



TOXICOLOGICAL IMPACT OF GLYPHOSATE BASED HERBICIDE ON THE SURVIVAL RATE OF DROSOPHILA MELANOGASTER

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ABSTRACT

The fruit fly, scientifically known as Drosophila melanogaster, has been widely used as an important model organism for many years in biomedical and toxicological studies due to its genetic and physiological similarities to humans (Bilder & Irvine, 2017; Dos Santos & Cochemé, 2024). This study explores the toxicological impact of glyphosate-based herbicide exposure on D. melanogaster mortality. Flies were subjected to three different concentrations of glyphosate-based solution: 5 ml, 10 ml, and 20 ml. A dose-dependent increase in mortality was observed, with the 20 ml group exhibiting the highest lethality. These findings suggest that elevated concentrations of glyphosate-based herbicides may significantly compromise survival in D. melanogaster, highlighting potential risks associated with environmental exposure to such compounds.

Keywords *Drosophila melanogaster, model organism, toxicity, non target, mortality, Glyphosate-based herbicide*

1 INTRODUCTION

Drosophila melanogaster has been a key model organism in genetics, with Thomas Hunt Morgan's work in the 1910s establishing transmission genetics and later studies in the 1970s exploring genetic control of development and behavior (Roberts, 2006). It has been widely used as a model organism due to its short lifespan and high genetic similarity to mammals, making it valuable for metabolic and signal transduction studies (Staats et al., 2018). Additionally, its maintenance is cost-effective, and there are fewer ethical concerns compared to rodent models (Staats et al., 2018)

Higher organisms require macromolecules for metabolism, unlike microbes that synthesize biomass from elements. *Drosophila melanogaster* needs carbohydrates for energy, amino acids for nitrogen and sulfur, sterols (cholesterol/ergosterol), choline, inosine, uridine, myo-inositol, and B-group vitamins (B1,

B2, B3) for optimal growth and function. Artificial diets have been used to study its nutritional physiology, allowing precise control over nutrient composition (Piper, 2017).

2 METHODS AND METHODOLOGY

2.1 Stock preparation

Wild *Drosophila* flies were caught from the local environment, and a suitable medium was prepared for culturing them. The required materials—8.3 g of maize, 2.5 g of sucrose, 0.5 g of dextrose, 1.5 g of yeast, 1.8 g of agar-agar, and 100 mL of distilled water—were weighed and placed in a beaker. Distilled water (100 mL) was added, and the contents were thoroughly mixed. The mixture was heated on a medium flame until it achieved a paste-like consistency. The medium was sterilized and then it was left in an autoclave for 15 minutes at 121°C. After sterilization, the medium was allowed to cool, and 0.68 mL of orthophosphoric acid and 4 mL of propanoic acid were added to it. The mixture was thoroughly stirred to ensure uniform distribution of the chemicals (National Centre for Biological Sciences, 2017). Finally, the prepared medium was poured into sterilized glass bottles, which were sealed and stored under sterile conditions until further use for *Drosophila* culture.

2.2 Test Chemical

Glyphosate (Fig. 1) is a broad-spectrum, systemic herbicide commonly applied in both agricultural and non-agricultural environments. Its mode of action involves blocking the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is crucial for the shikimate pathway in plants and some microorganisms, disrupting the production of aromatic amino acids. While initially considered safe for non-target organisms, its pervasive presence in food, water, and air has raised environmental and health concerns (Kanissery *et al.*, 2019). Recent studies suggest glyphosate's role in oxidative stress, endocrine disruption, and neurological effects, alongside its impact on ecosystems by affecting plants, insects, and dependent species. (Van Bruggen *et al.*, 2018).

Chemical Name: N-(phosphonomethyl) glycine

- **Molecular Formula:** $C_3H_8NO_5P$

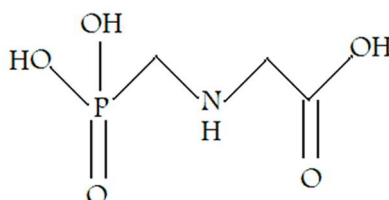


Figure 1: Molecular structure of glyphosate

The herbicide "Menaka 71," containing 71% SG ammonium salt of glyphosate, was used in this study due to its high glyphosate content and agricultural relevance. Its formulation includes 71% w/w ammonium salt of glyphosate, 12.5% w/w polyoxyethylene amine surfactant for enhanced efficacy, and ammonium sulfate as a stabilizing agent. This composition enables efficient herbicide delivery, making it ideal for evaluating glyphosate's toxicological impacts on non-target organisms like *Drosophila melanogaster*.

