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RESEARCH

Biological Activity of *trans*-2-Hexenal Against *Bradysia odoriphaga* (Diptera: Sciaridae) at Different Developmental Stages

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ABSTRACT. *trans*-2-Hexenal, one of the C6 green leaf volatiles, is potentially useful for the control of *Bradysia odoriphaga* Yang *et* Zhang. In this study, the biological activity of *trans*-2-hexenal on *B. odoriphaga* was assessed in the laboratory. *trans*-2-Hexenal was observed to kill *B. odoriphaga* in different developmental stages at a relatively low concentration under fumigation. The respiration rate in the male treatment group decreased from 131.44 to 4.07 nmol/g·min with a prolonged fumigation time, while the respiration rate in females decreased from 128.82 to 24.20 nmol/g·min. Male adults exhibited a more sensitive electroantennogram response at 0.05–500 μl/ml at the dose of 10.0 μl than female adults. Moreover, *trans*-2-hexenal had a repellent effect on adults based on the results with a Y-tube olfactometer at 10.0 μl, as shown by the deterrent rate of male and female adults with 96.67% and 98.33%, respectively. The results showed that *trans*-2-hexenal had good biological activity in different developmental stages of *B. odoriphaga*, which could reduce the need for, and risks associated with, the use of traditional insecticides and enable nonharmful management.

Key Words: *Bradysia odoriphaga*, *trans*-2-hexenal, fumigation toxicity, respiratory rate, electroantennogram response

Fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), are one of the most common insect pests of production nurseries and greenhouse plants (Harris et al. 1995, Jagdale et al. 2004). Severely injured plants generally lose their healthy appearance, become off-color, and dry out eventually. Larvae are capable of spreading fungal pathogens during feeding, and adult flies irritate people and disseminate fungal spores from plant to plant when they migrate in the greenhouse (Gillespie and Menzies 1993, Ludwig and Oetting 2001). The sciarid fly *Bradysia odoriphaga* is a serious pest of the Chinese chive, *Allium tuberosum Rottl. ex Spreng.*, in China. The larva gather in the roots and stems of the plant, which is hard to be controlled with common strategies and cause a huge yield loss of Chinese chives. Generally, applying of organophosphate insecticides, such as chlorpyrifos and phoxim, is the main method to control pest of Chinese chives. However, public concerning over the residual toxicity of organophosphate insecticides increased calls for new and less poisonous approaches to control this pest in recent years. Alternative control methods have been developed, including botanical secondary metabolites such as thermopsine, cytosine (Yu et al. 2003), the entomopathogenic nematode *Heterorhabditis indica* LN2 (Sun et al. 2004), and benzothiazole for trapping them (Chen et al. 2014). However, these methods do not provide adequate control; therefore, there is a need for safe insecticides or repellents for use on this fly.

Volatile organic compounds (VOCs), which exist widely in plant essential oils, are secondary metabolites produced in plant metabolism that have low toxicity to humans and wild life and are environmentally safe (Katz et al. 2008). They could interfere with the basic metabolic, biochemical, physiological functions, and behavior of insects (Mohamed and Abdelgaleil 2008). Because of the multiple action mechanisms, the probability of developing a resistant population is low (Abdelgaleil et al. 2008, Rattan 2010). Many VOCs had been investigated for potential use as fumigants against stored products insects (Tunç et al. 2000, Cosimi et al. 2009, Ilboudo et al. 2010, Li et al. 2013) and greenhouse pests (Çalmaşur et al. 2006, Yang et al. 2010). *trans*-2-Hexenal exists widely in green leaf volatile compounds (Tandon et al. 2000, Farag and Pare 2002, Tapia et al. 2007, Takayama et al. 2012) and can cause electroantennogram (EAG) and olfactory responses in pests (Stelinski et al. 2003, Park and Hardie 2004, Piesik et al. 2011). Fumigation of *trans*-2-hexenal has been reported to control mold on seedless table grapes *Vitis vinifera* L. (Archbold et al. 1999), but there are no previous reports on its effect as a poison when used in fumigation. Considering the significant biological activity exhibited by *trans*-2-hexenal and as part of an ongoing effort to discover environmental and efficient insecticidal VOCs for *B. odoriphaga*, *trans*-2-hexenal was found to have potential insecticidal and behavioral effects on *B. odoriphaga.*

In this article, we studied the biological activity of *trans*-2-hexenal against different developmental stages of *B. odoriphaga*. This study aimed to determine the biological activity of *trans*-2-hexenal on *B. odoriphaga* and provide a theoretical basis for use of this compound in ecological control of this pest.

