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Research on One Kind of Essential Oil Against Drugstore Beetle *Stegobium paniceum* (L.)

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1. Introduction

Insect pest management in stored products is facing a crisis due to several serious drawbacks of using insecticides, such as the development of resistance in the treated pest, toxic residues, and the increasing cost of application (Tapondjou et al 2002). Hence it is necessary to develop alternative pest control techniques for protecting stored commodities (Gunasekaran and Rajendran 2005). Toward this end, intensified efforts have lead to an increasing number of research studies to find safe, effective and viable alternatives (Tapondjou et al. 2002). Carbon dioxide gas (CO₂) and essential oils from plants have received considerable attention for the control of stored products insects, because of their relative safety to the non-target organisms (AliNiazee 1972; Bekele et al 1996; Juliana and Su 1983; Sudesh et al 1996; Bouda et al 2001).

Chinese medicinal materials (CMM) are widely available in China. While most of these products are stored prior to use for health protection or disease treatment, great losses often occur during storage due to infestation by insect pests. Drugstore beetle *Stegobium paniceum* (L.) is the most widely encountered insect causing serious damages to stored products. It is one of the dominant species found on stored CMM in Hubei, Guizhou and Shandong, causing huge economic losses (Can et al 2004; Guilin and Wangxi 1996; Zhaohui and Fangqiang 2001). A series of measures have been taken to control infestation (Nielsen 2001; Hashem 2000, Toh 1998; Platt et al 1998). However, concerns over health and safety associated with traditional synthetic insecticides have prompted the development of plant-based insecticides.

The present study explores the efficacy of essential oil from *Z. bungeanum* Maxim against larvae and adults of *S. paniceum*. The contact toxicity and fumigant toxicity were investigated, and the subsequent development of treated insects was recorded.

2. Materials and methods

2.1 Extraction of essential oil

The fruits of *Z. bungeanum* were collected from the mountains in the west of China. After cleaning and pounding into powder, the materials were soaked in clear water (1:5, w:v) at

60°C for 24h. After a 14 hour hydro-distillation, the crude oil was collected, dehydrated with anhydrous sodium sulfate and stored at 4°C until use.

2.2 Collecting and rearing insects

The insects were obtained from a local CMM storehouse. They were mass reared on *Euphorbia kansui* Liou in a growth chamber under controlled conditions (29±1°C; 75±5% r.h.; light for 14 h).

2.3 Fumigant toxicity of crude oil to drugstore beetle

The oil was introduced onto a filter paper (7cm diameter, surface 38.5cm²) placed in the center of a 1L glass jar. To prevent insects from contacting the oil, the insects and cultural medium were separated by a piece of gauze placed around the filter.

Larvae (25 to 30 days-old, n=20 in each of the three replicates) were exposed to concentrations of 12, 24, 48, 96 and 192 µl crude oil in the glass jars. Clear water was used as control. The jars were covered with fine gauze to prevent the insects from fleeing. The insects were incubated in the growth chamber at 29±1°C and 75±5% r.h.. During the exposure, the test insects were fed on *E. kansui* Liou. After exposure of 6 to 144 h, the insect mortalities were determined respectively. Mortality was corrected by Abbotts (1925) formula when the mortality of control insects is above 10%.

Similarly, adult insects (2 to 3 days-old, 20 per replicate) were exposed to concentrations of 12, 24, 48, 96 and 196 µl in 1L glass jars respectively. The glass jars were covered by gauze to prevent the insects from flying away and they were kept in the same conditions as the larvae. Insect mortalities were determined after exposure to oil for 3 to 72 h.

2.4 Contact toxicity of oil to drugstore beetle

The crude oil was diluted with clear water to 200, 400, 667, 1000, and 2000 ppm respectively for this test. One µl of each of these solutions was introduced onto the back of one insect. The glass jars were placed into growth chamber at 29±1°C and 75±5% r.h.. Three replicates of each dose and three controls treated with water were tested (n=20 per replicate). The toxicity to insects was assessed after treating for 12 to 144 h.

2.5 Observation of subsequent development

After LT₅₀ (The median mortality time) treatment, the living insect samples were transferred to similar sized jars and were incubated in the growth chamber, and untreated insects were transferred as a control group. They were under regular observation to assess their subsequent development in another day. The development time of the larvae and the number of eggs laid by each adult were recorded.

