International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 26-05-2026, Accepted: 25-06-2026, Published: 10-07-2026
Volume 11, Issue 3, 2026, Page No. 53-57

Evaluation of locomotor impairment in drosophila melanogaster following exposure to glyphosate-based herbicide

Ayesha Kaushar[1*], Reshma Sinha[2], Akanchha[1], Diksha Bhanu[1], Neha Manjari[1]
[1] Research Scholar, Department of Zoology, Patliputra University, Patna, Bihar, India
[2] Assistant Professor, Department of Zoology, JD Women's College, Patliputra University, Patna, Bihar, India
Corresponding Author: Ayesha Kaushar
DOI: https://doi.org/10.66856/ijer.2026.11.3.11231

Abstract
Glyphosate-based herbicides (GBHs) are widely used in agriculture and may affect non-target organisms. *Drosophila melanogaster* is a well-established model organism for studying chemical-induced behavioural alterations. The present study evaluated the effect of GBH exposure on locomotor behaviour of *Drosophila melanogaster*.

Adult flies were exposed to different concentrations of GBH (5%, 10%, and 20%) through GBH treated culture medium for 7 days. Locomotor activity was assessed using a manual grid-based assay by recording the number of grid squares crossed during the observation period. The results showed a concentration-dependent decline in locomotor activity following GBH exposure. The 5% treated group showed a slight reduction in movement, whereas significant impairment was observed at higher concentrations, with the maximum reduction recorded in the 20% treated group. One-way ANOVA revealed significant differences among the experimental groups.

The findings suggest that exposure to GBH adversely affects locomotor behaviour and motor performance in *Drosophila melanogaster*. This study highlights the usefulness of *Drosophila* as a model for assessing behavioural toxicity associated with pesticide exposure.

Keywords: *Drosophila melanogaster*, GBH, locomotor activity, behavioural toxicity, pesticide exposure

Introduction

Glyphosate is one of the most widely used broad-spectrum herbicides in modern agriculture due to its effective weed-control properties (Duke and Powles, 2008) [3]. The extensive application of GBH formulations has increased the possibility of their exposure to non-target organisms through environmental contamination (Van Bruggen *et al*., 2018) [11]. Glyphosate primarily acts by inhibiting the shikimate pathway enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is absent in animals; however, several studies have reported that glyphosate-based formulations may produce toxic effects through oxidative stress, cellular damage, and disruption of physiological processes (Mesnage *et al*., 2015; Van Bruggen *et al*., 2018) [5, 11].

The nervous system is considered a sensitive target for environmental contaminants because neuronal function depends on proper regulation of neurotransmission, cellular metabolism, and oxidative balance. Previous studies have suggested that GBH may affect neuronal function by inducing oxidative stress and altering neurotransmitter-related pathways (Cattani *et al*., 2014) [1]. These findings highlight the need to investigate the possible neurobehavioral effects associated with glyphosate exposure.

Among these behavioural endpoints, locomotor activity is considered a sensitive indicator of neurotoxic effects in Drosophila. Locomotor activity is a commonly used behavioural parameter in *Drosophila melanogaster* for evaluating changes in motor performance and nervous system function, as movement involves coordinated activity of neuronal circuits and muscular systems (Martin, 2004) [4].

Drosophila melanogaster is a widely used model organism in neurobiology and toxicology due to its short life cycle, genetic advantages, and conserved molecular pathways involved in nervous system function (Pandey and Nichols, 2011) [6]. Behavioural assays in *Drosophila* provide a reliable approach for assessing chemical-induced alterations in neurological performance.

Previous studies have demonstrated that exposure to environmental toxicants can alter locomotor behaviour and motor performance in *Drosophila*. GBH exposure has also been reported to produce toxic effects in *Drosophila melanogaster*, including concentration-dependent mortality responses (Talyn *et al*., 2019) [10]. However, the effects of GBH on locomotor behaviour remain comparatively less explored. Therefore, the present study was conducted to evaluate the effect of GBH exposure on locomotor behaviour of *Drosophila melanogaster* and to assess concentration-dependent changes associated with neurobehavioral performance.

Materials and methods

1. Experimental Organism and Maintenance

The fruit fly, *Drosophila melanogaster*, was selected as an experimental model organism for the present study to evaluate GBH-induced alterations in locomotor behaviour. *Drosophila melanogaster* is extensively used in toxicological and neurobiological investigations due to its short life cycle, ease of maintenance, high reproductive capacity, and conservation of several molecular pathways associated with human biological functions (Pandey and Nichols, 2011) [6].

The *Drosophila melanogaster* culture was maintained under controlled laboratory conditions. The flies were reared at 25

± 1°C temperature, 60–70% relative humidity, and a 12:12 h light–dark cycle. Adult flies were maintained in sterilized culture bottles containing freshly prepared culture medium. Newly emerged adult flies of similar age were selected for experimental exposure to minimize age-related variation.

2. Preparation of *Drosophila melanogaster* Culture Medium

A standard cornmeal-based medium was prepared for maintenance and rearing of *Drosophila melanogaster*. The composition of the culture medium is presented in Table 1.

