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EVALUATION OF STRESS OF ENVIRONMENTAL RELEVANT CONCENTRATION OF GLYPHOSATE PESTICIDE ON *LAMELLIDENS MARGINALIS*

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ABSTRACT

We evaluated glyphosate (GLP) toxicity on *Lamellidens marginalis* by using oxidative and genotoxic stress markers. The study provides evidence of oxidative stress and altered activities of antioxidative enzymes in bivalve (gill, foot, mantle, muscle, and hepatopancreas) upon exposure to an environmentally relevant concentration of glyphosate organophosphate pesticide (1 mg l^{-1}). The GLP exposure periods were 7 (T1) and 14 (T2) days, followed by a recovery period of 4 days (R1 & R2) respectively. GLP exposure showed a positive correlation between oxidative stress and duration of exposure. A catalase induction trend was observed in both the treated groups. Induction or inhibition of Super oxide dismutase (SOD) enzyme activities were tissue-specific after GLP exposure. The technical grade GLP has genotoxic potential, studied with the help of comet assay on gill tissues. Longer duration of exposure has significantly increased comet parameters such as tail length, tail DNA percentage as well as olive tail movement as compared to control bivalve. However, bivalves recovered significantly after the four days of the recovery period. The results suggested oxidative stress and genotoxic potential of GLP, evidenced by altered activities of antioxidative enzymes and induction of comet parameters. Even though, the animals experienced the stress of GLP exposure, however, recovery potential of bivalves was noteworthy after the removal of the stress.

KEY WORDS: Glyphosate, *Lamellidens marginalis*, Oxidative stress, Comet parameters Genotoxicity.

INTRODUCTION

Ideally, pesticides are toxic products designed to kill a target organism only; however, they also kill other non-target organisms such as the natural predators of the pest and also the organisms that are beneficial to health and to balance the ecosystem (WHO, 2003). Pesticides are biologically active substances, used for preventing, destroying, or controlling pests by interfering with their metabolic processes (Rice *et al.*, 2007).

Relatively a few pesticides' applications are made directly and exclusively on the target pests, and most application methods rely on the application of enough pesticides in the environment so that the exposure to the pest species reaches efficacious levels. It is known that less than 0.1 % of the applied pesticides actually reach the targeted pests, while the rest 99.9 % have the potential to move into other environmental compartments, including groundwater and surface water (Racke, 2003, Younos and Weigmann, 1988), which affect non-target organisms (Jarrell *et al.*, 2020).

As a part of the food chain *L. marginalis*, which acts as prey for birds and food for humans, accumulates a high concentration of pesticide than that of water (Dauberschmidt *et al.*, 1997), leads to the biomagnifications phenomenon.

The experimental animal *L. marginalis* is the most common bivalve found in the freshwater reservoirs around Pune, which is consumed as a major food item by a majority of the local population. Based on

the abundance of experimental animals and surrounding agricultural activities, Nandegaon and Kalubai temple sites of Mula River were selected as study sites. According to the survey of field region, it was found that glyphosate (GLP) was frequently used pesticides in this region.

GLP harms AchE activity, protein, carbohydrate, and lipid content of fairy shrimp at a sublethal concentration at different time intervals (Ali, 2013).

Native freshwater mussel, *Lampsilis siliquoidea*, glochidia, and juveniles were found to be sensitive to GLP. The growth of juveniles was also significantly affected by technical grade GLP (Bringolf *et al.*, 2007). GLP was found to be neurotoxic in *Cnesterodon decemmaculatus* at different concentrations including environmental relevant (1 mg l^{-1}) concentration (Menendez-Helman *et al.*, 2012).

GLP (CAS No. 1071-83-6) is available in many formulated forms like Roundup, Glysil, Glyphosate, Duster, Eraser, etc. GLP can injure or kill any plant it is sprayed upon. In the USA, GLP is used as a weed controller for the cultivation of genetically modified crops, which resulted in the widespread use of GLP. This increased use of GLP resulted in 430 µg l^{-1} residual level of GLP in river water, as well as in air during the application period (Chang *et al.*, 2011). Based on this background, the objectives of the present study were 1. to investigate the oxidative potential of GLP in freshwater bivalve *L. marginalis* by TBARS activity 2. to estimate alterations (enhancement/ inhibition) in antioxidative enzymes (SOD and CAT) in gill, foot, mantle, muscles and Hpt after independent exposure of the bivalve to GLP for 7 (T1) and 14 (T2) days. 3. to investigate the genotoxic potential of GLP exposure, *in vivo*, employing the gill cells using comet assay. 4. to evaluate the recovery potential of bivalves after 4 days of recovery (R1 and R2) respectively following each exposure.

