

Effects of salinity and temperature on the acute toxicity of the pesticides, dimethoate and chlorpyrifos in post-larvae and juveniles of the whiteleg shrimp

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Abstract

In this article, the acute toxicity of the organophosphorus pesticides (OPs), dimethoate (DMT) and chlorpyrifos (CPF) in post-larvae, and juveniles of *Litopenaeus vannamei* at different ambient salinity (5, 15 and 25) and temperature (15, 25 and 34 °C) regimes were compared with standard exposure conditions (temperature, 27 °C and salinity, 20). Chlorpyrifos was significantly more toxic to both life stages ($P < 0.05$). Post-larvae were more sensitive than juveniles to both OPs in all altered salinities and temperatures ($P < 0.05$). Acute toxicity indices (96 h LC₅₀ values) showed that lower salinities (5 and 15) and temperature (15 °C) induced significantly higher pesticide toxicity ($P < 0.05$) in both life stages. Very low acute toxicity indices of DMT and CPF at varying levels of salinity and temperature highlight the importance of monitoring OPs pollution in low-saline shrimp aquaculture ponds. Results of the present study provide baseline information for monitoring pesticide pollution in low-saline shrimp aquaculture ponds which are influenced by changes in climatic conditions.

Keywords: Dimethoate; Chlorpyrifos; Shrimp aquaculture; Climate change; Toxicity

1. Introduction

Organophosphorus pesticides (OPs) have largely replaced organochlorine compounds (OCs) for controlling insects in the intensive agriculture and horticulture because of their rapid degradability (Roque et al., 2005; Halappa and David, 2009). However, the effect of these rapidly degrading OPs on non-target organisms comparable OCs has been a major concern (Kumar et al., 2010; Joseph and Raj, 2011; Tu et al., 2012; Kumar et al., 2017). The primary mode of action of OPs is to inhibit the acetylcholinesterase enzyme which regulates the functioning of the nervous system (Díaz-Resendiz et al., 2015) leading to the hyperactivity and paralysis in the organisms, ultimately culminating into their death (Key and Fulton, 1993; Garcia-de la Parra et al., 2006). Many reports describing the detrimental effects of OPs on biological and physiological parameters in a wide range of economically important non-target freshwater and marine organisms, and abundance of benthic invertebrates in shrimp ponds is slowly beginning to emerge (Rao et al., 2005; Garcia-de la Parra et al., 2006; Eamkamon et al., 2012; Krishnapriya and Padmaja, 2014; Uddin et al., 2016; Hook et al., 2018; Pawar et al., 2019).

Recently, shrimp aquaculture has emerged as a fastest-growing food sector and has become an activity of economic importance in many countries of the world. Due to high demand in foreign markets, production of farm-raised shrimp, especially the whiteleg shrimp, *Litopenaeus vannamei*, has expanded rapidly worldwide. *L. vannamei* was the highest harvested crustaceans in the world with a total production of 4.16 million tonnes, contributing to 53% of the total crustacean production (~7.86 million tonnes) in 2016 (FAO, 2018). Owing to its remarkable ability to grow and survive in varying salinity and temperature conditions and relatively faster growth during shorter culture period (Ponce-Palafox et al., 1997; Roy et al., 2010), the farming of *L. vannamei* has expanded to inland low-saline areas in many Asian countries such as China, Thailand, Vietnam, Bangladesh and Indonesia including India. Such an extension in the farming of *L. vannamei* to inland low-saline regions often adjacent to the agriculture fields enhances the potential risk of pesticide toxicity to the farmed stock due to runoff and/or spray drift (Roque et al., 2005; Pawar et al., 2019).