2.3 Dose preparation

A stock solution was prepared by dissolving 1.000 g of Menaka herbicide (71% glyphosate) in 100 mL of the medium. From this stock solution, doses were derived to achieve final conc. of 5 mL/L, 10 mL/L, and 20 mL/L respectively in the food medium (Fig 2, Table 1), with 0 mL/L serving as the control. These concentrations were selected based on the LC₅₀ values reported by Talyn *et al.* (2019) ensuring sub-lethal effects with minimal mortality during experimentation.

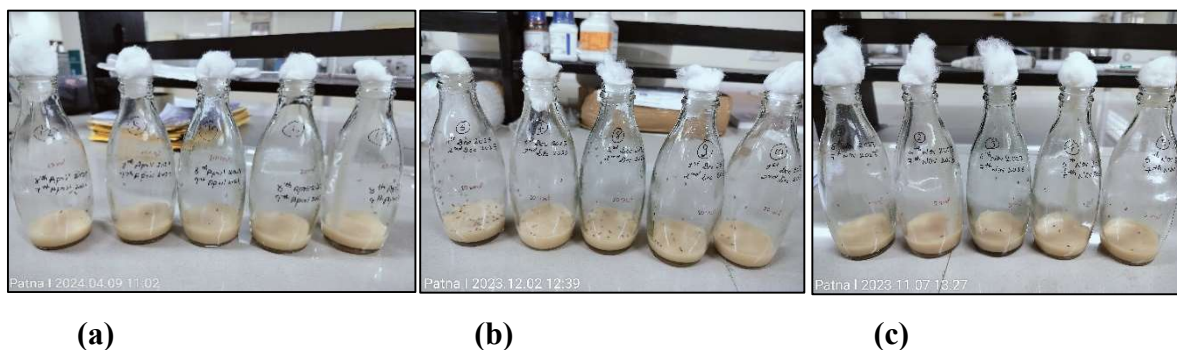


Figure 2: *Drosophila melanogaster* dosing bottles with different glyphosate concentrations – (a) 5ml, (b) 10ml and (c) 20ml

Group	Glyphosate Concentration (g/L)	Volume of Glyphosate Solution Added (mL)
Control	0.0	-
Treatment 1	0.5	5ml
Treatment 2	1.0	10ml
Treatment 3	2.0	20ml

Table 1. Preparation of different concentrations of glyphosate from the stock solution for the experimental groups. The control group received no glyphosate, while Treatment 1, Treatment 2, and Treatment 3 received glyphosate concentrations of 0.5 g/L, 1.0 g/L, and 2.0 g/L, respectively.

RESULTS

The impact of glyphosate-based herbicide on the mortality of *Drosophila melanogaster* was evaluated over a 15-day period using three different concentrations: 0.5 g/L (low dose), 1.0 g/L (medium dose), and 2.0 g/L (high dose). Each treatment group included approximately 55–60 flies. No mortality was recorded during the initial phase of exposure; however, deaths began to appear after Day 10 and progressively increased toward Day 15. The following table presents the cumulative number of deaths observed each day across the three treatment groups.

Day	0.5 g/L (55 flies)	1.0 g/L (58 flies)	2.0 g/L (60 flies)
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	1	1
6	0	1	1
7	0	2	2
8	0	2	3
9	1	3	4
10	1	4	5
11	2	6	7
12	3	10	10
13	4	13	13
14	6	14	15
15	7	15	17

Table 2. Cumulative mortality of *Drosophila melanogaster* over a 15-day exposure period to three different concentrations of glyphosate-based herbicide solutions (0.5 g/L, 1.0 g/L, and 2.0 g/L).

Following the 15-day exposure period, the total number of deaths in each group was used to calculate the mortality rate using the formula:

$$\text{Mortality Rate (\%)} = (\text{Number of Deaths} \div \text{Total Number of Flies}) \times 100$$

- 0.5 g/L: 7 deaths out of 55 flies $\rightarrow (7 \div 55) \times 100 = 12.73\%$
- 1.0 g/L: 15 deaths out of 58 flies $\rightarrow (15 \div 58) \times 100 = 25.86\%$
- 2.0 g/L: 17 deaths out of 60 flies $\rightarrow (17 \div 60) \times 100 = 28.33\%$

These findings indicate a clear dose-dependent increase in mortality, with higher concentrations of glyphosate resulting in greater fly mortality.

