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Acute toxicity effects of a herbicide, Glycel on freshwater ciliates *Paramecium caudatum*, *Oxytricha fallax* and *Blepharisma intermedium*

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ABSTRACT

The aim of this work was to assess the effect of Glycel on *Paramecium caudatum*, *Oxytricha fallax* and *Blepharisma intermedium* as this herbicide is mostly used in home, garden, and commercial applications. Acute toxicity tests were carried out for 3 hours and probit analysis is used to calculate the LC_{50} values which were 63.09 ppm, 977.23 ppm and 1047.12ppm against to *Paramecium caudatum, Blepharisma intermedium* and *Oxytricha fallax* respectively. *Paramecium caudatum* was found more sensitive among test species, *Blepharisma intermedium* was moderate and *Oxytricha fallax* was found relatively tolerant. Behavioural manifestations like speedy egesting of food vacuoles, erratic swimming, moving to corners of cavity block and loss of movement coordination were observed during acute exposure. The various sub-lethal concentrations of Glycel were used to evaluate food vacuole, contractile vacuole activity and macronuclear aberrations tests and these tests proved that protozoan ciliates can act as alternative models to pesticide toxicity studies.

Key words: *Eukaryotic ciliates, Glycel, In vitro toxicity, Water quality assessment*

Introduction

Glycel is a synthetic Organophosphorus compound (Glyphosate) used to control annual broadleaf weeds without harming the crop grasses, industrial, urban, forestry and aquatic landscapes (Çavas and Konen, 2007). The world-wide usage of Glycel was increased 100 fold from past three decades (Cressey 2015). Phosphonate group in this compound inhibits 5-enolpyruvylshikimate 3-phosphate synthase enzyme. In the year 2013, the German Federal Institute for Risk Assessment toxicology review stated that the exposure to glyphosate formulations were resulting in the risk of various cancers, Similarly, The World Health Organization's International Agency for Research on Cancer (IARC, 2015) studies classified Glycel as "carcinogenic to humans" (Cressey, 2015; Guyton *et al.,* 2015; IARC, 2015). European Food Safety Authority and The European Chemicals Agency (ECHA) recommended that the glycel based compounds are genotoxic causing damage to DNA and causing eye damage, carcinogenic, mutagenic, and toxic to reproduction system. Similarly extensive usage and high water solubility

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of glycel causes toxic effects to aquatic life (Tsui and Chu, 2003).

Materials and Methods

The test organisms selected are *Paramecium caudatum, Oxytricha fallax* and *Blepharisma intermedium* and the freshwater samples were collected from within the vicinity of Osmania University campus, Hyderabad, Telangana, India and the isolation and identification of ciliates was done as suggested by Edmondson (1959).

Culturing medium: These organisms are continuously grown in hay infusion by sub-culturing method after every 5 to 7 days at room temperature (25 ±2 ^{0}C). Hay infusion culturing media is used as a basic culture media for growing ciliates as it is the most suitable and appropriate culturing medium as suggested by Kirby (1950) and Log phase cultures were used throughout the study and Log phase differ from one organism to another i.e., *Paramecium caudatum* 5 days, *Oxytricha fallax* 5 - 6 days and *Blepharisma intermedium* 6-7 days.

Test compounds: Glycel stock solution of 100ppm was prepared using distilled water (APHA, 2007) and to carry out the experiments, 0.5 ml of Glycel was added to 4.5 ml of culture medium to achieve desired concentration of known concentration.

Acute tests: In cavity block 4.5 ml of hay infusion medium containing about 100 organisms was taken with the help of a pipette. To this 0.5 ml of known concentration of Glycel was added and the experiment was conducted for 3hours. Three sets were maintained for all different concentrations by taking control as reference. Counting was done at every 10 minutes interval during first one hour and there after 20 minutes interval during the next two hours to record the changes in cell structure, shape and mortality.

Food vacuole activity: One hundred organisms were exposed to different sub lethal concentrations of Glycel for 1 hour and stained with India ink for 10 minutes. The cells were picked with the help of micropipette on to a clean cavity slide and observed under the binocular microscope at 10X and the number of food vacuoles formed was calculated (Bozzone, 2000).

