Sociobiology 60(2): 169-173 (2013) DOI: 10.13102/sociobiology.v60i2.169-173

Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Effects of Hematoporphyrin Monomethyl Ether on Worker Behavior of Red Imported Fire Ant *Solenopsis invicta*

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Article History
Edited by:
Yi-Juan Xu, SCAU, China
Received 13 March 2013
Initial acceptance 24 April 2013
Final acceptance 13 May 2013

Keywords
Solenopsis invicta, Red imported fire ant, Hematoporphyrin Monomethyl Ether, Behavior.

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Abstract
The effect of hematoporphyrin monomethyl ether (HMME) activated under visible light on worker behavior of *Solenopsis invcita* was studied with the potter spray tower method. The results showed that greater than 10 mg/L HMME activated under visible light could reduce the walking, grasping, aggregation, and water and food recognition abilities of red imported fire worker ant significantly, but 100 mg/L HMME in darkness could not affect their abilities or behaviors significantly. Therefore, HMME may be a potential novel insecticide that can be used as a substitute for toxic insecticides for controlling red imported fire ants.

Introduction

The red imported fire ant, a pest newly introduced to mainland China in 2005 (Zhang et al., 2007) and widely distributed in South China, threatens households, agriculture and wildlife. During foraging, worker ants leave a chemical pheromone trail to guide additional worker ants to the food source. These additional worker ants retrieve the food and return to the colony, also marking the pheromone trail laid down by the first group of ants (Xu et al., 2007). The abilities to walk, grasp, aggregate and recognize food and water are important for forager ants or other worker ants that leave the nest. If they lose the ability to move and recognize, they will have difficulty living in the complicated external circumstances, which will directly cause a decrease in the food in their nest. This occurrence will eventually affect their population, and may even induce nestmates to become aggressive and bite each other.

Photosensitizer such as α-Terthienyl (α-T) can affect the walking behavior of worker ants and even kill them directly (Yan et al., 2012; Liu et al., 2011). However, α-T activated under UV light is not suitable for controlling worker ants because the worker ants leaving the nest usually wander under visible light.

Hematoporphyrin monomethyl ether (HMME), a novel photosensitizer activated under visible light, is the second generation of porphyrin-related photosensitizer; it consists of two monomer porphyrins, namely, 3-(1-methyloxyethyl)-8-(1-hydroxyethyl) deuteropor-phyrin IX and 8-(1-methyloxyethyl)-3-(1-hydroxyethyl) deuteropor-phyrin IX, that are mutually locational isomers (Chen et al., 2000). HMME possesses some excellent properties, such as strong photodynamic effect and fast removal from organs or cells. Moreover, HMME has been reported for the treatment of some cancers (Anderson et al., 2002; Ascencio et al., 2007; Moghissi et al., 2000; Yoshihiro et al., 1993). HMME may be useful for controlling worker ants.

To our knowledge, no research has been conducted on

ISSN: 0361-6525

the effect of HMME on red imported fire worker ants. This study aimed to investigate the effects of HMME activated under visible light on the behavior of red imported fire ants.

Materials and methods

Solenopsis invicta colonies were collected from the suburb of Guangzhou and maintained in the laboratory for bioassays. The collected ants were fed with a mixture of 10% honey and live insects (*Tenebrio molitor*). A test tube (25 mm×200 mm), which was partially filled with water and plugged with cotton, was used as a water source. The ants were maintained in the laboratory at 25±2 °C. Large worker ants used in the test were about 6 mm in length, whereas small worker ants were about 3 mm in length.

HMME was provided by the Institute of Red and Green Photosensitizer (Shanghai, China). The stock solution was prepared in ethanol at a concentration of 10 mg/mL and kept in the dark at -20 °C.

A 75 W bromine tungsten lamp source (provided by Zolix Instrument Co. Ltd., Beijing, China) provided spectral radiation from 350 nm to over 2500 nm, which falls within the entire visible range of wavelengths.

The stock solution was reconstituted at 5, 10, 25, 50 and 100 mg/L in acetone–water mixtures (3/7, v/v). Freshly prepared HMME solutions were kept from light at all times, except during actual measurement.

