

Effect of Glyphosate Formulation on Sperm Motility in Blue Gourami *Trichopodus Trichopterus*

¹Sandra, B.S. and ²Dr. Benno Pereira, F.G.*

¹Research Scholar, Department of Zoology,
University of Kerala, Kariavattom Campus,
Thiruvananthapuram -699581, Kerala, India
E-mail: sandra.balachandran@gmail.com

²Assistant professor, Department of Zoology,
University of Kerala, Kariavattom Campus,
Thiruvananthapuram -699581, Kerala, India
E-mail: bennopereira@gmail.com

*Corresponding author:

Sandra.B.S

Research scholar, Department of Zoology,
University of Kerala, Kariavattom Campus,
Thiruvananthapuram -699581, Kerala, India
E-mail: sandra.balachandran@gmail.com

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ABSTRACT:

Pesticides are the major environmental pollutants, which contaminate the elements of the aquatic and terrestrial environments they are applied in, such as crops and soil. Globally, herbicides account for approximately 50% of all pesticides used. The present study is undertaken to investigate the effects of invitro exposure of glyphosate formulation on sperm motility in the freshwater fish, *Trichopodus trichopterus*. The environmental concentrations of glyphosate ranged from 0.09 to 1.7mg mg a.e./L in pond water and 0.26 to 19 mg a.e./L in pond sediments. The glyphosate formulation used for the present study is Glyphosate 41% S. L (GLYPHO). The effect of glyphosate formulation on sperm motility was assessed by preparing stock solution of 500 ppm from which test solutions with 0.01, 0.1, 1, 10 and 20 ppm concentrations were prepared by serial dilution. The results of the study revealed that exposure to Glyphosate formulation drastically reduced percentage of motile sperms as well as duration of motility in the activation medium. In the control group, percentage of motile spermatozoa was 86% which immediately reduced to 76% in 0.01 ppm and lowest percentage of motile spermatozoa was 36% observed at 10ppm. The control group had duration of motility of 55.66± 2.80 secs which immediately dropped to 44.66± 2.67 secs in 0.01ppm and lowest duration of motility was obtained at 10ppm (34.33± 2.14secs). Therefore, results of the present study suggest that even low concentrations of the Glyphosate, causes deleterious effects on *Trichopodus trichopterus* spermatozoa, particularly in terms of percentage and duration of motility.

Keywords: Herbicide, Glyphosate, *Trichopodus trichopterus*, Sperm motility, Duration of motility

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INTRODUCTION

Glyphosate (N-[phosphonomethyl] glycine), an organic phosphate compound, acts as herbicide by inhibiting the synthesis of aromatic amino acids in plants (Boocock and Coggins, 1983). The primary mode of action of glyphosate is to target the shikimate pathway, specifically the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The shikimate pathway is responsible for the biosynthesis of compounds such as phenylalanine, tryptophan, tyrosine, indole, indole derivatives, tannins, flavonoids, lignin, alkaloids, and other aromatic metabolites. Glyphosate inhibits EPSPS, preventing chorismite production, which is the last common precursor in the process of biosynthesis of aromatic compounds in bacteria, fungi, and plants (Pérez et al., 2011; Stenersen, 2004).

Commercial glyphosate formulations vary in composition depending on the country and purpose. The concentration of glyphosate in the formulations, expressed as glyphosate acid equivalent (a.e.) is commonly used to compare their properties and toxicity. Glyphosate has a strong adsorption to soil and undergo microbial degradation. However, despite its widespread use, glyphosate and its associated components can enter aquatic environments through various pathways, such as rainfall immediately after application, flood events increasing river sediment load, surface runoff, overspray or drift during herbicide application, urban runoff, and wastewater treatment effluent (Solomon and Thompson, 2003). These sources contribute significant amounts of glyphosate to rivers, with reported concentrations worldwide reaching up to approximately ~10–15 µg/L in rivers (Byer et al., 2008; Struger et al., 2008). The highest environmental concentrations of glyphosate have been observed in pond water ranging from 0.09 to 1.7 mg a.e./L and in pond sediment ranging from 0.26 to 19 mg a.e./L (Giesy et al., 2000).