MATERIALS AND METHODS

Animal collection from the field

The freshwater bivalves *L. marginalis* (7-9 cm) were collected from the Mula River (N 18° 33' and E 073° 42'). Bivalves were transported to the laboratory in aerated water within 30 mins.

Analysis of water samples was done for estimation of physico-chemical parameters and determination of pesticide residues in this reservoir.

Acclimatization of bivalves to laboratory conditions

Bivalves were acclimatized to laboratory conditions (Temperature 27±1 °C, pH 7.4± 0.2 and DO 8± 1 mg l^{-1}) for twenty-one days in dechlorinated stored water. The bivalves were fed every day, according to the method of Amanullah (2010). The water was renewed after every 24 hr.

Chemicals

GLP (CAS No. 1071-83-6) technical grade is soluble in acetone; therefore, the stock solution is prepared in Acetone. Working solutions having < 0.05 % acetone concentration were prepared from stock solution and used immediately after preparation throughout the experimental period.

Experimental design

Bivalves were randomly divided into six groups containing 3 individuals per set. Freshwater bivalves, *L. marginalis*, were exposed to environmental relevant, 1 mg l^{-1} concentration of GLP for 7 days and 14 days, followed by four days of the recovery period in tap water following exposure period.

Bivalves from the first group were exposed to GLP (1 mg l^{-1}) for up to 7 days (T1). Bivalves from the second group were initially exposed to GLP (1 mg l^{-1}) for up to 7 days and then they were transferred to water without toxicant, up to 4 days for the assessment of recovery (R1). The third group was exposed to GLP (1 mg l^{-1}) for up to 14 days (T2). The fourth group involved bivalves, which were initially exposed to GLP (1 mg l^{-1}) for up to 14 days, and then they were transferred to water without toxicant, up to 4 days for the assessment of recovery (R2). The fifth and sixth group bivalves were maintained as the control groups in water and water with acetone solvent, respectively without the addition of any pesticide. Each treatment was carried out in triplicate. Water, with and without GLP, was replaced after every 24 hrs for each group. Bivalves from all groups were maintained in 1.5 l of water per individual and fasted throughout the experimental period (Janakidevi *et al.*, 2013). Bivalves were sacrificed after the respective period of exposure for further analysis.

Biochemical estimations

Lipid peroxidation (LPO)

LPO was evidenced using thiobarbituric acid

reactive substances (TBARS) assay, as described by Esterbauer and Cheeseman (1990). The tissues were homogenized in phosphate buffer (pH 7.4) over ice. Then absorbance of reaction mixture was checked at 532 nm.

Super oxide dismutase (SOD) activity

Tissue homogenates were prepared in phosphate buffer (pH 7) over ice. SOD (EC 1.15.1.1) activity of reaction mixtures was determined at 560 nm by the method of Beauchamp and Fridovich (1971).

Catalase (CAT) activity

CAT (EC 1.11.1.6) activity was determined by the method of Aebi (1984). Tissue homogenates were prepared in phosphate buffer (pH 7) over ice. The decreasing absorbance of reaction mixtures was measured at 240 nm for 3 minutes.

Genotoxicity by Comet assay

DNA strand breaks were detected using the alkaline comet assay as described by Singh *et al.* (1988) with minor modifications. Comet assay was standardized with hydrogen peroxide exposure in experimental animal, which showed dose dependent increase in tail DNA %, OTM [(Tail mean-Head mean) × Tail DNA %/100], and TL (Mundhe *et al.,* 2016). The slides were stained with ethidium bromide and examined under a fluorescence microscope (Carl Zeiss Axiovision, 400X, excitation filter 510-560 nm, barrier filter 590 nm). Randomly selected 100-gill cells (50 nuclei were analyzed on each duplicate slide) were analyzed for each sample. The experiment was repeated thrice.