Contractile vacuole activity: After exposing the cells to various sub lethal concentrations for 15 minutes, single individuals were picked normal in every respect and the rate of pulsation of posterior contractile vacuole was determined as suggested by Patterson and Sleigh (1977). The protamine coated slides were used to immobilize the ciliates (Marsot and Cuillard, 1973).

Cytological studies: Cytological studies were conducted to demonstrate general morphology of cell, nuclear shape and structure in ciliates after one hour exposure to different sublethal concentrations using Feulgen fast green technique. Schiff's reagent was prepared as suggested by De Tomasi (1936).

Statistical analysis

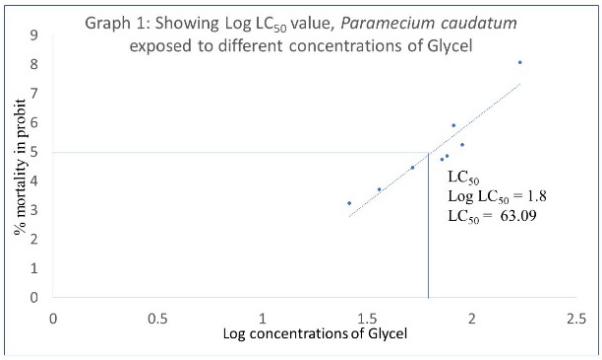
In acute toxicity studies, the LC_{50} value of Glycel was calculated by using probit analysis, (Finney, 1953). The results obtained from phagocytic activity, pulsatory vacuole activity and nuclear aberrations were analysed by One-way ANOVA to determine the significant difference. Value of $P \leq 0.05$ was considered statistically significant.

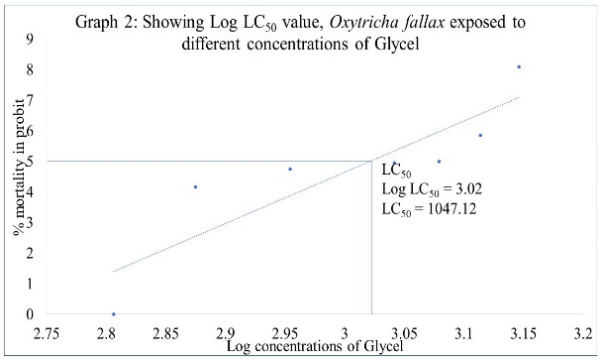
Results and Discussion

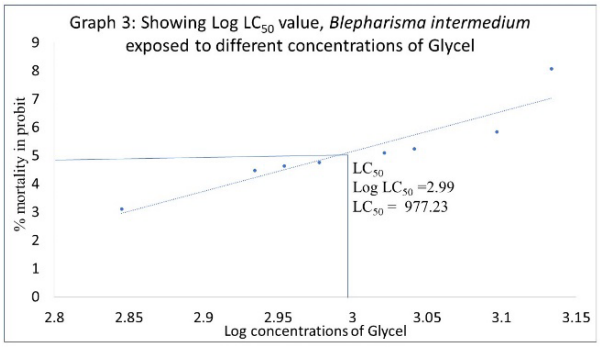
Acute toxicity studies

Acute bioassay studies were carried out for 3 hours exposure to establish the order of lethality. It includes immediate behavioral and cytopathological responses to toxicant such as changes in swimming pattern, motility, alterations in the body size, shape and ultrastructural deformities (Komala, 1992; Takiguchi *et al*., 2002; Shubham Singh and Tejashree, 2014; Maurya, 2019 and Sina, 2020, Amanchi and Ganta, 2021, Plattner, 2022). The acute toxicity test were done by using probit analysis to calculate the LC_{50}value, At the end of 3hrs acute toxicity test on *Paramecium caudatum*, *Oxytricha fallax* and *Blepharisma intermedium* exposed to Glycel, the obtained LC_{50} value was found to be 63.09 ppm, 1047.12 ppm and 977.23 ppm against mortality curve (Graph 1, 2 & 3). Worked out Sublethal concentrations for further studies were 12 ppm, 17 ppm and 22 ppm and 27ppm to *Paramecium*, 259 ppm, 289 ppm, 319 ppm and 349 ppm to *Oxytricha* and 235 ppm, 265 ppm, 295 ppm and 325 ppm to *Blepharisma. Paramecium caudatum* showed greater sensitivity to Glycel among the three experimental organisms tested and least sensitive organism was *Oxytricha fallax*. The acute effect of Glycel on cell membrane may be primarily altering its absorbency allowing entry of fluids into the cell until it leads to lysis. And it was observed that the gradual decrease

