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Sublethal Effects of Organophosphate Chlorpyrifos on Hemato-Immunological Parameters of the Gercacinid Crab, *Cardiosoma armatum* (Herklots, 1851)

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Abstract

Low insecticide exposure has been shown to cause profound effects on non-target organisms, including crabs. Therefore, the changes in hematological parameters, serum biochemistry and antioxidant enzymes in the Gercacinid Crab, *Cardiosoma armatum* were assessed during 28-day exposure to four concentrations of organophosphate chlorpyrifos (0.003, 0.006, 0.03 and 0.06 mg/l). The results showed a significant (P= 0.0) decrease in packed cell volume and total haemocyte count of the exposure crabs (except in 0.003 mg/l concentration) compared to control group. There were no significant changes in hemocyte sedimentation rate, granulocyte and agranulocyte, although all exposure groups increased in hemocyte sedimentation rate and agranulocyte with respective ranges of 3.00-3.02 mm/hr and 64.00-67.00 %. Except for alkaline phosphatase, there were no significant variation in the biochemical profile of both the control crabs and exposure crabs, although organophosphate chlorpyrifos exposure induced increase in all the measured biochemical parameters. The serum protein level and the activities of the enzymes (superoxide dismutase, catalase and malondialdehyde) were inhibited in exposure groups. The changes in these hemato-immunological parameters of the crabs were suitable biomarkers of a sub-lethal exposure to chlorpyrifos at the concentrations tested, and this will be useful in biomonitoring of aquatic environment.

Keywords: Antioxidant Enzymes, Chlorpyrifos, Crab, Contaminant, Hematology.

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INTRODUCTION

Organic and inorganic contaminants can suppress immune function in invertebrates. Among the insecticides commonly used in agriculture and houses to control the variety of insects is chlorpyrifos (0,0-Diethyl-0-3,5,6-trichloro-2-pyridylphosphorothioate), an organophosphate chemical. In general, organophosphate chemicals are not persistent in the environment as they break down quickly. Because of their relatively fast rate of degradation, they have been a suitable replacement for the more persistent organochlorine (Velmurugan *et al*., 2019). Exposure to low level of insecticides (between 26 µg L^{-1} and 33 mg L^{-1}) has attested to cause profound effects on non-target organisms (Paracampo *et al.,* 2015). Some aquatic organisms have the ability to live in contaminated regions, due to inducible defense mechanisms that allow detoxification and excretion of contaminants and protection by antioxidants from oxidative stress (Gomes *et al*., 2012). When these compensatory responses are activated, the survival potential of the organism may already have begun to decline because the ability of the organism to mount compensatory responses to new environmental challenges may have been compromised (Lawal-Are *et al*., 2019a; Moruf and Akinjogunla, 2019).

The use of biochemical and enzymatic biomarker measures as toxicity markers is constantly being developed and has the advantage of delineating symptoms prior to disease manifestation, thereby leading to better environmental risk assessment results (Bueno-Krawczyk *et al*., 2015; Usese *et al*., 2019). The most convincing explanation for the use of biomarkers is because, rather than simply quantifying their environmental levels, they may provide information on the biological effects of contaminants. While initiated as an adaptive reaction to destabilizing factors, stress response may have harmful consequences if prolonged by increasing susceptibility to infections through immune depression, causing mortality. (Moruf and Lawal-Are, 2018).

Among benthic communities, crabs are important members because a number of species are present for human consumption and a tremendous variety of species are useful as integrating sentinels of exposure to certain contaminants (Moruf and Lawal-Are, 2018). The Gercacinid crab (*Cardiosoma armatum*) is a useful sentinel, due to its biological and ecological characteristics (Lawal-Are *et al*., 2019b). Limited information is available on the effects of chlorpyrifos on haematological modulation in crabs, in particular with regard to sublethal concentrations. This research was therefore carried out to examine the hemato-immunological changes in *C. armatum* at various chlorpyrifos concentrations. The present study is a first attempt to understand the effects of chlorpyrifos on the immune functions and antioxidant system of an exposed crab.