Materials and Methods

Chemicals. *trans*-2-Hexenal was purchased from ACROS (Janssen Pharmaceuticalaan, Geel, Belgium, 99% purity).

Insects. *B. odoriphaga* eggs and larvae were collected from roots and subterraneous stems in Zhangqiu, Shandong Province, China. The eggs and larvae were reared on the stem of leeks at 25 ± 1°C, 70% humidity in the laboratory, Shandong Agricultural University. Rearing method of B. odoriphaga was referred to Mu et al. (2003).

Bioassay. All toxicity test and behavior experiment were performed at 20 ± 1°C, 60 ± 10% relative humidity in artificial climate chest.

Fumigation Toxicity of trans-2-Hexenal on Adults. Triangular glass flasks (3.75 liter) sealed with Parafilm were used for the bioassays. *trans*-2-Hexenal added in filter paper directly without any solvent. After the lethal concentration, range was determined by preliminary experiments. *trans*-2-Hexenal was serially designed into 0.15, 0.20, 0.25, 0.30, and 0.35 μl/liter and dispensed at an appropriate dose on filter paper (Whatman No. 1), and the filter paper was hung in a triangular flask. The control contained a similar filter paper but with nothing. The fumigation test was performed on adults that were divided into a female group and a male group. Approximately 50 new emerged adults were used per concentration and sex. At 0.5, 1, 1.5, and 2 h after treatment, the number of dead adults was recorded. The experiment was repeated for four times.

Fumigation Toxicity of trans-2-Hexenal on Eggs, Larvae, and Pupae. In a glass fumigation box (∼22 by 21.5 by 21 cm), a thin copper wire was fixed along the diagonal. Then, filter paper (cut into 2 cm by 2 cm pieces) with a quantitative *trans*-2-hexenal (concentration in egg, fourth-instar larvae, and pupae treatment group were 0.16, 0.21, 0.26, 0.31 and 0.36 μl/liter; 0.60, 0.80, 1.00, 1.20, and 1.40 μl/liter; 1.0, 1.5, 2.0, 2.5, and 3.0 μl/liter, respectively) was attached to the copper wire, avoiding contact with the bottom of the box. *trans*-2-Hexenal added in filter paper directly without any solvent and the control in each experiment contained a similar filter paper but with nothing. The eggs, larvae, and pupae were put in separate 9 cm Petri dishes in the bottom of the box. The eggs and pupae were fumigated with five concentrations separately for 24 h and then moved to normal rearing conditions. Egg hatchability was recorded after 3, 4, 5, and 6 d, and the emergence rate was counted after 2, 3, 4, and 5 d. In the larvae fumigation experiment, the fourth-instar larvae were treated with five concentrations of *trans*-2-hexenal. Mortality was recorded after 6, 12, 24, 48, and 72 h of exposure. Approximately 50 eggs per larvae per pupae were used for each concentration, and the experiment was repeated five times.

The Effect of trans-2-Hexenal on the Adult Respiratory Rate. Oxygen consumption was determined using a Clark-type electrode (Oxytherm oxygraph, Hansatech Instruments, Norfolk, England). Briefly, ∼100 nonsexed male and female adult insects were put in a triangular flask, respectively. Then, similar to the adult fumigation experiment, the fumigation concentrations (0.243 and 0.207 μl/liter for female and male, respectively) in this experiment were LC_{50} value after 2.0 h for female and male adults, respectively, while the control group did not receive *trans*-2-hexenal. Approximately 30 insects were transferred into the Oxytherm every 0.5 h to measure the respiratory rate over 2 min for males and females. Each experiment was repeated for four times.

