Low Salinity Decreases the Tolerance to Two Pesticides, Beta-cypermethrin and Acephate, of White-leg Shrimp, *Litopenaeus vannamei*

Xiaodan Wang, Erchao Li*, Zequan Xiong, Ke Chen, Na Yu, Zhenyu Du and Liqiao Chen*

School of Life Sciences, East China Normal University, 200241, Shanghai, China

**Abstract**

Acute toxic effects of two commonly used pesticides, beta-cypermethrin and Acephate, to the white-leg shrimp, *Litopenaeus vannamei*, were tested at ambient salinity 5.0% and 20.0%, by using a static renewal method. The results showed that the mean LC₅₀ values of beta-cypermethrin at 24, 48, 72 and 96 h were 0.437, 0.317, 0.203, and 0.170 µg/L to the white shrimp at 5.0%, and were 0.767, 0.440, 0.383, and 0.383 µg/L at 20.0%. The mean LC₅₀ values of Acephate at 24, 48, 72, and 96 h were 51.250, 38.007, 27.783, 18.247 mg/L at 5.0%, and were 59.853, 43.490, 34.220, 27.337 mg/L at 20.0%. *L. vannamei* is more sensitive to ambient beta-cypermethrin and Acephate toxicity at salinity 5.0% than at salinity 20.0%. Beta-cypermethrin is highly toxic to *L. vannamei* at either salinity, while acephate has low toxicity to *L. vannamei*.

**Keywords:** *Litopenaeus vannamei*; Beta-cypermethrin; Acephate; Toxicity; Salinity

**Introduction**

With the rapid development of green revolution in agriculture, pesticides are widely used in pest control. However, it could lead to high concentrations of these pesticides in surface water especially in intensive agricultural areas [1], which are hazards for aquatic species [2]. Most insecticides used in agriculture are organophosphate, Carbamate and synthetic Pyrethroid compounds [3] because of their relatively non-persistent characteristics in the environment. Though these pesticides can rapidly degrade in natural environment, their high acute toxicity to some non-target species has been demonstrated in many laboratory tests [4-7].

Beta-cypermethrin and Acephate are two commonly used pesticides worldwide. Beta-cypermethrin is a synthetic analog of Pyrethrins, extracted from the ornamental *Chrysanthemum Cinerariaefolium*, and widely used in field pest control [8]. Due to the Lipophilicity, Pyrethrroids have a high rate of gill absorption. So they have been reported to be extremely toxic to fish [8-12], but information in crustacean species is still limited. Acephate is a water soluble foliar spray insecticide of moderate persistence and is used on fruit and vegetable crops grown across the world [13]. A number of studies have been conducted on the toxicity of Acephate on different organisms and indicated as a potent Neurotoxicant [14]. It is also found to be mutagenic [15], carcinogenic [16], and Cytotoxic [17]. However, this compound is considered relatively non-toxic to fish with a median lethal concentration (LC₅₀) for goldfish of 9,550 mg/l and rainbow trout >1,000 mg/l over 96 h [13]. But potential hazard to aquatic crustaceans has been speculated for the heavy use of these compounds and deficiency in the immune system, since lower LC₅₀ values have been found in *Americanus bahia*, *Homarus americanus*, *Peneaus aztecus* and *P. duprurum* [18-19].

The white-leg shrimp, *Litopenaeus vannamei*, is a typical tropical crustacean that can adapt to a wide range of salinities ranging from 1 to 50.0% [20], and with the development of inland saline water farming, it has become one of the most popular shrimp for aquaculture in the Central and South American countries [21-22], Thailand [23] and China [24]. Toxicology of ambient pesticides on this species mainly focused on diazinon [25], Propiconazole [25], parathion [26], pyrazosulfuron-ethyl [27], permethrin [27], chlordane [28], DDT [28], lorsban [28] and lindane [28], while there is no report on the toxicology of beta-cypermethrin and Acephate to this species at either salinity, though environment characteristics may affect the toxicity of toxicants to aquatic organisms [29].

This study determined the lethal concentration values of beta-cypermethrin and Acephate to *L. vannamei* at two salinities (5.0% and 20.0%), and found that low salinity decreased the tolerance to these two pesticides in *L. vannamei*.