Salinity and temperature are two most prominent abiotic water quality parameters that affect the toxicity of xenobiotics to aquatic organisms (Brecken-Folse et al., 1994; DeLorenzo et al., 2009) because they regulate the bioavailability of a contaminant in the water. Changes occurring in the global climate particularly, variability in water temperature and salinity could exacerbate detrimental effects of pollutants on aquatic organisms and as a consequence of altered chemical fate and transport, and changes in physiological response may enhance their toxicity (Lin and Chen, 2003; DeLorenzo et al., 2009; Tu et al., 2012; DeLorenzo et al., 2013; Mehta, 2017). The fluctuations in

water temperature in natural water bodies and farming systems depend on air temperature, water depth, pond design, evaporation rate and water management. On the other hand, salinity varies in accordance with seasons, increases during the summer and decreases after rainfall (Tu et al., 2012). Temperature exerts serious physiological effects on living systems and directly influences the tolerance limits of aquatic biota to toxic substances (Mehta, 2017). Salinity is another critical factor that influences the distribution and maintenance of life in aquatic ecosystems (Wang et al., 2013). Therefore, the physiological effects induced by fluctuations in temperature and salinity may potentially altering ecotoxicity of pollutants (DeLorenzo et al., 2013; Velasco et al., 2019).

Sub-optimal temperature or unsuitable salinity may interact in an antagonistic, additive or synergistic manner with toxicants thereby significantly altering the tolerance of aquatic animals (DeLorenzo et al., 2009; Tu et al., 2012; Santos et al., 2014; Patra et al., 2015; Velasco et al., 2019). Influence of salinity and temperature on the tolerance to toxicity of pesticides in a variety of crustaceans species including shrimps has been reported (Staton et al., 2002; DeLorenzo et al., 2009; Tu et al., 2012; Wang et al., 2013). Due to their phylogenetic similarities with insects, crustaceans in general and the penaeid shrimps, in particular, have been proved to be more sensitive to ecotoxicological effects of pesticides (Nimmo et al., 1971; Couch, 1978; Cripe, 1994; Galindo-Reyes et al., 1996; Roque et al., 2005; Mello et al., 2011; Eamkamon et al., 2012; Pawar et al., 2019). Furthermore, higher sensitiveness of early life stages to pesticides than adults has also been widely documented (Juarez and Sanchez, 1989; Hutchinson et al., 1998; Pawar et al., 2019).

The ecotoxicity data for most commonly used pesticides conducted under standard test conditions is widely available. However, data derived from standard bioassays is often not comparable because of differences in test design, lack of information on the fluctuating environmental conditions, life stages of commercially important species. Furthermore, physico-chemical factors (salinity and temperature) may alter the bioavailability of chemicals and therefore affect the toxicity (Brecken-Folse et al., 1994). With this background, the present study examined the effects of varying salinity and temperature regimes on the acute toxicity of two commonly used OPs (dimethoate and chlorpyrifos) in post-larval (PL) and juvenile (JL) stages of *L. vannamei*. The outcome of the study has practical implications because of *L. vannamei* is a promising shrimp species for farming in low saline inland areas often influenced by diffuse source run-off from agriculture.

2. Materials and methods

2.1. Experimental shrimps and rearing conditions

Approximately 6000 healthy SPF (specific pathogen-free), 12 days old post-larvae (PL₁₂) stage) of the whiteleg shrimp, *Litopenaeus vannamei* (total length, 11.4 ± 0.2 mm; wet weight, 52 ± 0.5 mg) were procured from a commercial shrimp hatchery (Skyline Aqua Hatchery, Kumta, Karnataka). Post-larvae (PL₁₂) transported in oxygen-filled bags containing seawater (salinity, 20) to the Aquaculture Laboratory, CSIR-NIO, Goa (India). Upon arrival, shrimps were reared and acclimated under laboratory conditions in circular fibre reinforced plastic (FRP) tank (capacity, 2000 L) until they reached the PL₂₀ stage. The seawater used for the rearing of post-larvae and later for acute toxicity experiments was treated by rapid sand filtration, cartridge filtration (20 to 1 µm) and passed through ultraviolet radiation. During rearing of post-larvae, EPAC XL feed (INVE Aquaculture Inc., USA) was used.