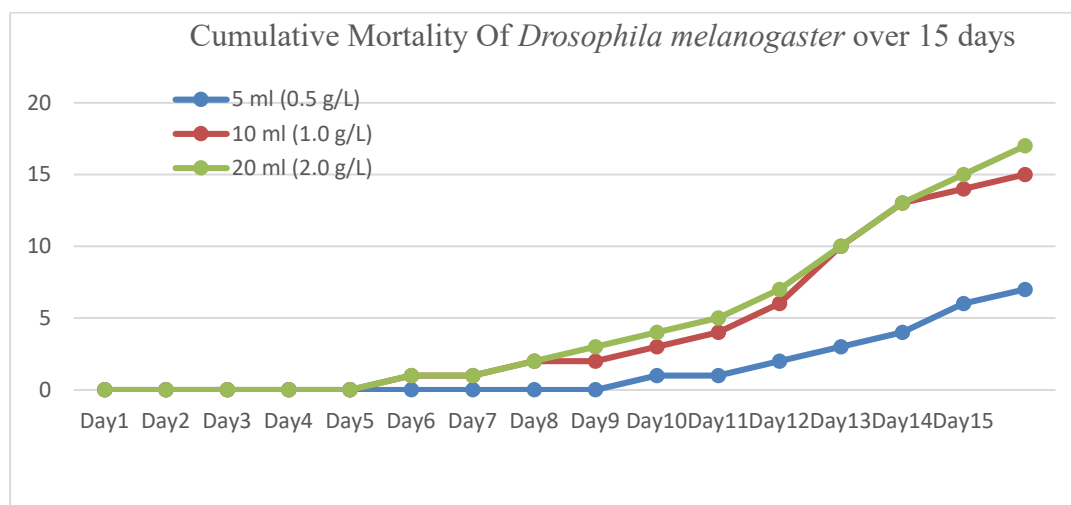


Figure 3. Line graph depicting cumulative mortality of *Drosophila melanogaster* over a 15-day period under exposure to three concentrations of glyphosate-based herbicide (0.5 g/L, 1.0 g/L, and 2.0 g/L respectively). Mortality began to increase notably after Day 10, with the highest cumulative deaths observed in the 2.0 g/L treatment group, indicating a concentration-dependent effect.

DISCUSSION

The findings of this study indicate a clear dose-dependent increase in mortality among *Drosophila melanogaster* exposed to glyphosate-based herbicide formulations. Mortality remained negligible during the initial exposure period but showed a marked increase after Day 10, with observed rates of 12.73% at 0.5 g/L, 25.86% at 1.0 g/L, and 28.33% at 2.0 g/L. This progressive rise in mortality suggests a cumulative toxic effect over time. These outcomes are consistent with those of R. R. Galin et al. (2019), who reported decreased lifespan and reduced progeny count in *D. melanogaster* at a concentration of 2.8 mg/ml of glyphosate. Such parallel findings reinforce the hypothesis that even low concentrations of glyphosate can compromise survival and developmental viability in fruit flies.

Beyond *Drosophila*, glyphosate has demonstrated toxic effects across a range of non-target species, emphasizing its ecological implications. Benamú et al. (2010) identified sublethal effects in spiders, including altered prey consumption and reproductive performance. Lucas Battisti et al. (2021) documented increased bee mortality across different life stages and exposure methods, while Rodríguez et al. (2021) revealed endocrine disruptions in crabs, such as decreased sperm count and oocyte reabsorption. These examples collectively suggest that glyphosate-based herbicides, even within recommended agricultural doses, may interfere with fundamental biological processes in non-target organisms. Given the neurological basis of climbing behavior in *Drosophila*, the observed impairments may result from neurotoxic or metabolic disruptions, as similarly proposed by Virginia Moser et al. (2022) in mammalian systems. Future investigations should aim to dissect the underlying physiological pathways involved, thereby deepening our understanding of glyphosate's broader ecological and biological impacts.

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