3. Results

3.1 Fumigant toxicity of essential oil to drugstore beetle

The percentages of mortality for insects exposed to different doses of oil are shown in Figures 1 and 2. In general, the higher mortality was achieved when the insects were

exposed to a higher dose of oil or a longer exposure period. The dose of 192 ul/L of oil achieved complete mortality of larvae after the pests were exposed for 96 h and the dose of 96 ul/L achieved the same result after exposure of 120 h. The dose of 48 ul/l needed 144 h or longer to achieve complete mortality. The lowest dosage of 12ul/l induced little mortality (<10%) when the pests were exposed for 6h or shorter.

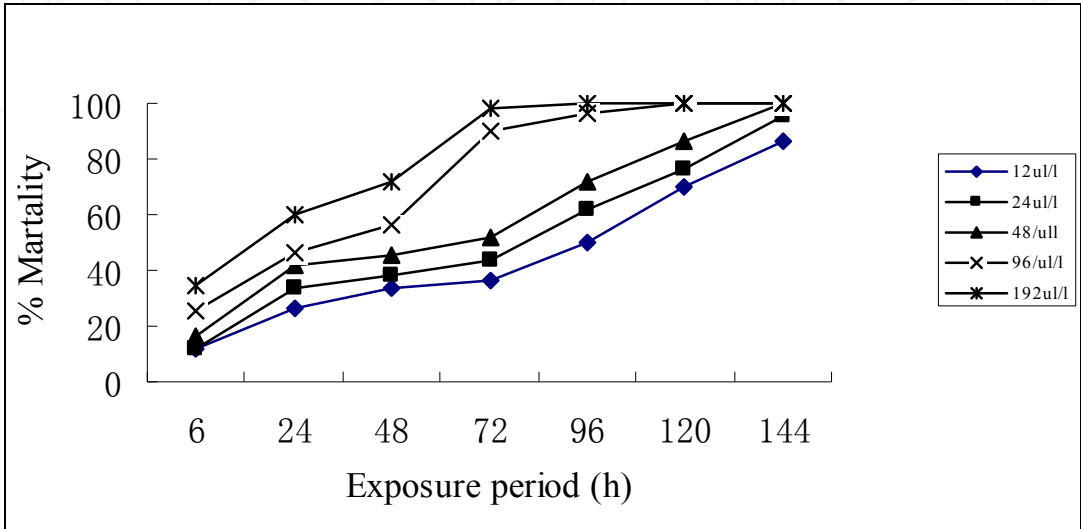


Fig. 1. Fumigant toxicity of essential oil to the larvae of drugstore beetle *S. paniceum*. Mortality was NOT corrected by Abbotts (1925) formula in all calculations because the mortality of control insect is <5%.