EAG Recordings. EAG equipment, produced by Syntech (the Netherlands), composed of an INR-5 micro-manipulator, IADAC-4 data acquisition controller, CS-55 odor stimulation control, and EAG record output device. Both flow velocity of the stimulatory odor and continuous flow were 20 ml/min, and the stimulation time was 0.5 s, with a 30 s interval between two stimuli. Because of *B. odoriphaga* has no EAG response to mineral oil (AMERSCO), so we use mineral oil as control stimulus. *trans*-2-Hexenal was diluted in mineral oil and 10 μl of each treatment (0.05, 0.5, 5, 50, and 500 μl/ml) was applied to a filter paper strip (4 cm by 0.5 cm), then inserted into a glass Pasteur pipette, constituting an odor cartridge. The control stimulus was a similar pipette containing a filter paper strip impregnated with a 10 μl aliquot of mineral oil. The antennae of unmated *B. odoriphaga* were cut from the base by sharp blade, and the tip of the antenna was cut off with a sharp razor blade. Two ends of the antennae were fixed on a gel electrode with a conductive adhesive, and the odor-mixing tube was 0.5 cm away from the antenna. For male and female adults, five series of *trans*-2-henxenal were applied to 10 antennae in the following order: standard stimulus, mineral oil control, series of concentrations of *trans*-2-hexenal, mineral oil control, and standard stimulus. Benzaldehyde can cause obvious EAG responses on *B. odoriphaga*, so it was used as the standard stimulus at the beginning and end of a recording series to confirm the activity of each antenna preparation.

The Effect of trans-2-Hexenal on Adult Olfactory Responses. A Y-tube olfactometer was used to test the ability of *trans*-2-hexenal to repel unmated females and males. An olfactometer (internal diameter: 1.0 cm; length: 10 cm; angle between arms: 50°) was designed for the response experiments. *trans*-2-Hexenal was injected at an appropriate dose (0.5, 2.5, 5, 7.5, 10 μl) on filter paper (2 cm by 2 cm) using a 10 μl pipetting gun (Eppendorf Research plus, Germany). A piece of filter paper was placed in the container of one arm, and the other container remained empty. Air was pushed through the two arms by a pump at a speed of 0.5 liter/min, and the adults used in the experiment were virgins. Check insect quantity of each tube after 5 min, each treatment was administered to 10 adults and was repeated six times.

Statistical Analyses. Absolute EAG = measured value of EAG – mean value of control stimulus

Standard EAG = mean value of standard stimulus – mean value of control stimulus

Normalized EAG = absolute EAG/Standard EAG

Repellence rate = adult population in control arm/total population

LC_{50} was calculated by SPSS program. For the analysis of the fumigation experiment, the data were arcsine transformed and respiratory rate data were square root transformed, then subjected to an analysis of variance and Duncan's multiple range test to determine significant differences between treatments for same treatment time and stages. The EAG and olfactory response were compared by two-sample *t* tests.

Results

Effect of Fumigation With trans-2-Hexenal on Different Insect Life Stages

Female Adults. *trans*-2-Hexenal caused 50% mortality after 2.0 h at a concentration of 0.243 μl/liter (Table 1). The mortality increased with increased doses of *trans*-2-hexenal and exposure time. *trans*-2-Hexenal was effective in controlling *B. odoriphaga.* Doses between 0.836 and 0.475 μl/liter achieved 95% lethality in female adults at fumigation times from 0.5 to 2.0 h. In all cases, a significant increase in the susceptibility of adults was observed with extended treatment.

Male Adults. Table 2 lists the effect of *trans*-2-hexenal on male adults at different times. The lethal concentration, LC_{50}, varied between 0.415 and 0.207 μl/liter for different times, while the LC_{95} was between 0.886 and 0.377 μl/liter. Similar to female adults, the LC_{50} value decreased with extended fumigation time. It is reasonable to conclude that increased fumigation time can enhance the effect of *trans*-2-hexenal. In addition, comparing Tables 1 and 2, female adults showed higher tolerance than male adults at the same time point.

Eggs. The results of the analysis of variance, showing the effect of *trans*-2-hexenal fumigation on egg hatchability, are shown in Fig. 1. There were significant differences ($P < 0.05$ level) between responses at 3, 4, 5, and 6 d. At a concentration of 0.36 μl/liter, 37.82% hatchability was observed after 6 d compared with 94.62% in the control. This

Table 1. The influence of *trans*-2-hexenal fumigation on the mortality of female *B. odoriphaga* adults

Treatment time[a]	Slope ± SE	*r*	LC_{50} (95% CL)[b]	LC_{95} (95% CL)[b]	df	χ^2
0.5	6.103 ± 0.757	0.963	0.450 (0.395∼0.554)	0.836 (0.653∼1.254)	3	1.270
1	5.018 ± 0.454	0.981	0.369 (0.342∼0.410)	0.785 (0.653∼1.108)	3	4.951
1.5	5.231 ± 0.390	0.983	0.301 (0.288∼0.319)	0.621 (0.547∼0.735)	3	6.719
2	5.302 ± 0.357	0.990	0.243 (0.234∼0.252)	0.496 (0.453∼0.558)	3	4.879

[a]Treatment time in hours of fumigation.