The Blue gourami (*Trichopodus trichopterus*) belongs to the order Anabantidae can be an ideal species for ecotoxicological study because of its wide environmental tolerances, ability to colonize anthropogenically disturbed habitats, trophic opportunistic nature and fast growth

rates. It is highly tolerant of hypoxic conditions because of the presence of auxiliary respiratory organ (McKinnoo and Liley, 1987). The Blue Gourami species is considered an excellent model for toxicity studies due to its year-round availability, voracious feeding habit, prolific reproduction, and adaptability to cultured environments. In India, there is a high likelihood of agrochemical exposure to Blue Gourami, as they are abundantly present in water bodies near agricultural fields. Sperm motility is one of the crucial characteristics to assess sperm quality as it is an essential pre-requisite for fertilization (Rurangwa et al., 2004). The present study aims to investigate the invitro effect of glyphosate-based herbicide on sperm quality parameters of freshwater fish *Trichopodus*

MATERIALS AND METHODS

Test Organism

The fish species selected for the study was *Trichopodus trichopterus* (Pallas, 1770), commonly known as the Blue Gourami. This species belongs to the order Anabantidae, which encompasses 16 genera and 50 species distributed across India, Southern Asia, and Central Africa. *Trichopodus trichopterus* collected from fish farms in Ernakulam, India, was used in the study.

Collection and Maintenance of Test Organism

Healthy adult *Trichopodus trichopterus* used in the study were purchased from fish farms in Ernakulam. Prior to the experiment, fishes were acclimatized in laboratory conditions for a period of two weeks. During the acclimatization period fishes were fed with commercial fish food twice daily. The physio-chemical parameters of the water were daily monitored using a standard procedure (APHA, 2005) and maintained constant conditions during the acclimatization period. The acclimatization was done at room temperature.

Chemical used for the study

Commercially available formula Glyphosate 41% S.L (GLYPHO), manufactured by National Pesticides and Chemicals, Amaravati, Maharashtra was used for the present study. The chemical composition of the glyphosate formulation was isopropyl amine salt of

glyphosate 41% w/w and inert materials 59% w/w (Polyoxyethylene amine surfactant 15%w/w, Manufacturing impurity 4.00%, water 40% w/w).

Effect of Invitro Exposure of Glyphosate Formulation on Sperm Motility in Blue Gourami *Trichopodus Trichopterus*

The effect of glyphosate formulation on sperm motility in spermatozoa of Blue Gourami *Trichopodus trichopterus* was analysed. Commercially available formulation, Glyphosate 41% S.L (GLYPHO), manufactured by National Pesticides and Chemicals, Amaravati, Maharashtra was used for the present study. The effect of glyphosate formulation on sperm motility was assessed by preparing stock solution of 500 ppm from which test solutions with 0.01, 0.1, 1, 10 and 20 ppm concentrations were prepared by serial dilution. The environmental concentrations of glyphosate ranged from 0.09 to 1.7mg mg a.e./L in pond water and 0.26 to 19 mg a.e./L in pond sediments (Giesy et al., 2000).

In the present study, the three lower concentrations selected are concentrations that can be expected to occur in the environment regularly (0.01 ppm) and during occasional peak contamination events (0.1, 1 ppm). The highest concentration tested (10 ppm) was the highest environmental concentrations of glyphosate acid equivalents a.e./L in pond sediments. Different media were mixed with the milt sample and the percentage of motile spermatozoa and duration

of motility were assessed (Goodall et al., 1989). Six samples were tested with each concentration of the activating medium. Three replications were done on each concentration.

RESULTS

Effect of In Vitro Exposure of Glyphosate Formulation on Sperm Motility in Blue Gourami *Trichopodus Trichopterus*

Exposure to Glyphosate formulation drastically reduced percentage of motile sperms as well as duration of motility in the activation medium. In the present study, the sperms exposed to different concentrations showed a decrease in the motility parameters and the lowest motility was obtained at 10ppm (Table 1; Fig 1,2).

The percentage of motile sperms vary inversely proportional to the concentration of glyphosate formulation. In the control group, percentage of motile spermatozoa was 86% which immediately reduced to 76% in 0.01 ppm. The lowest percentage of motile spermatozoa was 36% observed at 10ppm.

The duration of motility of sperms was also found to vary inversely to the concentration of glyphosate formulation. The control group had duration of motility of 55.66± 2.80 secs which immediately dropped to 44.66± 2.67 secs in 0.01ppm. The lowest duration of motility was obtained at 10ppm (34.33± 2.14).