2.2. Shrimps acclimation

Shrimps (PL₁₂) reared at salinity (20 ± 0.5) and temperature (27 ± 1 °C) were divided randomly into two batches. One batch of shrimps was further divided and adjusted gradually (stepwise decrease/increase in salinity of 2 per day) to three different target salinity regimes (5, 15 and 25) in three separate 800 L FRP tanks for each salinity group. Target salinities were obtained by a mixture of dechlorinated tap water with natural seawater. The selected salinity values fell within the range in which *L. vannamei* is cultured. Salinity was checked at regular intervals by hand-held refractometer (Atago, Japan). Another batch of acclimated shrimps was further divided randomly into three groups at the same temperature and then gradually adjusted to target temperatures (15, 25 and 34 °C). Temperature experiments were conducted in an air-conditioned room (air temperature, 16 °C). Target temperatures were achieved by stepwise decrease/increase in water temperature with the help of aquarium heaters (100 W, temperature range 22–34 °C, Protherm, Italy). The temperature of rearing water tanks was also monitored regularly with the help of calibrated thermometer.

For conducting acute toxicity experiment with juveniles of *L. vannamei*, acclimated post-larvae at three target salinities and temperature were further grown for four weeks with a photoperiod of 12 h light: 12 h dark. During this rearing period, shrimps were fed with commercial shrimp pellet feed (CP-Aquaculture, India; proximate composition, 38 – 40% protein; 5% lipids and 3% fibre) at the rate of 6% of total biomass split into three rations a day. Excreta, uneaten feed and sloughed exoskeletons were removed by siphoning every day. Temperature, dissolved oxygen (DO), salinity and pH were measured daily with a portable water quality meter (CyberScan Series 600, Eutech

Instruments, Singapore). All other water quality parameters ($\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$ and NH_3/NH_4) were analysed following methods described in APHA (2005).

2.3. Chemicals

The technical-grade of OPs, dimethoate (O, O-dimethyl S-methyl carbamoyl methyl phosphorodithioate; DMT) and chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothionate; CPF), respectively marketed under the brand names, 'TAFGOR' (Rallis India Ltd., Akola, India) and 'PYRIBAN' (AIMCO Pesticides Ltd., Ratnagiri, India) were used for the study. The technical-grade of DMT and CPF having effective concentrations of 30% and 20%, respectively were diluted with deionised water to prepare stock solutions of active DMT (2 mg L^{-1}) and CPF (2 mg L^{-1}).

2.4. Experimental design

For each pesticide, the tests were conducted under standard exposure conditions (temperature, 27 °C and salinity, 20) per life stage in triplicate. For altered exposure conditions, eighteen experimental tanks were obtained by combining three salinities (5, 15 and 25) and temperatures (15, 25 and 34 °C) with three replications per pesticide per life stage resulting in total of 72 experimental units. The parameters measured were the mortalities in post-larvae and juveniles of *L. vannamei* recorded at the end of 24, 48, 72 and 96 h of exposure.

2.5. Acute toxicity experiments

To determine acute toxicity indices (96 h LC_{50} values) for DMT and CPF in PL and JL stages of *L. vannamei*, aqueous semi-static renewal tests by following with the standard procedures of APHA (2005). For determining the acute toxicity indices (96 h LC_{50}) in post-larvae (PL_{20}) of *L. vannamei*, acclimated and adjusted shrimps (PL_{20} , $15.2 \text{ mm} \pm 0.3 \text{ mm}$; wet weight, $65 \pm 5 \text{ mg}$) to three salinities (5, 15 and 25) and temperatures (15, 25 and 34 °C) regimes were randomly selected. The average size of *L. vannamei* juveniles used for the experiment was of $53.4 \pm 6.4 \text{ mm}$ in total length and $1.79 \pm 0.6 \text{ g}$ in wet weight. Before being subjected to acute toxicity experiments, shrimps fasted for 24 h.