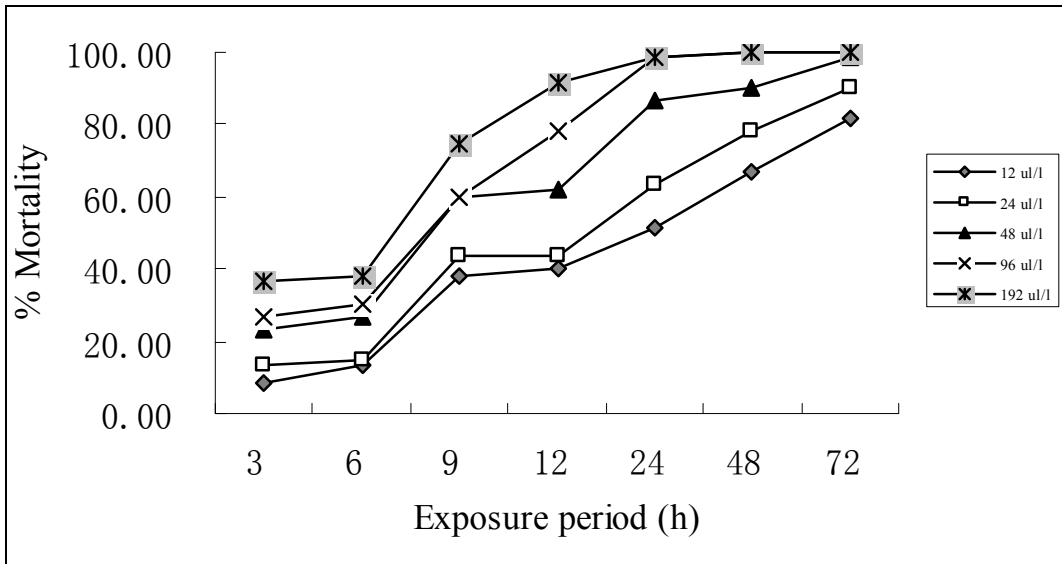


Fig. 2. Fumigant toxicity of essential oil to the adults of drugstore beetle *S. paniceum*. Figure 2 illustrates the fumigant toxicity of essential oil on the adults. The trend is similar to that shown in Figure 1. With the increase of concentration and exposure time, the

percentage of mortality improved. It reached 100% at the concentration of 96 or 192 ul/L of oil with the exposure time of 48 h. After 6 h treatment, the mortality rate was significantly higher than control group at all doses.

Significant differences of susceptibility were noted between adults and larvae under the same conditions. In fumigant test for 12h the calculated regression line equation was $Y=3.24+0.66X$ for larva and $Y=3.05+1.40X$ for adult, respectively (Probit analysis). Similar analysis of 24h treatment generated an equation $Y=3.61+0.70X$ for larvae and $Y=2.80+1.99X$ for adult. Comparison of LD₅₀ values for the two life stages showed that the adult was more susceptible (LD₅₀=24.57 ul/l for 12h; LD₅₀=12.64 ul/l for 24h) than larva (LD₅₀=485.84 ul/l for 12h; LD₅₀=98.92 ul/l for 24h) (Table 1).

Life stages	Fumigant toxicity 24h		Fumigant toxicity 12h	
	LD ₅₀ (ul/l)	Regression model	LD ₅₀ (ul/l)	Regression model
Larva	98.92	$y=3.61+0.70x,r=0.996$	485.84	$y=3.24+0.66x,r=0.988$
Adult	12.64	$y=2.80+1.99x,r=0.968$	24.57	$y=3.05+1.40x,r=0.976$

Table 1. LD₅₀ and regression model calculated for mortality within exposure period of 12 and 24 h to fumigant toxicity.

3.2 Contact toxicity of essential oil to drugstore beetle

Figure 3 represents contact toxicity of the larvae of different concentrations of essential oil solution. The dose of 2000 ppm of solution achieved complete mortality of larvae after the pests were exposed for 120 h. The dose of 1000 ppm or 667 ppm of the solution also achieved complete mortality after 144 h exposure (Fig 3). The percentage of mortality of adult reached 100% at the concentration of 2000 ppm and exposure of 144 h. It also achieved complete mortality at 1000 ppm of the solution for 144h (Fig. 4).

