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Deltamethrin affects the expression of voltage-gated calcium channel α1 subunits and the locomotion, egg-laying, foraging behavior of *Caenorhabditis elegans*

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ABSTRACT

Deltamethrin belongs to the class of synthetic pyrethroids, which are being widely used as insecticides in agricultural practices. Voltage-gated sodium channels (VGSCs) are the primary targets of these chemicals for toxicity to insects. *Caenorhabditis elegans* (*C. elegans*) does not have VGSCs but is susceptible to deltamethrin. Recent findings have suggested that pyrethroids can affect voltage-gated calcium channels (VGCCs). However, it remains elusive whether deltamethrin induces toxicity to *C. elegans* via modulating the activity of VGCCs. To identify the potential target of deltamethrin, we exposed *C. elegans* to different concentrations of deltamethrin and Ca^{2+} channel blockers for different times, characterized the behavioral toxicity of deltamethrin on *C. elegans*, and determined the expression of *egl*-19, *unc*-2, and *cca*-1, which encode the α1-subunit of the L-, R/N/P/Q-, and T-type VGCC, respectively. We found that deltamethrin inhibited the locomotion, egg-laying and foraging ability of *C. elegans* in a concentration dependent manner. We also showed that body length of worms on agar plates containing 200 mg L^{-1} deltamethrin for 12 h was not significantly different from controls, whereas the cholinesterase inhibitor carbofuran caused hypercontraction which is a characteristic of organophosphates and carbamates, suggesting that deltamethrin's mode of action is distinct from those nematicides. In addition, *unc*-2 was significantly up-regulated following 0.05 mg L^{-1} deltamethrin exposure for 24 h; while *egl*-19 and *cca*-1 were significantly up-regulated following 5 and 50 mg L^{-1} deltamethrin exposure for 24 h. Further tests of worms' sensitivity and expression of three α1-subunits of VGCC to Ca^{2+} channel blockers indicate that deltamethrin may induce toxic behavior *C. elegans* via modulation of the expression of the α1-subunits of VGCC. This study provides insights into the linkage between deltamethrin-induced toxic behavior and the regulation of α1-subunits of VGCC in *C. elegans*.

1. Introduction

Plant parasitic nematodes (PPN) are one of the most damaging pests of cultivated crop causing an estimated loss of at least $100 billion per annum [1,2]. Currently, the most successful approaches for controlling PPN are extensive use of chemical nematicides, including fumigant nematicide methyl bromide, carbamate- and organophosphate-based nematicides. However, these nematicides have now been banned or highly restricted due to their environmental and human health risks. There is an urgent need to identify potential target sites in nematodes that can be exploited for their control.

Pyrethroids are widely used pesticides and divided into two types according to the presence or absence of cyano group. Type-II pyrethroid has a cyano group at the α-position, while type-I does not. The primary targets for the action of pyrethroids are VGSCs [3,4]. Pyrethroids reduce the dynamic process of both the activation and inactivation of VGSCs, resulting in a prolonged depolarizing tail current that can lead to increased excitability [5,6]. Meanwhile, the difference of the length of delayed time for both the activation and inactivation of VGSC attributes to the different symptoms to type I and II pyrethroids [6]. Other targets, particularly VGCCs and chloride channels, have been implicated as alternative or secondary sites of action for a subset of pyrethroids [7,8]. Several studies have shown that pyrethroids affect VGCCs at the same concentration range as for VGSCs [8–12]. It has been reported that type II pyrethroid promotes the release of the synapse neurotransmitter via targeting VGCCs [13]. In addition, recent studies have demonstrated that pyrethroids including allethrin and deltamethrin inhibit T-, L-, P/Q- and N-type voltage-activated Ca^{2+} channels in oocytes [11,14]. Therefore, inhibition of calcium dynamics and VGCC activity plays an important role in the toxicity of pyrethroids.

Caenorhabditis elegans (*C. elegans*) as a popular model organism has conserved physiology and pharmacology with parasitic nematodes and has been exploited to provide insight into the mode of action of anthelmintic or nematicide [15–19]. The *C. elegans* genome contains five genes encoding putative VGCC α1 subunit *egl*-19, *cca*-1, *unc*-2, *nca*-1 and *nca*-

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2 [20]. Sequence analysis has shown that *egl*-19, *unc*-2, and *cca*-1 are homologues to the vertebrate α1 subunits conducting L-type, P/Q/R/N-type, and T-type VGCC, respectively [21–23]. The *unc*-2 is primarily expressed in motor neurons, several subsets of sensory neurons, and the HSN and VC neurons controlling egg-laying [24]. In situ characterization of voltage-gated calcium currents has shown that the α1 subunit *cca*-1 underlies a T-type current in pharyngeal muscle whereas *egl*-19 carries L-type currents both in pharyngeal and body muscles [23–26].

Several studies of neurotoxic pyrethroids utilizing *C. elegans* have found pyrethroids including cypermethrin and cyhalothrin elicit toxicity by affecting physiological parameters viz., egg laying, brood size, feeding rate and life span at sublethal concentrations [27–30]; However, the underlying mechanism remains to be determined. VGSC is the well-known target of pyrethroids, but has not yet been detected in *C. elegans*. We speculate that pyrethroids may exert their toxicity via targeting VGCC in *C. elegans*.

