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Evaluation of Plant Extracts from *Illicium verum* for the Control of Museum Insect Pest *Demestes maculatus*

Bo Zhang
Department of Natural History, Shenzhen Museum, Shenzhen 518027, China

Abstract: To assess the insecticidal activity of extracts from *Illicium verum* against museum insect pest *Demestes maculates*, mortalities of its larvae and adults under different dose or treatment time were investigated by bioassay methods. Results showed that both extracts (anise oil and (*E*)-anethole) were highly toxic to both life stages (larvae and adults) of *D. maculatus*. Furthermore, the insecticidal activity was dependent upon both dose and exposure time. With the increasing of the dose level and the exposure time, higher mortality was obtained. On the other hand, results also indicated that *D. maculatus* adults were more tolerant against the extracts from *I. verum* than larvae. At the same time, it was interesting to demonstrate that the toxicity of anise oil was more effective than (*E*)-anethole against *D. maculatus* under the same experimental condition. The highest mortality 95% was achieved at 72 h with anise oil against larvae at a dose of 32/cm^3. As naturally occurring insect-control agents, the *I. verum* extracts described could be useful for managing populations of museum pest *D. maculates*.

Key words: Fumigation, *Illicium verum*, museum pest, plant extracts

INTRODUCTION

Insect pest management for collections is a key work in natural history museum. Museum objects comprising mounted insect specimens and higher animals including stuffed birds and mammals, and cultural and historic properties having woolen or other animal materials and ethanographic items are commonly infested by different species (Rajendran and Hajira Parveen, 2005).The reason these species are able to flourish on museum objects is because both the food source (the museum object) and the environment (the storage room) are very favorable to their propagation and survival and these organisms can do a wide array of damage, ranging from the disfiguring of an objects surface to the total destruction of its structure. The variety of pests known to cause damage to museum collections is quite extensive. Beauchamp *et al*. (1981) lists 96 species of pests in the United States, to which 82 are insects. *Demestes maculates*, as recognized serious museum pest, was selected to tested pest for all kinds of pest control methods because adequate number of its life stages can be easily reared from culture (Linnie, 1987).

The use of chemicals to protect museum collections against damage by pests has a long tradition. However, concern over the health and safety implications of the use of chemicals in the museum environment, effectiveness, and potential adverse effects on museum specimens and associated materials has led to a reappraisal of collection protection policies (Dawson and Strang, 1992; Irwin, 1987; Linnie, 1996; Peltz and Rossol, 1983). Therefore, this has focused attention on alternative methods of controlling and eradicating pests from museums. There are now well reported alternatives which focus on a non-toxic approach which is beneficial to both the collections and the general public. These concerns have led to the development of non-chemical treatments such as freezing, heating (Berkouwer, 1994; Ketcham-Trosak, 1984; Strang, 1992) and the use of gamma, infrared and microwave radiation (Brower and Tilton, 1971; Hall, 1981; Kirkpatrick *et al*., 1973) and controlled atmospheres (Burke, 1996; Navarro, 1978). However, these physical techniques mainly focus on the direct treatment of known or suspected infestations rather than prevention and although welcome do not provide resistance to future attacks. These problems have highlighted the need for the development of new types of pesticides alternatives.

Some efforts searching for the new types of pesticides have been focused on the plant extracts (Chaubey, 2008; Chang and Ahn, 2002; Don-Pedro, 1989). Plants are a source of insect-control agents because they contain a range of bioactive chemical (Harborne, 1993), many of which are selective and have little or no harmful effect on the human and the environment (Arnason *et al*., 1989; Hedin *et al*., 1997). Star anise (*Illicium verum*) is an aromatic evergreen tree originally distributed in the tropic and subtropic areas of Asia and used as a traditional medicine as well as a commonly used spice (Loi and Thu, 1970). Numerous compounds including volatiles, seco-prezizaane-type sesquiterpenes, phenylpropanoids, lignas, flavonoids and other constituents have been identified from *I. verum* and modern pharmacology studies demonstrated that its crude extracts and active compounds possess wide

pharmacological actions, especially in antimicrobial, antioxidant, insecticidal, analgesic, sedative and convulsive activities (Wang *et al*., 2011). The insecticidal activity of *I. verum* extracts against the different life stages of *Tribolium castaneum*, *Sitophilus zeamais*, *Lasioderma serricorne*, *Sitophilus oryzae*, *Callosobruchus chinensis*, *Blattella germanica*, *Culex pipiens pallens* and *Musca domestica* was noted (Chang and Ahn, 2002). However, little work has been done on this basis to manage museum collections pest *D. maculatus*.

Therefore, this study described a laboratory study to assess the potential fumigant effects of both extracts (anise oil and (*E*)-anethole) from *I. verum* on both life stages (larvae and adults) of *D. Maculatus*.