**Materials and Methods**

*L. vannamei* were obtained from a farm with pond salinity of 20.0% in Haikou, Hainan, China. Shrimps were cultured in fiberglass tanks (60×50×50 cm) at a salinity of 20.0% for 1 week, then divided randomly into two groups and acclimated to the target salinities (5.0% and 20.0%) by changing 2.0% per day. After reaching the targeted salinities, each group was acclimated an additional week. Individual shrimp, weighing 4.54 ± 0.33 g, were selected for the toxicity experiments. During the pre-culture and acclimation period, shrimps were fed with a commercial feed containing 41.1% crude protein, 9.6% crude lipid, 12.0% ash, and 10.0% moisture. Seawater was pumped from the Xiuying Coast in Haikou City, and filtered through an activated carbon cartridge for at least 3 days before entering the culture system. The tap water was aerated before being added to the tank to adjust the salinity levels. The water quality parameters during the experiment were 27.7-29.2°C for temperature, 5.58-6.79 mg/L or dissolved oxygen, 8.3 ± 0.2 for pH value and <0.01 mg/L for total ammonia-nitrogen.

Beta-cypermethrin and Acephate (Yujing Bio-Tech Co. Ltd., Beijing City, China) stock solutions were prepared at the day of trial starting. As beta-cypermethrin was only slightly dissolved in water, it was prepared from the source are credited.

*Corresponding authors: Erchao Li, School of Life Sciences, East China Normal University, 200241, Shanghai, China, Tel: +86-21-54341002; E-mail: edli@bio.ecnu.edu.cn
Liqiao Chen, School of Life Sciences, East China Normal University, 200241, Shanghai, China, Tel: +86-21-54341002; E-mail: lqchen@bio.ecnu.edu.cn

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by diluting it in acetone to give the stock material. The control group received a same acetone volume used in the dilution of the dosing concentrations. For both pesticides, six concentrations with a control were used with triplicates for each treatment. The beta-cypermethrin stock solution was diluted to the following concentrations: 0 (control), 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 μg/L at 5.0%, and 0 (control), 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 μg/L at 20.0%. And the Acephate stock solution was diluted to 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 μg/L at 20.0%. The beta-cypermethrin concentrations used in this study were 0.0, 0.2, 0.4, 0.8, 1.6 μg/L at 20.0%. Ten shrimp were randomly assigned to each treatment and all the tanks were aerated continuously to keep oxygen at adequate levels. Tests solutions were renewed totally every 24 h. The experiment was conducted for 96 h and no feed was supplied to the shrimp during the test period. Observations on mortality and abnormal behavior of the shrimps were conducted at least two times each day. Death was assumed when juvenile white shrimps were non-motile and showed little movement when touched with a glass rod [30]. Dead shrimps were removed immediately when they were found. All treatments were run in triplicate with similar conditions.

Results and Discussion

Signs of toxicity in fish poisoned with beta-cypermethrin generally include the changes of loss equilibrium, color darkening and staying motionless [10]. Signs of toxicity in terrestrial animals poisoned with Acephate include muscular weakness, tremors, reduced activity [32], but reports on signs of toxicity in aquatic animals were limited. In this study, less toxic symptom can be observed for L. vannamei poisoned with both beta-cypermethrin and Acephate. Dying shrimp showed pronounced whiter body color compared with the alive, and the shrimp dying, when turned over, was nearly immobile on the bottom or showed little movement when touched with a glass stick.

The percentages of mortality recorded for each treatment are shown in Table 1. No mortality was observed in the controls at either salinity or in the treatments of 0.10 μg/L at 20.0% for beta-cypermethrin during the 96 h. However, 100% mortality occurred in the treatments of 101.25 mg/L at 5.0% and 151.88 mg/L at 20.0% for Acephate and 3.20 μg/L at 20.0% for beta-cypermethrin after 24 h of exposure. The mean LC50 values and the effect of salinity to the acute toxicity of beta-cypermethrin and Acephate at different exposure time was analyzed by the t-test.

| Salinity | Beta-cypermethrin | Acephate |
|----------|------------------|----------|
| 5%       |                  |          |
|          | Concentration    |          |
|          | (μg/L)           |          |
| 0.00     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 0.05     | 10.0 ± 0.0       | 10.0 ± 0.0|
| 0.10     | 16.7 ± 5.8       | 16.7 ± 5.8|
| 0.20     | 26.7 ± 5.8       | 26.7 ± 5.8|
| 0.40     | 33.3 ± 5.8       | 33.3 ± 5.8|
| 0.80     | 50.0 ± 5.8       | 50.0 ± 5.8|
| 20%      |                  |          |
|          | Concentration    |          |
|          | (mg/L)           |          |
| 0.00     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 0.10     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 0.20     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 0.40     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 1.60     | 0.0 ± 0.0        | 0.0 ± 0.0|
| 3.20     | 0.0 ± 0.0        | 0.0 ± 0.0|

Table 1: The mortality recorded for each concentration tested and for both drug at both salinities.

| Salinity | beta-cypermethrin (μg/L) | Acephate (mg/L) |
|----------|--------------------------|-----------------|
| 5%       | 0.05 0.10 0.20 0.40 0.80 1.60 | 0.05 0.10 0.20 0.40 0.80 1.60 |
| 20%      | 0.00 0.05 0.10 0.20 0.40 0.80 | 0.00 0.05 0.10 0.20 0.40 0.80 |

Table 2: LC50 values and 95% confidence interval of beta-cypermethrin and acephate at L. vannamei juveniles in renewal acute toxicity test exposed from 24 to 96 h.