in the swimming speed with increased time of exposure may be due to the effect of the Glycel on the cellular metabolism. The turn angle, swimming pattern and back word swimming were altered under pesticide stress due to sudden changes in pH and osmatic gradient of the media. Similarly, technical grade of glyphosate showed higher toxicity effect on several aquatic organisms such as *Tetrahymena pyriformis*, Euplotes and micro algae as reported by Tusi and Chu (2003) and Bonnet *et al.* (2007). And it was also stated that any change in the pH of the culturing medium during acute toxicity studies of glyphosate is directly proportional to the toxicity affect. This might be the reason for Glycel to cause severe changes in *Paramecium caudatum* in the present studies.







Food vacuole activity in *Paramecia, Oxytricha* and *Blepharisma* on treatment with different sub-lethal concentrations of Glycel

To show the toxicants were ingested in the organisms, the test species were stained with India ink, which are easily visible under microscope, the numbers of vacuoles containing stained particles were counted, mean and standard deviation were calculated and plotted against concentration (Fig. 4,5 & 6).The food vacuole activity in treated organisms has shown significant variation with respect to different sublethal concentrations of Glycel. The average number of food vacuoles recorded in *Blepharisma intermedium* with Mean & SD value is 5.3 ± 0.67 at 265ppm, 5.2 ± 0.78 at 295ppm, 6.0 ± 0.66 at 235ppm 2.5 ± 0.52. at 325ppm. *Oxytricha fallax* were 5.4 ± 0.69, 4.0 ± 0.66, 3.3 ± 0.67 and 2.6 ± 0.51 in concentrations of 259ppm, 289ppm, 319ppm and 349ppm respectively. Reduction in the formation of food vacuoles observed in *Paramecium* was recorded as 9.8 ± 0.78 at 12ppm, 8.1 ± 0.73 at 17ppm, 6.1 ± 0.99 at 22 ppm 5.5 ± 0.52 at 27 ppm. In the present study, the swimming velocity of ciliates was diminished after one-hour exposure and the organisms did not swim normally in a proper direction and were turning all around themselves with increasing concentration. When cells are exposed to different lower concentrations of Glycel, cells normally swim in a forward direction, reverse direction and swim backwards for a short time before starting to rotate. The irregularities in cilia beating and stress egestion of food vacuoles was also observed. The rate of food vacuole activity is directly proportional to activity of cilia. Similar findings were observed by Rao *et al.* (2007) in *Paramecium caudatum* exposed to 40.6mg/l concentration of monocrotophos and stated that to

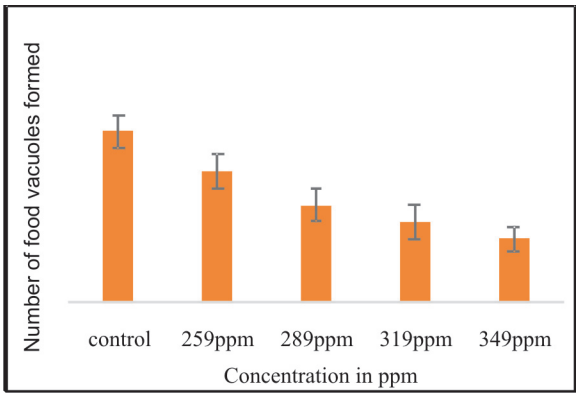
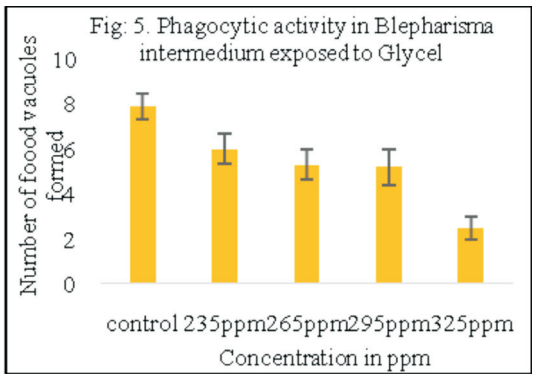
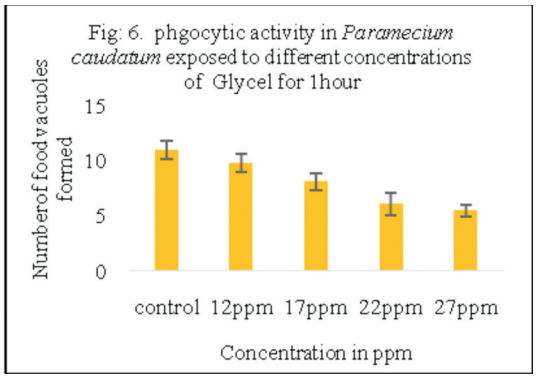