In this study, in order to define the role of VGCC in pyrethroid-induced behavior changes in *C. elegans*, we examined the behavioral toxicity of a well-studied type II pyrethroid deltamethrin on *C. elegans*, and assessed the expression of *egl*-19, *unc*-2, and *cca*-1, encoding for the α1-subunit of the L-, R/N/P/Q-, and T-type VGCC, respectively, under different dosages of chronic deltamethrin exposure.

2. Materials and methods

2.1. Reagents, C. elegans culture and drug treatment

Wild type *C. elegans* strain (N2) was used in this study and cultured at 20 °C on nematode growth medium (NGM) plates seeded with *Escherichia coli* strain OP50 as source of food according to a standard protocol [31]. Age synchronous populations of L4-larvae stage were obtained by collecting populations of eggs and allowing them to develop as described by Donkin and Williams [32]. Deltamethrin (technical grade, 95% purity) and carbofuran (technical grade, 97% purity) were provided by Jiangsu Yangnong Chemicals Ltd. (Yangzhou, China) and Hunan Research Institute of Chemical Industry (Changsha, China), respectively. Voltage-gated calcium channel blockers verapamil, diltiazem, nifedipine and mibefradil were purchased from Sigma-Aldrich.

Chemicals were first dissolved to a concentration of 1% (*w*/*v*) in acetone with tween-80 as emulsifier. Chemical emulsifiable concentrates were further diluted with K-medium (0.032 M KCl and 0.051 M NaCl) to different concentrations of stock solution. The stock solution was added to autoclaved NGM to final concentrations. The same volume of acetone as chemical stock solution was added as a vehicle control. Worms from synchronized culture (2 days after hatching) were incubated on NGM plate with different concentrations of chemical for 24 h, followed by analyses of mortality, behavioral toxicity and gene expression. Behavioral assays in liquid were conducted in M9 buffer (KH_2PO_4 3 g, Na_2HPO_4 6 g, NaCl 5 g, 1 M $MgSO_4$ 1 mL, in 1 L distilled water).

2.2. Lethal toxicity test

About one hundred L4 stage worms were transferred onto each NGM plates containing different concentrations of deltamethrin, carbofuran or vehicle control, and cultured for 24 h at 20 °C. Each compound was tested with five concentrations in four replicates. The tested concentrations of deltamethrin and carbofuran were between 5 and 200 mg L^{-1} based on the previous experiment results. The numbers of live and dead worms were then counted under a dissecting microscope by probing the worms with a platinum wire.

2.3. C. elegans behavioral toxicity analysis

Following the 24 h incubation with exposure to sublethal concentrations of deltamethrin and carbofuran, worms were removed and washed 3 times in M9 buffer, and then the thrashing rate, body bend frequency, egg laying rates and foraging behavior were evaluated.

To assay the head thrashes, washed worms was transferred into a microtiter well containing 60 μL of M9 buffer on the top of agar. Thrashes produced by each worm in a 1 min period were counted after a 2 min equilibration period. A thrash was defined as a change in direction of bending at the mid body as previously described [33]. To assay the body bends, worms were picked onto a second plate. The number of body bend generated in a 20 s time interval was counted. A body bend was defined as a change in the direction of propagation of the part of the worm corresponding to the posterior bulb of the pharynx along the *y*-axis, assuming the worm was traveling along the x-axis. 30 worms were examined per treatment, and three independent experiments were performed.

To assay the egg laying rates, worms were individually transferred to OP50/NGM plates that contained either vehicle or varying concentrations of deltamethrin. After 3 h, the adult worms were removed and discarded, and the number of laid eggs was counted. 10 worms were examined per treatment, and three independent experiments were performed.

The detection of foraging behavior was performed according to the method of Kohra [34]. In brief, *E. coli* was grown circularly within a 0.5 cm radius from the center of a chemical-free 9 cm NGM plate, and the washed worms those exposed to sublethal concentrations of deltamethrin for 24 h were transferred to plates for the foraging behavior assay. The worms were evenly placed 4 cm from the center. Each test was performed with plate loaded with 12 worms in five replicates. After 2, 4, 6, 8 and 24 h of incubation at 20 °C, the numbers of nematode that have reached the *E. coli* colony were counted in each plate. The attainment level of *C. elegans* was obtained by dividing the number of worm that reached the food source by the total number of worms on the plate.

2.4. Effect of deltamethrin on worm length

Assays were conducted as previously described [35]. The experimental L4 stage worms were imaged on a clean unseeded NGM plate before the treatment. Then, 10 worms were transferred to NGM plate containing 200 mg L^{-1} drugs. Further images were taken over 12 h. Software GSA Image Analyser that provides a skeleton image of the worm was used to measure its length. The lengths of individual worms on the drug (or control) plates were compared with the length of the same worm prior to treatment to give a fraction of the initial length of the worm. Measurements were normalized to the initial length for each individual worm.