Table 1: Composition of culture medium used for maintenance of *Drosophila melanogaster*

Ingredient	Quantity	Function
Maize flour	8.3 g	Nutritional source and larval growth support
Sucrose	2.5 g	Energy source
Dextrose	0.5 g	Readily available carbohydrate source
Yeast	1.5 g	Source of proteins, vitamins and amino acids
Agar-agar	1.8 g	Solidifying agent
Distilled water	100 mL	Solvent
Orthophosphoric acid	0.68 mL	pH regulation and contamination control
Propanoic acid	4.0 mL	Prevention of fungal contamination

All dry ingredients were mixed thoroughly with distilled water and heated until a uniform semi-solid consistency was obtained. After cooling, orthophosphoric acid and propanoic acid were added, and the prepared medium was transferred into sterile culture bottles. The medium was used for routine maintenance of fly cultures and preparation of experimental exposure groups. Standardized nutritional conditions are essential for obtaining reproducible growth and behavioural responses in *Drosophila* experiments (Piper *et al*., 2014) [7].

3. Glyphosate-Based Herbicide and Dose Preparation

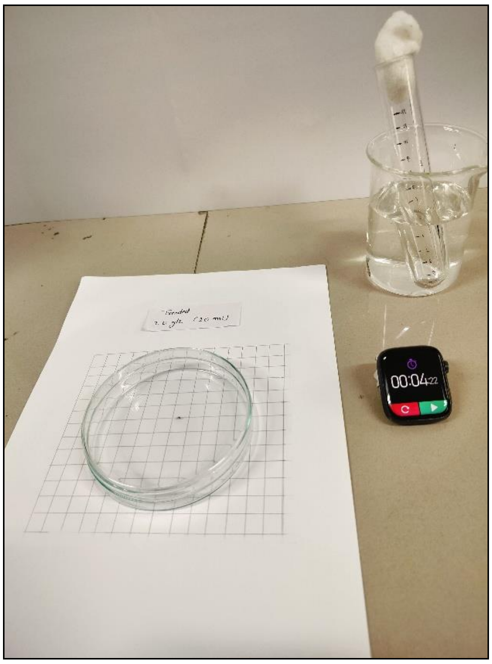
A commercial glyphosate-based herbicide formulation, Menaka 71 (71% glyphosate) manufactured by Crystal Crop Protection Pvt. Ltd., was used for experimental exposure. Commercial formulations were selected because environmental organisms are generally exposed to formulated herbicide products rather than pure glyphosate.

The stock solution was prepared by accurately weighing 1.000 g of GBH and dissolving it in 100 mL distilled water. The solution was mixed thoroughly to obtain a uniform preparation.

The treatment levels used in the present study were selected based on previously reported sub-lethal exposure studies demonstrating behavioural alterations while maintaining adequate survival of *Drosophila melanogaster* (Talyn *et al*., 2019) [10]. The prepared stock solution was subsequently used for the preparation of experimental treatment media. For preparation of treatment media, 5 mL, 10 mL, and 20 mL of stock solution were separately added to 100 mL of freshly prepared *Drosophila* culture medium, representing increasing levels of herbicide exposure. Medium without herbicide addition was maintained as the control group. The details of experimental groups are presented in Table 2.

Table 2: Preparation of Dose

Experimental group	Volume of stock solution added (per 100 mL medium)	Exposure level
Control	0 mL	Herbicide-free medium
Treatment I	5 mL	Low exposure
Treatment II	10 mL	Moderate exposure
Treatment III	20 mL	High exposure



All prepared treatment media were mixed thoroughly to ensure uniform distribution of the herbicide before introducing flies.

4. Experimental Design and Herbicide Exposure

Newly emerged adult *Drosophila melanogaster* flies were randomly divided into control and treatment groups. Flies were transferred into culture bottles containing either untreated medium or GBH medium. Each experimental group was maintained under identical laboratory conditions.

The flies were exposed to three different GBH treatment levels for 7 days. Following the exposure period, locomotor activity was assessed to determine the effect of herbicide exposure on motor performance.

5. Locomotor Activity Assay

Locomotor activity was assessed to evaluate the effect of GBH exposure on spontaneous movement and motor coordination of *Drosophila melanogaster*. The assay was performed using a manual grid-based method following previously described behavioural assessment approaches in *Drosophila* (Martin, 2004) [4].

After completion of the 7-day exposure period, adult flies from control and GBH-treated groups were used for locomotor assessment. A clean Petri dish was placed over a white background marked with a grid pattern consisting of 1 cm × 1 cm squares. Individual flies were gently transferred into the Petri dish without the use of chemical anaesthesia to avoid possible alterations in normal behavioural activity.

A representative image of the locomotor activity assay setup is shown in Figure 1. The assay was performed by observing individual flies on a grid-marked Petri dish, where locomotor performance was assessed based on the number of grid squares crossed during a fixed observation period. The schematic representation illustrates the principle of grid-based movement measurement used for evaluating locomotor activity.

