TOXICOLOGICAL EFFECTS OF CHLORPYRIFOS ETHYL ON THE FRESHWATER CILIATE *Paramecium tetraurelia*

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Abstract. *Paramecium tetraurelia* is the most vulnerable freshwater ciliated protest to the pesticide-contaminated aquatic environment and is commonly used as a valuable biological model in studies investigating the monitoring of environmental quality. The present study was undertaken to screen the physiological, biochemical, and antioxidant responses of *Paramecium tetraurelia.* exposed to chlorpyrifos-ethyl (an insecticide). Paramecia received increasing concentrations of chlorpyrifos ethyl (0.04, 0.05, 0.06 and 0.07 mg/L) for 24, 48, 72 and 96h. Results revealed concentration- and time-dependent variations in growth and response percentage in exposed cells. In addition, protein and malondialdehyde levels and catalase activity were significantly increased in a concentration-dependent manner in treated cells compared with controls. However, the enzymatic activity of acetylcholinesterase and glutathione level showed a concentration-dependent decrease in treated paramecium cells compared with controls. In conclusion, chlorpyrifos-ethyl, especially at higher concentrations, proved to induce marked changes in the physiological and antioxidant profiles of *Paramecium tetraurelia.* which are promised to be used as an efficient monitoring organism for environmental water pollutants.

Key words: insecticide; Chlorpyriphos-ethyl; *Paramecium tetraurelia.*; antioxidants; growth; Acetylcholinesterase (ACHE).

INTRODUCTION

Pesticides contaminating the water environment are a worldwide concern and are believed to be an important risk factor for aquatic organisms [29]. Organophosphorus pesticides (OPs) are the most efficient, highly toxic, and environmentally biodegradable insecticides commonly used throughout the world to protect crops against pests [37]. Additionally, they are persistent and have effective insecticidal activity, as well as nutritional benefits [18] and antibacterial activity [15, 16]. The large use of organophosphorus and its release into the aquatic environment can negatively affect the aquatic non-target species, especially unicellular organisms [19]. The adverse effects of OPs on freshwater ciliates may result in the disruption of cell growth and induction of oxidative stress reactive oxygen species (ROS) leading to lipid peroxidation of the cell membrane, and alterations in the cell antioxidants [29]. Furthermore, chlorpyrifos ethyl is among the potent organophosphorus insecticides commonly applied to control foliage and soil-borne insect pests on a variety of crops including vegetables, fruits, cereals, and tomatoes [6, 32]. Nevertheless, the half-life of chlorpyrifos ethyl in water and sediment is between 14 and 120 days but may last as 365 days depending on climatic conditions and other factors [14]. For instance, considerable amounts of chlorpyrifos ethyl were detected in various sediment samples in the tropical aquatic environment resulting into its inclusion in the different priority list of compounds to be analyzed in water [27, 30]. On the other hand, the large use of this chemical adjacent to water body surfaces can cause severe toxicity to aquatic organisms at concentrations between 0.035-1.1 $\mu g \cdot L^{-1}$ [23, 25]. The primary actions of chlorpyriphos ethyl are the inhibition of acetylcholinesterase (ACHE) activity [24, 26, 35], the growth kinetics, and the antioxidant defense systems in non-target organisms [17, 21]. Among these, non-target organisms, ciliate protists freshwater *Paramecium tetraurelia* are freshwater eukaryotic unicellular organisms of slipper-like shape with beating cilia [34], as well as feed on bacteria, algae, and yeasts. Furthermore, the changes in direction and frequency of the ciliary beating are the main response ways that paramecia exhibited to various stimuli [3]. Thus, paramecia are interesting aquatic organism models for the toxicological assessments of many environmentally hazardous chemicals. In this regard, the present study is the first to investigate the concentration-dependent toxicity of an organophosphorus insecticide (chlorpyrifos ethyl) on the physiological and antioxidant profiles of *Paramecium tetraurelia*

MATERIALS AND METHODS

Chemical material

The pesticide chlorpyrifos-ethyl (CE) commercially named Pychlorex 48 EC was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

Biological material

Freshwater ciliated protozoa *Paramecium tetraurelia* provided by the team of cell toxicology laboratory of the Annaba University of Algeria were used in the present study [4].