2.5. RT-PCR analysis of VGCC gene expression in C. elegans

Total RNA from *C. elegans* was extracted using Trizol reagent (Invitrogen) according to the manufacturer's protocol. Total RNA was quantified by a Nanodrop ND-1000 (Nanodrop Technologies, Wilmington, DE, USA). First-strand cDNA was synthesized from 1 μg of each RNA sample in a 20 μL reaction volume with Oligo dT-Adaptor Primer using the RNA PCR Kit (AMV) Ver. 3.0 (TaKaRa, Dalian, China). Real-time PCR was conducted using SYBR® Premix Ex Taq™ II (Perfect Real Time) (TaKaRa) in a typical 20 μL PCR mixture that included 10 μL of SYBR® Premix Ex Taq™ II, 2 μL of template cDNA, and 0.4 μM of each PCR primer. Cycling conditions were 95 °C for 30 s, 40 cycles at 95 °C for 3 s, 58 °C for 30 s, followed by dissociation curve analysis at 95 °C for 15 s, 60 °C 1 min and 95 °C for 15 s to verify the amplification of a single product. PCR reactions were run on an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The results were analyzed using the ΔΔCt method [36]. Actin mRNA was used for expression-level normalization of the studied genes. Primers were designed using the Primer express 3.0 software and are listed in Table 1.

Table 1
Primer information for the 4 tested genes in *C. elegans*.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
egl-19	TCGACCGCTTCGTCTTGTATC	GGCAATGTGGAGCAAAGGA
cca-1	ATGAGTCCTTGGTTTGACCGAATA	ACATCCCGAGGGTTACACAATT
unc-2	CACTATTCATGCCGGAAGACAA	AAACGGCGGTGATGTAACCA
act-1	GTCGGAAGACCACGTCATCAA	GGGGCAACACGAAGCTCATT

2.6. Data analysis

Median lethal concentration (LC_{50}) was derived through Probits analysis. Statistical analysis was conducted using DPS v12.01 software for Windows 7. The data are presented as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) was used for comparison between the control and treatment groups in thrashing rates, body bend frequency, egg-laying rates, attainment levels and gene expression levels. If there was a significant difference among treatment groups at 0.05 and 0.01 significant levels, least significant difference (LSD) of multiple comparisons was conducted to compare the mean of each group.

3. Results

3.1. C. elegans are sensitive to deltamethrin

To test the toxicity of deltamethrin to *C. elegans*, 100 of L4 stage worms were exposed to NGM plates containing different concentrations of deltamethrin (12.5–400 mg L^{-1}) or vehicle control for 24 h. The numbers of live and dead worms were counted under a dissecting microscope. After 24 h, deltamethrin showed a significant effect on the survival of *C. elegans* in a concentration dependent manner. At concentration of 400 mg L^{-1} deltamethrin, the entire population worms were dead. Acute toxicity was studied using LC_{50} derived through Probit analysis (Table 2). The half lethal concentration of deltamethrin to *C. elegans* was 55.8 mg L^{-1}. While, under the same conditions, the LC_{50} of carbofuran to *C. elegans* was 29.8 mg L^{-1}.

3.2. Deltamethrin decreases the thrashing and body bend frequency of C. elegans

To assess *C. elegans* behavioral toxicity following exposure to deltamethrin, worms were treated with sub-lethal concentrations of deltamethrin for 24 h. The thrashes of each worm in a 1 min period were counted after a 2 min equilibration period. As shown in Fig. 1, deltamethrin exposure at concentrations 0.5 mg L^{-1} significantly decreased the thrashing of L4 *C. elegans* larvae. And the head thrashing frequency was 64.3 ± 1.2 times per min. Furthermore, exposure at 5 mg L^{-1} carbofuran, the head thrashing frequency was 52.3 ± 2.8 times per min.

The body bend is the change in the direction of propagation of the part of the worm corresponding to the posterior bulb of the pharynx and an important index of behavioral toxicity of worm. Deltamethrin at concentrations >0.5 mg L^{-1} significantly reduced the body bend frequency of L4 *C. elegans* larvae (Fig. 2). And the body bend frequency was 21.4 ± 0.4 times per 20 s. While, the body bend frequency was 12.6 ± 0.8 times per 20 s at 5 mg L^{-1} carbofuran. These results demonstrated that deltamethrin exposure led to decreased thrashing and body bend frequency of L4 *C. elegans* larvae in a concentration-dependent manner.

Table 2
LC_{50} of deltamethrin in *C. elegans*.