Fig. 4. Phagocytic activity in *Oxytricha fallax* exposed to Glycel

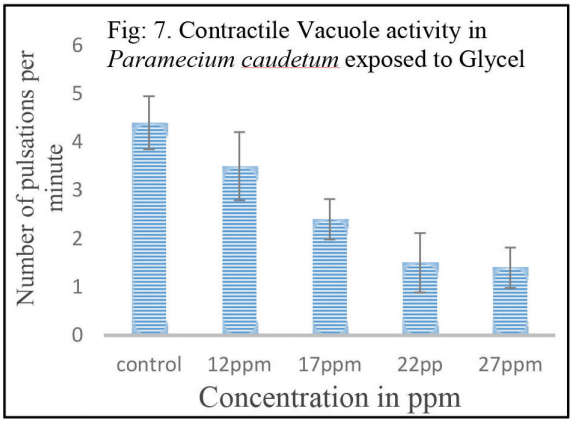
avoid toxic conditions, the organism undergo starvation which results in inhibition of food vacuoles formed, it is one kind of adaptive strategy in protozoan ciliates. Diminished food vacuole activity in *Tetrahymena* was studied by Rebandal and Karpinska (1981) on exposure to antibiotics colistin and Pencilin V&G along with culturing medium. In higher concentrations, pesticides cause severe damage on the cytostome region and internal contents of the organism as suggested by Amanchi and Masood (2012).

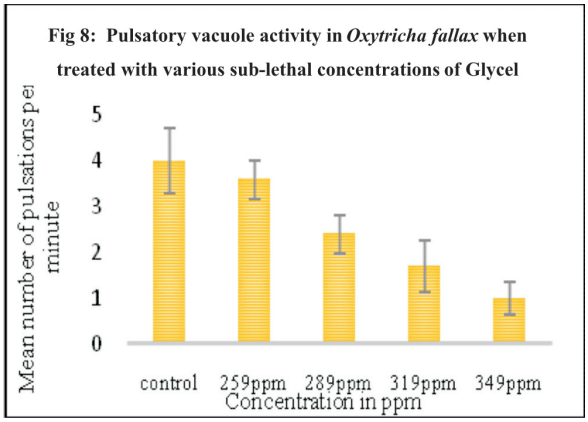


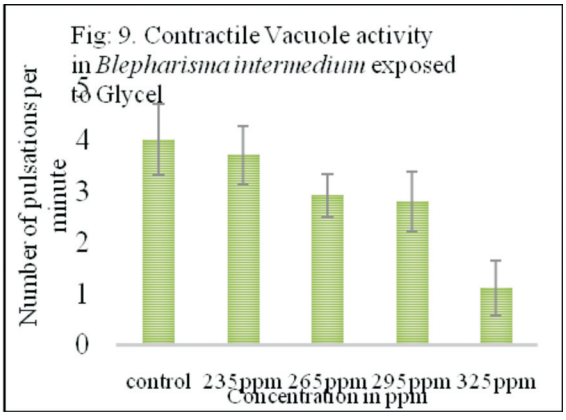


Contractile vacuole activity in *Paramecium*, *Oxytricha* and *Blephrisma on* treatment with various sub-lethal concentrations of Glycel