Statistical analysis

Statistical data analysis was carried out using one-way ANOVA; Tukey's pair-wise-multiple comparison test was used for biochemical estimations. Data were presented as mean ± standard deviation (SD).

RESULTS

Bivalves from both, the control group of tap water only and the control group of tap water with acetone solvent were compared. There were no significant differences in all the studied parameters. Pesticide residues in reservoir water from the collection site were below the limit of quantification (0.01-1.01 ppb) as demonstrated by the results of GC-MS analysis.

Lipid peroxidation (LPO)

Upon exposure to GLP, in T1 group, LPO (Table 1) was induced significantly (P≤0.05) in the mantle (2396 %), gill (2051 %), foot (1175 %), Hpt (796 %) tissues, while it increased to some extent in muscle (529 %) tissue. In R1 group, LPO in only Hpt (134 %) tissue recovered significantly (P≤0.05), but in gill (1907 %), mantle (1562 %), foot (620 %), and muscle (273 %), non-significant recovery was observed.

In T2 group, LPO was induced significantly (P≤0.05) in the foot (4500 %), gill (3702 %), Hpt (3475 %), muscle (3078 %), and mantle (2892 %) tissues as compared to control. In R2 group, LPO in gill (2361 %), mantle (1974 %), Hpt (1551 %), foot (1016 %), and muscle (446 %) tissues recovered significantly (P≤0.05).

When we compared both the duration of exposure, the percentage of oxidative stress was increased as the duration of exposure increased in all the tissues. It was also observed that only Hpt recovered significantly in R1 group, while all the other tissues recovered significantly in R2 group. Though there was a significant recovery in all the tissues of the R2 group, the percentage of LPO was higher in R2 than that of the R1 group. It may be concluded that, as the duration of exposure increased, bivalve became more susceptible to GLP.

Super oxide dismutase (SOD) activity

SOD activity (Table 1) was altered after 7 days of GLP exposure (T1) in gill (229 %), foot (139 %), mantle (137 %), Hpt (130 %), and muscle (46 %). Gill, foot and Hpt showed significant (P≤0.05) induction of SOD activity. Recovery of SOD activity was significant (P≤0.05) in gill, mantle, Hpt and muscle, while in case of foot tissue, it was non-significant. In T1 group, muscle tissue showed significant (P≤0.05) inhibition of SOD activity, followed by significant (P≤0.05) recovery.

After 14 days of GLP exposure (T2), significantly (P≤0.05) elevated SOD activity was observed in foot (180 %), muscle (129 %), and Hpt (110 %). In R2 group, foot, Hpt, gill, mantle, and muscle showed recovered SOD activity to some extent after 4 days of the recovery period.

Catalase (CAT) activity

CAT activity (Table 1) showed an increasing trend after both the duration of exposure (T1 and T2), in Hpt (1341 % and 550 %), gill (606 % and 811 %), mantle (465 % and 355 %), foot (418 % and 219 %)

Table 1. LPO, SOD and CAT activities in different tissues of *L. marginalis* upon exposure to GLP (1 mg l^{-1}) for 7 days and 14 days