	Regression equation	24 h LC_{50} (mg L^{-1})	95% Confidence interval
Deltamethrin	y = 2.6574 + 1.3414×	55.8	45.5–68.4
Carbofuran	y = 1.9386 + 2.0528×	29.8	24.5–39.2

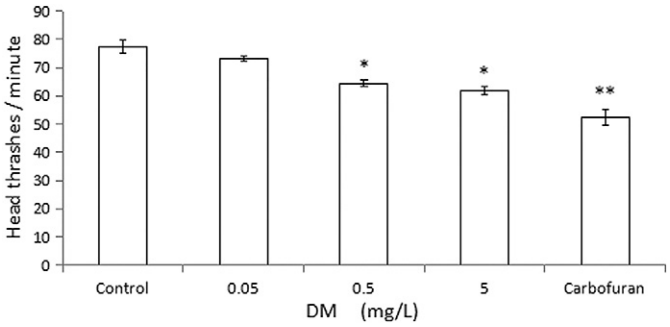

Fig. 1. Deltamethrin reduced the head thrashes of *C. elegans* in a concentration dependent manner. 100 worms were treated with the indicated concentrations of deltamethrin for 24 h. The thrashes of each worm in a 1 min period were counted after a 2 min equilibration period. The head thrashing frequency was determined by the total number of thrashes divided by 2 min (times/min). The data shown is the average of three independent experiments and statistically analyzed using ANOVA. Differences between the data were considered significant at $P \le 0.05$. Error bars represent mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$. DM is the abbreviation for deltamethrin and the same below.

3.3. Deltamethrin reduces the egg-laying of C. elegans

To check deltamethrin on the ability of egg-laying of *C. elegans*, L4 stage worms were individually transferred to OP50/NGM plates (10 worms per plate) that contained either vehicle or varying concentrations of deltamethrin. After 3 h, the number of laid eggs was counted. As shown in Fig. 3, exposure to 5 mg L^{-1} deltamethrin for 24 h caused a significant reduction in the egg laying when compared to the control. The number of eggs laid per 10 worms in control group was 25.0 ± 1.1, while that in worms exposed to 5 mg L^{-1} deltamethrin was 19.8 ± 1.2.

3.4. Effect of deltamethrin on foraging behavior in C. elegans

To assess the foraging behavior of worms following deltamethrin exposure, worms were transferred to chemical-free 9 cm NGM plates containing *E. coli* grown circularly within a 0.5 cm radius from the center. After 2, 4, 6, 8 and 24 h of incubation at 20 °C, the numbers of nematode that have reached the *E. coli* colony were counted in each plate. As shown in Fig. 4, the attainment levels decreased significantly after deltamethrin exposure at 5 mg L^{-1} for 2 h and 24 h compared to the control. However, there were no significant differences between treatments and controls at 4, 6, and 8 h. Comparatively, *C. elegans* have a lower attainment level at 5 mg L^{-1} carbofuran.

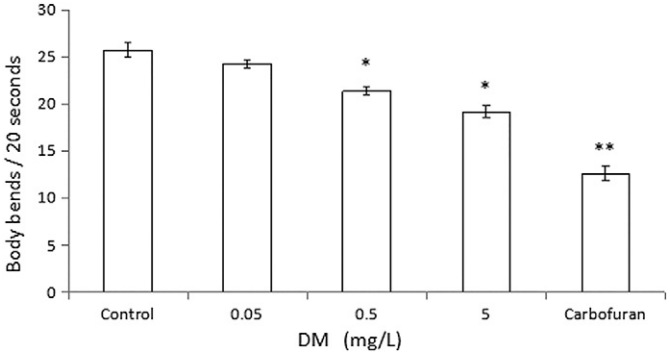

Fig. 2. Deltamethrin decreased body bend frequency of *C. elegans* in a concentration dependent manner. 100 worms were treated with the indicated concentrations of deltamethrin for 24 h. The number of body bend generated in a 20 s time interval was counted. The data shown is the average of three independent experiments and statistically analyzed using ANOVA. Differences between the data were considered significant at $P \le 0.05$. Error bars represent mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$.

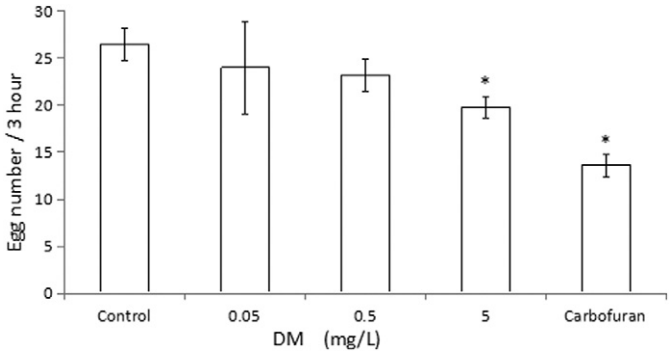

Fig. 3. Effect of deltamethrin on the egg-laying of *C. elegans*. L4 stage worms were individually transferred to OP50/NGM plates (10 worms per plate) that contained either vehicle or the indicated concentrations of deltamethrin. After 3 h, the adult worms were removed and discarded, and the number of laid eggs was counted. The data shown is the average of three independent experiments and statistically analyzed using ANOVA. Differences between the data were considered significant at $P \leq 0.05$. Error bars represent mean ± SEM, $^{*}p < 0.05$.