MATERIALS AND METHODS

Experimental setup

Thirty two (32) samples of active *C. armatum*, 15-21cm in length; 58-75g in weight, were obtained from the mangrove area of University of Lagos Lagoon front (latitude 6°26°N and 6°39°N and longitude 3°39°S and 3°50°S), western axis of the Lagos Lagoon (Moruf and Lawal-Are, 2015). The experiment was carried out at the Department of Marine Sciences of University of Lagos, Nigeria. The crabs were selected and randomly stocked into eight small plastic tank (length × width × depth = 8 m × 8 m × 1.5 m) at a 4 crabs per tank. Acclimatization was 21 days. They were fed with trash fish (*Sardinella aurita*) once daily, the amount of which was approximately 2.2 % of the total weight of crabs held in the tank. However, feeding was terminated 24h prior to the range-finding and toxicity test. The organophosphate chlorpyrifos (480EC, Batch No: 20140620), was purchased from an agrochemical shop in Ilasa, Lagos State, Nigeria and stored at ambient temperature (27°C).

Bioassay procedure

The crabs were subjected to sub-lethal concentration of organophosphate chlorpyrifos at 0, 0.003, 0.006, 0.03, 0.06 mg/l concentrations in triplicates with 5 L of lagoon water. The exposure period lasted for 28 days during which each plastic container was well aerated. The LC_{50} for chlorpyrifos is 2.10 µg/L for crustaceans (Duarte-Restrepo *et al*., 2020).

Analytical procedures

Crab haemolymph was drawn with a 23G Syringe from the juncture between the bases of the ischium of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM Sodium citrate and 26 mM Citric acid), transferred into a 5 mL lithium heparin bottle kept in an ice chest for immediate analysis.

Total haemocyte counts (THC) of haemocyte population were determined using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973). One of the aliquots of the haemolymph of individual crabs was transferred into the haemocytometer and counted manually. Haemocyte morphotypes were identified and a total number of 100 cells from each slide were counted. The percentage of each counted cell type was calculated and multiplied by total haemocyte population count to obtain absolute count. The serum was assayed for transaminases such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the phosphatase alkaline phosphatase (ALP) activities according to methods described by Coles (1986). Samples of excised muscle tissues of crabs stored at −20 °C were later thawed and homogenized for the assays of reduced glutathione, catalase, superoxide dismutase and levels of proteins following the protocol described by Lushchaks *et al.* (2005) and Bertholdo-Vargas *et al.* (2009).

Statistical Analysis

Analysis of variance (ANOVA) and Duncan multiple post hoc tests were used to compare the differences between means at $p < 0.05$ level of significance. All statistical analyses were conducted using SPSS version 17.

RESULTS AND DISCUSSION

Hematological parameter of Gercacinid crab, *Cardisoma armatum*

No mortality was observed during the exposure for all treatments. According to Adewumi *et al.* (2018), sub-lethal concentrations of toxicants in the aquatic ecosystem will not certainly result in the outright death of aquatic organisms. However, the bioaccumulation of these contaminants over an era of time may create potential health threats not only to the aquatic animals like crab but also on higher trophic level. The haematological parameter of *C. armatum* exposed to sub-lethal concentrations of Chlorpyrifos is presented in Table 1. There was a significant ($P<0.05$) decrease in packed cell volume (PCV) and total haemocyte count (THC) of the exposure crabs (except in 0.003 mg/l concentration) compared to control group, with 7.00±0.01 % and 40.15±0.05 mL for PCV and THC respectively. The PCV and THC are vital indicators of oxygen conveyance capacity of crab thus making it possible to create a relationship with the oxygen concentration present in the habitat and the health status of the crab. Adewumi *et al.* (2018) reported a significant reduction in PCV of *C. gariepinus* exposed to Chlorpyrifos. The significant decrease in PCV may be ascribed to gill damage and/or damaged osmoregulation in aquatic animal. Reports have indicated that the increase in number of circulating haemocytes under hypoxic condition is a compensatory response to maintain oxygen tissue perfusion in crabs (Sussarellu *et al.,* 2012).

In the present study, no significant changes ($P>0.05$) were observed in haemocyte sedimentation rate (HSR), granulocyte and agranulocyte, although all exposure groups increased in HSR and agranulocyte with respective ranges of 3.00-3.02 mm/hr and 64.00-67.00 %. The higher HSR in the exposed groups can be related to inflammatory reactions in tissues leading to faster cell aggregation or an increase in the percolation of cells. Also, the higher level of granulocytes in exposed groups may be a stress response to unfavorable environmental conditions. Granulocytes have been reported to play a significant role in the crustacean defense system because of their antibacterial activity and function in secreting extracellular matrix proteins that stops the action of invading organisms, when the host is attacked by either extremely large particles or numerous tiny particles (Moruf and Lawal-Are, 2018).