Paramecia culture

Paramecia cells were cultured in a medium prepared following the protocol of [4]. Ten gram (10g) of lettuce, 5 g of hay, 5 g of wheat, 5 g of cucumber, 5 g of potato, and 2 g of peanuts were boiled for 1 h in 1.5 L of distilled water. Once the mixture becomes

cool, it was filtered and then sterilized by boiling at 100°C for 30 min in a heat-resistant bottle. The pH of the resulting mixture was adjusted to a value of 6.5, and the mixture was afterward kept away from light at a temperature close to 25°C. The prepared medium was incubated in an oven (Memmert UM 400) at temperature ranges between 28 and 16°C. Paramecia can be well microscopically observed after adding a few short stems of hay with distilled water to the culture medium and left in a warm, dark, and well-ventilated place. The paramecia cells were sub-cultured in the culture medium every three days to get a yellow culture.

Experimental design

Paramecium cells were treated with increasing concentrations of chlorpyrifos-ethyl (CE) (0.04, 0.05, 0.06, and 0.07 mg/L), including untreated control cells prepared in an appropriate volume of distilled water. These concentrations were chosen based on the median lethal concentration (LC_{50}) previously found to be 0.154 mg/L after 24 h in insecticide- exposed *Paramecium caudatum* [22]. All experiments were performed in three replicates.

Physiological evaluations

Determination of Percentage of Response (PR)

The growth inhibition of paramecium cells after 72 h of exposure to the test chemical was calculated by the estimation of the cell percentage of response (PR), where the positive values and negative values of percentage response indicate respectively inhibition and stimulation of cell growth [38]. The PR is calculated according to the following equation:

$$PR = 100 \times \frac{Cn - En}{Cn}$$

where: *PR* is the protozoa percentage of response (%), *Cn* is the cell control number (cell/mL), and *En* is the treated cells number (cell/mL).

Growth kinetic study

The growth kinetics of the treated paramecia cells was determined daily for one week by counting the number of living cells in a culture medium using an optic microscope (OPTICA Axiom 2000), and then few drops of Lugol were added to microscope slides containing paramecia [31].

Biochemical evaluation

Protein levels in purified *paramecium tetraurelia* were calorimetrically quantified at 620 nm using Coomassie Blue G-250 dye as previously described [5]. Acetylcholinesterase activity was determined using Ellman's colorimetric method [10], with slight modifications [36] where the kinetics of acetylthiocholine iodide hydrolysis by acetylcholinesterase were spectrophotometrically determined.

Antioxidant evaluation

The reduced GSH content was determined as previously determined using the color indicator, 5,5′-dithiobis-(2-nitrobenzoicacid) (DTNB), and the absorbance was read at 412 nm on a spectrophotometer (UV-1601 PC; Shimadzu, Japan). GSH content was expressed as nanomol per liter (nmol·L^{-1}). Catalase activity was assayed spectrophotometrically as previously described [1] based on the measurement of the light absorption at 240 nm of the decomposed H_2O_2 and expressed as µmol of H_2O_2 / min. / mg protein. Malondialdehyde (MDA), a lipid peroxidation marker, was measured as previously reported [11] with 2-thiobarbituric acid by a colorimetric method at 532 nm.

Statistical analysis

Experiments were performed three times, and results were displayed as mean ± SE of the mean. Comparisons between multiple groups were tested by one-way ANOVA using GraphPad Prism. $P<0.05$ was considered significant.