3.5. Effect of deltamethrin on VGCC gene expression in C. elegans

To explore the underlying mechanism of deltamethrin on *C. elegans* behavioral toxicity, we treated worms with different concentrations of deltamethrin for 24 h and determined the mRNA levels *egl*-19, *cca*-1 and *unc*-2 by RT-PCR. Compared with control, the mRNA levels of *egl*-19 were up-regulated significantly by 6.3 fold in nematodes exposed to 5 mg L^{-1}, and down-regulated significantly by 9.7 fold at 0.5 mg L^{-1}; the mRNA levels of *cca*-1 genes were increased significantly by 13.6 fold at the concentration of 50 mg L^{-1}, and decreased at 0.5 and 0.05 mg L^{-1}; the expression levels of *unc*-2 gene were up-regulated significantly by 8.3 fold at concentration of 0.05 mg L^{-1}, and down-regulated significantly by 5.7 fold at 0.5 mg L^{-1} (Fig. 5). These results demonstrated that the expression of the subunits of VGCC was differentially altered by different concentrations of deltamethrin in *C. elegans*.

3.6. Comparison of the effects of deltamethrin and the carbamate carbofuran

Both deltamethrin and carbofuran inhibit *C. elegans* motility, egg laying and foraging (Figs.1, 2 and 3). To further support these observations, measurements were made from images captured of L4 larvae before and after exposure to either 200 mg L^{-1} carbofuran or deltamethrin for 12 h. We found that carbofuran elicited shortening in contrast to deltamethrin, which there was no overall change in the body length. The results indicate that its mode of paralysis is distinct from that of carbofuran as hyper-contraction was not observed (Fig. 6).

3.7. The sensitivity of C. elegans to VGCC blockers

To assess the toxicity of VGCC blockers to *C. elegans*, we exposed worms to 500, 50 and 5 mg L^{-1} VGCC blockers for 24 h and then counted the mortality rate of the worms. Table 3 showed the mortality rate of worms treated with mibefradil was the same as that treated with deltamethrin, and higher than those treated with verapamil, diltiazem and nifedipine at 5, 50 and 500 mg L^{-1}. In addition, after 24 h exposure, worms were washed with ddH_2O and transferred to plates, and the morphology of dead worms was observed. The characteristic shape of worms killed by deltamethrin was straight (Fig.7B) or bent (Fig.7C), and very few showed a wavy shape, which was similar to those killed by VGCC blockers nifedipine, verapamil and mibefradil. These similarities suggest that the occurrence of the straight shape was related and might represent a similar in toxicity.

3.8. Effect of Ca^{2+} channel blockers on VGCC gene expression in C. elegans

Since the direct target of Ca^{2+} channel blockers are the VGCC, we tested the expressions of three α1-subunits of VGCC genes under sublethal concentrations of Ca^{2+} channel blockers (500 mg L^{-1} for verapamil, diltiazem and nifedipine, 50 mg L^{-1} for mibefradil). Fig. 8 showed the fold change in gene expression of three α1-subunits of VGCC gene. Of these genes, *egl*-19 was significantly down-regulated at 500 mg L^{-1} verapamil (L-type Ca^{2+} channel blockers) and 50 mg L^{-1} mibefradil (T-type Ca^{2+} channel blockers) treatment; *unc*-2 was down-regulated at 500 mg L^{-1} verapamil or diltiazem, and 50 mg L^{-1} mibefradil treatment. Interestingly, there was no significant difference of *cca*-1 expression at T-type Ca^{2+} channel blockers treatment, but was up-regulated by 2.1 and 3.2 fold at 500 mg L^{-1} diltiazem and nifedipine. However, *cca*-1 was significantly up-regulated at 50 mg L^{-1} deltamethrin treatment (Fig. 5).