Serum biochemical parameters of Gercacinid crab, *Cardisoma armatum*

The serum biochemical profile of *C. armatum* exposed to sub-lethal concentrations of chlorpyrifos is shown in Table 2. With the exception of ALP, there were no significant changes in the biochemical profile of both the control crabs and crabs in exposure doses, although organophosphate chlorpyrifos exposure induced increase in all the measured biochemical parameters (AST, ALT, ALP and Albumen). The increase may be a direct consequence of stress induced protein metabolism in the tissue of the crab. According to Sanni *et al.* (2020), changes in AST could be attributed to interference in the immune system of the crab, resulting to cell damage or a way in which the crabs are reacting to the exposure of contaminant. This finding is in agreement with earlier investigators who have observed similar results after exposure of crabs to chemicals such as Chloroform (Aliko *et al.,* 2015; Qyli and Aliko, 2016), pharmaceuticals (Anguirre-Martinez *et al.,* 2013) and copper sulphate (Jacobo *et al..* 2016).

Table 1. Sublethal effect of organophosphate chlorpyrifos on hematological parameters of Gercacinid crab, *Cardisoma armatum*

	0.000 mg/l	0.003 mg/l	0.006 mg/l	0.030 mg/l	0.060 mg/l
PCV (%)	(7.00-.80) 7.00±0.01[a]	(3.00-3.04) 3.00±0.01[b]	(3.00-3.03) 3.00±0.01[b]	(3.04-3.10) 3.00±0.01[b]	(8.00-8.10) 3.00±0.01[a]
Haemocyte Sedimentation Rate (mm/hr)	(2.00-2.35) 2.00±0.01[a]	(3.00-3.12) 3.00±0.01[a]	(3.00-3.12) 3.00±0.01[a]	(2.03-2.06) 2.00±0.01[a]	(3.03-3.05) 3.00±0.01[a]
Granulocyte (%)	(32.00-32.02) 32.00±0.01[a]	(29.00-29.23) 29.00±0.01[a]	(31.00-31.13) 31.00±0.01[a]	(30.02-30.41) 30.00±0.01[a]	(28.00-28.07) 28.00±0.01[a]
Agranulocyte (%)	(63.00-63.02) 63.00±0.01[a]	(64.00-64.31 64.00±0.01[a]	(66.00-66.14) 66.00±0.01[a]	(65.00-65.01) 65.00±0.01[a]	(67-67.07) 67.00±0.01[a]

Keys: Range in bracket. Mean±Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

Table 2. Sublethal effect of organophosphate chlorpyrifos on serum biochemical parameters of Gercacinid crab, *Cardisoma armatum*

	0.000 mg/l	0.003 mg/l	0.006 mg/l	0.030 mg/l	0.060 mg/l
AST (μl^{-1})	(1.62-1.99) 1.81±0.18[a]	(2.05-2.16) 2.11±0.06[a]	(2.90-2.93) 2.90±0.01[a]	(1.76-3.20) 2.48±0.72[a]	(2.48-2.65) 2.57±0.09[a]
ALT(μl^{-1})	(6.23-6.55) 6.39±0.16[a]	(7.30-7.74) 7.52±0.22[a]	(7.82-7.83) 7.83±0.01[a]	(6.92-7.88) 7.40±0.48[a]	(7.28-7.35) 7.32±0.03[a]
ALP (μl^{-1})	(63.48-66.24) 64.86±1.38[a]	(74.51-93.84) 84.18±9.66[b]	(80.04-80.05) 80.05±0.01[b]	(74.5-82.8) 78.65±4.15[b]	(71.75-72.06) 71.91±0.16[b]
Albumen (gl^{-1})	(1.91-1.94) 1.91±0.01[a]	(1.73-3.96) 2.85±1.12[a]	(4.10-4.11) 4.11±0.01[a]	(2.73-4.28) 3.51±0.78[a]	(5.84-5.86) 5.85±0.01[a]

Keys: AST- Aspartate Aminotransferase, ALT- Alanine Aminotransferase, ALP - Alkaline phosphatase, ALB- Albumen. Mean ± Standard Error. Values with different superscripts across row are significantly different at ($P < 0.05$).

