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Toxicity of Phenol on *Macrobrachium rosenbergii* (de Man) Eggs, Larvae, and Post-Larvae

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Literature on the toxicities of phenol on aquatic organisms is very limited (Hellawell 1986; Buikema *et al.* 1979). USEPA (1987) reported that the acute and chronic toxicities of phenol to freshwater aquatic life occur at concentrations as low as 10.2 mg/L and 2.56 mg/L, respectively, while for the saltwater aquatic life the acute toxicity occurs at concentrations as low as 5.8 mg/L. No data are available for the chronic toxicity of phenol to saltwater aquatic life.

Sublethal concentrations of phenol have significant effects on the physiological and histological processes of the aquatic organisms: such as gill necrosis (Mitrovic *et al* 1968); destruction of erythrocyte cells (Waluga 1966); inhibition of sexual activities (EIFAC 1973; Colgan *et al. 1982);* suppression of growth and reduction of resistance to diseases (EIFAC 1973).

Macrobrachium rosenbergii(de Man) is the sole freshwater prawn cultured in Malaysia. Occasionally, the hatcheries are unable to produce the post-larvae because of undefined pollutants present in the water supplies. It has been observed that the use of cracked fiberglass tanks for larvae rearing is correlated with high mortality. This high mortality is probably due to the toxicity of the phenolic compounds which are leached out from the fiber glass tank into the water. This study was undertaken to evaluate the toxicity of phenol on eggs, larvae and Post-larvae of *M. rosenbergii* and to set the water quality criteria of phenol for the said species.

MATERIALS AND METHODS

Procedure for preparation of the detached and individual *M. rosenbergii eggs* and the bioassay method for egg hatchability followed the same technique described by Law (1995). Fertilized egg masses were removed from the buried

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M. rosenbergii prawn. The eggs were dispersed by introducing a gentle jet of air into the water. The single and detached eggs were collected and rinsed with 12 g/L salinity brackish water to remove any attached organic matters. Thirty detached eggs were randomly removed and spread out at the bottom of a 100 ml glass beaker containing the phenol test solution. Freshly prepared test solution was pumped into the beaker continuously at a rate of 12 ml/hr for a period of 12 days. The unhatched eggs were used as the criterion for this bioassay determination.

M. rosenbergii larvae and post-larvae were obtained from the University Pertanian Malaysia (UPM) hatchery. The larval stage was identified according to the classification of Uno and Kwon (1969). At specific stages they were removed and stocked into the 100 ml glass beakers for experimentation.

The same flow-through bioassay system for egg hatchability (Law 1995) was used for the larvae acute toxicity tests. Ten larvae per bioassay bottle were used for each experiment. The larvae were fed with newly hatched *Artemia* at the rate of 10 *Artemia/larva/day.* Brackish water of 12 g/L salinity with pH 7 was used for this study.

For post-larvae acute toxicity tests, the phenol test solution was changed after 48 hour exposure time. The compartmentalized tank measured 61cm x 17.8cm x 15cm (length: width: height) with 30 compartments measuring 5.1cm x 5.1cm x 15.3cm (length: width:height). Each compartment contained one post-larva. The phenol test solutions ranged from 20 mg/L to 60 mg/L were used for the toxicity tests. Mortality of the post-larvae was recorded at 24, 48, 72 and 96 hr exposure time. Chlorine free tap water was used for the experiments.

A flow-through bioassay system with the compartmentalized tank described previously was employed for the post-larvae chronic toxicity tests. The sublethal phenol concentrations used were 0%, 1%, 10%. and 30%. of the 96h LC_{50} phenol values obtained from the acute toxicity tests for the post-larvae. The post-larvae were fed with a UPM 30% protein prawn feed at the rate of 5%. body weight twice daily. Growth rate of the post-larvae was measured as change of body weight over time. Growth rate of the post-larvae over a period of 6 weeks was used as the criterion for testing the effect of the sublethal phenol concentration on the prawns. The turnover rate of the testing solution in the tank was set at 4 times per day. The tests were run in chlorine free tap water.