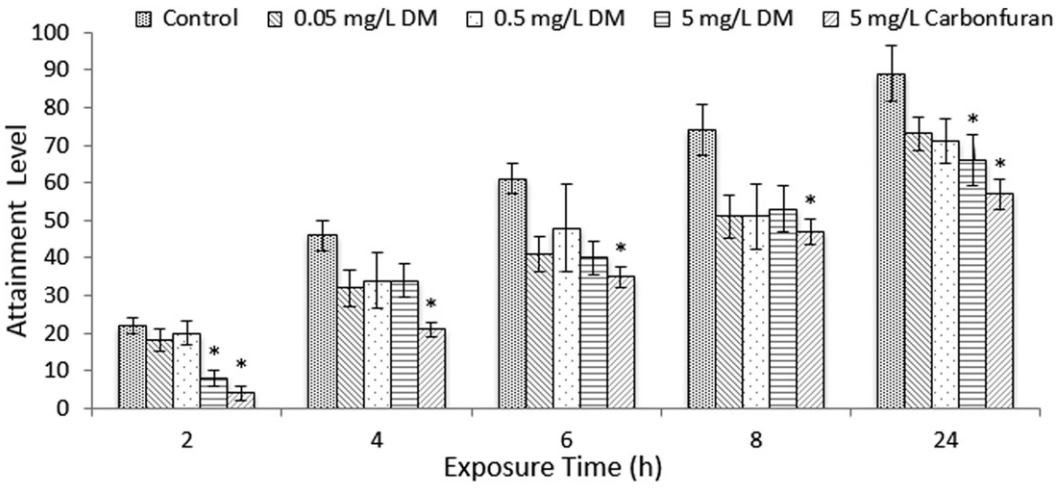

Fig. 4. Effects of deltamethrin on foraging behavior in *C. elegans*. Following the 24 h incubation with NGM plates containing sublethal concentrations of drugs for 24 h, L4 worms were removed and washed 3 times in M9 buffer, and then transferred to chemical-free 9 cm NGM plates containing *E. coli* grown circularly within a 0.5 cm radius from the center. After 2, 4, 6, 8 and 24 h of incubation at 20 °C, the numbers of nematode that have reached the *E. coli* colony were counted in each plate. The detection of foraging behavior was performed according to the method of Kohra [34]. The data shown are the average of five independent experiments and statistically analyzed using ANOVA, $n = 12$ worms. Error bars represent mean ± SEM , $^{*}p < 0.05$. DM, deltamethrin.

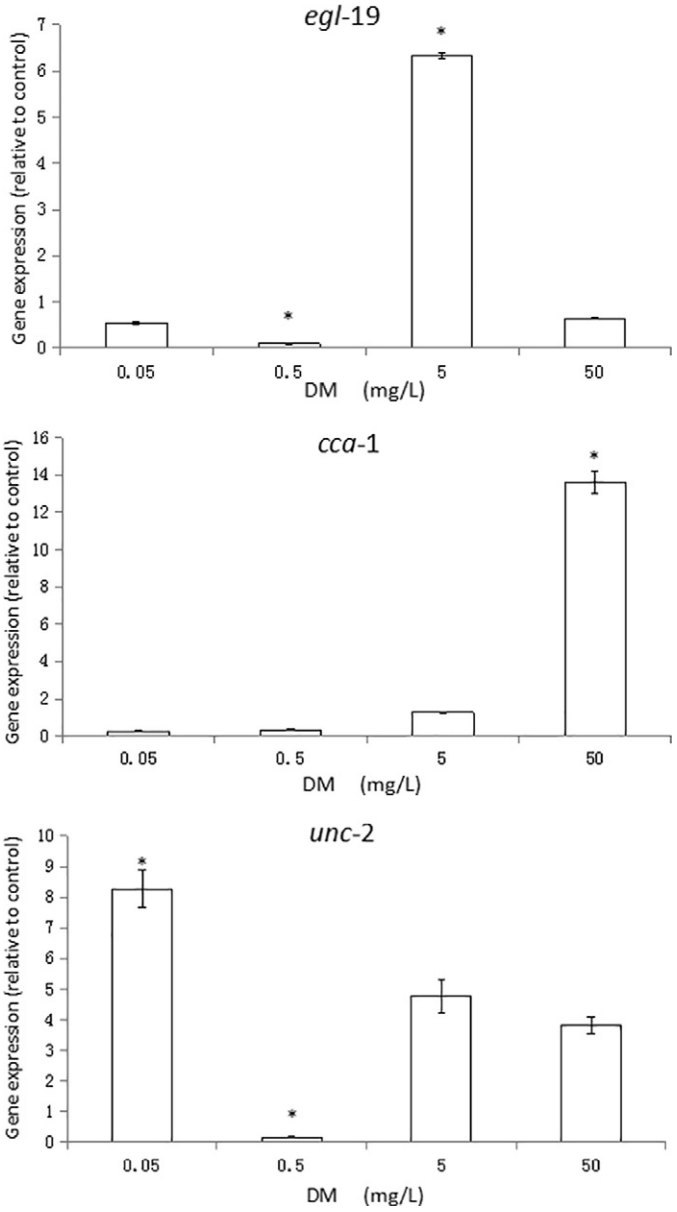

Fig. 5. The effect of deltamethrin on different gene expression in *C. elegans*. L4 larvae of *C. elegans* were treated with the indicated concentrations of deltamethrin for 24 h. The mRNA levels *egl*-19, *cca*-1 and *unc*-2 by real-time RT-PCR with actin as internal control are presented as relative units compared to control (control = 1, dosed in K-medium; $n = 3$). Differences between the data were considered significant at $P \leq 0.05$. Error bars represent mean ± SEM, $^{*}p < 0.05$.

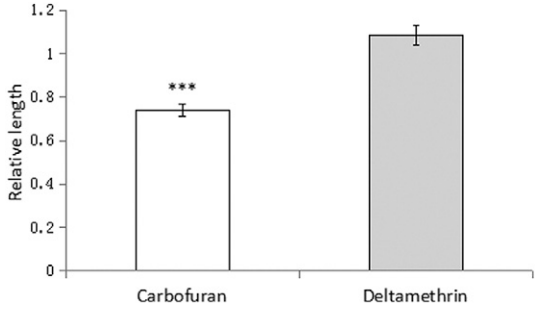

Fig. 6. Effect of deltamethrin and carbofuran on length of *C. elegans*. Ten worms were treated on the drug plate which contained deltamethrin or carbofuran with the final concentration of 200 mg L^{-1}. Both the initial stage and the after treatment stage length were recorded. (Initial stage length = 1, dosed in NGM; $n = 10$ worms; mean ± SEM). Asterisks indicate statistical significance ($^{***}p < 0.001$) between control and chemical plates using ANOVA.

