry plant powders (30 g) were subjected to steam distillation to get the oil water mixture. All mixtures were collected and extracted by the petroleum ether. The petroleum ether extract was concentrated in the rotary evaporation machine to reach the maximum yield. The essential oils were collected in sealed brown bottles and refrigerated in the dark at 0 -4℃ until their use.

Fumigant bioassays of 14 Essential Oils

The sealed conical flask fumigant method used by Deng et al.[8] was adopted. Filter paper was cut to strips (1 cm wide 4 cm long) and we passed a thread through each strip. Then the thread was stuck to the middle of a plastic film. Thirty *S. zeamais* adults were introduced into a 250 mL conical flask, and 14.7 μL/L essential oil was dropped on the filter strip. The flask was sealed using plastic film and the strip hung in the center of the flask. Experiments were repeated four times for each essential oil. Control flasks contained no essential oil. All treatments were kept in the dark insect bioassay room at 27 ±1℃ and 70% ±5% relative humidity. The number of the dead insects was observed in terms of treatment time, and mortality was corrected for the control mortality. After comparing the fumigant results of all 14 essential oils, two were selected for further bioassays.

Fumigant Bioassay of Selected Essential Oils

The sealed conical flask fumigant method was adopted (see above). There were three exposure periods of each treatment (24, 48 and 72 h). Seven concentrations in the range of 2 - 32 μL/L were used for each exposure time and the Lethal Concentration 50 (LC_{50}) was determined. Each experiment was repeated fourtimes.

Statistical Analysis

Mortality = (Number dead/Total number) ×100%

Abbott's formula was used to correct the mortality:

Corrected mortality = (Treatment mortality - Control mortality)/(1 - Control mortality) × 100%

Mortality data were subjected to analysis of variance (ANOVA) and Fisher's Protected LSD was used to compare effects among treatments (SPSS 14.0 for Windows). LC_{50}, LC_{95} values were calculated using to probit analysis.

Results and Discussion

Results of Extraction of Essential Oils

After using steam distillation and evaporating the petroleum ether solvent, the extract rate was obtained for each of the eight species (Table 2). The resulting extract rates presented important differences. The extract rates of only two species reached 1%, i.e. *I. verum* with 1.87% and *C. longa* with 1.16%. The extract rates of the other six species were all under 1%, with a minimum of 0.08% for *C. annuum*.

Fumigant Effect of 14 Essential Oils

The experiment against *S. zeamais* was conducted at 27 ± 1℃ and 70% ± 5% relative humidity with a fumigant concentration of 14.7 μL/L. The corrected mortality of *S. zeamais* exposed for 24 and 48 h is shown in Table 3. It is obvious that the fumigant effect of plant essential oils against *S. zeamais* varies with the exposure time and species. As expected, mortality was significantly higher after 48 h exposure ($P < 0.05$). The species effect was significant too ($P < 0.05$). At 24 h, eight oils were not significantly different from the control. At 48 h, six oils among these eight had again no effect. The two other oils (*A. tatarinowii* and *B. juncea*) presented weak effects on *S. zeamais* mortality after 48 h exposure. *M. haplocalyx* oil and *M. spicata* oil presented significantly the most important fumigant effect after both 24 and 48 h exposure time. After 24 h, *M. spicata* oil and *M. haplocalyx* oil caused 65.83 and 86.67% mortality respectively, indicating the rapid availability of these two oils for *S. zeamais*. At 48 h, mortality from *M. haplocalyx* oil had reached 100%, and *M. spicata* oil values reached 79.17%. Compared with the 24 h exposure, corrected mortality from *I. verum* oil, *A. officinarum* oil, *M. haplocalyx* oil, *M. spicata* oil and *M. fragrans* oil increased more than 10%. The increase of toxicity of *I. verum* oil was especially obvious with over 30% mortality.

Table 2. Extract rate of essential oil of plants

Plant	Quantity of dry powder (g)	Quantity of essential oil (g)	Extraction rate (%)
I. verum	280	5.24	1.87
C. longa	360	4.17	1.16
M. fragrans	360	3.58	0.99
A. tatarinowii	220	1.38	0.63
A. aofficinarum	680	2.66	0.39
B. juncea	320	0.89	0.28
M. haplocalyx	720	2.02	0.28
C. annuum	600	0.48	0.08

Extract rate = Quantity of essential oil/Quantity of dry plant powder 100%.