Note: values are means ± standard error (S. E.) of three replicates (%)
values of beta-cypermethrin at 24, 48, 72 and 96 h were 0.437, 0.317, 0.203, 0.170 μg/L to the shrimp at 5.0%, and were 0.767, 0.440, 0.383, 0.383 μg/L at 20.0%. While for Acephate, the mean LC₅₀ values at 24, 48, 72 and 96 h were 51.250, 38.007, 27.783, 18.247 mg/L at 5.0%, and were 59.853, 43.390, and 34.220, 27.337 mg/L at 20.0% (Figure 1 and Table 2). The results indicated that L. vannamei at low salinity is more sensitive to the ambient beta-cypermethrin and Acephate toxicity. Meanwhile, beta-cypermethrin had higher toxicity than Acephate to L. vannamei at either salinity.

The LC₅₀ values for beta-cypermethrin and Acephate to aquatic animals reported in the existing literatures are shown in Table 3, which support the finding of higher toxicity of beta-cypermethrin to white animals reported in the existing literatures. The LC₅₀ values of both pesticides varied in different aquatic organisms. When compared with the toxicity of other pesticides reported to L. vannamei (Table 4), except for permethrin, beta-cypermethrin is more highly toxic to L. vannamei than some other commonly used pesticides, such as diazinon [25], propiconazole [25], parathion [26], pyrazosulfuron-ethyl [27], chlordane [27], DDT [28], lorsban [28], and lindane [28]. While the toxicity of Acephate to L. vannamei is less than that of other pesticides except for pyrazosulfuron-ethyl (Table 4). According to the determination of toxicity for different pesticides [33], beta-cypermethrin is extremely toxic to L. vannamei (96 h LC₅₀<0.1 mg/L), while Acephate is low toxic (96 h LC₅₀=10-100 mg/L).

Besides, the 96 h LC₅₀ values of beta-cypermethrin to L. vannamei at either 5.0% or 20.0% in this study were much lower than the LC₅₀ values reported in fishes (Table 3), such as Cyprinus carpio [9,11], Oncorhynchus mykiss [9], Salmo trutta [9], Salmo salar [8], Pocella reticulata [10], Gambusia affinis [8], Cynprinodon macularius [8]. For Acephate, the toxicity to L. vannamei at either salinity and to other crustacean species, including A. bahia [19], H. americanus [19], P. aztecus [18], and P. duorarum [19] were also much lower than the values reported in fishes [18,19,34]. The susceptibility of crustacean species to these pesticides are due to the relatively undeveloped detoxification system which cannot degrade most toxic compounds efficiently [35], and the open vascular system of crustacean species make ambient toxicants enter their body easily.

L. vannamei is more susceptible to ambient beta-cypermethrin and Acephate at 5.0% than at 20.0%, and significant differences were both found at 24 h, 72 h and 96 h (P<0.05). Similar findings were reported in the toxic effects of ambient ammonia, boron and nickel to L. vannamei. The 96 h LC₅₀ with 95% confidence limit of L. vannamei at 3.0% to ambient ammonia-N was 9.33 mg/L [36], which was significantly lower than the previous results [37], which reported that the LC₅₀ of ammonia to L. vannamei at 15 and 25% were 24.39 and 35.40 mg/L respectively. The mean LC₅₀ values of boron at 24 h, 48 h, 72 h and 96 h were 552.55, 153.35, 50.14, 25.05 mg/L to the shrimp at 3.0%, and were 598.13,
219.52, 147.8, 80.06 mg/L at 20.0% [38]. The 96 h LC50 values were 41 μmol/L and 362 μmol/L for L. vannamei at 5% and 25%, respectively [39].

Therefore, lower mean LC50 values of beta-cypermethrin and Acephate to white shrimp were observed at 5.0% when compared with those at 20.0% in the present study.

Overall, ambient beta-cypermethrin and Acephate can pose an acute toxic effect on L. vannamei. Beta-cypermethrin is extremely toxic to L. vannamei, while Acephate is toxic. L. vannamei at low salinity was more sensitive to the toxicity of these two insecticides.

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Competing Interests
The authors declare that they have no competing interests.

Authors’ Contributions
Conceived and designed the experiments: EL, LC, NY. Performed the experiments: XW, ZX, EL, KC. Analyzed the data: ZX, XD, EL. Wrote the paper: XW, EL, ZD, LC. All authors read and approved the final manuscript.

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