RESULTS

Growth kinetic of *Paramecium tetraurelia*

Between 0h and 72h, the growth rate in the form of increasing cell number in treated cells increased progressively (about up to 15.000 cells/mL) in a time-dependent manner, but lesser and slower than that of control, showing high cell number and quick growth rate (about up to 50.000 cells/mL). Whilst, the cells number was decreased between 72 h and 96 h in control cells to reach about 23.000 cells/mL, in chlorpyrifos-ethyl treated cells at 0.04-0.06 mg/L (about 8000 cells/mL), and chlorpyrifos-ethyl treated cells at 0.07 mg/mL (about 2000 cells/mL). Moreover, the cells number between treatment groups were significantly decreased in increasing chlorpyriphos-ethyl concentrations (Fig. 1).

The percentage of response (PR) showed a highly significant concentration-dependent increase ($P<0.01$)

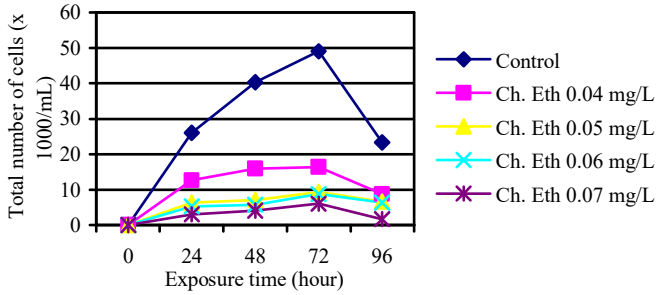

Figure 1. Kinetics of growth of the control paramecia, and those treated with chlorpyriphos-ethyl (Ch.Eth) at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L, and exposure time of 24, 48, 72, and 96 hours.

in cells treated with chlorpyrifos-ethyl at concentrations; 0.05; 0.06 and 0.07 mg/L as compared with those of 0.04 chlorpyrifos-ethyl treated cells after 24 and 48 hours, and significant concentration-dependent increase (P<0.05) after 72 and 96 hours, since the RP of cells treated chlorpyrifos-ethyl at 0.07 mg/L was highly significantly increased (P<0.01) compared with 0.04 mg/L chlorpyrifos-ethyl treated cells after 96 hours (Fig. 2).

Results of morphological abnormalities

The control paramecia showed a normal morphological structure evidenced by normal cell shape with an intact plasma membrane (Fig. 3a). Whilst, chlorpyriphos-ethyl dose depedent histological changes were observed compared with controls. These morphological changes are shown by round and oval-shaped cells, and swelling (Fig. 3b, 3c), in addition to the the cell membrane distruction as a result of cell necrosis (Fig. 3d, 3e).

Protein levels in purified paramecia cells

As displayed in Figure 4, protein levels in paramecia cells were concentration dependent significantly increased (P<0.05) in chlorpyrifos-ethyl at 0.04 and 0.05 mg/L, and highly significantly increased (P<0.01) in a concentration-dependent manner in chlorpyrifos-ethyl at 0.06 and 0.07 mg/L as compared with untreated control cells.

Chlorpyriphos-ethyl-induced acetylcholinesterase inhibition

Figure 5 displays concentration dependent significantly decreased (P<0.05) in chlorpyrifos-ethyl at 0.04 and 0.05 mg/L, and highly significantly decreased (P<0.01) in a concentration-dependent manner in chlorpyrifos-ethyl at 0.06 and 0.07 mg/L as compared with untreated control cells.

Antioxidant results

The content of MDA in purified *paramecium tetraurelia* was highly significantly concentration

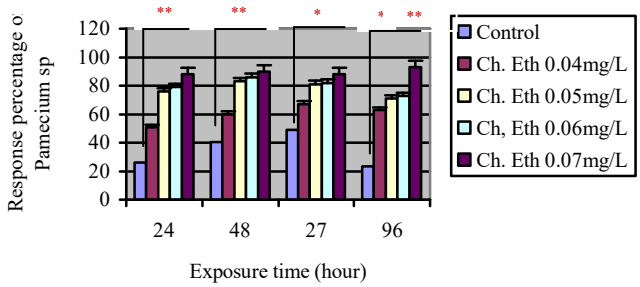

Figure 2. Evolution of the percentage of response of *paramecium tetraurelia* of the control paramecia, and those treated with chlorpyriphos-ethyl (Ch. Eth) at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L, and exposure time of 24, 48, 72, and 96 hours. Data are given as mean ± SD. Values with superscripts are statistically different p values. * P<0.05 and ** P<0.01 are statistically different from control of each exposure time.