Table 3. Toxicity to adults of *S. zeamais* of different species of essential oil vapours *

Treatment	Concentration (μL/L)	Corrected mortality after different treated periods (%) (Mean ± SE)	
		24h	48h
M. haplocalyx oil	14.7	86.67 ± 1.36 a	100.00 ± 0.00 a**
M. spicata oil	14.7	65.83 ± 0.83 b	79.17 ± 0.83 b
I. verum oil	14.7	21.67 ± 0.96 c	58.33 ± 3.19 c
M. fragrans oil	14.7	29.17 ± 2.10 d	42.50 ± 0.83 d
A. officinarum oil	14.7	18.33 ± 0.96 d	33.33 ± 1.36 e
C. parthenoxylon oil	14.7	11.67 ± 6.45 e	20.83 ± 2.50 f
A. tatarinowii oil	14.7	4.17 ± 0.83 f	5.00 ± 0.96 g
B. juncea oil	14.7	3.33 ± 1.36 f	5.00 ± 1.67 g
C. annuum oil	14.7	0.83 ± 0.83 f	4.17 ± 1.60 gh
L. cubeb oil	14.7	0.00 ± 0.00 f	3.33 ± 1.36 gh
C. longa oil	14.7	2.50 ± 0.83 f	2.50 ± 0.83 gh
A. princeps oil	14.7	0.00 ± 0.00 f	2.50 ± 1.60 gh
P. cablin oil	14.7	0.00 ± 0.00 f	1.67 ± 0.96 gh
C. citratus oil	14.7	0.00 ± 0.00 f	1.67 ± 0.96 gh
Control	14.7	0.00 ± 0.00 f	0.00 ± 0.00 h

* Each datum represents mean of four replicates.

** Means followed with different letters within the same column are significantly different at 5% level ($P < 0.05$) by Fisher's Protected LSD.

Toxicity of *M. haplocalyx* oil and *M. spicata* Oil at Different Exposure Periods

The fumigant toxicity experiment was conducted at 27 ± 1℃ and of 70% ± 5% relative humidity. In relation to exposure period and concentration, the toxicity of *M. haplocalyx* oil and *M. spicata* oil to adult *S. zeamais* is illustrated in Tables 4 and 5. The linear regression equation between probit mortality (Y) and the logarithm of concentration (x) is shown in Ta-

bles 6 and 7. The longer of the exposure period, the lower the LC_{50} values for *M. haplocalyx* oil and *M. spicata* oil. Values of LC_{50} for *M. haplocalyx* oil against *S. zeamais* were respectively 11.53, 9.49 and 7.93 μL/L after 24, 48 and 72 h. The LC_{50} value for 72 h exposure was 1.45 times lower than the LC_{50} value for 24 h, and range of decrease range was small. The linear regression equation between LC_{50} value (y) and exposure period (t) is: $y = 13.250 - 0.075t$; $r = 0.997$; $df = 1, 1$; $F = 168.750$; $P < 0.05$. In the fumigant experiment using *M. spicata* oil, the LC_{50} for 24 h exposure was 13.43 μL/L, decreasing to 11.36 μL/L after 48 h exposure. The LC_{50} value for 72 h exposure was 9.20 μL/L, which was 1.45 times lower than the LC_{50} value for 24 h exposure, and range of decrease was small. The linear regression equation between LC_{50} value (y) and exposure period (t) is: $y = 15.560 - 0.088t$; $r = 1.000$; $df = 1, 1$; $F = 6627.000$; $P < 0.05$. LC_{95} reached 16.54 μL/L and 21.40 μL/L after 72 h exposure time for *M. haplocalyx* oil and *M. spicata* oil. These results show the greater efficacy of *M. haplocalyx* oil compared to *M. spicata* oil also observed in the previous experiment.

Table 4. Toxicity to adults of *S. zeamais* of *M. haplocalyx* oil vapour*

24 h		48 h		72 h	
Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)
18	99.17	16	100.00	16	100.00
16	88.33	14	85.83	14	93.33
14	64.17	12	65.83	12	78.33
12	49.17	10	45.00	10	64.17
10	28.33	8	27.50	8	47.50
8	17.50	6	12.50	6	18.33
6	2.50	4	6.67	4	14.17
CK	0.00	CK	0.00	CK	0.00

* Each datum represents mean of four replicates.

Table 5. Toxicity to adults of *S. zeamais* of *M. spicata* oil vapour

24 h		48 h		72 h	
Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)
32	99.17	28	100.00	24	100.00
28	97.50	24	100.00	20	94.17
24	97.50	20	89.17	16	84.17
20	83.33	16	75.00	12	76.67
16	60.83	12	65.00	8	23.33
12	51.67	8	12.50	4	5.83
8	3.33	4	1.67	2	2.50
CK	0.00	CK	0.00	CK	0.00

Table 6. Toxicity to adults of *S. zeamais* of *M. haplocalyx* oil vapour

Exposure period (h)	Regression equation	LC_{50} (μL/L) (95% Confidence limits)	LC_{95} (μL/L)	DF	X^2
24	$Y = -7.9148 + 7.4528x$	11.53 (10.52 ~ 12.59)	19.17	5	20.444*
48	$Y = -5.5443 + 5.6724x$	9.49 (7.91 ~ 11.23)	18.51	5	41.384*
72	$Y = -4.6311 + 5.1503x$	7.93 (6.63 ~ 9.18)	16.54	5	29.507*