HMME solution was applied to the worker ants with a potter spray tower (Burkard Manufacturing Co. Ltd., UK) using methods similar to those described by Harris *et al* (1962). Worker ants were placed in a glass Petri dish (120 mm in diameter) whose vertical wall was coated with a Fluon emulsion. The ants were then placed in the spray tower and sprayed with 2 ml HMME solution. The treated worker ants were transferred into a clean 500 ml beaker, whose vertical wall was coated with a Fluon emulsion (the same as below), and then placed in darkness immediately and incubated at 25 °C for 30 h. They were then exposed to visible light emitted by the bromine tungsten lamp (20 cm in height above the 500 ml beaker) for 10 min. The treated worker ants were placed in darkness immediately and incubated at 25 °C for 1h, and then their behaviors were observed. Each treatment was replicated three times, and each replicate included 30 to 40 worker ants.

In the following methods used to observe the behavior of worker ants regarding water and food recognition, the worker ants were placed in darkness without food and water for 10 h before treatment.

Controls were similar to the above steps, except that 10 min of light treatment was replaced with 10 min of dark treatment.

Behavior observation on walking ability of worker ants

Worker ants were placed on an A4 paper. They were regarded as possessing walking ability if they could walk continuously for 10 cm and did not fall down.

The formula was as follows: walking rate = number of worker ants possessing walking ability/number of worker ants per replicate × 100.

Behavior observation on worker ants' aggregation

Worker ants were placed in a 500 ml beaker, and 20 min later, worker ant aggregation was observed. Aggregation was considered present if over five worker ants gathered into an aggregate mass.

The formula was as follows: aggregation rate = number of worker ants in aggregate mass/number of worker ants per replicate × 100.

Behavior observation on grasping ability of worker ants

Worker ants were placed on an A4 paper, and 10 s later, the A4 paper was turned over 180 degrees gently. They were regarded as possessing grasping ability if they would not fall down from the A4 paper.

The formula was as follows: grasping rate = number of worker ants possessing grasping ability/number of worker ants per replicate × 100.

Behavior observation on water recognition of worker ants

A water-soaked cotton ball (1 g) and 20 worker ants (the distance between the cotton ball and the ants was 25 cm) were placed on the midcourt line of a porcelain tray (20 cm × 30 cm × 5 cm) whose vertical wall was coated with a Fluon emulsion (the same as below). Then, the water drinking behavior of worker ant was observed within 30 min. Worker ants were regarded as having water recognition ability if they continuously touched the cotton ball with their mouth for greater than 10 s. A worker ant was removed from the porcelain tray if it had drunk water.

The formula was as follows: drinking water rate = number of worker ants drank water/number of worker ants per replicate × 100.

Behavior observation on food recognition of worker ants

The treatment method was the same as that used for the behavior observation on the water recognition of worker ants, but the cotton ball was replaced with a dead larva of *Tenebrio molitor*. Worker ants were regarded as possessing food recognition ability if they continuously touched the dead larvae with their mouth for greater than 10 s.

The formula was as follows: food recognition rate = number of worker ants could recognize the dead larvae/number of worker ants per replicate × 100.

Statistical Analysis

Data were reported as means ± SE based on three independent experiments. The percentage values were transformed into arc sin of square root of the percentages prior to the analysis, and three-factor ANOVA with worker size, light treatment, and concentration as the main effects were conducted. Moreover, the differences between the data were assessed by Duncan's multiple range test (SAS 1989) with $P < 0.05$ regarded as statistically significant. The figures were produced using Microsoft Office Excel 2007.

Results

Three-factor ANOVA with worker size, light treatment, and concentration as the main effects, as well as two and three-way interactions between these effects, was conducted. Results show that all three main effects and partial interactions were significant (Table 1.). Significant differences in walking, aggregation, grasping, and water and food recognition abilities were observed from the different treatment concentrations, light treatment, and size of the worker ants (Table 2.).