The results of the range-finding experiments for standard exposure conditions and altered exposure conditions, were used as criteria for the definitive tests to select five nominal exposure concentrations of DMT and CPF. The exposure concentrations ($\mu\text{g L}^{-1}$) of each OPs used for assessing acute toxicity in the PL stage of *L. vannamei* at different salinity and temperature regimes are presented in Table 1. Each treatment had separate controls (without pesticides) in triplicate. Post-

larvae (PL₂₀) were exposed in 3 L glass jar with 2 L medium with 20 PL jar⁻¹, whereas juveniles (JL) were exposed in 21 L glass aquaria containing 10 L medium with 10 JL aquaria⁻¹. Each jar was covered with a glass disc and aerated via 2 mm glass tubing. During the exposure period of 96 h, each test medium was renewed every 24 h under the same conditions (Buikema et al., 1982). Shrimps were not fed during the exposure period and experiments were conducted in a natural light cycle (12 h light: 12 h dark).

Water quality parameters such as temperature, salinity, DO and pH were recorded daily, before each media change, whereas NO₃-N, NO₂-N and NH₃/NH₄ were measured at the beginning and the end of the experimental period. Probes of portable water quality meter were thoroughly rinsed with double distilled water before every use to remove traces of pesticide adhering to the probes. According to the schedule at the end of 24, 48, 72 and 96 h of exposure, mortalities in post-larvae and juveniles were recorded during the 96 h exposure period (Table 1). The dead organisms, if any were removed immediately on detection, in order to avoid any type of bacterial contamination. The criteria for proof of mortality were total lack of body movement, immobility and showed no response when touched with a glass rod (Lin and Chen, 2003). The cumulative mortality rates at the end of exposure periods of 24, 48, 72 and 96 h were calculated using the formula as defined by Abbott (1925). The concentration response of test organisms was determined for LC₅₀ value (median lethal concentration) for each treatment and their 95% confidence limits for an exposure period of 96 h for each treatment were calculated with a computer-based programme described by Finney (1971).

2.6. Data presentation and analysis

All the experiments were performed in three replicates per treatment. Mortality data obtained from the experiments are presented as mean ± SD (Table 1). Measured variables were tested to normality and homogeneity by Cochran's test (Winer et al., 1991). Prior to statistical analysis, acute toxicity data were log₁₀ (*x* + 1) transformed. One-way analysis of variance (ANOVA, Underwood, 1997) was used to assess the differences in acute toxicity indices between standard exposure and altered exposure conditions and within different treatments. If ANOVA found significant differences, multiple comparisons between different means amongst treatments were then made by Tukey–Kramer test high significant differences (HSD) (Zar, 1996). Statistical analysis was performed by using computer-based GraphPad PRISM 5.0 software (Graph Pad, San Diego, CA, USA). Significance in all statistical tests was judged at a ‘*P*’ =0.05 level.

3. Results

No significant variations in water quality parameters were observed in control and treated tanks during the entire experimental routine (*P* > 0.05). The results showed that the significant water

quality parameters during the experiment fell within the optimal limits for *L. vannamei*. No mortality observed in the control group either in salinity or temperature based acute toxicity experiments ensured that the observed effects were due to the action of OPs. The 96 h LC₅₀ values at standard test conditions (temperature, 27 °C and salinity, 20) for DMT and CPF to post-larvae were 257.84 and 0.68 µg L⁻¹, respectively. Relatively higher 96 h LC₅₀ values of 463.61 and 0.73 µg L⁻¹, respectively for DMT and CPF were obtained for juveniles of *L. vannamei* (Table 2). The 96 h LC₅₀ values of DMT and CPF to PL stages were 127.2, 137.44 and 386.59 µg L⁻¹ and 0.31, 0.32 and 0.92 µg L⁻¹ at 5, 15 and 25 salinities, respectively (Table 2). The 96 hrs LC₅₀ values at different temperatures (15, 25 and 34 °C), respectively were 103.11, 321.26 and 182.76 µg L⁻¹ for DMT and 0.17, 0.62 and 0.46 µg L⁻¹ for CPF (Table 2).

The 96 h LC₅₀ values of DMT and CPF obtained for altered salinity and temperature regimes for two life stages of *L. vannamei* differed significantly ($P < 0.05$) from standard exposure conditions (Table 2). A comparison of 96 h LC₅₀ values for post-larvae of *L. vannamei* between adjacent salinities showed the statistical difference ($P < 0.5$) between all tested salinities in case of DMT (Table 2). However, differences in 96 h LC₅₀ values were significant ($P < 0.5$) between salinities 5 and 25 but not between salinities 5 and 15 ($P < 0.5$) for CPF. On the other hand, a comparison of 96 h LC₅₀ values obtained for both DMT and CPF was significant ($P < 0.5$) in all tested temperature regimes (Table 2).