[b]Concentration in μl/liter of *trans*-2-hexenal. Parentheses contain the 95% lower and upper confidence intervals, CL means confidence limit.

[c]Data are the means of four replications.

finding shows that *trans*-2-henxenal had a good fumigation effect on egg hatching.

Larvae. There was a concentration-dependent increase in mortality of fourth-instar larva treated with *trans*-2-hexenal (Table 3). LC_{50} varied between 1.908 and 0.633 µl/liter for different treatment times, while the LC_{95} was between 6.430 and 2.077 µl/liter. Moreover, a significant feeding decrease was found in the treatment group but not the control group.

Pupae. The results showed that *trans*-2-hexenal had a good insecticidal effect on pupae emergence (Fig. 2). The emergence rate was obviously affected by concentration and treatment time. The effect of concentration at each treatment time was highly significant different ($P < 0.05$ level). At the highest dose of 3.0 µl/liter of *trans*-2-hexenal at 5 d, an 11.20% emergence rate was recorded compared with 97.60% in the control.

Respiratory Rate. Figure 3 shows the respiratory rate of female and male adults after 2.5 h of treatment with 0.243 and 0.207 µl/liter, respectively. The results showed that the initial respiratory rate of the treatment group was higher than that of control group, then the rates equalized, and the final respiratory rate of females and males were 24.20 and 4.07 nmol/g·min, respectively. The maximum value in the male and female treatment group appeared at 0.5 h. However, the respiratory rate of the control group remained unchanged.

Electrophysiological Responses to trans-2-Hexenal. The olfactory responses of insects depend on the interaction of chemicals with antennal sensillae. In this study, we tested the EAG response of each sex of *B. odoriphaga* at five concentrations. The data were compared with Student's *t*-test to determine the significance of EAG responses between female and male adults. All of the concentrations elicited significant EAG responses in unmated male and female *B. odoriphaga*, with male adults exhibiting a greater EAG response than females (*P* values of Student's *t*-test at 0.05–500 µl/ml were all 0.0001). The EAG

Table 2. The influence of *trans*-2-hexenal fumigation on the mortality of male *B. odoriphaga* adults

Treatment time[a]	Slope ± SE	*r*	LC_{50} (95% CL)[b]	LC_{95} (95% CL)[b]	df	χ^2
0.5	5.040 ± 0.513	0.999	0.415 (0.377~0.476)	0.886 (0.715~1.213)	3	0.242
1	4.327 ± 0.374	0.983	0.322 (0.303~0.348)	0.772 (0.648~0.986)	3	5.071
1.5	4.375 ± 0.349	0.983	0.271 (0.259~0.285)	0.644 (0.560~0.778)	3	5.808
2	6.312 ± 0.378	0.993	0.207 (0.199~0.214)	0.377 (0.356~0.405)	3	4.610

[a]Treatment time in hours of fumigation.
[b]Concentration in µl/liter of *trans*-2-hexenal. Parentheses contain the 95% lower and upper confidence intervals, CL means confidence limit.
[c]Data are the means of four replications.

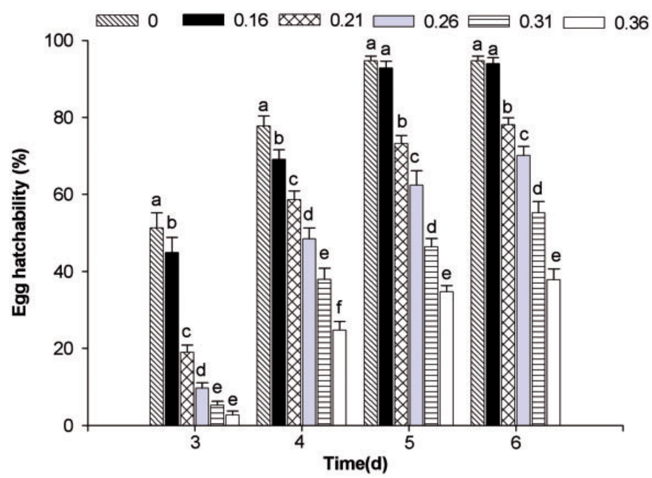

Fig. 1. Percentage inhibition of egg hatchability (±SE) of *B. odoriphaga* by different concentrations of *trans*-2-hexenal and different hatch days. Data in the same treatment time followed by different letters are significantly different at the 0.05 level by Duncan's multiple range test. The same is true for Fig. 2.