Small and big worker ants possessed good walking, aggregation, and grasping abilities after treated with HMME in darkness. After treated with 5, 10, 25 and 50 mg/L HMME, the walking rates of small and big worker ants were greater than 96.0%. The walking rates were 86.67% and 98.89% at 100 mg/L, respectively (Fig. 1A, Table 2.). All the aggregation rates of small and big worker ants were greater than 92.0% (Fig. 1B, Table 2.), and all their grasping rates were greater than 82.0% at the treatment concentration in darkness (Fig. 1C, Table 2.).

HMME activated under visible light could significantly affect the walking, aggregation, and grasping abilities of red imported worker fire ants. After treated with 10, 25, 50 and 100 mg/L HMME activated under visible light, the walking rates of small worker ants were 82.22%, 53.33%, 14.44% and 0.0%, respectively, and those of big worker ants were 92.22%, 81.11%, 34.44% and 0.0%, respectively, which were significantly different from the values obtained from the treatments in darkness ($P < 0.05$) (Fig. 1A, Table 2.). The aggregation rates of small worker ants were 70.83%, 50.83%, 26.67% and 2.50%, respectively, and those of big worker ants were 92.50%, 80.00%, 52.50% and 8.33%, respectively, which were significantly different from the values obtained from the treatments in darkness ($P < 0.05$) (Fig. 1B, Table 2.). The climbing rates of small worker ants were 45.56%, 21.11%, 2.22% and 0.0%, and those of big worker ants were 68.89%, 35.56%, 6.67%, and 0.0%, respectively, which were significantly different from the values obtained from the treatments in darkness ($P < 0.05$) (Fig. 1C, Table 2.).

Small and big worker ants possessed good water and food recognition abilities after treated with HMME in darkness. After treated with HMME at test concentration in darkness, the drinking water rates of small and big worker ants were greater than 73.00%, and their food recognition rates were greater than 90.00% (Fig. 2, Table 2.).

The visible light-activated HMME could affect the water and food recognition abilities of worker ants significantly. After treated with 5, 10, 25, 50 and 100 mg/L HMME activated under visible light, the drinking water rates of small worker ants were 51.11%, 43.33%, 34.44%, 13.33% and 5.56%, respectively, and those of big worker ants were 84.44%, 78.89%, 61.11%, 32.22% and 14.44%, respectively, which were significantly different from the values obtained from the treatments in darkness ($P < 0.05$) (Fig. 2A, Table 2.). The food recognition rates of small worker ants were 80.00%, 56.67%, 44.44%, 20.00% and 12.22%, respectively, and those of big worker ants were 93.33%, 86.67%, 58.89%, 27.78% and 10.00%, respectively, which were significantly different from the values obtained from the treatments in darkness ($P < 0.05$) (Fig. 2B, Table 2.).