MATERIALS AND METHODS

Experimental time and site: The experiment was carried out from April 16th, to June 28th, 2011 at the Animal & Ecology Test Lab, School of Life Sciences, Sun Yat-sen University, China.

Test insects: Stock cultures of strains of *D. maculatus* were obtained from School of Life Sciences, Sun Yat-sen University, China. Adult beetles of *D. maculatus* were selected from stock cultures and were provided with 200 g of ground culture media. Water was provided daily for five days by pipetting a small amount onto folded filter paper placed on the surface of media. Fully grown larvae of known age (~30 days) and similar size (10-12 mm) were obtained from serial cultures and provided with a small amount of media (25 g). Adults of known age group (14-20 days) were selected from serial cultures using a spatula. Mature adults were selected to avoid immature cuticle susceptibility inherent in young beetles (Parkin, 1966). These were transferred to petri dishes and provided with media and access to moistened filter paper.

The extracts from *Illicium verum*: The extracts of anise oil (96%) and (*E*)-anethole (94%) from *I. verum* were obtained from South China Botanical Garden, Chinese Academy of Sciences.

Bioassay: The susceptibility of *D. maculatus* larvae and adults to the fumigant action of anise oil and (*E*)-anethole was investigated according to the method of Kin and Ahn (2001) with some modifications. Dosed used 0.5, 1, 2, 4, 8, 16 and 32, respectively for anise oil and (*E*)-anethole, dissolved in 1 mL mehanol and applied to a piece of filter paper (Whatman, 3.0 cm diameter) which was dried in air for 5 min and was placed in a glass bottle (1 L). In the control, only 1 mL mehanol was applied to filter paper. Forty larvae and adults were kept in small glass tubes (40 0 mm) with open ends covered with 40-mesh copper mesh. The tubes were hung at the geometrical centre of the glass bottles, which were then sealed with air-tight lids. The insects were exposed and mortality counts at 6, 12, 24, 36, 48 and 72 h, respectively after treatment with the series of concentrations. All treatments were replicated three times. Insects were considered to be dead if appendages did not move when prodded with a camel's hair brush. Data were corrected for control mortality (Abbott, 1925):

Corrected mortality = (Total mortality-Control mortality)/Total number of insect used×100

whole experiment was held at 25ºC, 70-80% relative humidity (RH).

Statistical analysis: The analysis of the corrected mortality of test insects by one-way ANOVA and the dose-mortality response by probit analysis was conducted by using SPSS 16.0 statistical software. Significant differences among the treatments were compared by LSD test. Differences were considered significant at the level of 0.05.

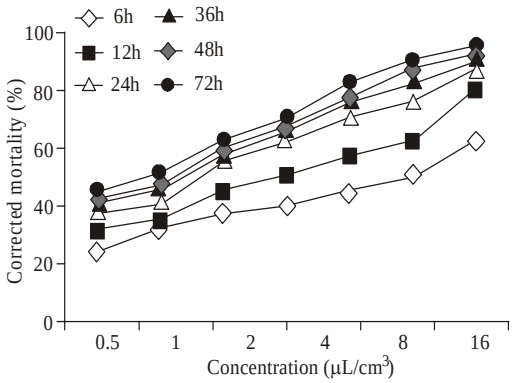

Fig. 1: Corrected mortality of *Demestes maculatus* larvae fumigated with anise oil at different exposure time and concentrations

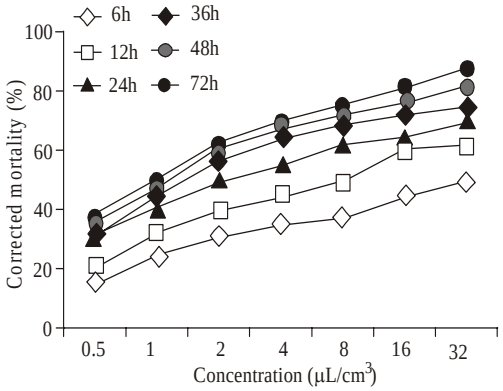

Fig. 2: Corrected mortality of *Demestes maculatus* larvae fumigated with (*E*)-anethole at different exposure time and concentrations

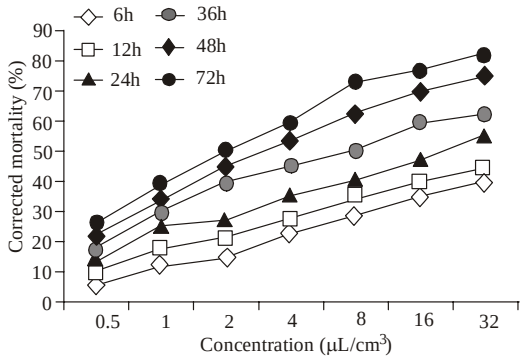

Fig. 3: Corrected mortality of *Demestes maculatus* adults fumigated with anise oil at different exposure time and concentrations