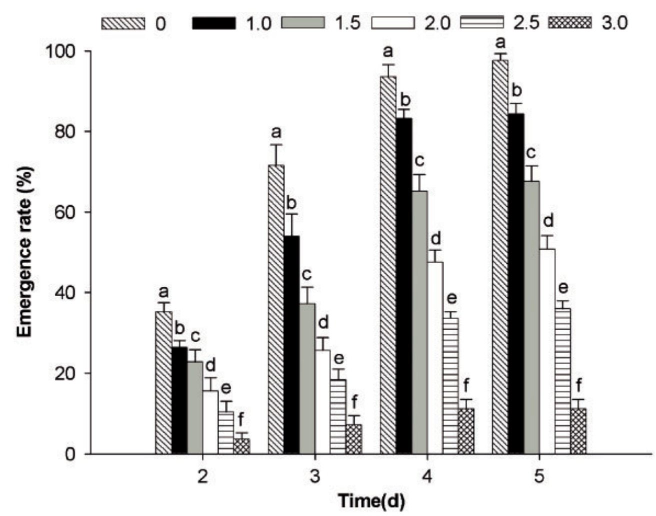

Fig. 2. Percentage inhibition of emergence rate (±SE) of *B. odoriphaga* by different concentrations of *trans*-2-hexenal and different treatment times.

Table 3. The influence of *trans*-2-hexenal fumigation on the mortality of fourth-instar larvae of *B. odoriphaga*

Treatment time[a]	Slope ± SE	*r*	LC_{50} (95% CL)[b]	LC_{95} (95% CL)[b]	df	χ^2
6	3.117 ± 0.400	0.988	1.908 (1.640-2.437)	6.430 (4.364-12.258)	3	1.494
12	3.185 ± 0.351	0.983	1.425 (1.303-1.622)	4.682 (3.512-7.293)	3	2.905
24	2.806 ± 0.319	0.981	1.033 (0.968-1.112)	3.984 (3.053-6.034)	3	3.067
48	2.909 ± 0.320	0.965	0.821 (0.756-0.877)	3.018 (2.442-4.169)	3	6.119
72	3.186 ± 0.334	0.973	0.633 (0.557-0.692)	2.077 (1.799-2.566)	3	4.717

[a]Treatment time in hours of fumigation.
[b]Concentration in µl/liter of *trans*-2-hexenal. Parentheses contain the 95% lower and upper confidence intervals, CL means confidence limit.
[c]Data are the means of four replications.

responses increased as the *trans*-2-hexenal concentration increased from 0.05 to 500 µl/ml (Fig. 4). The results showed that male adults were more sensitive to *trans*-2-hexenal than females in the electrophysiological experiment.

Olfactory Response. A "Y" shape olfactometer was used to measure the behavioral responses of unmated male and female adults to *trans*-2-hexenal (Fig. 5). The results demonstrated a significant repellent response of unmated male and female *B. odoriphaga* adults to *trans*-2-hexenal, with the repellence rate increasing at higher doses. At least 68.33% and 96.67% of male adults were repelled by 0.5 µl and 10.0 µl *trans*-2-hexenal (Fig. 4a). Meanwhile, 70.00% and 98.33% of female adults chose the control arm to avoid *trans*-2-hexenal at 0.5 and 10 µl, respectively (Fig. 6). *trans*-2-Hexenal shows a good repellent activity at a relatively low dose. In addition, *B. odoriphaga* exhibited a dose-dependent increase choice behavior in the behavior experiment, in which the repellence rate increased with the dose.

