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Lio-Po, G. D.; Lavilla, C. R. & Trillo-Llobrera, A.

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G. D. Lio-Po, C. R. Lavilla
and A. Trillo-Llobrera

Larval rearing of *Penaeus monodon* in the Philippines is adversely affected by *Lagenidium* and protozoan infestations. The use of malachite green is being considered and this study aims to determine the effect of the chemical on the survival and development of the zoeae, myses and postlarvae of *P. monodon*.

The zoeae, myses and postlarvae of *P. monodon* were taken from the larval stock hatched and reared at SEAFDEC. Fifty larvae were stocked in each glass aquarium at a stocking density of 10 larvae/L seawater. They were acclimated for 2 hours in the test containers and given adequate aeration before chemical treatment was initiated. The feeds consisted of unialgal cultures of *Chaetoceros* for the zoeae, and *Artemia* and *Chaetoceros* for the myses and postlarvae. Feeding throughout the experiment was at the rate of 10,000 to 50,000 *Chaetoceros* and 3-5 *Artemia*/mL of rearing water.

Zinc-free malachite green (Merck) was used in a static bioassay scheme. The doses tested were 10, 50, 100, 500 and 1,000 micrograms/L for the zoeae and 0.1, 1.0, 10, 100, 1,000 and 10,000 micrograms/L for myses and postlarvae. An initial concentration of 1 mg/mL, diluted further in a liter of seawater, was used to arrive at the desired dosages. For each concentration tested, there were four trials for the postlarvae and zoeae and five trials for the myses; each trial consisted of two replicates including controls. The following physico-chemical parameters of the culture water were monitored daily until the termination of the experiment: salinity, temperature, pH and ammonia and nitrite levels.

Mortality counts were made at 24, 48, 72 and 96 hours. The absence of any movement or response after gentle prodding served as the criterion of death of the larvae and postlarvae. Two live and two dead specimens from each aquarium were taken at random for the microscopic examination of morphological defects and inability to molt. The larvae were returned to their respective aquaria after examination.

In addition to the TL_{50}, the TL_5 and TL_{95} values were estimated likewise as a guide to minimum and maximum concentrations that may exert an effect on the larvae. Values of zero and 100 percent were assumed for experiments with no data on mortalities less than 5 or more than 95 per cent, respectively. Furthermore, the two-way analyses of variance at the 0.05 per cent level of significance and the Duncan's multiple range test were employed to determine variations among the data on mortality. The relationships between concentration levels and the physico-chemical parameters were estimated by the least squares regression analysis.

Malachite green at 10 micrograms/L caused the least mortality of the zoeae of *P. monodon* (Fig. 1A). A progressive rise in larval death followed as dose and exposure time increased. The differences between the control and doses of 50-100 micrograms/L at an exposure time of 24 to 72 hours were statistically significant. Treatment for 96 hr likewise resulted in a notably greater larval loss at 10 micrograms/L compared with the untreated group. The dose of 500 micrograms/L was fatal to the larvae.

The mortality rate for the myses at 0.1 to 10 micrograms/L parallels that of the control at exposure periods of 48 to 72 hr (Fig. 1B). There was a substantial decrease in survival rate at doses of 0.1 to 100 micrograms/L after 24 hr. At 96-hr exposure, mortality at 1 microgram/L was significantly high. More than half of the larval population died in 24 hr at a dose of 10,000 micrograms/L.

The experimental runs for the postlarvae gave relatively variable results (Fig. 1C). At 0.1 to 100 micrograms/L, dead postlarvae after 48 to 72 hr significantly outnumbered those in the control. Mortality after 24 hr was significantly high at 0.1 to 1 and 100 to 1,000 micrograms/L. A dose of 10,000 micrograms/L was immediately harmful.

Microscopic examinations revealed no adverse effects of malachite green on the physical development and molting capacity of the zoeae, myses and postlarvae of *P. monodon*. The physico-chemical parameters of the rearing water were within acceptable limits and regression analyses (Table 1) revealed that they had no significant linear relationship with the increasing concentrations of malachite green even at prolonged exposures.