Acute toxicity experiment conducted with juveniles of *L. vannamei* at three different salinities (5, 15 and 25) and temperatures (15, 25 and 34 °C) resulted in relatively higher 96 h LC₅₀ values for DMT compared to CPF (Table 2). Juveniles of *L. vannamei* were found to be less sensitive to both OPs than post-larvae of *L. vannamei* in all tested salinity and temperature regimes. The 96 h LC₅₀ values of DMT and CPF pesticides at 25 salinity and 25 °C temperature differed significantly ($P < 0.05$) when compared to salinities of 5 and 15 and temperatures of 15 and 34 °C. Relatively, CPF was found to be more toxic compared to DMT to both life stages (PL and JL) of *L. vannamei* in all tested salinity and temperature regimes (Table 2).

4. Discussion

The organophosphate pesticides, DMT and CPF are widely used to restrict insect pests in a range of commercial crops. As these products are extensively used in the market, many toxicological effects on economically important non-target freshwater, marine organisms and abundance of benthic invertebrates in shrimp have been reported (Rao et al., 2005; Garcia-de la Parra et al., 2006; Eamkamon et al., 2012; Krishnapriya and Padmaja, 2014; Uddin et al., 2016; Hook et al., 2018;

Pawar et al., 2019). However, their direct or indirect effects on commercially important shrimp species, particularly under variable salinity and temperature was first addressed in the present study. The 96 h LC₅₀ values of DMT and CPF in PL and JL stages of *L. vannamei* recorded during present study are roughly comparable with those previously laboratory-derived for other crustacean species (Roast et al., 1999; Montagna and Collins, 2007; Kumar et al., 2010; Satapornvanit et al., 2009; Narra et al., 2013; Pawar et al., 2019) because of differences in size and life stage, type of rearing water (freshwater or seawater/brackishwater), duration of exposure and interaction of pesticides with biotic/abiotic factors (DeLeronzo et al., 2014; DeLeronzo, 2015).

The two tested OPs significantly differed in their toxicity to both early life stages (PL and SL) of *L. vannamei* in all salinity and temperature regimes. CPF was a highly toxic pesticide indicated by the low acute toxicity indices. CPF was found to be an approximately 400 to 500 fold and an approximately 400 to 600 fold more toxic to PL and JL stages of *L. vannamei* compared to DMT in altered tested salinity and temperature conditions. Such a vast intra-species variation for the OPs tested may be due to the lipophilic nature of CPF (Vlahovic et al., 2017). As a lipophilic molecule, CPF's permeation into the lipid-rich tissues of aquatic animal is relatively higher. This lipophilic nature of CPF results in elevated levels of bioaccumulation and causes severe damage or death of the organism (Essumang et al., 2009; Banaee, 2012).

Post-larvae of *L. vannamei* were more sensitive to both OPs than juveniles. Post-larvae showed 1–2 orders magnitude higher sensitive than juveniles to both OPs. The sensitivity of shrimp larvae to pesticides has been observed to be 1–3 orders magnitude higher than adults (Nimmo et al., 1971; Mohammed, 2013; DeLorenzo et al., 2014). Our results are in agreement with previous studies. Significantly higher toxicity levels in grass shrimp (*Palaemonetes pugio*) larvae compared to adults following exposure to CPF has been reported (Key and Fulton, 1993). The sensitivity of crustacean larvae is relatively more prone to toxicants than adults as a consequence of increased moulting frequency during their early life stages (Rao et al., 1985). Therefore, higher toxic levels recorded during the present study in post-larvae of *L. vannamei* might be due to cumulative stress effect induced by changing salinity and temperature in addition to the toxicity of DMT and CPF.