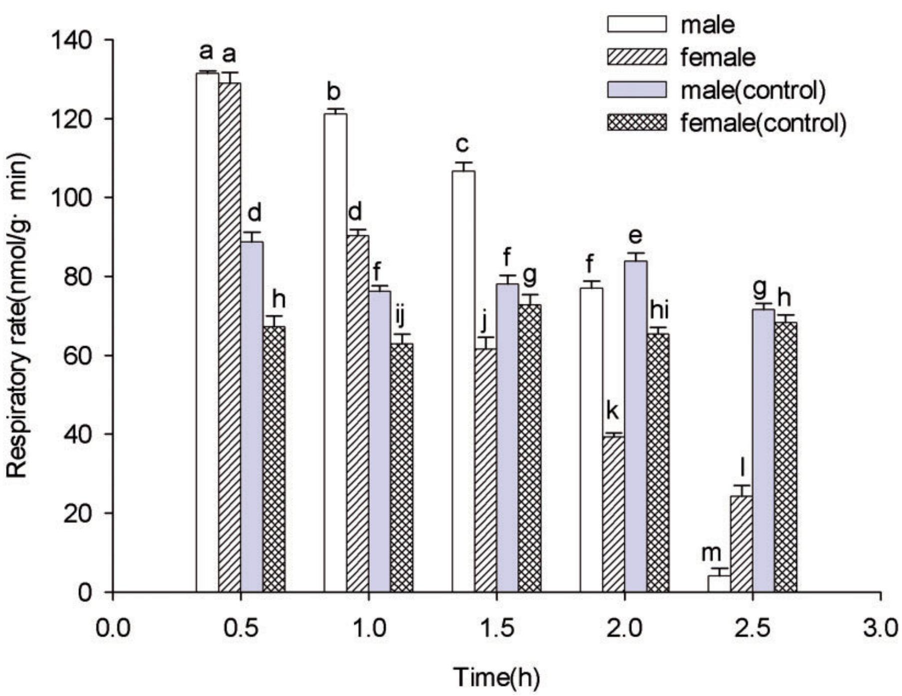

Fig. 3. Effect of *trans*-2-hexenal on the respiratory rate of male and female *B. odoriphaga* adults. Data in the figure are the mean ± SD, and bars with different small letters above them are significantly different at the 0.05 level at the same time point comparing the fumigation treatment and blank control by Duncan's test.

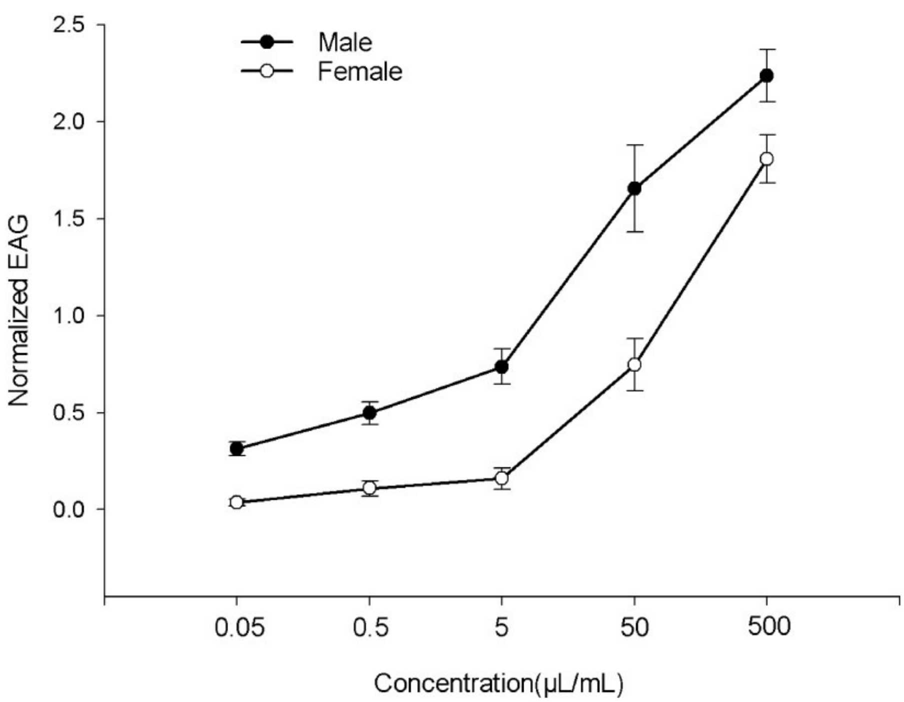

Fig. 4. EAG dose-response curves to different doses of *trans*-2-hexenal for male and female *B. odoriphaga*. The EAG responses were normalized (± SE) to mineral oil.