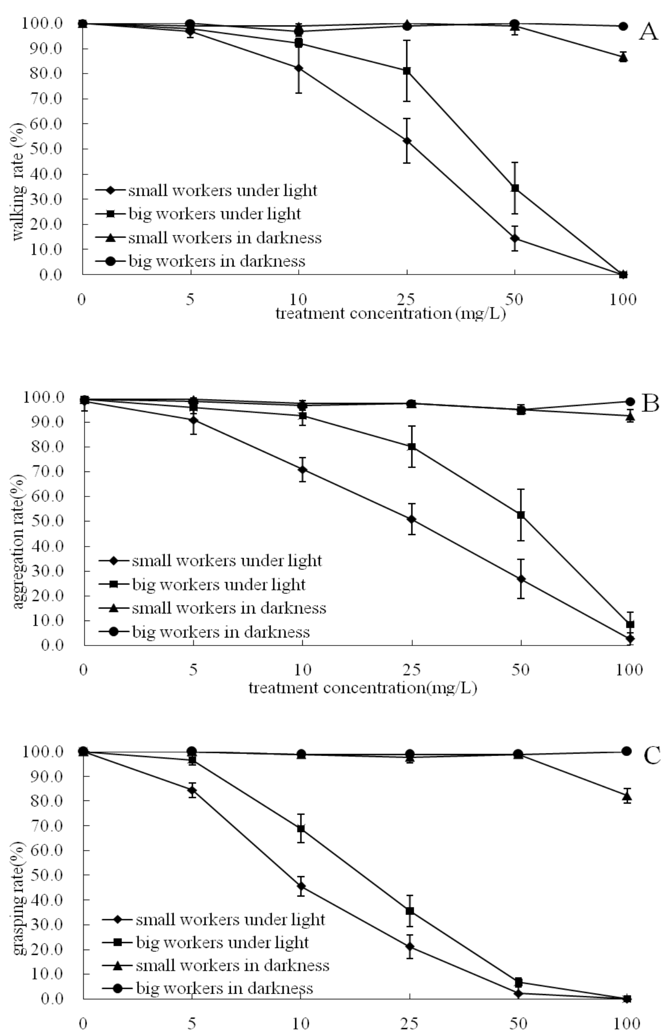

Fig. 1 The effect of visible light-activated HMME on walking ability (A), aggregation (B), and grasping ability (C) of worker ants.

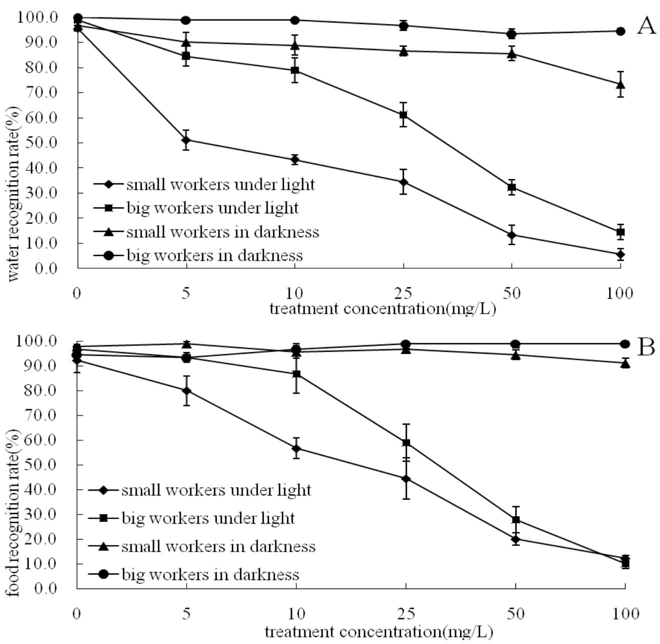

Fig. 2 The effect of visible light-activated HMME on water (A) and food (B) recognition of worker ants.

Discussion

This study showed that HMME concentration greater than 10 mg/L activated under visible light could reduce the walking, grasping, aggregation, and water and food recognition abilities of red imported worker fire ants. However, 100 mg/L HMME in darkness does not affect these abilities or behaviors of worker ants. This finding suggests that HMME could be transmitted successfully in an ant nest, which is the key factor for effectively controlling red imported fire ants.

HMME activated under visible light is a novel photosensitizer that has been effectively used for solid tumors, which means that it is relatively safe for humans. HMME is also safe for the environment because of its photodegradation characteristics. As a formulation for controlling red imported fire ants, HMME can be used as bait or as powder applied in the morning or late afternoon. Thousands of worker ants living in one nest will then carry it into the nest, resulting in effectively decreasing the labor and photodegradation of HMME, and thus, producing good controlling effect.

In conclusion, this study showed that HMME is a potential novel alternative for moderately and highly toxic insecticides to control red imported fire ant.

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Table 1. ANOVA for main factors that affect the behaviors of red imported fire ants.

Factors	Walking ability		Aggregation		Grasping ability		Water recognition ability		Food recognition ability	
	F values	P values	F values	P values	F values	P values	F values	P values	F values	P values
A	235.870	0.0001	82.473	0.0001	204.860	0.0001	96.551	0.0001	88.786	0.0001
B	990.869	0.0001	384.242	0.0001	1505.817	0.0001	607.761	0.0001	668.217	0.0001
C	22.714	0.0001	20.820	0.0001	30.094	0.0001	129.990	0.0001	18.087	0.0001
A×B	159.554	0.0001	51.779	0.0001	134.497	0.0001	31.030	0.0001	51.749	0.0001
A×C	2.450	0.0468	0.879	0.5023	2.144	0.0761	1.764	0.1383	3.606	0.0075
B×C	4.233	0.0451	13.727	0.0005	2.496	0.1207	0.981	0.3268	9.766	0.0030
A×B×C	7.790	0.0001	1.418	0.2350	7.290	0.0001	1.010	0.4222	1.301	0.2793

A: Concentration, B: Light treatment, C: Worker size (P=0.05)

Table 2. Influences of different treatments on behaviors of red imported fire ants .