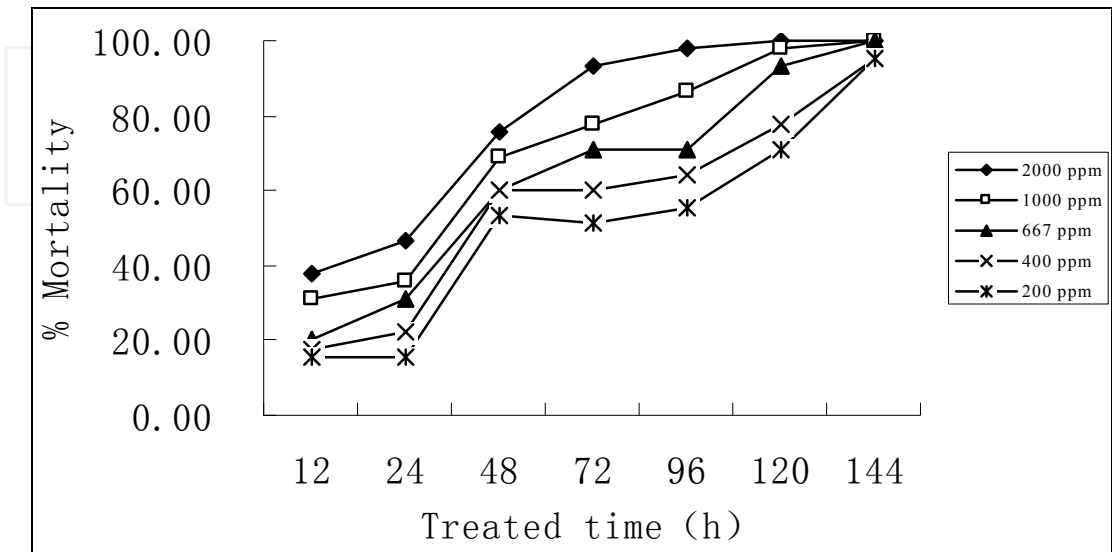


Fig. 3. Contact toxicity of essential oil to the larvae of drugstore beetle *S. paniceum*.

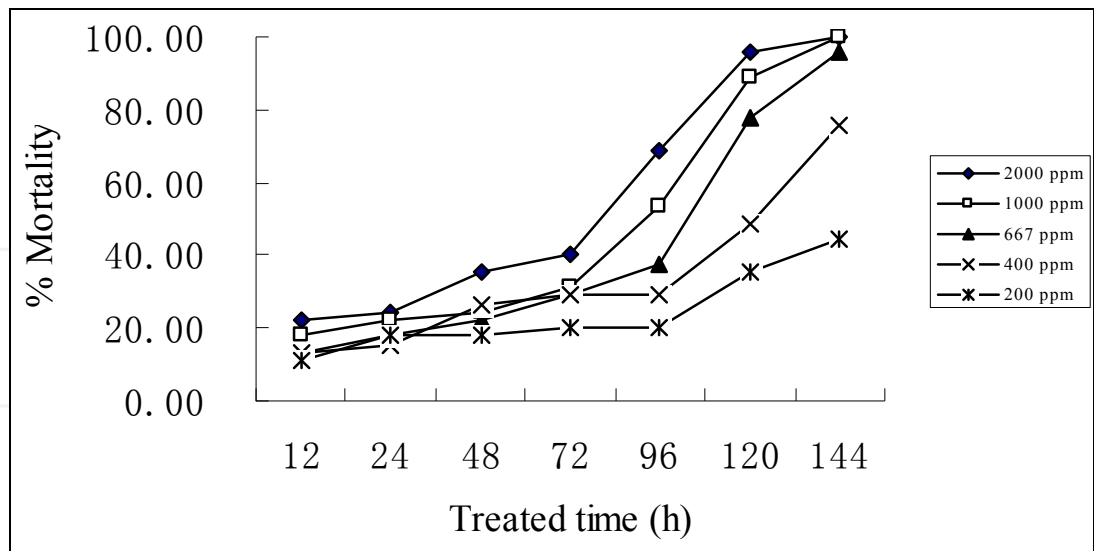


Fig. 4. Contact toxicity of essential oil to the adults of drugstore beetle *S. paniceum*.

The mortality of larvae was significantly higher than that of adults (Fig 3 and Fig 4) when treated for the same period and concentration. According to Probit analysis, the calculated regression line equation of 24 h for larva was $Y=1.82+0.91X$ and for adult was $Y=2.35+0.60X$. Similar trend was found with the 48 h data. Comparison of LD₅₀ values for life stages showed that the larva was more susceptible (LD₅₀=3175.91 ppm for 24h; LD₅₀=292.13ppm for 48h) than adult (LD₅₀=23610.74 ppm for 24h; LD₅₀=6784.18 ppm for 48 h) (Table 2).

Life stages	Contact toxicity 24h			Contact toxicity 48h		
	LD ₅₀ (ppm)	Regression model		LD ₅₀ (ppm)	Regression model	
Larva	3175.91	$y=1.82+0.91x$	$r=0.948$	292.13	$y=2.92+0.85x$	$r=9.981$
Adult	23610.74	$y=2.35+0.60x$	$r=0.967$	6784.18	$y=2.09+0.76x$	$r=0.986$

Table 2. LD₅₀ and regression model calculated for mortality within treated time of 24 and 48 h for contact toxicity.