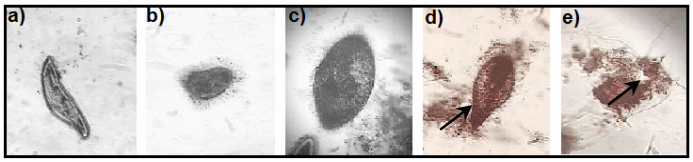

Figure 3. Light microscopic (Gr x40) showing altered morphological structures in control paramecia (a) and those exposed to chlorpyriphos-ethyl (Ch.Eth) at increasing concentrations; 0.04 (b) ; 0.05 (c) ; 0.06 (d) , and 0.07 (e) mg/L. Arrows indicate plasma membrane destruction.

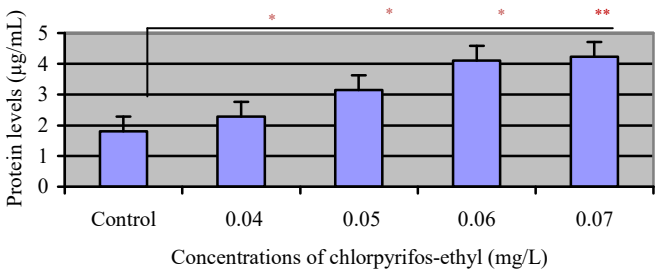

Figure 4. Levels of protein in control and treated *paramecium tetraurelia* with chlorpyriphos-ethyl at increasing concentrations; 0.04; 0.05; 0.06 and 0.07 mg/L. Data are given as mean ± SD. Values with superscripts are statistically different p values. * P<0.05 and ** P<0.01 are statistically different from control.

dependent increased (P<0.01) in chlorpyrifos-ethyl treated cells compared with control cells (Fig. 6). In contrast, the results revealed a highly significant (P<0.01) concentration-dependent decrease in GSH content in 0.04 and 0.05 mg/L, and a very highly significant decrease (P<0.001) in 0.06 and 0.07 mg/L chlorpyrifos-ethyl treated cells when compared with controls (Fig. 7). In addition, the enzymatic activity of catalase in *paramecium tetraurelia* showed a marked concentration-dependent increase in chlorpyrifos-ethyl

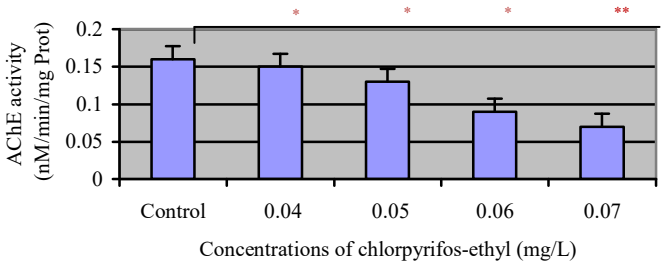

Figure 5. Acetylcholinesterase activity in control, and treated *paramecium tetraurelia* with chlorpyriphos-ethyl at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L. Data are given as mean ± SD. Values with superscripts are statistically different p values. * P<0.05 and ** P<0.01 are statistically different from control.

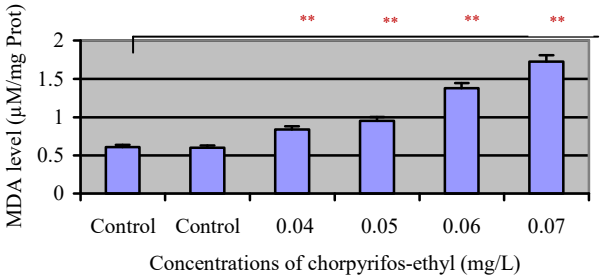

Figure 6. MDA content in control, and treated *paramecium tetraurelia* with chlorpyriphos-ethyl at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L. Data are given as mean ± SD. Values with superscripts are statistically different p values. ** P<0.01 are statistically different from control.

