Acute Toxicity of Niclosamide on Creeper Shell (*Cerithidea cingulata*) and Pacific White Shrimp (*Litopenaeus vannamei*) Postlarvae

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ABSTRACT

The efficacy of niclosamide for controlling creeper shell (*Cerithidea cingulata*) was studied. Static bioassay was used to determine the median lethal concentration of niclosamide needed to kill creeper shell within 96 hours (96-hr LC_{50}). Water parameters were pH 7.0, 7.5, 8.0 and 8.5, with the 96-hr LC_{50} at 0.22, 0.33, 0.34 and 0.48 ppm. Toxicity of niclosamide decreased when the pH increased. The 48-hr LC_{50} of niclosamide on Pacific white shrimp (*Litopenaeus vannamei*) postlarvae 12 (PL12) was 1.36 ppm. The concentration of niclosamide at 1.0 ppm was more than two times higher than the concentration which caused 100% mortality of creeper shell in 96 hours, and had proven safe for PL 12. It is inferred from this experiment that niclosamide at 1.0 ppm can be used for water preparation, in order to eradicate creeper shell before stocking the PLs into the ponds.

Keywords: Acute toxicity, niclosamide, creeper shell, Pacific white shrimp

INTRODUCTION

One problem in shrimp farming these days is the introduction of another organism to the shrimp pond, specifically creeper shell (*Cerithidea cingulata*). Creeper shells are often found in large numbers in the feeding areas in shrimp ponds. If the outbreak of creeper shell is severe, it can reduce shrimp production by more than 90% due to mortality. Limsuwan (2000) reported that creeper shell could be found everywhere, from the edge and to the bottom of the pond. Large populations of these shells can cause problems by using calcium to build up their shells which causes the water alkalinity to drop in the early culture cycles. This in turn causes severe mortality among shrimp as they die during molting.

At present, shrimp farmers have been facing problems with creeper shells in Pacific white shrimp (*Litopanaeus vannamei*) ponds. It has been found that once creeper shells become established in a pond, they still remain even when the next generation of shrimp is introduced. In ponds that have a large number of creeper shells, slow growth in shrimp occurs. Once the pond is infected with these intruders, it is difficult to maintain water quality. Some farmers have tried to scoop out all the creeper shells daily and

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leave them on the pond dikes to dry out and die. The use of various chemicals have been tried to eradicate creeper shells during pond preparation, such as brestan-60 (Sanprasert and Piromchart, 1975), tea seed cake (Tharnbuppa, 1981), chlorine (Chitmon, 1995), copper sulfate and benzalkoniumchloride (Pongpanit *et al*., 1995). However, these chemicals must be used in relatively high concentrations which may be harmful to shrimp (Tharnbuppa, 1981; Rattanarpa, 1997).

In the past, farmers used niclosamide to get rid of snails. This chemical was used effectively to control golden apple snail (*Pomacea canaliculata*) in paddy fields (FAO, 2004). The toxicity of niclosamide to aquatic animals and plants is relatively low and it decomposes quickly in nature. The objectives of this study were to determine the optimum concentration of niclosamide that would result in a maximum efficacy in the eradication of creeper shells without causing harm to *L. vannamei* postlarvae.

MATERIALS AND METHODS

Animal preparation

Creeper shells were collected from shrimp farms located in coastal areas of Thailand. They were reared in 500-liter fiberglass tanks filled with 100 liters of disinfected saline water with 25 ppt salinity and aerated continuously. The snails were left to acclimate for one week before the experiments.

One thousand white shrimp postlarvae (PL 12) were obtained from a private hatchery. They were reared in 500-liter fiberglass tanks filled with 400 liters of disinfected saline water with a salinity of 25 ppt. The water was continuously aerated and shrimp were left to acclimate for one week before the experiments.

Chemical preparation

The chemical used in this experiment was niclosamide. It is manufactured by Bayer Thai Company, Limited, with 70% active ingredients. A stock solution was prepared in distilled water to achieve the concentration of 100 ppm.

Acute toxicity of niclosamide on creeper shells (96 hour LC_{50}) at different pH levels

Static bioassay method was used to determine the concentration of niclosamide that would kill 50 % of the creeper shells during the 96 hour test period (96-hours LC_{50}). Niclosamide was added only one time at the beginning of the experiment. The animals were not fed during the 96 hour toxicity test. This experiment consisted of two steps.

The first step was to establish the lowest concentration that killed 100% of the test animals and the highest concentration at which 100% of the test animals survived. This was defined by a broad range of Niclosamide concentrations at different pH levels (7.0, 7.5, 8.0 and 8.5). Various concentrations of niclosamide were added in 5-liter glass chambers filled with 3 liters of water. Twenty test animals were stocked in each chamber. The experiment was run with three replicate chambers per treatment.

The test animals were observed and the mortality rate was recorded after 96 hours. The concentration range defined in this stage was used to make a more detailed test in the next step.

The second step used the concentration range determined in the previous step, which was divided into 6 levels on a logarithmic scale, with each level tested with a control group. Each test had three replications. The behavioral reactions of the test animals were observed and the mortality rate was recorded after 96 hours. The animals were considered dead if they were not moving and did not react if prodded with a glass rod for 2-3 seconds (Throp and Lake, 1974). Dead animals were recorded and removed.

At the end of the experiment, the data were analyzed statistically according to the Finney (1971). Data were plotted on logarithmic-probability paper. Results were analyzed statistically to determine the 96-LC_{50} with 95% confidence interval and the slope function.