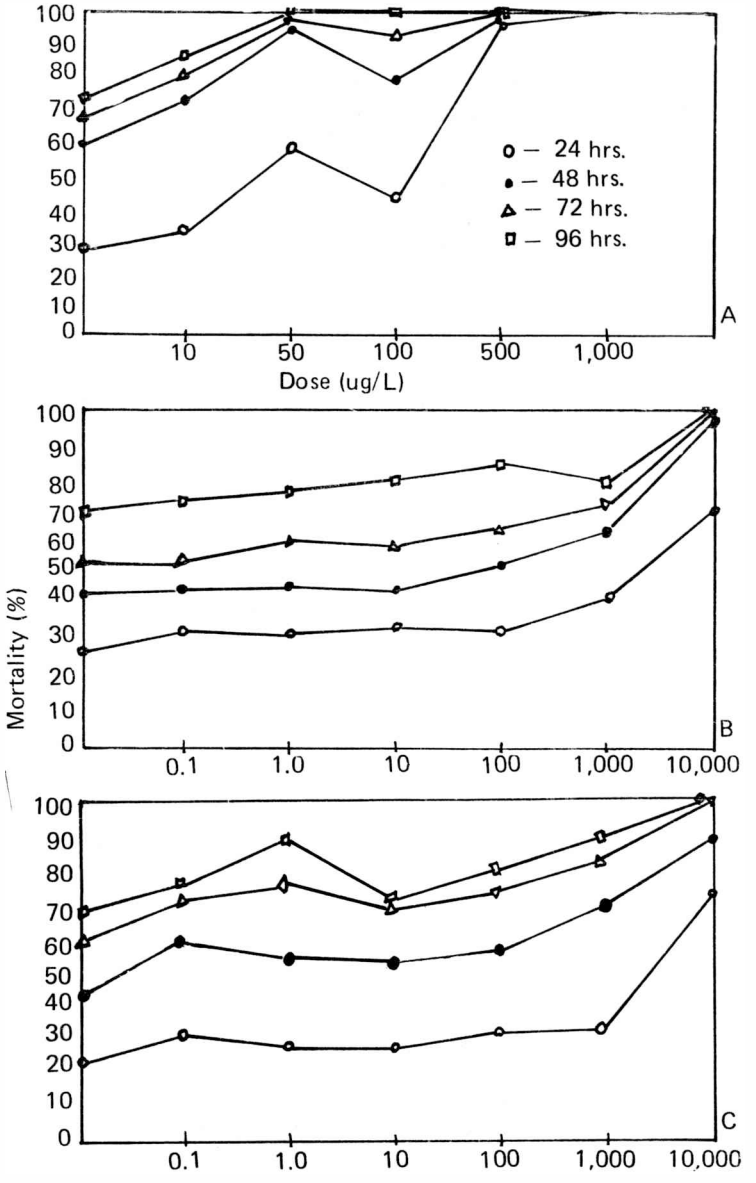

Fig. 1. Mortality rates (%) of *P. monodon* (A) zoea (B) mysis and (C) postlarva treated with malachite green for 24 to 96 hours.

Table 1. Coefficients of determination (r^2), physico-chemical parameters of larval rearing water treated with malachite green for 24-96 hours.

Parameter	Exposure time (hr)			
	24	48	72	96
pH	2.62×10^{-2}	5.35×10^{-2}	5.71×10^{-2}	1.60×10^{-2}
Temperature	3.47×10^{-2}	9.53×10^{-3}	1.46×10^{-2}	3.15×10^{-2}
Salinity	3.12×10^{-4}	2.25×10^{-4}	4.58×10^{-3}	3.03×10^{-3}
Nitrite	1.14×10^{-3}	2.15×10^{-2}	1.17×10^{-1}	5.12×10^{-7}
Ammonia	1.80×10^{-4}	1.92×10^{-3}	5.37×10^{-3}	1.21×10^{-2}

Table 2. Mortality rates (%) of *Penaeus monodon* larvae treated with varying concentrations of malachite green.