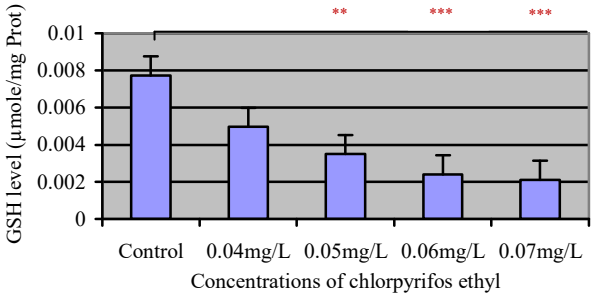

Figure 7. GSH content in control, and treated *paramecium tetraurelia* with chlorpyriphos-ethyl at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L. Data are given as mean ± SD. Values with superscripts are statistically different p values. ** P<0.01 and *** P<0.001 are statistically different from control.

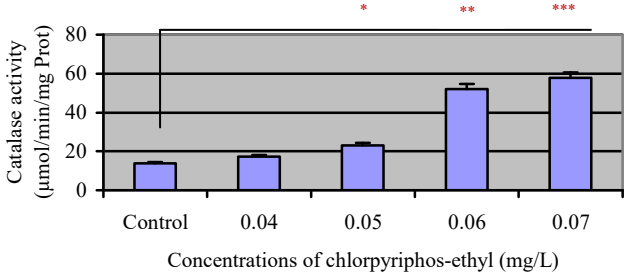

Figure 8. Catalase activity in control, and treated *paramecium tetraurelia* with chlorpyriphos-ethyl at increasing concentrations; 0.04; 0.05; 0.06, and 0.07 mg/L. Data are given as mean ± SD. Values with superscripts are statistically different p values. * P<0.05, ** P<0.01 and *** P<0.001 are statistically different from control.

treated cells as compared with control cells. This increase was significant in 0.04 mg/L ($P<0.05$), highly significant ($P<0.01$) in 0.05 mg/L, and very highly significant in ($P<0.001$) in 0.06 and 0.07 mg/L chlorpyrifos-ethyl treated cells (Fig. 8).