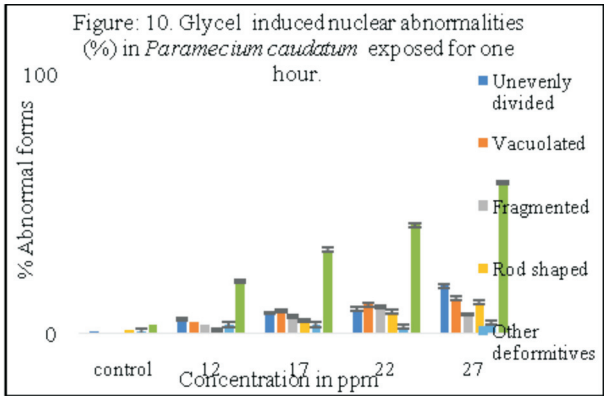
Fig 7, 8 and 9 were showing pulsatory vacuole activity in exposed groups of *Paramecium*, *Oxytricha* and *Blepharisma.* Glycel exerted an obvious inhibitory effect in pulsatory activity in all the concentrations below the control level. Reduction in pulsatory activity of *Paramecium* with Mean and SD values such as12ppm (3.5±0.7), 17ppm (2.4±0.41), 22ppm (1.5± 0.61) and 27ppm (1.4±0.41). Pulsatory vacuole activity of *Oxytricha* was observed at different sublethal concentrations to be 259ppm, 289ppm, 319ppm and 349ppm were3.6±0.41, 2.4±0.41, 1.7±0.57 and 1.0±0.35respectively. Pulsatory activity of *Blepharisma* with Mean & SD values was recorded as 3.7±0.57, 2.9±0.41, 2.8±0.57, & 1.1±0.54 at 235ppm, 265ppm, 295ppm and 325ppm respectively. One way ANOVA was performed to obtain the p value which is 0.00 and was significant at 5% level. Organisms showed reduced vacuolar activity by increasing concentration, this is due to sudden variations in external osmolarity of the culturing medium and also damage caused to the structure of the contractile vacuole, thus disturbing the rate of vacuole pul-

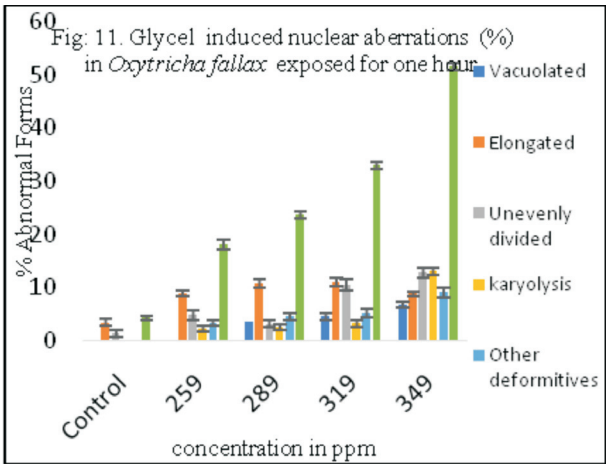






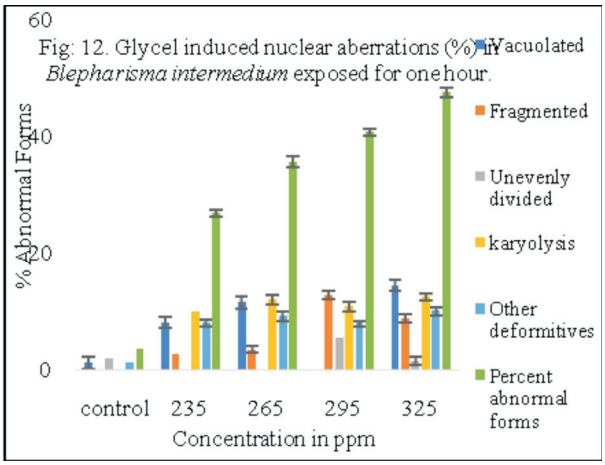
sations and may cause the arrest of the expulsions. Delfin showed a significant effect on the contractile vacuole activity of *Paramecium caudatum* as reported by Amanchi and Masood (2008). An alteration in the osmotic pressure of the culturing medium which is induced by the toxicant causes the osmotic imbalance (Amanchi and Masood, 2008, Busa *et al.*, 2019). The herbicide, Diuron and Carbofuran toxicity increased the reactive oxygen species (ROS) level resulting in oxidative stress and also inhibition in respiration, disintegration of the cell membrane leading to death of the organism according to Catala (2006), Tenda *et al.* (2012) and Mansano *et al.* (2014). Experimental observations conclude that pulsation in ciliates gets altered by external environment.