3.3 The subsequent development of test insects

Subsequent observation on the adult pests revealed that the essential oil also prevented adults from laying eggs normally. Significant differences were observed on the number of eggs and development time of larvae between the treated and untreated pests ($p<0.05$). The development time of the treated larvae was 29.77 ± 6.27 (d) ($n=13$), and untreated was 16.77 ± 2.17 (d) ($n=13$) (mean \pm SE). The mean number of eggs laid by treated adult was 14.21 ± 3.28 ($n=27$), comparing with 63.50 ± 23.94 ($n=27$) (mean \pm SE) by the untreated insects.

4. Discussion

Contact and fumigant insecticidal actions of plant essential oils have been well demonstrated against pests in stored products (Murray B. Isman 2000). Several investigations have demonstrated contact, fumigant and antifeedant effects of a range of

essential oil constituents against the red flour beetle *Tribolium castaneum* and the maize weevil *Sitophilus zeamais* (Ho et al 1994, 1995, 1997; Huang and Ho 1998). Essential oils from many plants have been developed as pest control agents. Perhaps the most attractive benefit of using essential oils (or their constituents) as protectants is their low mammalian toxicity (Murray B. Isman 2000).

Insecticidal activity of *Z. bungeanum* Maxim volatile oil was reported before. The results showed that *Z. bungeanum* Maxim volatile oil had high repellent and insecticidal activity against *Sitophilus zeamais* and *Tribolium castaneum*. The volatile constituents of the essential oil were identified by their retention index and mass spectrum in comparison with those of standard synthetic compounds (Zhi'an et al 2001). The major chemical constituents are C₉, C₁₀ alcohols and alkenes. The most abundant constituent was 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl) (33.578%), followed by Eucalyptol (15.656%) and Benzene,1-methoxy-4-(1-propenyl) (8.33%). Among those chemical components, beta-Phellandrene (23.2%), alpha-Pinene (1.89%) (Jun et al 2001) could be the key factors to control the pests.

The infestation of stored Chinese medicinal materials by drug store beetle is a serious problem. The use of insecticides is avoided because of toxicity. In this study, we investigated the effects of the essential oil from *Z. bungeanum* on the control of drug store beetle and found that it not only prolonged the development time of larvae but can also prevented adults from laying eggs successfully. Effective contact and fumigant insecticidal actions of such oil were demonstrated, and the essential oil may help control expansion of drugstore beetle populations. Adult drugstore beetles were more susceptible than larvae to fumigant action of the oil. Adults showed higher mortalities at the same concentration than larvae, likely cause by suffocation. However, larvae were more susceptible than adults to contact treatment. These results are likely because the respiration of adults is higher than that of larvae and the adults' hard elytra may have prevented the infiltration of oil. The distinct responses to oil at these two life stages may be attributed to the morphological and behavioral differences (Tapondjou 2002). It is important to note that the susceptibility varies during the life cycle when using these oils as pest control regime.

Essential oil from *Z. bungeanum*, as a plant product, is unlikely to affect the nutritional or medicinal composition of stored CMM. Its use can be beneficial for human health and environment safety (Murray B. Isma 2000). The effectiveness and safety of the essential oil supported its use as a natural pest control agent.

5. Acknowledgements

This research was sponsored in part by Guizhou provincial Modernization of Chinese Material Medical project (No.QKHZYZ[2011]5049), Organization Department of CPC Guizhou Committee [TZJF-2009-02] and Guiyang city science and technology bureau [No. 2010(1-4)].

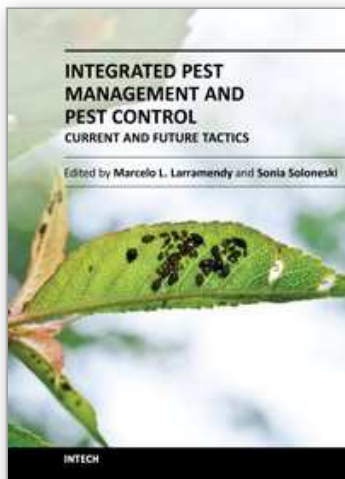
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Edited by Dr. Sonia Soloneski

ISBN 978-953-51-0050-8

Hard cover, 668 pages

Publisher InTech

Published online 24, February, 2012

Published in print edition February, 2012

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