DISCUSSION

Microorganisms are the most sensitive to hazardous chemicals in water environments since paramecia were reported to be more resistant to elevated concentrations of contaminants [33]. In addition, paramecia are considered valuable organisms in monitoring the pesticides contaminated aquatic environment [19]. Thus, to assess the individual chemicals at laboratory conditions, paramecia would be an effective specie model able to exhibit the stress and toxicity on aquatic biota. To our knowledge, the present study is the first to investigate the toxicological effects of chlorpyrifos-ethyl on the physiological, biochemical and antioxidant profiles in *paramecium tetraurelia*. Here, the test chemical induced inhibitory cell growth kinetics in accordance with marked time and concertation-dependent increase in percentage of response as compared with control untreated cells, showing no effect either on cell growth or percentage of response. This concords with previous studies reporting the effect of organophosphorus on paramecium physiology and behavior [2, 21]. According to our data, the decreased cell growth of control cells after 72h is likely explained by the change in some external factors, including food and temperature, while the drop in cell growth in treated cells after 72h may explain the increased number of dead cells following the long exposure time. Overall, the impairment in cell division results in cell growth inhibition and induction of cell death processes [2], and hence, chlorpyrifos-ethyl used in our study, is believed to act as a potent genotoxic chemical to ciliate models, especially at concentrations higher than 0.05 mg/L. It was reported that the induction of oxidative stress-mediated generation of reactive oxygen species (ROS) is the main toxic action of organophosphorus on ciliate *paramecium* [29]. In addition, results showed a significant increase in protein and MDA contents in treated cells compared with controls. This finding was similarly reported in organophosphorus-exposed fishes [7, 32]. The increased protein level in paramecia cells can be explained by the induction of oxidative cell injury resulting in cell lysis evidenced by rupture of cell membranes, and mixing up of the cell contents with protoplasm, appearing thus as coagulation of proteins [37]. Whilst, the high MDA content is referred to as increased free radical production and membrane lipid peroxidation [19]. Further, the marked inhibition in AChE activity found in our results was similarly reported in previous studies [12, 19], suggesting AChE as the main OPs target enzyme, where they irreversibly link the AChE molecule by the phosphate group [37]. The ChE activities found in *paramecium tetraurelia* may contribute to regulating cell-to-cell communication culminating in cell-to-cell adhesion [18]. Moreover, cells protect themselves against chemicals-induced oxidative cell injuries by developing their powerful antioxidant defense system against oxidative stress-induced cellular damage [9]. Accordingly, in this study, the marked decrease in GSH level, an abundant peptide whose oxidation is ensured by glutathione peroxidase, in chlorpyrifos-ethyl treated cells as compared with controls, proves the involvement of GSH in chemicals-induced oxidative stress by scavenging free radicals leading to cellular oxidative injuries [8]. Additionally, our result of increased enzymatic activity of CAT in chlorpyrifos-ethyl treated paramecia is consistent with some previously reported findings of fishes exposed to organophosphorus compounds [20, 28]. The increased catalase activity is an antioxidant protection mechanism against ROS [13], as well as is attributed to increased levels of endogenous hydrogen peroxide (H_2O_2), known as the main cellular precursor of the hydroxyl radical (HO^-), the highly reactive and toxic form of ROS [14]. This increase in CAT activity was not enough to reduce ROS-induced oxidative damage to paramecia cells. In contrast, other studies have reported that the increased activity of CAT can explain the reduction of its synthesis, as well as consumption during detoxifying process against reactive oxygen species (ROS) production in the cell [29].

Paramecia sp proved to be a highly sensitive aquatic unicellular organism to chlorpyrifos-ethyl toxicity as evidenced by inhibition of cell growth associated with an increase in percentage of response, increased protein, and MDA levels, inhibition of AChE activity, and increased enzymatic activity of catalase in purified paramecium cells.

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Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Aebi, H., (1984): Catalase *in vitro*. Methods in Enzymology, 105: 121-126.

[2] Amanchi, N.R., Hussain, M.M., (2010): Cytotoxicity assessment of monocrotophos in *Paramecium caudatum* and *Oxytricha fallax*. Journal of Environmental Biology, 31(5): 603-607.

[3] Amaroli, A., Benedicenti, A., Ferrando, S., Parker, S., Selting, W., Gallus, L., Benedicenti, S., (2016): Photobiomodulation by infrared diode laser: effects on intracellular calcium concentration and nitric oxide production of Paramecium. Photochemistry and Photobiology, 92(6): 854-862.

[4] Azzouz, Z., Berrebbah, H., Djebar, M.R., (2011): Optimization of *Paramecium tetraurelia* growth kinetics and its sensitivity to combined effects of azoxystrobin and cyproconazole. African Journal of Microbiology Research, 5(20): 3242-3250.

[5] Bradford, M.M., (1976): A rapid and sensitive method for the quantitation of microgram quantities of rotein utilizing the principle of protein-dye binding. Analytical

Biochemistry, 72: 248-254.

[6] Cengiz, M.F., Catal, M., Erler, F., Bilgin, K., (2015): The effects of heat treatment on the degradation of the organophosphate pesticide chlorpyrifos-ethyl in tomato homogenate. Quality Assurance and Safety of Crops & Foods, 7(4): 537-544.

[7] Clasen, B., Loro, V.L., Cattaneo, R., Moraes, B., Lopes, T., de Avila, L.A., Baldisserotto, B., Zanella, R., Reimche, G.B., Baldisserotto, B., (2012): Effects of the commercial formulation containing fipronil on the non-target organism *Cyprinus carpio*: Implications for rice−fish cultivation. Ecotoxicology and Environmental Safety, 77: 45-51.