Glycel induced maconuclear abnormalities in Paramecium, Oxytricha and Blepharisma treated for one hour

Macro nuclear changes are due to failure of cell division, genotoxicity of pesticides. Dose-related nuclear abnormal forms were detected in treated group of *Paramecium, Oxytricha* and *Blepharisma*on exposure to different sublethal concentrations of Glycel, which include rod shaped,marginalization, vacuolated, Fragmented, Unevenly divided and karyolysis and total lysis of nucleus with increasing concentration of Glycel. *Paramecium* excerted highest percent deformities at 27ppm (58.2±0.63%) and lowest percent abnormalities at 12ppm (20.1±0.73%). The percent abnormal forms recorded in *Oxytricha fallax* were 18±0.67, 23.8±0.79, 33±0.67 and 51.5±0.71 at 259 ppm, 289 ppm, 319 ppm and 349ppm respectively. *Blepharisma intermedium,* maximum number of abnormal forms was recorded at 325ppm 47.5±0.85%. Among this vacuolated were 14.4±0.7%, fragmented were 8.8±0.63%, unevenly divided were 1.5±0.71%, karyolysis 12.4±0.52 % and othere deformities were 10±0.67%. Minimum number of abnormal forms was observed at 235ppm (26.8±0.63 %). One way ANOVA was performed to calculate total percent abnormal forms showing significant difference between mean sources of concentrations and the calculated p-value were significant at 5% level. Pesticide effect on the structure, shape and size of macronucleus in microorganisms especially protozoan ciliates is very scanty to provide substantial evidence regarding their mode of action. Extrusion of nucleus and hypertrophy due to pesticide stress on microtubular material in test species, has suggested that these forms are degenerative in nature, leading to death. Nuclear aberrations were more conspicuous in treatment to organophosphates due to the presence of phosphate group in the Glycel. Toxicants may bind to phosphate groups and base sites on nucleic acids leading to changes in the constancy of nucleic acids.



Conclusion

This study concludes that the exposure of ciliates to acute and sublethal concentrations of Glycel has significantly altered the food vacuole activity, pulsatary vacuole activity and macronuclear structure. Further, Glycel is a highly toxic to *Paramecium caudatum* and presence of Glycel even at very low concentrations in the aquatic environment may cause deleterious effect to non-target microorganisms like protozoan ciliates. Based on the data generated, we conclude that, present studies can ascertain a safer level of Glycel in aquatic environments.

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Conflict of interest

Both authors declare that there is no conflict of interest in publication of the data generated directly or indirectly.

References

Amanchi, N.R. and Ganta, A. 2021. Invitro toxicity of Pendimethalin using Ciliate Protozoa *Paramecium caudatum* and *Blepharisma intermedium*. *Indian Journal of Ecology.* 48(4): 1191-1197.

Amanchi, N. R. and Hussain, M. M. 2008. Cytotoxic effects of Delfin insecticide (*Bacillus thuringiensis*) on cell behaviour, phagocytosis, contractile vacuole activity and macronucleus in a protozoan ciliate *Paramecium caudatam*. *African Journal of Biotechnology.* 7(15): 2637–2643.

Amanchi, N.R. and Hussain M.M. 2012. Ecophysiological and cytopathological impact of Delfin insecticide (Bacillus thuringiensis) to an unicellular ciliate protozoan, Euplotes patella. *Research Journal of Recent Sciences.* 1: 64-67.

APHA, 2007. Standard methods for the examination of water and wastewater. 21st ed. American Public Health Association, Washington DC, Port City Press. Baltimore, Maryland.

Bonnet, J.L., Bonnemoy, F., Dusser, M. and Bohatier, J. 2007. Assessment of the potential toxicity of herbicides and their degradation products to nontarget cells using two microorganisms, the bacteria Vibrio fischeri and the ciliate Tetrahymenapyriformis. *Environ. Toxicol.* 22 (1): 78-91.

Busa, S., Amanchi, N.R., Ganta, A. and Bestha, S. 2019. Invitro toxicity responses of *Blepharisma intermedium* to commonly used insecticide deltamethrin. *International Journal of Recent Scientific Research.* 10(02): 31028-31031.

Catala, A. 2006. An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *Int. J. Biochem. Cell. Biol.* 38: 1482e1495.

Catala, A. 2006. An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *Int. J. Biochem. Cell. Biol.* 38: 1482-1495.