Discussion

The Chinese chive grows in sheltered ground mainly in a sunlight greenhouse and is usually no higher than 1 meter. This makes it difficult for conventional spray application of insecticides; therefore, fumigation is a good alternative method of application. In this study, the insecticidal activity of *trans*-2-hexenal increased with increasing concentration and exposure times. The relationship between mortality and fumigation time was similar to those reported by Collins et al. (2005). Furthermore, female adults showed better tolerance to *trans*-2-hexenal than males (Tables 1 and 2), which may be caused by the weight difference between these two sexes, given that females are normally three times heavier than males. *trans*-2-Hexenal caused significant mortality on fourth larvae (2.077 µl/liter killed 95% of the larvae) at 72 h (Table 3) and had an obvious inhibitory effect on eggs and pupae at 0.36 and 3.0 µl/liter after 5 d of exposure (Figs. 1 and 2). So the *trans*-2-hexenal has a relatively high insecticidal activity against different life stages of *B. odoriphaga* and is suitable for use in a sunlight greenhouse, and *trans*-2-hexenal can apply to field by processing into microcapsule suspension which with remarkable sustained-release property to control *B. odoriphaga.* The mode of action of *trans*-2-hexenal on egg hatch inhibition and pupae emergence has not been known but it may be due to suffocation and inhibition of various biosynthetic processes of the insect (Chaubey 2008). In addition, compared with other chemical insecticides, *trans*-2-hexenal might be less harmful to humans given that it has been identified in many fruits and foods (Burdon et al. 2004, Frank et al. 2007, Song et al. 2011) and has been applied as edible flavor in the food industry.

Respiratory metabolism is one of most important physiological and ecological characteristics of insects. Changes in respiratory rate can reflect the influence of toxic drugs or environmental pressure on insects (Dingha et al. 2005, Santos et al. 2011, DeVries and Appel 2013). In our study, the treatment group exhibited a significantly higher respiratory rate than the control group at 0.5 and 1 h (Fig. 3), which is similar to the results of other published studies, such as those on insecticides (Kestler 1991), toxic plant extracts (Harak et al. 1999, Sibul et al. 2004), and handling stress (Harak et al. 1998). With extended fumigation time, the respiratory rate was significantly reduced to 4.07 and 24.20 $nmol \cdot g^{-1} \cdot min^{-1}$ (male and female, respectively) at 2.5 h, which

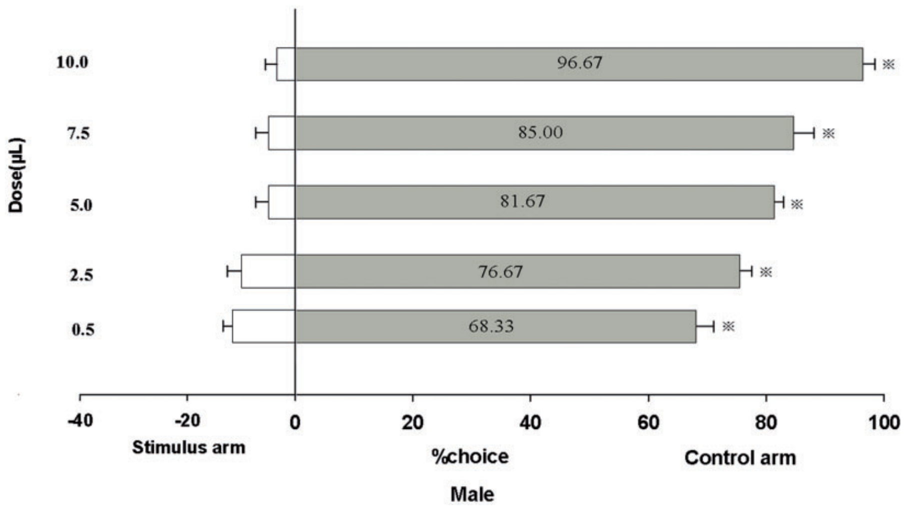

Fig. 5. Response of unmated male *B. odoriphaga* to *trans*-2-hexenal treatments in a Y-tube olfactometer. The responses of unmated male adults when given a choice between air (control) and different doses of *trans*-2-hexenal stimuli. Gray bars indicate the percentage responses to the control; white bars indicate the percentage responses to the tested stimuli. $N = 10$ individuals per choice test. Asterisks (*) indicate significant differences within a choice test ($P < 0.05$, Student's *t*-test). The same notation is used in Fig. 6.