The phenol test solutions were prepared daily. The actual phenol concentration in the test solution was determined by using the ultra-violet absorption method developed in this study; absorption at 194 nm for fresh water and 210 nm for brackish water of 12 g/L salinity.

Temperature and dissolved oxygen were determined by using an Oxygen Meter (YSI Model 57). pH was estimated with a Metrohm pH meter (Model 620). Total ammonia nitrogen and alkalinity were determined according to the Standard Methods (APHA 1989). Salinity was determined with a refractometer (Atago).The water quality of the experiments was monitored routinely and their ranges were as follows: Temperature 26-27 °C; dissolved oxygen 6.6-7.1 mg/L; pH 7.0-7.3; alkalinity 25.3-28.50 mg/L $CaC0_3$; total ammonia-nitrogen 0.05-0.07 mg/L. These values are within the safety levels for larvae and post-larvae rearing of this prawn (Ang and Cheah 1986).

The lethal concentration values for larvae and post-larvae mortality (LC) and for egg hatchability (EC) were estimated by using the probit analysis (Wardlaw 1985). Growth rates were analyzed for statistical significance by one-way ANOVA and those values which were significantly different were analyzed with Duncan's Multiple Range test (SAS Inc. Cary, North Carolina; USA).

RESULTS AND DISCUSSION

The acute toxicities of phenol on larval and post-larval stages of *M. rosenbergii* are given in Table 1. The 48h LC_{50} phenol values for different stages of larvae and post-larvae exhibited a progressive increment with age of the organisms; ranging from 11.83 mg/L phenol for stage 2 larvae to 29.92 mg/L for post-larvae PL42. This clearly showed that the earlier life stage of *M. rosenbergii* is more sensitive than the later stages to phenol. Similar phenomenon had been observed by Piyan *et al.* (1985) on the toxicity of mercury to different larval stages of this prawn. The 96h LC_{50} phenol values for post-larvae PL21, PL28 and PL42 were also age dependent. There was a significant increase in the 96h LC_{50} value from the PL21 to the PL28 but only a slight increase from PL28 to PL42.

Table 1. Median lethal concentration (LC_{50}) values of phenol for different larval and post-larval stages of *M.rosenbergii.*

Stages	LC_{50} (mg/L) 48 hr	96 hr
Larval		
2	11.83(8.94-15.76)1	-2
4	16.46(12.03-35.77)	-
10	19.48(15.51-48.03)	-
Post-larval		
21	22.25(19.04-24.76)	16.66(10.61-19.91)
28	27.39(23.41-30.72)	22.03(17.76-25.16)
42	29.04(24.64-34.19)	23.04(18.93-26.15)

1: 95% confidence limits. 2: not conducted

The effect of phenol on hatching rate of *M. rosenbergii eggs* is shown in Table 2. By using the probit regression analysis, the estimated 12-d EC_{50} phenol value is 0.961 mg/L. A 100% inhibition of the embryonic development was observed at phenol concentration at 5.0 and 10.0 mg/L. A significant effect on the hatching rate of eggs was detected even at the phenol concentration of 0.05 mg/L. These indicated that the embryonic development of *M. rosenbergii* was extremely sensitive to phenol. The 12d EC_{50} value was eleven times lower than the 48h LC_{50} value of the stage 2 larvae. This suggests that phenol is more toxic to the eggs of *M. rosenbergii* than to the larvae and post-larvae.

Studies on the effect of phenol on embryonic development of aquatic organisms are very limited. Buikema *et al.* (1979) reported that generally fish embryonic development was not affected by phenol level that was less than 25 mg/L. This may indicate that embryonic development of *M. rosenbergii* is at least 20 to 30-times more sensitive to phenol than that of fish.