[8] Croute, F., Vidal, S., Dupouy, D., Soleilhavoup, J.P., Serre, G., (1985): Studies on catalase, glutathione peroxidase and superoxidismutase activities in aging cells of *Paramecium tetraurelia*. Mechanisms of Ageing and Development, 29(1): 53-62.

[9] Di Giulio, R.T., Washburn, P.C., Wenning, R.J., Winston, G.W., Jewell, C.S., (1989): Biochemical responses in aquatic animals: a review of determinants of oxidative stress. Environmental Toxicology and Chemistry, 8(12): 1103-1123.

[10] Ellman, G.L., Courtney, K.D., Andres, Jr.V., Featherstone, R.M., (1961): A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7: 88-95.

[11] Esteribauer, H., Cheeseman, K., (1991): Determination of aldehydic lipid peroxidation products: Malonaldehyde on related aldehyde. Free Radical Biology and Medicine, 11: 81-128.

[12] Falugi, C., Amaroli, A., Evangelisti, V., Viarengo, A., Corrado, M.U.D., (2002): Cholinesterase activity and effects of its inhibition by neurotoxic drugs in *Dictyostelium discoideum*. Chemosphere, 48(4): 407-414.

[13] Hernando, M., Houghton, C., Giannuzzi, L., Krock, B., Andrinolo, D., Malanga, G.F., (2016): Oxidative stress in *Microcystis aeruginosa* as a consequence of global climate change. Biocell, 40(1): 23-25.

[14] Hossain, M.S., Peng, M., Small, B.C., (2021): Optimizing the fatty acid profile of novel terrestrial oil blends in low fishmeal diets of rainbow trout (*Oncorhynchus mykiss*) yields comparable fish growth, total fillet n-3 LC-PUFA content, and health performance relative to fish oil. Aquaculture, 545: 737230.

[15] Howard, P.H., (1991): Handbook of environmental fate and exposure data: for organic chemicals. Routledge. NY, 1St Ed, 712 p.

[16] Jones Jr, G.S., Daly, J.S., (1993): Antibacterial organophosphorus compounds: Phosphoranili-dohydrazones of 5-nitro2-fural dehyde. Journal of Pharmaceutical Sciences, 82(7): 755-757.

[17] Kavitha, P., Rao, J.V., (2008): Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. Environmental Toxicology and Pharmacology, 26(2): 192-198.

[18] Koureas, M., Tsakalof, A., Tsatsakis, A., Hadjichristodoulou, C., (2012): Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. Toxicology letters, 210(2): 155-168.

[19] Kuzikova, I., Rybalchenko, O., Kurashov, E., Krylova, Y., Safronova, V., Medvedeva, N., (2020): Defense responses of the marine-derived fungus *Aspergillus tubingensis* to alkylphenols stress. Water, Air, and Soil Pollution, 231(6): 1-18.

[20] Maduenho, L.P., Martinez, C.B.R., (2008): Acute effects of diflubenzuron on the freshwater fish Prochilodus lineatus. - Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology, 148: 265-272.

[21] Mansano, A.S., Moreira, R.A., Pierozzi, M., Oliveira, T.M.A., Vieira, E.M., Rocha, O., Regali-Seleghim, M.H., (2016): Effects of diuron and carbofuran pesticides in their pure and commercial forms on *Paramecium caudatum*: The use of protozoan in ecotoxicology. Environmental Pollution, 213: 160-172.

[22] Mansano, A.S., Moreira, R.A., Dornfeld, H.C., Freitas, E.C., Vieira, E.M., Daam, M.A., Seleghim, M.H.R., (2020): Individual and mixture toxicity of carbofuran and diuron to the protozoan *Paramecium caudatum* and the cladoceran *Ceriodaphnia silvestrii*. Ecotoxicology and Environmental Safety, 201: 110829.

[23] Nguyen, T.T., Berg, H., Nguyen, H.T.T., Van Nguyen, C., (2015): Effects of chlorpyrifos ethyl on acetylcholinesterase activity in climbing perch cultured in rice fields in the Mekong Delta, Vietnam. Ecotoxicology and Environmental Safety, 117: 34-40.