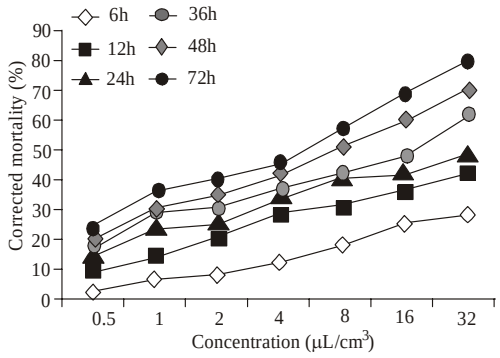

Fig. 4: Corrected mortality of *Demestes maculatus* adults fumigated with (*E*)-anethole at different exposure time and concentrations

RESULTS

Effects of anise oil and (*E*)-anethole against *Demestes maculatus* larvae: The vapor-phase toxicity of plant derived compounds anise oil and (*E*)-anethole on the mortality of mature larvae of *D. maculates* was studied and showed significant effect responses varied according to dose and exposure time. At the highest dose of anise oil 32/cm^3 and 72 h of exposure, 95% mortality of the larvae was achieved; at the lowest dose (0.5/cm^3) and 72 h of exposure, anise oil caused over 45% mortality against larvae of *D. maculatus* (Fig. 1). (*E*)-anethole caused over 87% mortality against the larvae at the highest dose of 32/cm^3 and 72 h of exposure (Fig. 2). *D. maculatus* larvae were more susceptible against anise oil than (*E*)-anethole.

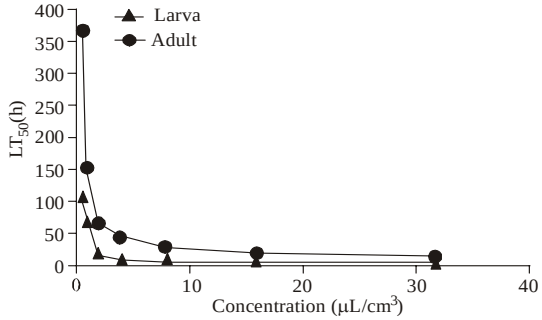

Fig. 5: Probit mortality for lethal time of *Demestes maculatus* larvae and adults fumigated with arise oil at different concentrations

Effects of anise oil and (*E*)-anethole against *Demestes maculatus* adults: The fumigant toxicity of anise oil and (*E*)-anethole on the mortality of *D. maculatus* adults was studied and showed significant effect. Responses varied according to dose and exposure time. Anise oil caused over 82% mortality of the adults at 32/cm^3 and 72 h of exposure, while at the lowest dose (0.5/cm^3) and 72 h of exposure anise oil caused only 26% mortality against *D. maculatus* adults (Fig. 3). (*E*)-anethole caused over 70% mortality against the adults at the highest dose of 32 /cm^3 and 72 h of exposure (Fig. 4). *D. maculatus* adults were more tolerant against (*E*)-anethole than anise oil.

Probit result of anise oil against larvae and adults of *Demestes maculatus*: Probit mortality values of anise oil against larvae and adults of *D. maculatus* at different exposure time and doses are summarized in Table 1 and Fig. 5. LT_{50} values of anise oil were 105.78, 67.79, 18.34, 11.59, 7.65, 6.18 and 2.88 h against larvae, and 365.65, 152.17, 68.89, 43.23, 26.36, 17.93 and 13.32 h against adults at 0.5, 1, 2, 4, 8, 16 and 32/cm^3 doses, respectively (Fig. 5). LD_{50} values for the anise oil against *D. maculatus* larvae were 1.55, 1.03 and 0.86/cm^3 and 18.83, 3.34 and 2.07/cm^3 against adults at 24, 48 and 72 h of exposure, respectively (Table 1). The larvae of *D. maculatus* were more susceptible to anise oil than adults.

Probit results of (*E*)-anethole against larvae and adults of *Demestes maculatus*: LT_{50} values of (*E*)-anethole were 177.71, 61.67, 24.13, 15.85, 11.96, 7.23 and 6.16 h against larvae, and 430.48, 185.25, 170.43, 123.66, 68.11, 43.48 and 26.16 h against adults at 0.5, 1, 2, 4, 8, 16 and

Table 1: Probit mortality for the anise oil against *Demestes maculatus* fumigated at different time intervals

Stage	Exposure (h)	LC_{50} (/cm^3)	Lower-uppe r (95%CL)	Slope E	X^2	Probability
Larva	24	1.55	0.77-2.50	0.77 .14	0.80	0.89
	48	1.03	0.51-1.61	0.91 .18	0.72	0.92
	72	0.86	0.43-1.35	0.98 .15	0.94	0.66
Adult	24	18.83	9.71-68.97	0.61 .12	0.74	0.42
	48	3.34	2.04-5.26	0.80 .13	0.55	0.95
	72	2.07	1.23-3.12	0.88 .11	0.76	0.91