Table 3
Mortality of *C. elegans* after exposure to NGM plates containing different concentrations of VGCC blockers. Mortality is expressed as a percentage of the initial number of live worms in the sample. Data are mean ± SEM, $n = 3$ replicate plates with about 100 worms for each treatment group.

Test compounds	VGCC blocker type	%Mortality		
		5 mg L^{-1}	50 mg L^{-1}	500 mg L^{-1}
Deltamethrin	–	6.3 ± 0.9	59.6 ± 1.7	100
Nifedipine	L	0	4.5 ± 0.8	45.6 ± 2.3
Verapamil	L	0	0	24.8 ± 2.6
Diltiazem	L	0	0	10.4 ± 0.4
Mibefradil	T	3.7 ± 0.7	47.0 ± 1.4	100

4. Discussion

In this study, we showed that deltamethrin reduced the survival of *C. elegans* in a concentration dependent manner. Moreover, deltamethrin decreased the thrashing and body bend frequency, egg-laying and

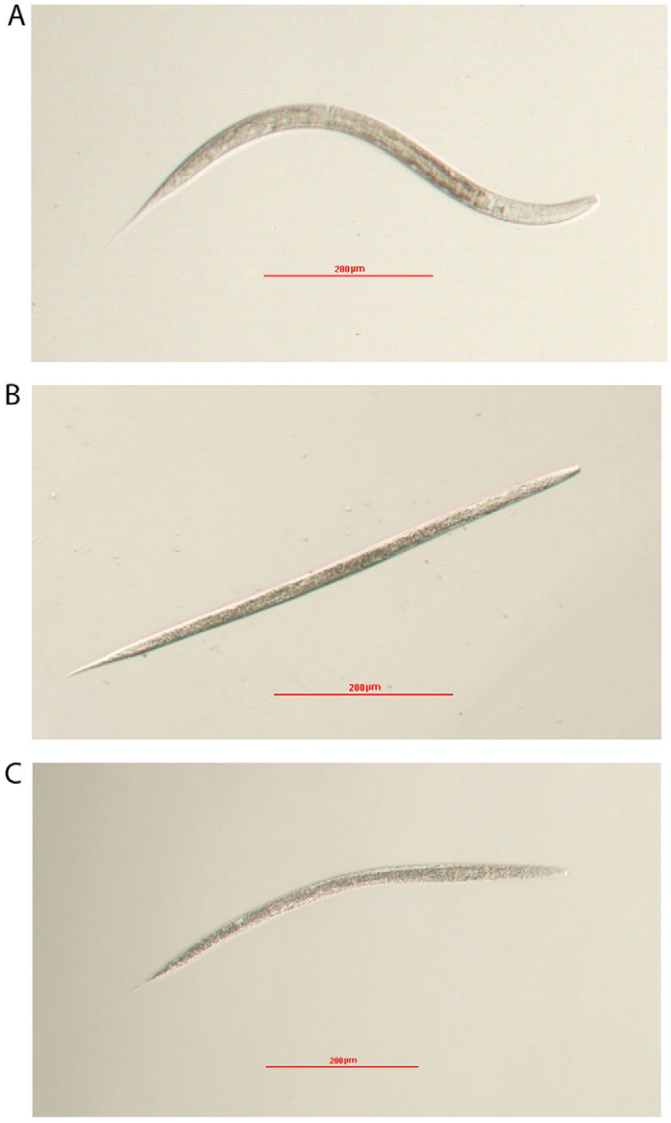

Fig. 7. Representative images of *C. elegans* after 24 h in the presence of 500 mg L^{-1} deltamethrin, verapamil, diltiazem, nifedipine, mibefradil. A: Untreated; B, C: Treated with 500 mg L^{-1} deltamethrin, and VGCC blockers (verapamil, diltiazem, nifedipine, mibefradil) for 24 h. Characteristic shapes of dead nematodes: straight (B); bent (C).

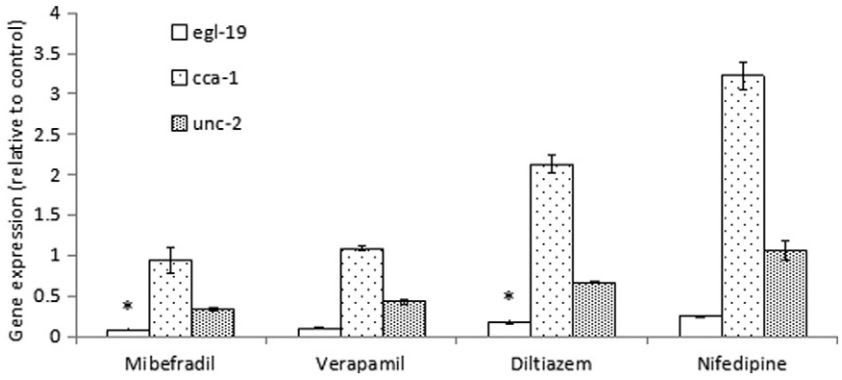

Fig. 8. The effect of VGCC blockers on different gene expression in *C. elegans*. L4 larvae of *C. elegans* were treated with the indicated VGCC blockers (500 mg L^{-1} verpamil, 500 mg L^{-1} ditiazem, 50 mg L^{-1} mibefradil and 500 mg L^{-1} nifedipine) for 24 h. The mRNA levels *egl*-19, *cca*-1 and *unc*-2 by RT-PCR with actin as internal control are presented as relative units compared to control (control = 1, dosed in K-medium; $n = 3$). Differences between the data were considered significant at $P \leq 0.05$. Error bars represent mean ± SEM, $^{*}p < 0.05$.