[24] Ntiendjui, L.G., Tamungang, S.A., Ngoula, F., Ateufack, G., Tchoumboue, J., (2009): Effects of Maneb and Chlorpyrifos-Ethyl pesticide toxicity on a freshwater fish, *Oreochromis niloticus*. International Journal of Biological and Chemical Sciences, 3(1): 48-53.

[25] Odenkirchen, E.W., Eisler, R., (1988): Chlorpyrifos hazards to fish, wildlife, and invertebrates: a synoptic review. Fish and Wildlife Service, US Department of the Interior, pp. 16-24.

[26] Oruç, E.Ö., (2010): Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pesticide Biochemistry and Physiology, 96(3): 160-166.

[27] Ossana, N.A., Pérez-Iglesias, J.M., Soloneski, S., Larramendy, M.L., (2019): Auxinic herbicides induce oxidative stress on *Cnesterodon decemmaculatus* (Pisces: Poeciliidae). Environmental Science and Pollution Research, 26(20): 20485–20498.

[28] Persch, T.S.P., da Silva, P.R., Dos Santos, S.H.D., de Freitas, B.S., Oliveira, G.T., (2018): Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®). - Environmental Toxicology and Pharmacology, 58: 170-179.

[29] Ramzan, U., Shakoori, F.R., Shakoori, A.R., Abbas, S.Z., Wabaidur, S.M., Eldesoky, G.E., Rafatullah, M., (2022): Biodegradation and decolorization of Reactive Red 2 azo dye by *Paramecium jenningsi* and *Paramecium multimicronucleatum* in industrial wastewater. Biomass Conversion and Biorefinery, 13 (5): 1–9.

[30] Readman, J.W., Kwong, L.L.W., Mee, L.D., Bartocci, J., Nilvé, G., Rodriguez-Solano, J.A., Gonzalez-Farias, F., (1992): Persistent organophosphorus pesticides in tropical marine environments. Marine Pollution Bulletin, 24(8): 398–402.

[31] Sauvant, N.P., Pepin, D., Piccinni, E., (1999): Tetrahymena pyriformis: a tool for toxicological studies. Chemosphere. 38: 1631–1669.

[32] Sharma, D., Nagpal, A., Pakade, Y.B., Katnoria, J.K., (2010): Analytical methods for estimation of organophosphorus pesticide residues in fruits and

vegetables: A review. Talanta. 82: 1077-1089.
[33] Takahashi, T., Yoshii, M., Kawano, T., Kosaka, T., Hosoya H., (2005): A new approach for the assessment of acrylamide toxicity using a green paramecium. Toxicology *in vitro*. 19: 99-105.
[34] Tassin, A.M., Lemullois, M., Aubusson-Fleury, A., (2015): Paramecium tetraurelia basal body structure. Cilia, 5: 1-6.
[35] Timperley, C.M., Sellers, D.J., Waters, M.J., (2001): Fluorinated phosphorus compounds: Part 4. A lack of anticholinesterase activity for four tris (fluoroalkyl) phosphates. Journal of Fluorine Chemistry, 107: 155-158.
[36] Villescas, R., Ostwald, R., Morimoto, H., Bennett, E.L., (1981): Effects of neonatal undernutrition and cold stress on behavior and biochemical brain parameters in rats. Journal of Nutrition, 111: 1103-1110.
[37] Wan, L., Wu, Y., Ding, H., Zhang, W., (2020): Toxicity, biodegradation, and metabolic fate of organophosphorus pesticide trichlorfon on the freshwater algae *Chlamydomonas reinhardtii*. Journal of Agricultural and Food Chemistry, 68(6): 1645-1653.
[38] Wong, C.K.C., Cheung, R.Y.H., Wong, M.H., (1999): Toxicological assessment of coastal sediments in Hong Kong using a flagellate, *Dunaliella tertiolecta*. Environmental Pollution, 105: 175-183.

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