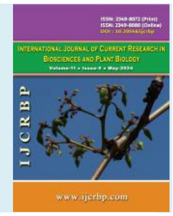
EXCELLENT PUBLISHERS

International Journal of Current Research in
Biosciences and Plant Biology

Volume **11** • Number **5** (May-2024) • ISSN: 2349-8080 (Online)

Journal homepage: www.ijcrbp.com



Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2024.1105.001

Glyphosate herbicide effects on biochemical composition of freshwater Cyclopoid copepods

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Article Info

Keywords:
Aquatic toxicology
Biochemical profiling
Cyclopoid copepods
Glyphosate

Abstract

Modern agricultural practices often employ herbicides, such as glyphosate, to manage pests. This study investigates the toxic effects of glyphosate on freshwater zooplankton, particularly Cyclopoid copepods. Using a range of concentrations (0.1–0.5 mg/ml) and exposure durations (30–180 minutes), the LC_{50} value was determined to be 0.042 mg/ml. Cyclopoid copepods exposed to a sub-lethal concentration (0.014 mg/ml) for 20 days showed significant biochemical alterations. Findings highlight the adverse effects of glyphosate on metabolic profiles and emphasize the need for monitoring herbicide impacts on aquatic ecosystems.

• *Received: 15 February 2024* • *Revised: 6 April 2024* • *Accepted: 26 April 2024* • *Published Online: 6 May 2024*

Introduction

Modern agricultural practices heavily depend on synthetic herbicides to mitigate pest-related crop losses. However, these herbicides pose significant risks to freshwater ecosystems, affecting both target and non-target organisms. Herbicides may exert direct effects, altering species-specific interactions, and indirect effects, impacting species not directly exposed. Recent studies have highlighted the detrimental effects of herbicides and pollutants on zooplankton populations, including Cyclopoid copepods, and other aquatic organisms (Choung et al., 2011). The presence of toxic chemicals and metals in water has degraded water quality, threatening aquatic biodiversity. Organo phosphates, widely used for pest control due to their high efficacy and low mammalian toxicity, have been shown to induce both lethal and sub-lethal effects on zooplankton (Altenburger et al., 2000).

Aquatic toxicology extensively explores the impacts of pollutants, such as herbicides, on aquatic organisms like zooplankton. The effects of herbicides span molecular interactions, tissue and organ damage, and disruptions at population and community levels (Choung et al., 2010). Glyphosate, a commonly used herbicide, is a focus in ecotoxicological research due to its persistence in aquatic environments and widespread use. Herbicides such as organophosphates and organo fluorines disrupt aquatic ecosystems by contaminating surface waters, adversely affecting growth, survival, and reproduction in aquatic organisms (Detenbeck et al., 1996).

Toxicity assessments often employ indicators like LD_{50} (lethal dose for 50% mortality) and LC_{50} (lethal concentration for 50% mortality) to measure herbicide impacts. Persistent contamination of freshwater ecosystems by herbicides leads to both direct and indirect effects on zooplankton, such as Cyclopoid copepods. Direct effects involve mortality or physiological disruptions, while indirect effects impact higher trophic levels by altering primary producer populations (Dewey, 1986; Hildebrand et al., 1982).

Cyclopoid copepods are crucial predators of mosquito larvae and play a vital role in mosquito control efforts (Fairchild et al., 1994). Zooplankton, small aquatic animals distributed across lakes and oceans, are essential components of freshwater ecosystems, facilitating energy transfer from primary producers to higher trophic levels, such as fish (Austin et al., 1991). These communities, often comprising over 20 species, are sensitive to toxic chemicals, including herbicides, which affect individuals, populations, and community structures (Baruah et al., 2018).

Cyclopoid copepods exhibit the highest protein levels among zooplankton species, underscoring their importance as a primary live feed source in aquaculture. Understanding their biochemical composition is essential for studying the ecological impacts of herbicide exposure. Protein levels were assessed using the Lowery method (2018), while lipid content was measured following Roe's methodology (2020). This study aims to evaluate the toxic effects of glyphosate herbicide on Cyclopoid copepods, focusing on mortality, biochemical changes, and their broader ecological implications.

Materials and methods

Zooplankton collection and herbicide preparation

Freshwater zooplankton specimens were collected from Chengalpattu Lake during early morning hours and transported to the laboratory under live conditions. Samples were acclimated to laboratory conditions before experimental use. Cyclopoid copepod species were sorted using a stereomicroscope and nourished with yeast as part of the experimental setup (Menon et al., 2020). For toxicity evaluation, 50 Cyclopoid copepod individuals were introduced into each of three replicate beakers containing glyphosate herbicide concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml. Incubation durations were set at 30, 60, 90, 120, 150, and 180 minutes to study the toxic effects of glyphosate on copepods. The lethal concentration (LC_{50}) was calculated, and the sub-lethal concentration was determined. Further, the biochemical profile (protein, lipid, and amino acid levels) was analyzed using standard methodologies (Ganapati, 2017).