The two tested OPs was significantly more toxic to both PL and JL stages of *L. vannamei* at 5 and 15 salinities and 15 °C and 34 °C temperatures than at 25 salinity and 25 °C temperature ($P < 0.05$), respectively. A decrease in the toxicity tolerance on exposure to two pesticides (beta-cypermethrin and acephate) in *L. vannamei* at lower salinity levels (5 vs. 20) have been reported by Wang et al. (2013). Similarly, increase in herbicide, atrazine toxicity with decreasing salinity in copepod nauplii (*Eurytemora affinis*) has been reported by Hall et al. (1994). Induction of osmotic stress and consumption of higher oxygen in *L. vannamei* reared at lower salinity (1) and higher salinity (37) as

well as lower temperature (20 °C) has been reported (Bett and Vinatea, 2009). In contrast, increase in toxicity in larval and adult estuarine grass shrimp, *P. pugio* on exposure to fungicide chlorothalonil and the insecticide Scourge[®] concomitant with the increase in salinity and temperature has been reported by DeLorenzo et al. (2009). Furthermore, an increase in salinity resulted in enhancement of toxicity of pesticides and insecticides such as DMT, aldicarb and tebufenozide in brine shrimp (*Artemia* sp) (Song and Brown, 1998). These results suggest that the interactive effects between salinity and pesticides, particularly in crustaceans, are far from clear. Therefore, it appears that the changes in salinity altering the toxicity of pesticides would depend on exposure factors such as duration, acclimation, species, life stage and the specific characteristic of the individual pesticide (DeLorenzo, 2015).

Being one of the regulatory processes of physiological activities, the temperature affects the intensity of biochemical reaction, molecular mobility and the membrane exchange processes (Ozoh, 1992). A comparison of 96 h LC₅₀ values for both and JL stages of *L. vannamei* between adjacent temperatures revealed that an increase in temperature from 15 to 25 °C decreased the acute toxicity of both DMT and CPF. However, further increase in rearing temperature from 25 to 34 °C resulted in enhanced toxicity of DMT and CPF to PL and JL stages of *L. vannamei*. Increasing temperature resulting in enhanced toxicity on grass shrimp (*Palaemonetes* spp.) on exposure to OPs has been reported (Breken-Folse et al., 1994). An increase in metabolic rate at higher temperatures results in increased water movement across the gills and increased pesticide uptake (DeLorenzo, 2009). Higher temperatures have been reported to significantly affect the species' ability to cope with stress induced by toxic substances (Cherkasov et al., 2006; Lanning et al., 2006) and the regulation of associated physiological processes involved in the detoxification of hazardous substances. Furthermore, the deleterious effect in shrimps as a consequence of thermal and osmotic stress has been reported (Chien et al., 2003; Moser et al., 2012). The increase in water temperature triggering an outbreak of the *Vibrio* has been reported. This may be due to weakening the immune response of shrimp, and an accompanied reduction in aspartate aminotransferase and alanine aminotransferase activities (Chien et al., 2003; Moser et al., 2012).

The findings of the study suggest that changes in salinity and temperature altered the toxicity of DMT and CPF in *L. vannamei* and the degree of the effect depended on its life stage and type of OP. The potential effect of pesticides in farming systems is expected to be much higher than these laboratory-derived results as the presence of sediment enhances the toxicity of pesticides (Holmes et al., 2008). There may be a build-up of OPs in ponds over time and may pose a risk to shrimp aquaculture, due to partitioning of CPF to sediment (Giddings et al., 2014). Owing to their burrowing

nature into sediments and detritivore feeding habit, all stages of shrimp are susceptible to sediment-bound contaminants (Hook et al., 2018), thus posing a greater risk to shrimp culture. In conclusion, acute toxicity to PL and JL stages occurred at very low concentrations of OPs, especially at lower salinities calls for precaution in extending the cultivation of *L. vannamei* in low-salinity areas adjacent to traditional agriculture fields.

Acknowledgment

The authors are grateful to the Director, CSIR-National Institute of Oceanography, Goa (India) and the head, Zoology Department, Goa University, Goa for encouragement and use of facilities. This represents contribution No. Xxxx of the CSIR-National Institute of Oceanography, Goa (India).