Tissue		Gill	Foot	Mantle	Muscle	Hpt
TBARS activity	Control	0.39±0.03	0.24±0.08	0.27±0.09	0.41±0.07	0.52±0.07
(nmol mg	T1	8.00±2.72[a]	2.82±0.29[a]	6.47±1.39[a]	2.17±0.43	4.14±1.02[a]
protein^{-1})	T2	14.44±0.89[a]	10.80±1.52[a]	7.81±0.30[a]	12.62±4.55[a]	18.07±2.96[a]
	R1	7.44±1.61	1.49±0.53	4.22±0.70	1.12±0.71	0.70±0.14[b]
	R2	9.21±0.42[b]	2.44±0.71[b]	5.33±1.02[b]	1.83±0.46[b]	8.07±1.10[b]
SOD activity	Control	1.55±0.24	1.06±0.02	1.71±0.34	1.17±0.02	2.04±0.04
(unit mg	T1	3.55±0.11[a]	1.47±0.24[a]	2.34±0.38	0.64±0.09	2.66±0.13[a]
protein^{-1})	T2	1.82±0.02	1.91±0.28[a]	0.98±0.16	1.52±0.28[a]	2.25±0.10[a]
	R1	1.96±0.41[b]	1.12±0.02	1.90±0.07[b]	0.86±0.07[b]	1.82±0.21[b]
	R2	0.90±0.10[b]	1.19±0.04[b]	1.07±0.11	1.00±0.10[b]	0.57±0.23[b]
CAT activity	Control	1.13±0.01	1.09±0.02	1.16±0.08	1.09±0.03	1.42±0.07
(unit mg	T1	6.85±1.44[a]	4.56±1.03[a]	5.40±2.60[a]	1.42±0.25	19.05±1.95[a]
protein^{-1})	T2	9.16±0.73[a]	2.39±0.21	4.12±0.70	3.00±1.32[a]	7.81±0.48[a]
	R1	2.46±0.22[b]	0.98±0.58[b]	1.01±0.38[b]	1.27±0.25	6.61±0.94[b]
	R2	5.25±0.47[b]	1.17±0.35	1.24±0.80	1.85±0.29	1.37±0.33[b]

T1: 7 days exposed, R1: 4-day recovery (mean ± SD) a there are significant differences (P≤ 0.05) between the control and exposed groups, b there are significant differences (P≤ 0.05) between the exposed and 4-day recovery T2: 14 days exposed, R2: 4-day recovery (mean ± SD) a. there are significant differences (P≤ 0.05) between the control and exposed groups, b. there are significant differences (P≤ 0.05) between the exposed and 4-day recovery

and muscle (130 % and 274 %). Four days of recovery (R1 and R2) following both the exposure durations, resulted in some recovery of CAT activity in Hpt (465 % and 96 %), gill (217 % and 465 %), muscle (116 % and 169 %), foot (90 % and 107 %) and mantle (87 % and 107 %) tissues.

All the tissues except muscle, showed significant (P≤0.05) elevation of CAT activity after 7 days (T1) of exposure followed by significant (P≤0.05) recovery (R1). After 14 days of exposure (T2), CAT activity in gill, muscle, and Hpt showed significant (P≤0.05) elevation; this was followed by significant (P≤0.05) recovery (R2) in gill and Hpt.

When we compared both the duration of exposure, gill and Hpt showed significant elevation of CAT activity followed by significant recovery.

Comet assay

Exposure of GLP for 7 days, showed non-significant elevated tail DNA percentage (169 %) (Figure 2a), tail length (155 %) (Figure 2b) and OTM (346 %) (Figure 2c). Four days of recovery (R1) showed lower tail DNA percentage (69 %), tail length (87 %) and OTM (107 %) in gill cells. However, the recovery was not significant.

After exposure of GLP for 14 days, significant (P≤0.05) increase in tail DNA percentage (216 %) (Figure 2a), tail length (192 %) (Figure 2b) and OTM (559 %) (Figure 2c) was observed. Significant (P£0.05) recovery in R2 group of gill cells was observed in tail DNA percentage (131 %), tail length (113 %), and OTM (209 %).

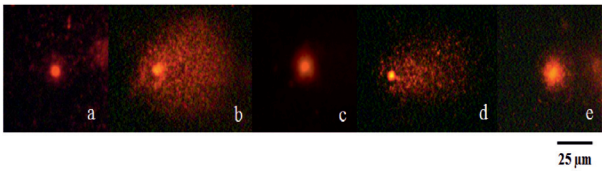

Fig. 1. Images of comet (400X), stained with ethidium bromide (a) Control, (b) T1, (c) R1, (d) T2 and (e) R2

DISCUSSION

It was found that pesticide residues were below detection level (10 µg l^{-1}>) in the water collected from the field (SF1). Physico-chemical parameters were within the range of acceptable limits (SF2).

GLP (1 mg l^{-1}) exposure concentration is selected for the present study and it is considered to be an environmentally relevant concentration based on a study, which also detected GLP in rainwater samples from Mississippi and Iowa (<0.1 to 2.5 mg l^{-1}) (Menendez-Helman *et al.*, 2012). GLP has also been detected at sublethal levels up to 328 ug L^{-1} in some water bodies in the USA (Battaglin *et al.*, 2009) and between 0.36 and 2.16 mg l^{-1} in Brazil (Rodrigues and Almeida, 2005).