Table 1. Mean Percentage and duration of motility of spermatozoa of *Trichopodus trichopterus* across various concentrations of glyphosate

Concentration of Glyphosate (ppm)	Percentage of motile spermatozoa (%)	Duration of motility (s)
0	86.67±4.92	55.66± 2.80
0.01	76.66± 4.92	44.66± 2.67
0.1	66.66± 4.92	43.83± 3.27
1.0	56.66± 4.92	41.00±2.56
10	36.66±4.92	34.33± 2.14
20	19.67±3.11	23.12± 4.15

⁻x±SD of 6 observations

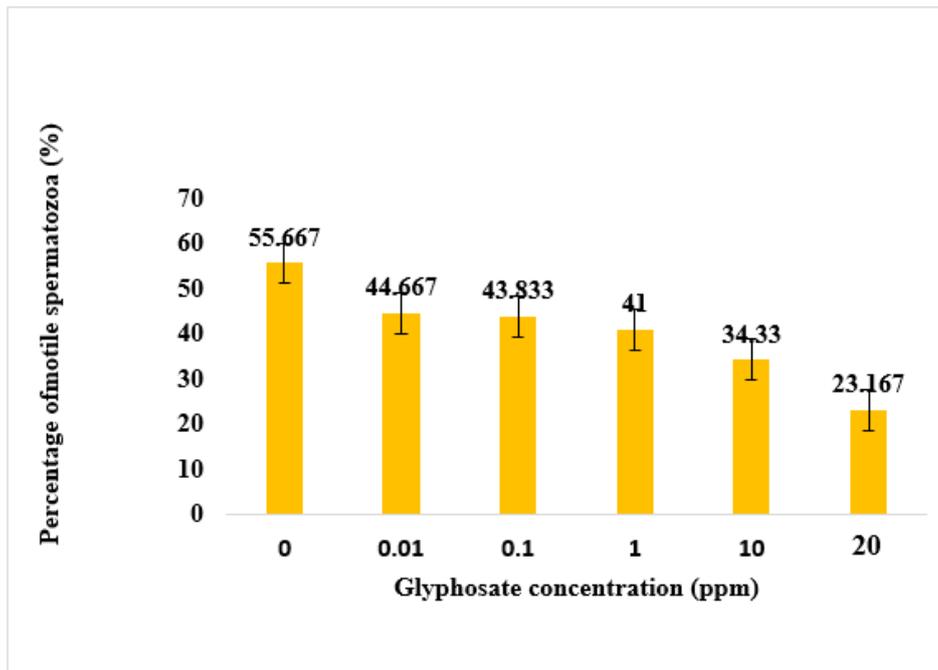


Figure 1: Mean Percentage of motile spermatozoa of *Trichopodus trichopterus* across various concentration of glyphosate

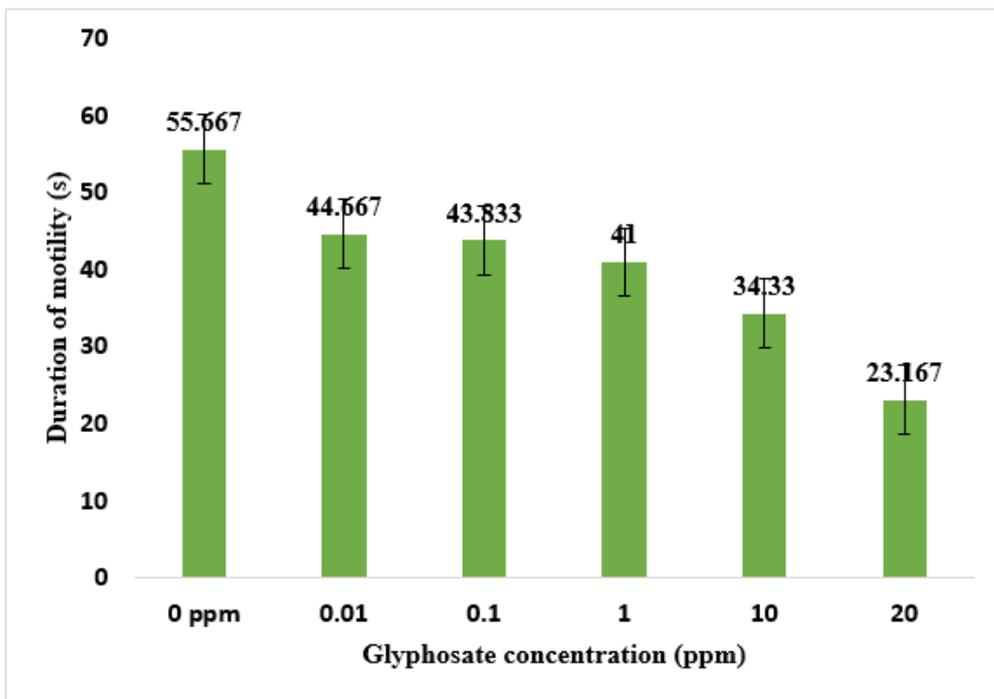


Fig 2. Mean duration of motility of spermatozoa of *Trichopodus trichopterus* across various concentrations of glyphosate