Çavas, T. and Könen, S. 2007. Detection of cytogenetic and DNA damage in peripheral 'ERDerythrocytes of goldfish (*Carassiusauratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis.* 22: 263–268

Cressey, D. 2015. Widely used herbicide linked to cancer. Nature.

Edmondson, W.T. 1959. Freshwater Biology,2nd edition. John Wiley & Sons, Inc., New York.

Finney, D.J. 1953. Probit Analysis, second ed., Cambridge University Press, Cambridge, England.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H. and Straif, K. 2015. "Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate". *The Lancet. Oncology.* 16 (5) : 490–1.

IARC Monographs 2015.evaluation of five organophosphate *insecticides and herbicides" (PDF). International Agency for Research on Cancer, World Health Organization. Volume 112.

Janapala, V.R., Gunda, V.G., Srikanth, K. and Arepalli, S.K. 2007. Acute toxicity bioassay using *Paramecium caudatum* a key member to study the effects of monocrotophos on swimming behaviour, morphology and reproduction. *Toxicology & Environmental Chemistry.* 89(2) : 307-317.

Kirby, P.L. 1950. Metetrials and methods in in the study of protozoa. University of California press, Barkeley and Los Angeles, California.P:1-55.

Komala, Z. 1992. Toxicity of Fastac 10EC, a pyrethroids insecticide to *Paramecium primaurelia* and *Tubifex sp*. Folia Biol. (Krakow). 40(3): 109-112.

Mansano, A.S., Hisatugo, K.F., Hayashi, L.H. and Regali-Seleghim, M.H. 2014. The importance of protozoan bacterivory in a subtropical environment (Lobo-Broa Reservoir, SP, Brazil). *Braz. J. Biol.* 74 (3): 569-578.

Marsot, P. and Couillard, P. 1973.T he use of Protamine-coated slides for immobilizing protozoa. *Journal* of *Protozoology.* 20: 105-106.

Maurya, R., Dubey, K., Singh, D., Jain, A. K., & Pandey, A. K. 2019. Effect of difenoconazole fungicide on physiological responses and ultrastructural modifications

in model organism *Tetrahymenapyriformis*. *Ecotoxicology and Environmental Safety*. 182: 109375.

Patterson, D.J. and Sleighm, A. 1971. Behavior of the contractile vacuole of *Tetrahymenapyriformis.* W: A redescription with comments on the terminology. *Journal of Protozoology.* 23: 410-417.

Plattner, H. 2022. Ciliate research: From myth to trendsetting science. *Journal of Eukaryotic Microbiology*. 69 (e12926) https://doi.org/10.1111/jeu.12926

Rebendal, H. and Karpinska, A. 1981. Toxic action of Colistin and penicillin "V" and "G" on Tetrahymena. P. Inhibition of phagocytic activity. *Acta Protozool.* 20: 291-298.

Shubhamsingh, T. and Tejashree, S. 2014. Effect of Neurotoxic Insecticide Dimethoate on Unicellular Freshwater Protozoan Ciliate Paramecium sp. *International Research Journal of Biological Sciences ISSN 2278-3202* 3(5): 62-66.

Sina N. Volz , Jonas Hausen, Kilian Smith, Richard Ottermanns, Andreas Schaeffer, Sabrina Schiwy, Henner Hollert, 2020. Do you smell the danger? Effects of three commonly used pesticides on the olfactory-mediated antipredator response of zebrafish (*Daniorerio*). *Chemosphere.* 241: 124963.

Takiguchi, N., Tajima, T., Asayama, K., Ikeda, T., Kuroda, A., Kato, J. and Ohtake, H. 2002. Behavioral responses of the ciliated protozoan Paramecium caudatum to 2,4-dichlorophenoxyacetic acid and its analogues. *J. Biosci. Bioeng*. 93: 416–420.

Tenda, H., Alves-Pereira, I. and Ferreira, R. 2012. Diuron determines Saccharomyces cerevisiae UE-ME3 survival at beginning of exponential phase. In: Mendez-Vilas, A. (Ed.), Microbes in Applied Research: Current Advances and Challenges. World Scientific, Singapore, pp. 80e83.

Tsui, M.T.K. and Chu, L.M. 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere.* 52: 1189–1197.