Aquatic invertebrate, *Daphnia magna*, was exposed to GLP and also its formulated herbicides.

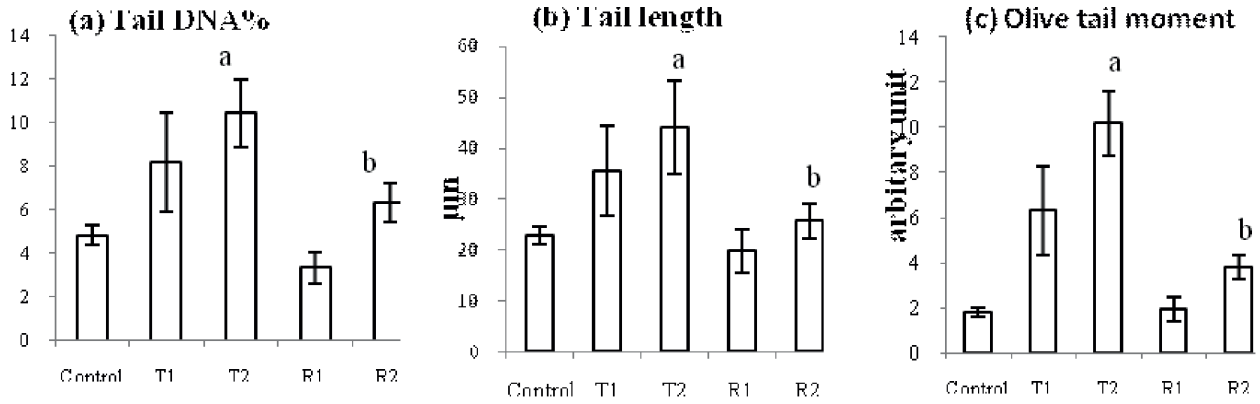

Fig. 2. DNA damage (a) Tail DNA percentage, (b) Tail length, and (c) Olive tail moment parameters in gill cells of control, T1, T2, R1, and R2 groups, respectively in *L. marginalis* after GLP exposure (1 mg l^{-1}). Comet parameters were reported as mean ± SD. a: significant differences ($P \leq 0.05$) between the control and exposed groups, b: significant differences ($P \leq 0.05$) between the exposed and 4-day recovery

EC50 values of isopropylamine salt of GLP and Roundup were 3.7 to 10.6 mg l^{-1} and 1.4 to 7.2 mg l^{-1}, respectively. Results indicated that formulated GLP was more toxic than isopropylamine salt of GLP (Cuhra *et al.*, 2013). Chronic exposure of GLP to *Daphnia magna* showed no observable effect at a concentration of 50 mg l^{-1} (McKee *et al.*, 1986).

To know the oxidative and genotoxic potential gill, foot, mantle, muscle, and Htp tissues were used (El-Shenawy *et al.*, 2009, Mundhe *et al.*, 2014). Significantly elevated LPO was observed in all the tissues after 7 (T1) and 14 (T2) days of GLP exposure. After the comparison of both durations, it was observed that the percentage of LPO activity increased with the increasing duration of the exposure. Thus, it revealed that there is a positive correlation between oxidative stress and the duration of exposure (Romero *et al.*, 2011).

Lipid peroxidation is an index of antioxidant status and oxidative stress (Pandit *et al.*, 2013). GLP or its formulation can induce oxidative stress in male albino rats (El-Shenawy, 2009).

SOD activity is induced after 7 days exposure in gill and Hpt, whereas SOD activity is enhanced in foot and muscle with an increasing duration of exposure of 14 days. The extended duration of exposure negatively affected the recovery potential of the SOD activity.

In GLP exposure, gill and Hpt showed higher CAT activity after 7 and 14 days. Our results suggested that gill tissue is more sensitive to the toxicant and Hpt is a detoxicant organ. We may conclude that as the duration of exposure increased, bivalves adapted themselves to GLP stress. Catalase and super oxide dismutase activities increased in dose-dependent manner in *Chlorella kessleri*, exposed to increasing concentration of glyphosate (Romero *et al.*, 2011).