DISCUSSION

The success of fertilization, whether in natural or artificial spawning, is influenced by the percentage of sperm motility and sperm velocities, which serve as predictors of semen quality. Sperm quality plays a crucial role in ensuring the correct process of natural or artificial spawning. Therefore, research on semen biology holds significant importance, particularly within the realm of fish reproduction. Sperm quality parameters such as motility and concentration are vital for successful fertilization (Krol et al., 2006).

Several studies proved that glyphosate have negative effects on fertilization rates and the quality of gametes, leading to hindrances in reproductive success. The progressive sperm motility velocities were found to be directly correlated with fertilization rates in various fish species. Silveira et al., (2019) demonstrated that the majority of kinetics parameters of *Odontesthes humensis* spermatozoa were significantly decreased after glyphosate exposure (7.8 mg/L). Similarly, exposure to Roundup (0.5 mg/L) for 96 hours resulted in disruption of sperm quality, including sperm motility and concentration, in live-bearing *Jenynsia multidentate*. Studies on zebrafish (*Danio rerio*) reported a reduction in sperm motility and motility period after exposure to pure glyphosate at concentrations of 5 and 10 mg/L for 24 and 96 hours, respectively (Lopes et al., 2014). Furthermore, Harayashiki et al. (2013) observed a reduction in sperm motility in adult guppies (*Poecilia vivipara*) following exposure to Roundup, a glyphosate-based herbicide

Numerous studies by different authors have demonstrated that both glyphosate and its commercial formulation have the potential to disrupt the levels of antioxidant defense in various organisms, resulting in oxidative stress (Langiano and Martinez, 2008; Contardo-Jara et al., 2009; Ferreira et al., 2010). Excessive levels of reactive oxygen species can have detrimental effects on cells, leading to processes such as lipid peroxidation, amino acid oxidation, enzyme inactivation, co-factor oxidation, and DNA damage (Brooker, 2011).

Based on the in vitro experimental results, exposure to Glyphosate formulation drastically reduced the percentage of motile sperms as well as the duration of motility in the activation medium. The percentage of motile sperms and duration of motility vary inversely proportional to the concentration of glyphosate formulation. This might be associated with increased reactive oxygen species production. Studies conducted by various authors have indicated that both glyphosate and its formulations have the potential to disrupt antioxidant defense mechanisms in different organisms, leading to oxidative stress (Langiano and Martinez, 2008; Contardo-Jara et al., 2009; Ferreira et al., 2010). Excessive reactive oxygen species can be detrimental to cells, resulting in processes such as lipid peroxidation, oxidation of amino acids, enzyme inactivation, co-factor oxidation, and DNA damage. Findings from the present study along with those already described in the literature support the idea that fish reproduction could be impaired in aquatic environments contaminated with Glyphosate based herbicides even at environmentally realistic concentrations.

CONCLUSION

In summary, the results of the present study suggest that even low concentrations of the Glyphosate, causes deleterious effects on *Trichopodus trichopterus* spermatozoa, particularly in terms of percentage and duration of motility. The percentage of motile sperms and duration of motility decreased with increase in herbicide concentration in the activating medium. Even environmentally realistic concentrations of this herbicide could have long-term negative effects on the reproduction of *Trichopodus trichopterus*, potentially leading to changes in fish populations in environments contaminated with Glyphosate.