The effects of long-term sublethal phenol concentrations on growth rate of the post-larvae indicated that reduction of growth rate was significant even at phenol concentration as low as 0.23 mg/L ($P < 0.05$). At phenol concentrations of 0.23 mg/L, 2.30 mg/L, and 6.80 mg/L, the percentage of growth reduction were 11.76%, 29.41%, and 47.06%, respectively. Theoretically, the safety level of phenol in water is the concentration where there will be no effect on the growth rate. However, Table 3 revealed that a phenol concentration that is infinitely small or approaching zero will satisfy this condition. It would be almost impossible to achieve such a condition in naturally occurring water. For aquiculture management purpose, a safety level of phenol is required for water quality management for culturing this prawn. We would like to suggest the safety level for phenol which will cause a reduction in growth of 10% or less. As such, the safety phenol concentration estimated for the post-larvae at the age of PL28 is 0.196 mg/L. This safety value is very close to the safety value of 0.22 mg/L calculated from multiplying the 96h LC_{50} of 22.03 mg/L phenol value with a safety factor of 0.01 (Table 2). Therefore, a safety factor of 0.01 could be applied for obtaining the safety level for larvae from the 48h LC_{50} phenol value; the value obtained is 0.118 mg/L. The 12d EC_{50} value of phenol obtained from this study is 0.96 mg/L. From the probit equation with 10% failure of egg hatchability the estimated safety level of phenol for hatching the eggs is 0.088 mg/L.

McKee and Wolf (1963) suggested a 0.2 mg/L criterion of phenol for aquatic life. AWRC (1984) recommended a criterion of 0.1 mg/L phenol for aquatic organisms. USEPA (1987) reported that the acute toxicity of phenol on saltwater organisms occurred at concentrations as low as 5.8 mg/L.

The criteria obtained from this study, i.e. 0.196 mg/L for post-larvae, 0.118 mg/L for larvae and 0.088 mg/L for eggs of *M. rosenbergii* are comparable to those values recommended by McKee and Wolf (1963) and AWRC (1984), but are much

Table 2. Effect of phenol on the hatching rate of *M. rosenbergii* eggs.

Phenol (mg/L)	No of eggs used	Hatching rate (%)
0.0	180	97.22±2.51(6)[1]
0.0	90	87.78±1.92(3)
0.1	90	82.22±3.85(3)
0.2	90	74.45±6.94(3)
0.5	90	73.33±3.34(3)
1.0	90	61.11±5.09(3)
3.0	90	35.56±1.93(3)
5.0	90	0(3)
10.0	90	0(3)

1: Mean±SD. (no of replicates)

lower than that reported by USEPA (1987). This reveals that *M. rosenbergii* is more sensitive to phenol when compared to other aquatic organisms. DOE-MU (1986) recommended an interim standard of phenol of not exceeding 9.9 mg/L at any time for the aquatic life. The phenol level in Malaysian waters ranged between below 0.01 mg/L and 3.5 mg/L (DOE-MU 1986). In order to protect and to propagate this indigenous prawn, a more stringent phenol standard should be adopted and implemented. Perhaps, the water quality standard for phenol in the Malaysian natural waters should be less than 0.1 mg/L.

Table 3. Effect of phenol on growth rate of PL28 *M. rosenbergii* in a 42-days flow-through experiment

	Mean Weight (g)			
Growth	Phenol Conc. (mg/L).			
Period (d)	0	0.23±0.03	2.30±0.08	6.80±0.09
0	0.067±0.008	0.072±0.001	0.070±0.004	0.074±0.004
14	0.089±0.003	0.082±0.002	0.087±0.005	0.088±0.003
28	0.112±0.007	0.112±0.002	0.104 0±005	0.097±0.005
42	0.137±0.004	0.133±0.006	0.121±0.007	0.111±0.004
Growth rate (g/d)	0.0017[a] ±0.0004	0.0015[b] ± 0.0004	0.0012[c] ±0.0003	0.0009[c] ±0.0001

1: Mean ± S.D.(n=21); 2: Mean ±S.D. (n=30);
a,b,c: Means in the horizontal row followed by different letters are significantly different ($p<0.05$).

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