Antioxidant enzyme activity

The antioxidant enzyme activity in *C. armatum* as induced by sub-lethal concentrations of organophosphate chlorpyrifos is illustrated in Table 3. The protein level and the activities of the enzymes (Superoxide dismutase, catalase and malondialdehyde) were inhibited in exposure groups. The observed lower protein content of exposure groups (25.85-32.21 g/l) could be attributed to interference with and/or modulation of their participation in various biological processes that were altered by exposure to organophosphate chlorpyrifos. The activity level of superoxide dismutase (SOD) observed in the exposure groups (142.89 -1189.33 min/mg pro) was lower when compared to the control (180.64-214.59 min/mg pro). SOD is the first antioxidant enzyme, which scavenges superoxide radicals (Ighodaro and Akinloye, 2018). Similar to this finding, Singaram *et al.* (2013) reported a decrease in protein level and lower SOD activity in mercury-exposed crab (*Scylla serrata*) and correlated it with a decrease in cell size due to the chemical's adverse effects.

In the present study, the activity level of catalase (CAT) in the exposure groups ranged from 1.05 to 2.10 min/mg pro, while that of the control group was 1.53 to 2.20 min/mg pro.). Also, the level of malondialdehyde activity (traditionally been used as a primary indicator of lipid peroxidation) in the exposure groups (7.27-9.15 nmol/ml) was lower than the level in the control group (9.36±0.61 µmol/ml). In contrast, there was an increase in glutathione (GSH) level in exposed crab groups (4.93-7.79 µmol/ml) when compared to the control (5.00±0.13µmol/ml). In the gills and hepatopancreas of *L. vannamei* exposed to chlorpyrifos, CAT also showed a dose-dependent elevation; 5.12%, 15.38%; and 35% and 100% (Duarte-Restrepo *et al.,* 2020). The lesser activity of CAT in exposure group could be attributed to greater production of superoxide anion radical, which has been reported to inhibit CAT activity. GSH anchors a vital role in the scavenging of cellular radical

oxygen species as part of the cellular first layer defense system that shields organisms from oxidative stress. An increase is often indicative of initiation of cellular defense mechanism in response to increasing concentration of free radical in the cell while a decrease may indicate an overrun of the antioxidant defense. According to Jacobo *et al.* (2016), exposure of crabs to different stressors is likely to increase the energy expenditure, which leads to an increase of metabolic rate in order to fuel the homeostatic regulation process.

Table 3. Sub-lethal effect of organophosphate chlorpyrifos on antioxidant enzyme activity of Gercacinid Crab, *Cardisoma armatum*

	0.000 mg/l	0.003 mg/l	0.006 mg/l	0.030 mg/l	0.060 mg/l
PRO (g/l)	(33.9-34.42) 34.16±0.26[a]	(25.85-32.21) 29.03±3.18[a]	(29.45-31.78) 30.62±1.17[a]	(26.06-30.30) 28.18±2.12[a]	(29.56-30.29) 29.93±0.37[a]
SOD (min/mg pro)	(180.64-214.59) 187.62±26.97[a]	(182.29-189.33) 185.81±46.48[a]	(148.96-149.01) 148.99±0.02[b]	(175.64-196.21) 185.93±10.29[a]	(142.89-143.43) 143.16±0.27[b]
CAT (min/mg pro.)	(1.53-2.20) 1.86±0.33[a]	(1.43-1.70) 1.56±0.13[a]	(1.05-1.23) 1.14±0.09[a]	(1.64-2.10) 1.67±0.38[a]	(1.45-1.54) 1.49±0.04[a]
MDA (µmol/ml)	(8.75-9.96) 9.36±0.61[a]	(8.21-9.15) 8.68±0.47[a]	(8.62-8.76) 8.69±0.07[a]	(7.27-9.15) 8.21±0.94[a]	(8.80-9.02) 8.91±0.11[a]
GSH (µmol/ml)	(4.86-5.13) 5.00±0.13[a]	(4.93-5.49) 5.21±0.28[a]	(7.54-7.79) 7.67±0.12[a]	(94.60-7.743) 6.17±1.57[a]	(6.92-7.34) 7.13±0.21[a]

Keys: PRO – Protein, SOD- Superoxide dismutase, CAT- catalase, MDA- malondialdehyde, GSH- Glutathione. Mean±Standard Error. a, b, c, means on the same row with different superscripts are statistically different (P>0.05)

CONCLUSION

Exposure of the Gercacinid crab, *Cardisoma armatum* to Organophosphate Chlorpyrifos caused drastic changes in immune-related parameters, especially the strong inhibition of the superoxide dismutase, catalase and malondialdehyde. This study clearly indicates that the presence of chlorpyrifos in water body, even in small concentration, could cause deleterious effects on crab physiology and may potentially disturb their survivability in the natural environment. Therefore, controlling measures should be taken to prevent the possible contamination of the aquatic environment by such toxic pesticides.

Conflict of Interest

Authors have no conflict of interest to declare.

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