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REFERENCES

- Boocock. M. R., Coggins. J. R. (1983). Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate, *FEBS Letters*, Vol.154 (1),127-133. doi:10.1016/0014-5793(83)80888-6
- Brooker. R. J. (2011). *Genetics: Analysis and principles* (4th ed). New York: McGraw-Hill Science.
- Byer. J. D., Struger. J., Klawunn. P., Todd. A., Sverko. E. D. (2008). Low-cost monitoring of glyphosate in surface waters using the ELISA method: An evaluation, *Environmental Science and Technology*, Vol.42 (16), 6052-6057. doi:10.1021/es8005207
- Contardo-Jara. V., Klingelmann. E., Wiegand. C. (2009). Bioaccumulation of glyphosate and its formulation Roundup ultra in *Lumbriculus variegatus* and its effects on biotransformation and antioxidant enzymes, *Environmental Pollution*, Vol.157(1), 57-63. doi:10.1016/j.envpol.2008.07.027
- Ferreira. D., da Motta. A. C., Kreutz. L. C., Toni. C., Loro. V. L., Barcellos. L. J. G. (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals, *Chemosphere*, Vol. 79(9), 914-921. doi:10.1016/j.chemosphere.2010.03.024
- Giesy. J. P., Dobson. S., Solomon. K. R. (2000). Ecotoxicological risk assessment for roundup (R) herbicide, *Reviews of Environmental Contamination and Toxicology*, New York: Springer, 35-120. doi:10.1007/978-1-4612-1156-3_2
- Goodall. J. A., Blackshaw. A. W., Capra. M. F. (1989). Factors affecting the activation and duration of motility of the spermatozoa of the summer whiting (*Sillago ciliata*), *Aquaculture*, Vol. 77(2-3), 243-250. doi:10.1016/0044-8486(89)90206-8
- Harayashiki. C. A. Y., Varela. A. S., Machado. A. A. S., Cabrera. L. C., Primel. E. G., Bianchini. A., Corcini. C. D. (2013). Toxic effects of the herbicide Roundup in the guppy *Poecilia vivipara* acclimated to fresh water, *Aquatic Toxicology*, Vol.142-143, 176-184. doi:10.1016/j.aquatox.2013.08.006
- Krol. J., Glogowski. J., Demska-Zakes. K., Hliwa. P. (2006). Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period, *Czech Journal of Animal Science*, Vol.51 (5), 220-226. doi:10.17221/3932-CJAS
- Langiano. V. C., Martinez. C. B. R. (2008). Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*, *Comparative Biochemistry and Physiology, Part C*, Vol. 147, 222-231.
- Lopes. F. M., Varela Junior. A. S. V., Corcini. C. D., da Silva. A. C., Guazzelli. V. G., Tavares. G., da Rosa. C. E. (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*, *Aquatic Toxicology*, Vol.155, 322-326. doi:10.1016/j.aquatox.2014.07.006
- McKinnon. J. S., Liley. N. R. (1987). Asymmetric species specificity in responses to female sexual pheromone by males of two species of *Trichogaster* (Pisces: Belontiidae), *Canadian Journal of Zoology*, Vol.65 (5), 1129-1134. doi:10.1139/z87-176
- Pallas. P. S. (1770). *Spicilegia zoologica: Quibus novae imprimis et obscurae animalium species iconibus, descriptionibus atque commentariis illustrantur*. Berolini, prostant Apud Gottl. August, Lange, Vo.8, 1-56.
- Pérez. G. L., Vera. M. S., Miranda. L. (2011). Effects of herbicide glyphosate and glyphosate-based formulations on aquatic ecosystems. In A. Kortekamp (Ed.), *Herbicides and Environment*. Rijeka, Croatia: InTech Publications.
- Rurangwa. E., Kime. D. E., Ollevier. F., Nash. J. P. (2004). The measurement of sperm motility and factors affecting sperm quality in cultured fish, *Aquaculture*, Vol.234 (1-4), 1-28. doi:10.1016/j.aquaculture.2003.12.006
- Silveira. T., Varela Junior. A. S., Corcini. C. D. (2019). RoundupVR herbicide decreases quality parameters of spermatozoa of silversides *Odontesthes humensis*. *Bull Environ Contam Toxicol*, Vol.102 (1):1-6.

Solomon. K. R., Thompson. D. G. (2003). Ecological risk assessment for aquatic organisms from over-water uses of glyphosate, *Journal of Toxicology and Environmental Health. Part B, Critical Reviews*, Vol.6 (3), 289-324. doi:10.1080/10937400306468

Stenersen. J. (2004). *Chemical pesticides: Mode of action and toxicology*, CRC Press, BocaRaton p. 296. FL.

Struger. J., Thompson. D., Staznik. B., Martin. P., McDaniel. T., Marvin. C. (2008). Occurrence of glyphosate in surface waters of southern Ontario, *Bulletin of Environmental Contamination and Toxicology*, Vol.80 (4), 378-384. doi:10.1007/s00128-008-9373-1