Acute toxicity (48-hour LC_{50}) of niclosamide on white shrimp postlarvae stage 12 (PL12)

Pacific white shrimp postlarvae stage 12 (PL12) was used in this experiment. Static bioassay method was used to determine the concentration of niclosamide that killed 50 precent of PL12 during 48 hour test period (48-hour LC_{50}) at salinity of 25 ppt (pH 8.3). Niclosamide was added only one time at the beginning of the experiment. The animals were not fed during the 48-hour toxicity test. The experiment followed the previous method used for the acute toxicity test (96 hour LC_{50}) of niclosamide on creeper.

Safety period of niclosamide on Pacific white shrimp postlarvae

Niclosamide with a concentration of two times more than the concentration which killed 100 % of creeper shells in the 96 hour test, was added into the 5-liter glass chamber containing 3 liters of water. The experiment was carried out with three replicates per treatment. The water was continuously aerated. Twenty postlarvae were stocked in each chamber every day from the first day of the experiment. Mortality rate was observed and recorded to determine the safe level of niclosamide after 24 and 48 hours.

RESULTS AND DISCUSSION

Acute toxicity of niclosamide on creeper shell (96-hour LC_{50}) at different pH levels

The behavior of creeper shells was observed after the application of niclosamide at various concentrations. Creeper shells not exposed to niclosamide (control group) were creeping around the bottom or side of the glass jar. The moving creeper shells were showing out their head, tentacle, eyes and siphon. The creeper shells exposed to niclosamide at a low concentration exhibited behavior similar to the control group. However creeper shells exposed to niclosamide at a high concentration had responses of a shrinking head, retracted tentacle, eyes and the siphon also closed

operculum and released more mucous due to the irritation from niclosamide. The exposure to niclosamide continued for another 24 hours. The result showed extended organs that would not shrink back into the shell.

The 96-hr LC_{50} of niclosamide for creeper shell at different pH levels are shown in Table 1. The more neutral the pH, i.e. at pH 7.0, the lower the 96-hr LC_{50}, which was 0.2234 ppm. The concentration for the 96-hr LC_{50} of niclosamide increased as the pH increased. Niclosamide had effectively eradicated the creeper shell because niclosamide disturbs oxygen consumption of animals (Ruangpan and Tabkaew, 1996). Niclosamide is effective at a very low concentration compared to other chemicals, such as copper sulfate and tea seed cake, which have effective concentrations of 2 and 100 ppm, respectively (Pongpanit *et al*., 1995).

Table 1. 96-hr LC_{50} (ranges in parenthesis) of niclosamide on creeper shells at different pH levels.

pH levels	96-hr LC_{50} (ppm)
7.0	0.223 (0.193-0.291)
7.5	0.333 (0.268-0.569)
8.0	0.336 (0.306-0.382)
8.5	0.477 (0.330-0.690)

Acute toxicity of niclosamide on Pacific white shrimp postlarvae (PL12) (48-hour LC_{50})

The behavior of PL12 after exposure to niclosamide was studied. For the first 24 hours, most of the shrimp which were still alive exhibited a normal behavior, but some shrimp died and settled at the bottom of the glass chamber. Dead shrimp showed pronounced whitish or opaque body color compared to live shrimp. Moribund shrimp became nearly immobile and showed little movement when touched with a glass rod.

The 48-hr LC_{50} of niclosamide for PL12 was 1.3586 (0.8244-2.239) ppm, which was higher compared to the findings of Lavanamal (2003), who used niclosamide on black tiger shrimp PL15 and obtained a 48-hr LC_{50} of 0.52 ppm.

Safety period of niclosamide on Pacific white shrimp postlarvae

A concentration of 1.0 ppm niclosamide was prepared. This was more than two times higher than the niclosamide concentration responsible for the 100% mortality of creeper shell in 96 hours. Niclosamide at 1.0 ppm was applied and left for 5 days. None of the shrimp died during the first 4 days (Table 2). This experiment has a decay time which was nearly similar to Lavanamal (2003), who used niclosamide on black tiger shrimp PL15 at the concentration of 14.98 ppm, where it remained an active substance for 6 days. But the study of Muir and Yarechewski (1982) reported niclosamide was effective for 1- 4 days.

Table 2. Percentage mortality of Pacific white shrimp with the application of 0 and 1 ppm niclosamide at different times

Duration of exposure to niclosamide (days)	Concentration (ppm)	The number of dead animals at 48 hours		Percent mortality
		24	48	
0	0 (control)	0	0	0
	1.0	4	10	50
1	0 (control)	0	0	0
	1.0	3	8	40
2	0 (control)	0	0	0
	1.0	1	4	20
3	0 (control)	0	0	0
	1.0	0	1	5
4	0 (control)	0	0	0
	1.0	0	0	0
5	0 (control)	0	0	0
	1.0	0	0	0

CONCLUSION

Niclosamide is the compound of choice to eradicate creeper shells since it has been considered an effective substance in killing the creeper shell and is still safe to use (not harmful to nature). This chemical proved to be extremely toxic to creeper shells in waters with low pH. This information will help extension personnel to formulate safer and more effective application rates for niclosamide in shrimp farming. These results indicated that niclosamide was more toxic at low pH and can be used at the rate of 1.0 ppm to prepare the water in culture ponds before stocking of PLs to prevent creeper shell infestation during the rearing period.

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