GLP induced significant DNA damage after 14 days of exposure in *L. marginalis*. However, 4 days recovery period was found to be sufficient for significant recovery of the damage.

When freshwater fish, *Channa punctatus*, was exposed to sub-lethal concentrations of the commercial formulation of GLP for different durations, the genotoxicity of GLP was highest after the 14th day, while DNA damage declined afterward (21st, 28th, and 35th days). Thus the result reflects the repairing ability of fish and protective mechanisms for excreting GLP (Nwani *et al.*, 2013). In the present study, the percentage of DNA damage was significant after the 14 days (T2) of GLP exposure. Genotoxicity increased with the increased duration of exposure, but 4 days of recovery period was found to be sufficient to repair the damage. This may reflect the repairing ability of the bivalve (Mundhe *et al.*, 2016). According to Kier (2015), GLP and GLP-based formulations have negligible genotoxicity risk. Also, GLP did not bioaccumulate in the aquatic organism (Giesy *et al.*, 2000), which might be responsible to reduce its toxicity.

Aquatic organisms, being an important source of food for humans, can prove to be a major health risk if exposed to environmental toxicants like genotoxic and carcinogenic substances (Harvey, 1991).

CONCLUSION

GLP exposure showed duration-dependent oxidative stress and DNA damage. The results

suggested oxidative stress and genotoxic potential of GLP, evidenced by altered activities of antioxidative enzymes and induction of comet parameters. Even though, the animals experienced the stress of GLP exposure, however, recovery potential of bivalves was noteworthy after the removal of stress.

Non-target aquatic organisms like bivalves being an important source of food for humans can prove to be a major health risk if exposed to toxicants like pesticides, as bivalves have the ability to bio concentrate and being edible, toxicants are conveyed to other organisms including human beings. GLP must be used with awareness in agriculture to prevent natural water resource contamination by way of runoff. The judicious application of pesticides is of utmost importance in order to protect non-target edible animals, which affect the food chain, ultimately causing hazards to human health.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests

REFERENCES

Aebi, H., 1984. Aebi, Hugo. ed." Catalase *in vitro*. *Meth Enzymol*. 105: 121-126.

Ali, A.J. 2013. Toxic impacts of two organophosphorus pesticides on the acetylcholinesterase activity and biochemical composition of freshwater fairy shrimp *Streptocephalus dichotomus*. *International Journal of Pharma and Bio Sciences*. 4(2): 966-972.

Amanullah, B., Stalin, A., Prabu, P. and Dhanapal, S., 2010. Analysis of AchE and LDH in mollusc, *Lamellidens marginalis* after exposure to chlorpyrifos. *Journal of Environmental Biology*. 31(4): 417-419.

Battaglin, W.A., Rice, K.C., Focazio, M.J., Salmons, S. and Barry, R.X. 2009. The occurrence of glyphosate, atrazine, and other pesticides in vernal pools and adjacent streams in Washington, DC, Maryland, Iowa, and Wyoming, 2005-2006. *Environmental Monitoring and Assessment*. 155(1): 281-307.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*. 44(1): 276-287.

Bringolf, R.B., Cope, W.G., Mosher, S., Barnhart, M.C. and Shea, D. 2007. Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae). *Environmental Toxicology and Chemistry: An International Journal*. 26(10): 2094-2100.

Chang, F.C., Simcik, M.F. and Capel, P.D. 2011. Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environmental Toxicology and Chemistry*. 30(3): 548-555.

Cuhra, M., Traavik, T. and Bohn, T. 2013. Clone-and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. *Ecotoxicology*. 22(2): 251-262.

Dauberschmidt, C., Dietrich, D.R. and Schlatter, C. 1997. Esterases in the zebra mussel *Dreissena polymorpha*: activities, inhibition, and binding to organophosphates. *Aquatic Toxicology*. 37(4): 295-305.

dos Santos, K.C. and Martinez, C.B. 2014. Genotoxic and biochemical effects of atrazine and Roundup®, alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicology and Environmental Safety*. 100: 7-14. doi: http://dx.doi.org/10.1016/j.ecoenv.2013.11.014

El-Shenawy, N.S. 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. *Environmental Toxicology and Pharmacology*. 28(3): 379-385.