Larval stage	Dose ug/L	Corrected mean mortality (per cent)				Cumulative mortality (per cent)[a]			
		24 hr	48 hr	72 hr	96 hr	24 hr.	48 hr.	72 hr	96 hr
Zoea[b]	10	6	34	38	48	3	20	29	44
	50	44	86	96	98	27	63	82	94
	100	20	44	74	92	46	75	89	97
	500	98	100	100	100	97	100	100	100
	1,000	100	100	100	100	100	100	100	100
F values: exposure time, 9.74 * ; dose, 9.12*									
Mysis[c]	0.1	10	4	0	8	2	1	0	2
	1.0	10	8	14	22	5	3	4	10
	10	10	4	12	34	10	6	10	25
	100	10	18	22	44	16	18	25	47
	1,000	22	32	36	34	34	48	57	68
	10,000	58	96	100	100	74	98	100	100
F values: exposure time, 83.82 * ; dose, 39.04 *									
Postlarvae[d]	0.1	12	28	30	26	2	7	9	8
	1.0	8	18	42	64	5	13	21	27
	10	6	16	20	4	8	21	31	32
	100	10	22	36	30	14	36	53	54
	1,000	12	44	52	64	28	63	79	84
	10,000	64	80	100	100	76	91	100	100
F values: exposure time, 125.39 * ; dose, 29.55 *									

* Significant at 5% level

[a] Computed according to Reed – Muench's Method

Table 3 Tolerance limits of *Penaeus monodon* larvae to short-term exposures of malachite green.

Tolerance limit level	Stage	Time of exposure (hr)			
		24	48	72	96
TL_5	Zoea	11.77	2.15[b]	0.02[b]	0.01[b]
	Mysis	1.06	4.11	1.34	0.23
	Postlarvae	1.20	0.06[b]	0.04[b]	0.01[b]
TL_{50}	Zoea	112.80 (80-159)[c]	30.61 (20-46)	18.91 (13-27)	12.27 (9-18)
	Mysis	2,502.65 (1,169-5,357)	1,105.63 (1,036-1,180)	610.09 (598-1,205)	139.01 (60-325)
	Postlarva	2,900.17 (1,558-5,401)	339.28 (145-764)	70.85 (30-170)	66.60 (26-173)
TL_{95}	Zoea	471.97	364.46	242.33	68.12
	Mysis	64,146.53[b]	8,870.54	7,662.43	6,956.97
	Postlarva	62,288.50[b]	26,907.58[b]	5,787.22	4,884.96

a

[a] TL_5, TL_{50}, TL_{95} – doses that will produce 5%, 50%, 95% kill of the population, respectively.

[b] Computed on assumed values.

[c] Estimated 95% confidence interval (micrograms/L).

The sensitivity to malachite green varied with the different larval stages but the latter are all susceptible to the chemical. Statistical tests and corrected mean mortality and estimated cumulative mortality data (Table 2) confirmed these findings.

The zoeae seemed to be the least tolerant to malachite green especially during the first 2 days of exposure. At an exposure time of 48 to 96 hr, on the other hand, the resistance of the postlarvae was considerably lower compared to that of the myses. The postlarvae of *P. monodon* became cannibalistic and benthic at this stage, thus increasing their uptake of the chemical through ingestion of dead larvae and other residues at the bottom. Besides, they molt during the first four days of development, a condition that could render them more susceptible to the toxic action of the dye.

Results suggest that acute toxicity of malachite green is the primary cause of larval death in *P. monodon*. This toxicity is unrelated to the salinity, temperature, pH and nitrite and ammonia concentrations of the rearing water (Table 1).

The results of this experiment also allow consideration of the prophylactic potentials of malachite green in the control of the *Lagenidium* and *Zoothamnium* infesting the *P. monodon* larvae. Following Perkins mathematical method the following sublethal doses have been arrived at: 0.61 for the zoeae, 6.95 for the myses and 3.99 micrograms/L for the postlarvae. The fungitoxic level of 6 microgram/L can be applied safely only to the mysis stage. For the control of *Zoothamnium*, a dose of 500 microgram/L would be lethal. At any rate, should larval treatment become imperative, the TL_5 and TL_{95} values (Table 3) may provide ample guidance regarding the probable larval response to malachite green. Toxicity risks may be reduced by applications between ecdyses or by the removal of the dye by filtration through activated carbon.

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