Competing Interests

The authors have declared that no competing interests exist.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

All relevant data are within the manuscript.

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Table 1. The recorded mortality (%) in post-larvae and juveniles of *Litopenaeus vannamei* for each tested concentration ($\mu\text{g L}^{-1}$) of dimethoate (DMT) and chlorpyrifos (CPF) at three different salinities and temperatures after 96 h of exposure. Mortality data of three replicates (mean \pm SD).

Post-larvae of <i>Litopenaeus vannamei</i>									
Treatment	DMT		CPF		Treatment	DMT		CPF	
Salinity	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)	Temp ($^{\circ}\text{C}$)	Conc ($\mu\text{g L}^{-1}$)	Mortality (%)	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)
5	100	43.3 \pm 3.1	0.1	13.3 \pm 1.1	15	50	30.0 \pm 1.9	0.1	30.0 \pm 2.1
	200	60 \pm 4.2	0.2	20.0 \pm 1.5		100	40.0 \pm 2.5	0.2	50.0 \pm 3.1
	300	83.3 \pm 6.1	0.3	50.0 \pm 2.4		150	60.0 \pm 4.1	0.3	70.0 \pm 4.2
	400	90.0 \pm 7.1	0.4	60.0 \pm 3.1		200	80.0 \pm 5.9	0.4	90.0 \pm 5.1
	500	100.0 \pm 8.1	0.5	83.3 \pm 5.9		300	100.0 \pm 8.1	0.5	100.0 \pm 6.7
15	100	40 \pm 2.3	0.1	10.0 \pm 1.0	25	50	10.0 \pm 1.1	0.2	10.0 \pm 1.00
	200	63.3 \pm 3.5	0.2	20.0 \pm 1.1		100	10.0 \pm 1.1	0.4	30.0 \pm 1.14
	300	70.0 \pm 2.9	0.3	40.0 \pm 1.7		150	20.0 \pm 1.5	0.6	40.0 \pm 2.87
	400	90.0 \pm 5.5	0.4	60.0 \pm 2.8		200	40.0 \pm 3.1	0.8	63.3 \pm 4.1
	500	100.0 \pm 6.5	0.5	80.0 \pm 4.8		300	50.0 \pm 2.7	1.0	80.0 \pm 6.1
25	100	10.0 \pm 1.1	0.2	23.3 \pm 1.2	34	50	20.0 \pm 1.6	0.2	20.0 \pm 1.4
	200	20.0 \pm 2.3	0.6	30.0 \pm 2.1		100	30.0 \pm 2.7	0.4	30.0 \pm 2.1
	300	30.0 \pm 2.5	1.0	40.0 \pm 2.9		150	40.0 \pm 3.5	0.6	63.3 \pm 6.0
	400	50.0 \pm 2.6	1.4	60.0 \pm 3.8		200	50.0 \pm 4.2	0.8	80.0 \pm 6.6
	500	70.0 \pm 3.1	1.8	80.0 \pm 4.7		300	70.0 \pm 5.3	1.0	100.0 \pm 8.0
Juveniles of <i>Litopenaeus vannamei</i>									
Treatment	DMT		CPF		Treatment	DMT		CPF	
Salinity	Conc ($\mu\text{g L}^{-1}$)	Mortality (%)	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)	Temp. ($^{\circ}\text{C}$)	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)	Conc. ($\mu\text{g L}^{-1}$)	Mortality (%)
5	100	30.0 \pm 1.9	0.1	10 \pm 0.59	15	50	10.0 \pm 1.1	0.1	30.0 \pm 2.1
	200	43.3 \pm 3.2	0.2	13.3 \pm 1.1		100	30.0 \pm 2.1	0.2	40.0 \pm 2.5
	300	60.0 \pm 4.7	0.3	40 \pm 2.9		150	40.0 \pm 3.1	0.3	53.3 \pm 3.5
	400	80.0 \pm 6.1	0.4	53.3 \pm 3.7		200	60.0 \pm 4.2	0.4	70.0 \pm 4.5
	500	90.0 \pm 7.1	0.5	60.0 \pm 4.9		300	90.0 \pm 6.2	0.5	90.0 \pm 6.5
15	100	30.0 \pm 2.1	0.1	10.0 \pm 1.0	25	100	13.3 \pm 1.2	0.2	10.0 \pm 1.0
	200	40.0 \pm 2.9	0.2	20.0 \pm 1.3		200	20.0 \pm 1.1	0.4	20.0 \pm 1.1
	300	50.0 \pm 3.7	0.3	40.0 \pm 3.1		300	30.0 \pm 1.9	0.6	30.0 \pm 2.1
	400	70.0 \pm 4.7	0.4	50.0 \pm 4.7		400	40.0 \pm 2.5	0.8	50.0 \pm 2.97
	500	90.0 \pm 6.1	0.5	70.0 \pm 5.1		500	60.0 \pm 3.6	1.0	70.0 \pm 4.8
25	100	0 \pm 0.00	0.2	10.0 \pm 1.0	34	100	20.0 \pm 1.3	0.2	0 \pm 0.00
	200	10.0 \pm 0.9	0.6	20.0 \pm 1.7		200	40.0 \pm 2.2	0.4	23.3 \pm 2.1
	400	20.0 \pm 1.2	1.0	30.0 \pm 2.7		300	60.0 \pm 5.5	0.6	40.0 \pm 3.5
	600	30.0 \pm 2.1	1.4	40.0 \pm 3.3		400	70.0 \pm 6.2	0.8	60.0 \pm 4.6
	800	70.0 \pm 4.2	1.8	70.0 \pm 5.1		500	80.0 \pm 6.5	1.0	83.3 \pm 7.1