El-Shenawy, N.S., Moawad, T.I., Mohallal, M.E., Abdel-Nabi, I.M. and Taha, I.A. 2009. Histopathologic biomarker response of clam, Ruditapes decussates, to organophosphorous pesticides reldan and roundup: a laboratory study. *Ocean Science Journal*. 44(1): 27-34.

Esterbauer, H. and Cheeseman, K.H. 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Methods in Enzymology*. 186: 407-421.

Giesy, J.P., Dobson, S. and Solomon, K.R. 2000. Ecotoxicological risk assessment for Roundup® herbicide. *Reviews of Environmental Contamination and Toxicology*. 35-120.

Harvey, R.G. 1991. *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*. CUP Archive.; http:/

/sitem.herts.ac.uk/aeru/footprint/en/ index.htm.

Janaki Devi, V., Nagarani, N., Yokesh Babu, M., Kumaraguru, A.K. and Ramakritinan, C.M. 2013. A study of proteotoxicity and genotoxicity induced by the pesticide and fungicide on marine invertebrate (*Donax faba*). *Chemosphere*. 90(3): 1158-1166.

Jarrell, Z.R., Ahammad, M.U. and Benson, A.P. 2020. Glyphosate-based herbicide formulations and reproductive toxicity in animals. *Veterinary and Animal Science*. 10: 100126.

Kier, L.D. 2015. Review of genotoxicity biomonitoring studies of glyphosate-based formulations. *Critical Reviews in Toxicology*. 45(3): 209-218; doi: 10.3109/10408444.2015.1010194

McKee, M.J. and Knowles, C.O. 1986. Effects of fenvalerate on biochemical parameters, survival, and reproduction of Daphnia magna. *Ecotoxicology and Environmental Safety*. 12(1): 70-84.

Menéndez-Helman, R.J., Ferreyroa, G.V., dos Santos Afonso, M. and Salibián, A. 2012. Glyphosate as an acetylcholinesterase inhibitor in *Cnesterodon decemmaculatus. Bulletin of Environmental Contamination and Toxicology*. 88(1): 6-9. doi 10.1007/s00128-011-0423-8

Mundhe, A.Y. and Pandit. S.V. 2014. Assessment of toxicity of monocrotophos in freshwater bivalve, *Lamellidens marginalis*, using different markers. *Toxicol.Int*. 21(1): 51-56.

Mundhe, A.Y., Bhilwade, H. and Pandit, S.V. 2016. Genotoxicity and oxidative stress as biomarkers in fresh water mussel, *Lamellidens marginalis* (Lam.) exposed to monocrotophos. *Indian J. Exp. Biol*. 54(12): 822-828.

Nwani, C.D., Nagpure, N.S., Kumar, R., Kushwaha, B. and Lakra, W.S. 2013. DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus. Environmental Toxicology and Pharmacology*. 36(2): 539-547.

Pandit, S.V. and Mundhe, A.Y. 2013. Monocrotophos induced behavioral stress, biochemical and histological alterations in *Lamellidens marginalis* (Lamarck). *The Bioscan*. 8: 1053-1056.

Racke, K.D. 2003. Release of pesticides into the environment and initial concentrations in soil, water, and plants. *Pure and Applied Chemistry*. 75(11-12): 1905-1916.

Rice, P.J., Rice, P.J., Arthur, E.L. and Barefoot, A.C. 2007. Advances in pesticide environmental fate and exposure assessments. *Journal of Agricultural and Food Chemistry*. 55(14): 5367-5376.

Rodrigues, B.N. and Almeida, F.S. 2005. *Guide to Herbicides,* 5th Edition, IAPAR, Londrina, 592.

Romero, D.M., de Molina, M.C.R. and Juárez, Á.B. 2011. Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of *Chlorella kessleri. Ecotoxicology and Environmental Safety*. 74(4): 741-747. DOI: https://doi.org/10.1016/j.ecoenv.2010.10.034

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*. 175(1): 184-191.

WHO (world health organization) 2003. Climate change and human health. http://www.who.int/globalchange/ecosystems/biodiversity/en/index.html

Younos, T.M. and Weigmann, D.L. 1988. Pesticides: a continuing dilemma. *Journal (Water Pollution Control Federation)*. 60(7): 1199-1205.