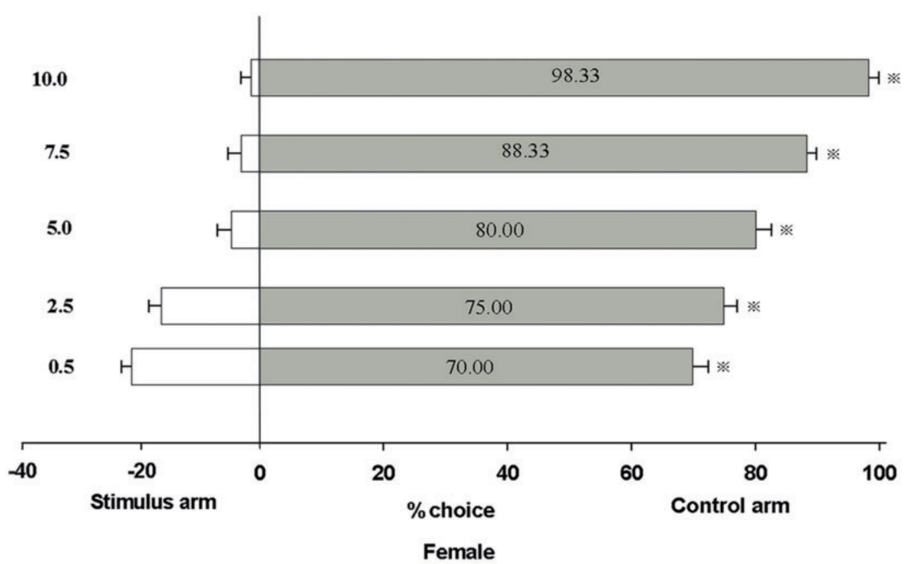

Fig. 6. Response of unmated female *B. odoriphaga* to *trans*-2-hexenal stimuli treatments in a Y-tube olfactometer.

is lower than that of the control group. Unlike respiratory inhibitors such as rotenone and nitric oxide, where the respiratory rate is significantly reduced after application, the change in the respiratory rate in this experiment indicated that *trans*-2-hexenal is not a respiratory inhibitor. Its mode of action requires further research.

With the concept of plant protection transforming from kill to management, the influence of volatile compounds on insect behavior has received more and more attention. As a result, there appears to have been successful use of plant-derived repellents against sanitary, stored-product insect pests, and arthropods under laboratory conditions (Liu and Ho 1999, Odalo et al. 2005, Nerio et al. 2010, Yoon et al. 2011).

EAG experiments are a convenient method to assess the overall sensitivity of insects to volatile compounds at physiologically relevant concentrations. *trans*-2-Hexenal can cause an obvious EAG response in many types of insects (Han and Han 2007, Ngumbi et al. 2010, Chi et al. 2011). In our EAG test, *B. odoriphaga* adults had a strong EAG response to 500 µl/ml *trans*-2-hexenal (Fig. 4), and males were more sensitive than females. These results verified again that *trans*-2-hexenal may play an important role in the behavior regulation on insects. So, it has the potential ability to control pests by changing the way of behavior and our behavior experiment verify this point.

There are many reports on the effect of *trans*-2-hexenal on insect behaviors (Pope et al. 2004, Han and Han 2007). However, there are no reports concerning the repellent activity of *trans*-2-hexenal against *B. odoriphaga*. The results of the Y-tube olfactometer bioassays in this study demonstrated strong repellence of both sexes. We also found that female *B. odoriphaga* had a stronger olfactory response and more active selective behavior than males when the dose was higher than 5.0 µl. The fundamental reason for this selectivity may be that the antennae receptors in male and female adults have gender-related differences or qualitative differences in olfactory physiology. We can take advantage of the repellent activity to mask the odors of the Chinese chive, so that pests are not able to detect the presence of food and oviposition sites. Furthermore, *trans*-2-hexenal could also be used to treat sheltered ground to flush out hidden *B. odoriphaga* before fresh Chinese chive plants are introduced.

Traditional volatile compounds such as methyl bromide, phosphine, chloropicrin mainly used for control storage insect pests and soil fumigation before cultivation due to their low crop safety, so it is not suitable to use in perennial Chinese chives field. This work provides new information on plant volatile compound component *trans*-2-hexenal, which is toxic to all stages of *B. odoriphaga* and also has a strong repellent effect on adults of both sexes. These characteristics suggest that *trans*-2-hexenal could act as a fumigant in sunlit greenhouses or as protective bands to drive pests away from Chinese chives.

For commonly used as flavors and fragrances, the cost for agriculture pest control of many VOCs, including of *trans*-2-hexenal, are higher than that of traditional chemical insecticides. Large-scale application depends on the exploration of their application effect and scope in future.

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