Table 7. Toxicity to adults of *S. zeamais* of *M. spicata* oil vapour

Exposure period(h)	Regression equation	LC_{50} (μL/L) (95% Confidence limits)	LC_{95} (μL/L)	DF	X^2
24	Y = −7.2037 +6.3864x	13.43(11.68 −14.99)	24.29	5	19.444*
48	Y = −6.2449 +5.9164x	11.36(9.57 −12.98)	21.55	5	22.313*
72	Y = −4.3236 +4.4861x	9.20(5.95 −12.38)	21.40	5	64.935*

Conclusion

This research indicates that eight of 14 essential oils had fumigation activity on the adult of *S. zeamais*. These were the oils from *M. haplocalyx*, *M. spicata*, *I. verum*, *M. fragrans*, *A. officinarum*, *C. parthenoxylon*, *A. tatarinowii* and *B. juncea*. The oils of *M. haplocalyx* and *M. spicata* were better than the others, especially *M. haplocalyx* oil. Further research on the toxicity of *M. haplocalyx* and *M. spicata* oils against adult *S. zeamais* during different exposure period and concentration showed that LC_{50} values decreased with increase of exposure period, which showed that these two essential oils had longer persistence. When the concentration of the essential oil is persistent, the fumigant effect is better at longer of exposure periods. Therefore prolonging the exposure period can reduce the quantity of the essential oil required.

There have been several reports describing research on control of *S. zeamais* with essential oils. Huang *et al.* [12] tested the effect of *Elletaria cardamomum* oil against *S. zeamais* and *Tribolium castaneum*, and the result indicated that the sensitivity of *S. zeamais* to *E. cardamomum* oil was double than the sensitivity of *T. castaneum*. Hou and Zhang [7] studied the fumigant effect and population inhibiting activity of 24 essential oils against *S. zeamais*, and found that *M. spicata* oil and *I. verum* oil had high fumigant activity. The research of Deng *et al.* [8] on the fumigant effect of nine essential oils against adult *S. zeamais* showed that *C. parthenoxylon* oil, *Melaleuca alternifolia* oil, *Citrus limonum* oil, *M. spicata* oil and *Pinus tabulaeformis* oil had the best fumigant effect, especially *C. parthenoxylon* oil. In the current study, the fumigant efficacy of *I. verum* oil and *C. parthenoxylon* oil were not very good, possibly for the reason that the temperature and the fumigation method are different in the two studies. Also, the toxicity data collected in this study were obtained in flasks without grain, and the data are likely to vary with the species and the quantity of grain. Thus the influence of temperature and grain on the fumigant efficacy of essential oils needs more study.

References

[1] Yang CJ, Deng WX, Yang ZH. Rapeseed oil treatment for the control of *Sitophilus zeamais* during grain storage. *Journal of Huazhong Agricultural University*, 1993, 12(1): 87 −89

[2] Ho SH, Koh J, Ma Y, *et al*. The oil of garlic, Alliumsativum L. (Amaryllidaceae), as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motschulsky). *Postharvest Biology and Technology*, 1996, 9(1): 41 −48

[3] Shen ZP. The occurrence and prospect of fumigant in stored grain. *Heilongjiang Grain*, 2004, (6): 33 −36.

[4] Yu YF. The discussion of fumigant effect of phosphine. Science and Technology of Cereals, *Oils and Foods*, 2005, 13(2): 19 −20

[5] Xiao L. The resistance of phosphine fumigant. *Pesticide of World*, 2001, 23(2): 46 −48

[6] Li LS. The retrospect and prospect of storage pest research and control. *Entomological Knowledge*, 2000, 37(2): 84 −88

[7] Hou HM, Zhang X. Research progress on the insecticidal activity of plant essential oil. *World Agriculture*, 2001, (4): 263 −265

[8] Deng YX, Wang JJ, Ju YM, et al. Comparison of fumigation activities of nine kinds of essential oils against the adults of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae). *Chinese Journal of Pesticide Science*, 2004, 6(3): 85 −88

[9] Lu CB, Xue M, Liu YQ, et al. Study on effect of volatile oils of *Vitex negundo* against *Sitophilus zeamais* (Motschulsky). *Grain Storage*, 2005, (6): 13 −16

[10] Yao YJ, Xue D, Yang CJ. Research advances in the control of *Sitophilus zeamais* by botanical insecticides. *Journal of the Chinese Cereals and Oils Association*, 2005, 20(3): 83 −88

[11] Yao YJ, Xue D, Yang CJ. Bioactivities of extracts from 21 species of plants against maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae). *Acta Entomologica Sinica*, 2005, 48(5): 692 −698

[12] Huang Y, Lam SL, Ho SH. Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton to *Sitophilus zeamais* (Motschulsky) and *Tribolium castaneum* (Herbst) *Journal of Stored Products Research*, 2000, 36(1): 107 −11