Table 2. Acute toxicity values with 95% confidence limits (in parentheses) for post-larvae and juveniles of *Litopenaeus vannamei* on dimethoate (DMT) and chlorpyrifos (CPF) at different salinities and temperatures. 96 h LC₅₀ values for each test condition is based on percent control mortality. Different superscript letters in the same column significantly differ ($P < 0.05$) from standard conditions (temperature, 27 °C and salinity, 20) and between tested salinities and temperatures.

Post larvae of <i>Litopenaeus vannamei</i>					
Salinity	DMT ($\mu\text{g L}^{-1}$)	CPF ($\mu\text{g L}^{-1}$)	Temperature (°C)	DMT ($\mu\text{g L}^{-1}$)	CPF ($\mu\text{g L}^{-1}$)
5	127.2 (71.2–150.0) ^a	0.31 (0.16–0.44) ^a	15	103.11 (92.11–125.36) ^a	0.17 (0.12–0.23) ^a
15	137.44 (110.13–160.71) ^b	0.32 (0.24–0.46) ^a	25	321.26 (286.45–395.51) ^b	0.62 (0.56–0.68) ^b
25	386.59 (300.53–692.15) ^c	0.92 (0.75–1.32) ^b	34	182.76 (156.46–221.47) ^c	0.46 (0.32–0.60) ^c
Standard	257.84 (278.21–311.20) ^d	0.68 (0.50–0.87) ^c	Standard	257.84 (278.21–311.20) ^d	0.68 (0.50–0.87) ^d
Juveniles of <i>Litopenaeus vannamei</i>					
Salinity	DMT	CPF	Temperature (°C)	DMT	CPF
5	196.69 (161.64–229.51) ^a	0.40 (0.35–0.53) ^a	15	151.45 (110.18–212.00) ^a	0.22 (0.12–0.31) ^a
15	218.86 (185.22–298.41) ^b	0.37 (0.33–0.42) ^a	25	479.20 (404.02–619.27) ^b	0.77 (0.60–1.39) ^b
25	691.51 (560.44–832.42) ^c	1.68 (1.54–2.52) ^b	34	236.74 (209.48–264.86) ^c	0.65 (0.49–0.93) ^c
Standard	463.61 (356.11–576.35) ^d	0.73 (0.69–1.06) ^c	Standard	463.61 (356.11–576.35) ^d	0.73 (0.69–1.06) ^d