attainment levels in *C. elegans*. Furthermore, deltamethrin exposure resulted in differential expression of the subunits of VGCC in *C. elegans*. These results suggest that deltamethrin may induce toxic behavior on *C. elegans* via regulating the expression of the α1-subunits of VGCC.

Elucidation of the toxic effect of neurotoxic pesticides on the behavior change of *C. elegans* may advance our understanding of the mechanisms underlying the toxicity that is induced by these chemicals. Neurotoxic insecticides usually cause locomotion, egg laying and foraging behavior defects in *C. elegans* when its sensory neurons and nerve conduction are impaired, and cuticule, vulval and pharyngeal muscles are inhibited. *C. elegans* moves in a sinusoidal fashion by generating waves of alternating dorsal and ventral muscle contraction [37]. Egg-laying are controlled by two types of neurons and two sets of muscles [38]. We demonstrated in this study that, both type-II pyrethroid insecticide deltamethrin and carbofuran altered the behaviors in *C. elegans*, including thrashing, body bend frequency, egg laying and foraging behavior. Taken together these observations suggest that deltamethrin and carbofuran might have a similar mechanism of action. However, the deltamethrin treated worms showed no hyper-contraction. Carbofuran is an anticholinesterase. In the presence of carbofuran, acetylcholine accumulates in the synaptic cleft as a result of the inability of acetylcholinesterase to break down acetylcholine. This build-up of acetylcholine leads to over-activation of cholinergic receptors, muscle hyper-contraction, paralysis, and eventually cell death [16,39]. Thus, deltamethrin and carbofuran showed the difference on body length, indicating the profile of actions of deltamethrin in *C. elegans* is distinct from carbofuran.

While some studies have shown that the above behaviors are controlled by multiple factors. For example, the hermaphrodite-specific neurons (HSNs) control 16 egg-laying muscles via electrical connection between the vulval and the uterine muscles, although the HSNs make synapses directly only onto the vulval muscles [40,41]. Foraging is a rapid, side-to-side movement of the nose generated by *C. elegans* as it explores its environment. Several neurons, including odorant-sensing AWA and AWC neurons, the OLQ and IL1 sensory neurons and the RMG motor neurons, have been shown to be required for this behavior [42,43]. Pharyngeal neurons MC, M2, M4, and I1 form multiple direct and indirect excitatory pathways in a robust network for control of pharyngeal pumping [44]. Therefore, whether deltamethrin is toxic to these neurons needs further investigation.

In addition, the expression levels of 3 genes encoding the α1-subunits of VGCC were changed in *C. elegans* after 24 h exposure of L4 worms to different concentrations of deltamethrin. The results suggest that modulations of VGCC were not specific to individual subunits of VGCC: *cca*-1, which is expressed in pharyngeal muscle, was specifically up-regulated in the 50 mg L^{-1} treatment. Likewise, *egl*-19, which is expressed in pharyngeal and body muscles, was significantly up-regulated at 5 mg L^{-1} deltamethrin, and *unc*-2, which is required in the HSNs and/or VCs to negatively regulate egg-laying, was significantly up-regulated at 0.05 mg L^{-1}. The results suggest that *egl*-19, *cca*-1, and *unc*-2 may be potential regulators of deltamethrin-dependent behaviors at different concentration of deltamethrin. Interestingly, all the three α1-subunits of VGCC were down-regulated in *C. elegans* following 0.5 mg L^{-1} deltamethrin exposure. It remains to be determined whether low level and chronic exposure of deltamethrin is associated to desensitization of VGCC receptors.

To further identify which VGCCs are involved, we selected 4 different VGCC blockers (three were L-type, one was T-type) and tested their relative nematicidal activity and effects on three α1-subunits of VGCC expression in *C. elegans*. Among 4 VGCC blockers, mibefradil (T-type) demonstrated the highest lethal activity, followed by nifedipine (L-type). Further analysis of the expression of α1-subunits of VGCC following Ca^{2+} channel blockers treatment revealed that *egl*-19 was significantly down-regulated at both deltamethrin and four Ca^{2+} channel blockers.

In summary, we revealed that deltamethrin was toxic to and reduced the locomotion, egg laying and foraging behavior of *C. elegans* by modulating the expression of α1-subunits of VGCC, *egl*-19, *cca*-1, and *unc*-2. Our findings may provide insights into the linkage between deltamethrin-induced toxic behavior and the regulation of α1-subunit of VGCC.

Conflict of interests

The authors declare that they have no competing interests.

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