Treatments			Percentage (Means ± SE, %)				
Concentration (mg/L)	Light	Worker size	Walking ability	Aggregation	Grasping ability	Water recognition	Food recognition
0	Light	Small	100.00±0.96a	98.33±3.91abc	100.00±0.00a	95.56±1.11bcd	92.22±5.09bcde
		Big	100.00±0.00a	99.17±1.38a	100.00±0.00a	98.89±1.11ab	96.67±1.57abc
	Darkness	Small	100.00±0.96a	99.17±0.72a	100.00±0.00a	96.67±1.92abc	97.78±0.96ab
		Big	100.00±0.00a	99.17±1.38a	100.00±0.00a	100.00±0.00a	97.78±1.11ab
5	Light	Small	96.67±2.48b	90.83±5.83cd	84.44±2.94b	51.11±4.01ij	80.00±5.98f
		Big	97.78±1.57ab	95.83±2.47abc	96.67±1.92a	84.44±4.01efg	93.33±1.84bcde
	Darkness	Small	98.89±0.96ab	99.17±0.72a	100.00±0.00a	90.00±3.85cdef	95.56±0.96abcd
		Big	100.00±0.00a	98.33±1.38ab	100.00±0.00a	98.89±1.11ab	98.89±1.84a
10	Light	Small	82.22±10.11d	70.83±4.77e	45.56±4.01d	43.33±1.92jk	56.67±4.16gh
		Big	92.22±1.84c	92.50±3.80bc	68.89±5.77c	78.89±4.84fg	86.67±7.86ef
	Darkness	Small	98.89±0.96ab	97.50±1.18abc	98.89±1.11a	88.89±4.01def	93.33±2.48bcde
		Big	96.67±1.84b	96.67±1.86abc	98.89±1.11a	98.89±1.11ab	95.56±2.22abcd
25	Light	Small	53.33±8.80e	50.83±6.28f	21.11±4.84e	34.44±4.84k	44.44±8.37h
		Big	81.11±12.11d	80.00±8.28de	35.56±6.19d	61.11±4.84hi	58.89±7.43g
	Darkness	Small	100.00±0.00a	97.50±1.18abc	97.78±2.22a	86.67±1.92def	97.78±1.11ab
		Big	98.89±0.96ab	97.50±1.18abc	98.89±1.11a	96.67±1.92abc	96.67±1.84abc
50	Light	Small	14.44±4.81g	26.67±7.91g	2.22±4.84g	13.33±3.85lm	20.00±2.48ij
		Big	34.44±10.23f	52.50±10.37f	6.67±1.92f	32.22±2.94k	27.78±5.30i
	Darkness	Small	98.89±3.64ab	95.00±1.18abc	98.89±1.11a	85.56±2.94efg	96.67±1.84abc
		Big	100.00±0.00a	95.00±1.86abc	98.89±1.11a	93.33±1.92cde	94.44±1.57bcde
100	Light	Small	0.00±0.00h	2.50±2.50h	0.00±0.00g	5.56±2.22m	12.22±1.11j
		Big	0.00±0.00h	8.33±5.07h	0.00±0.00g	14.44±2.94l	10.00±1.92j
	Darkness	Small	86.67±1.92cd	92.50±2.50bc	82.22±2.94b	73.33±5.09gh	90.00±1.92def
		Big	98.89±1.11ab	98.33±0.83abc	100.00±0.00a	94.44±1.11cde	91.11±1.11cde

[a] Sharing same letters means not significantly different from each other (P>0.05, Duncan's Multiple Range Test).