Probit analysis

The percentage mortality was calculated and transformed into a probit scale for analysis.

Test for sub-lethal concentration

Experimental design

A 500-ml beaker was prepared with water containing glyphosate at a concentration of 0.5 µg/ml. Ten 25-ml beakers were set up, each filled with 20 ml of water, and divided into two groups: five for duplicate treatments and five as controls. Herbicide was added at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml.

Fig. 1: a) Chengalpattu Lake b) Zooplankton Net, c) Herbicide (Glyphosate), d) Sorting of Cyclopoid copepods

The mortality of Cyclopoid copepods was monitored at different time intervals (30, 60, 90, 120, 150, and 180 minutes). Mortality data were used to analyze sub-lethal concentrations and their impact on copepod survival (Jhingran, 2022).

1. Collect Cyclopoid copepod samples from the pond.
2. Separate Cyclopoid copepods using a stereomicroscope.
3. Expose copepods to various concentrations of glyphosate herbicide.

4. Calculate LC_{50} and determine sub-lethal concentrations.
5. Analyze biochemical profiles, including amino acid, protein, and lipid levels.

Results and discussion

Zooplankton mortality under herbicide exposure

The impact of herbicide exposure on the mortality of Cyclopoid copepods was evaluated using varying concentrations of glyphosate (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) over different time intervals (30, 60, 90, 120, 150, and 180 minutes). Fifty Cyclopoid copepods were used per treatment group to assess mortality rates.

Percentage mortality of cyclopoid copepods

The percentage mortality of Cyclopoid copepods at various herbicide concentrations and time intervals is summarized in Table 1. The mortality increased with herbicide concentration and exposure duration, as shown by the mean mortality percentages and standard deviations at each treatment level.

Table 1. Mortality of Cyclopoid Copepods treated with herbicide at different concentrations.

Herbicide Conc.	30 min (%)	60 min (%)	90 min (%)	120 min (%)	180 min (%)
500 ppm	0.0	0.0	15.56	43.33	67.78
1000 ppm	0.0	12.22	63.33	73.33	91.11
1500 ppm	18.89	58.89	100.0	67.78	100.0
2000 ppm	31.11	78.89	100.0	100.0	100.0
2500 ppm	64.44	100.0	100.0	100.0	100.0

Statistical analysis

Analysis of variance (ANOVA) demonstrated significant differences in mortality across concentrations and time intervals. Probit analysis was conducted to calculate lethal and sub-lethal concentrations, yielding an LC_{50} of 0.042 mg/ml at 180 minutes of exposure.

Biochemical profile

Protein estimation

Protein content was analyzed using Bradford's method, and the results indicated a significant reduction in protein levels in herbicide-exposed Cyclopoid copepods compared to control samples.

Table 2. Protein content in control and herbicide-treated samples.

Sample	Protein Content (mg/ml)
Control	0.859
Herbicide Sample 1	1.198
Herbicide Sample 2	1.370
Herbicide Sample 3	1.299

Lipid analysis

Lipid content was determined using Roe's method and revealed alterations in lipid profiles of copepods exposed to sub-lethal herbicide concentrations. Lipid extraction and analysis using gas chromatography confirmed the biochemical impact of glyphosate exposure.

Probit analysis

The probit model was applied to estimate the mortality probabilities at various herbicide concentrations and exposure times. The 95% confidence intervals for LC_{50} at 180 minutes are shown in Table 3.

Table 3. Probit Analysis for LC_{50} (180 min).

Probability	Herbicide Concentration (mg/ml)	Lower Bound	Upper Bound
50%	0.042	0.035	0.048

Conclusions

The study evaluated the impact of glyphosate herbicide on Cyclopoid copepods collected from Chengalpattu Lake. Mortality rates were concentration- and time-dependent, with an LC_{50} of 0.042 mg/ml after 180 minutes. Sub-lethal herbicide exposure induced significant changes in the biochemical profiles of copepods, notably in protein and lipid levels. Glyphosate herbicide poses a potential threat to Cyclopoid copepods by affecting their survival and biochemical composition. Despite observed changes, the overall ecological impact remains moderate. However, these findings underscore the importance of monitoring herbicide contamination in freshwater ecosystems and assessing its broader ecological consequences.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

The authors are thankful to Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chengalpattu.

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How to cite this article:
Karthikeyan, V., Abishek, N M., Uthamaraj, V., Sanjana, H., Akash, R., Anuradha, M., 2024. Glyphosate herbicide effects on biochemical composition of freshwater cyclopoid copepods. Int. J. Curr. Res. Biosci. Plant Biol., 11(5): 1-4. doi: https://doi.org/10.20546/ijcrbp.2024.1105.001