Table 2: Probit mortality for the (*E*)-anethole against *Demestes maculatus* fumigated at different time intervals

Stage	Exposure(h)	LC_{50}(/cm^3)	Lower-upper (95% CL)	Slope E	X^2	Probability
Larva	24	2.78	1.26-5.18	0.57 .16	0.61	0.75
	48	1.19	0.48-2.05	0.70 .11	0.82	0.58
	72	1.00	0.44-1.68	0.79 .13	0.43	0.91
Adult	24	63.10	20.94-1774.64	0.49 .14	0.66	0.83
	48	11.47	6.51-28.81	0.65 .13	0.23	0.67
	72	6.51	3.96-12.26	0.71 .17	0.67	0.96

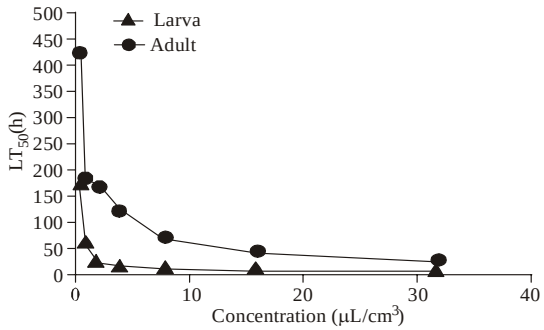

Fig. 6: Probit mortality for lethal time of *Demestes maculatus* larvae and adults fumigated with (*E*)-anethole at different concentrations

32/cm^3 doses respectively. LD_{50} values for the (*E*)-anethole against *D. maculatus* larvae were 2.78, 1.19, 1.00/cm^3, and 63.10, 11.47 and 6.51/cm^3 against adults at the exposure of 24, 48 and 72 h, respectively (Table 2 & Fig. 6). (*E*)-anethole was more effective against larvae than adults of *D. maculatus*.

DISCUSSION

The present results showed that the extracts from *I. verum*, either anise oil or (*E*)-anethole was found to be highly toxic against both the larvae and adults of *D. maculatus*. The previous studies also obtained similar results. Fumigant activity against B. germanica, S. oryzae, C. chinensis and L. serricorne have been also reported in (*E*)-anethole (Kin and Ahn, 2001; Chang and Ahn, 2002). It was suggested that the (*E*)-anethole was one of the effective components from *I. verum* for eradicating pests. In this study, however, the toxicity against *D. maculatus* of (*E*)-anethole was less than that of anise oil under the same test condition. Perhaps other chemical ingredients among anise oil, such as limonene, linalool and α-pinene, having ability to control pests, would also play some role during the course against *D. maculatus* (Wang *et al*., 2011). The mechanism and other details obtained required further study in the future.

Basing on the analysis of LD_{50} and LC_{50} given by the study, *D. maculatus* adults were more tolerant against the extracts from *I. verum* than larvae. The results support the findings of Fasakin and Aberjo (2002), who reported that larval stages of *D. maculatus* were more susceptible than adult stages to some kinds of plant materials including *Tithonia diversifolia*, *Aframomum melegueta*, *Nicotiana tabacum*, *Monodora myristica* and *Psidium Guineense*. Similar results have also been reported for the toxicity of β-asarone to adults of *S. oryzaw* and *L. serricorne* (Park, 2000). Such difference maybe related with the different respiratory metabolism in different life stages of pests (Linnie and Michael, 2000).

In the present study, both the concentration of extracts and the exposure time were the key factors affecting the mortality of *D. maculatus*. With the increasing of the dose level of the extracts and the exposure time, higher mortality was obtained. These results are in agreement with the finding of previous reports which indicated that the adulticidal activity of (*E*)-anethole against *S. oryzae*, *C. chinensis* and *L. sericorne* was dependent upon both dose and exposure time (Ha, 2000; Kin and Ahn, 2001). It maybe related to both the high volatility of the extracts and the insecticidal methods of the closed boxes. Study has stated that the toxic effects of pesticides was largely penetrating the insect body via the respiratory system, and recommended fumigant action as the important insecticidal methods for stored-product insects (Linnie and Michael, 2000).

Some factors including the feature of the collection and the condition of insecticidal chamber should be considered if the results from the present study was applied for control of pests in museum, because *D. maculatus* was dealed with freely museum collections during the course of the experiment. Studies illustrated that the insulating properties of heavy material such as wood and/or wool could protect the pest from the controlling action (Linnie and Michael, 2000). Furthermore, the temperature, the HR and other environmental factors would also impact on the toxicity performance of the extracts from *I. verum* (Wang, 2011). Therefore, prior to the insecticidal action of valuable collections it is advisable to consult the literature and the expertise of conservators.

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