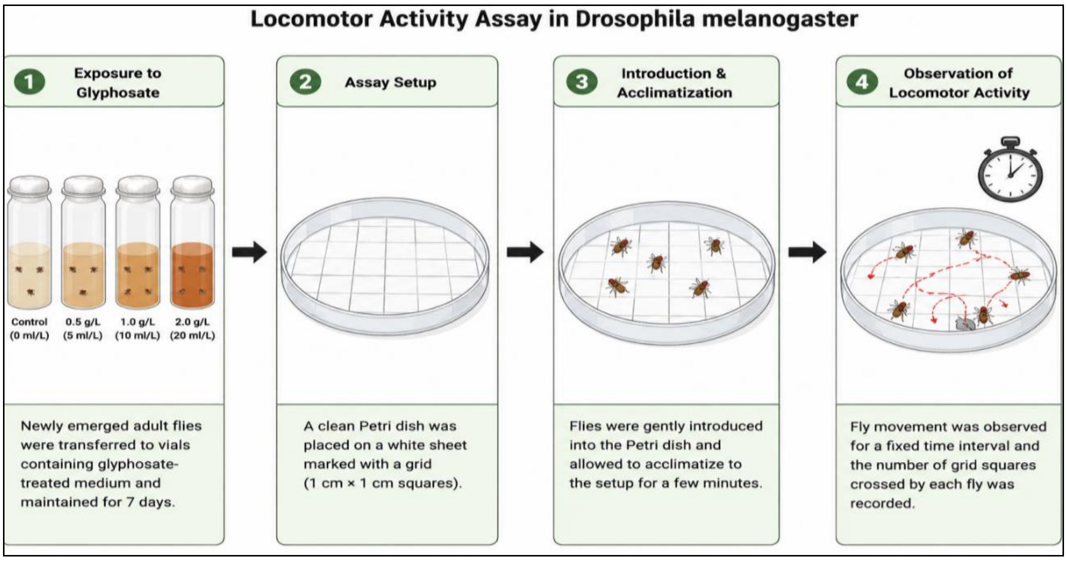


Fig 1: Representative image of manual grid-based locomotor activity assay in *Drosophila melanogaster*. (A) Original photograph showing adult flies on a grid-marked Petri dish used for locomotor assessment. (B) Schematic representation of the locomotor assay showing grid-based measurement of movement activity**.**

Following acclimatization, the movement of flies was recorded for a fixed observation period. The number of grid squares crossed by each fly was manually counted. Locomotor activity was calculated as the number of grid crossings per unit time using the following formula:

$$\text{Locomotor Activity} = \frac{\text{Number of grid squares crossed}}{\text{Observation time (minutes)}}$$

The mean locomotor activity was calculated for each experimental group. A reduction in locomotor activity in GBH-treated groups compared with control flies was considered indicative of impaired movement behaviour and altered motor performance.

6. Statistical Analysis

The experimental data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a Tukey's HSD post hoc test. Differences among groups were considered statistically significant at $p < 0.05$.

Results

1. Effect of Glyphosate-Based Herbicide on Locomotor Activity of *Drosophila melanogaster*

The effect of GBH exposure on locomotor behaviour of *Drosophila melanogaster* was evaluated using a manual grid-based locomotor assay. The locomotor activity of control and GBH-treated groups is presented in Fig 2.

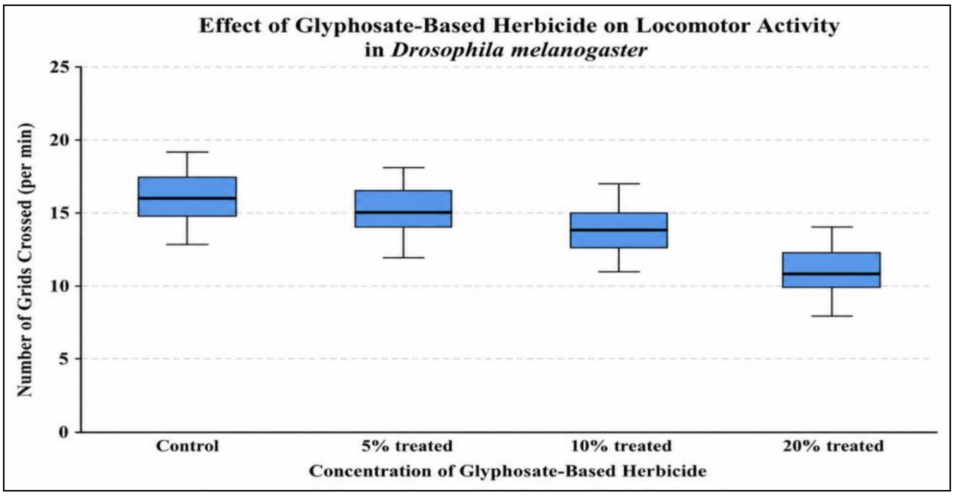


Fig 2: Effect of different concentrations of glyphosate-based herbicide on locomotor activity of *Drosophila melanogaster*.

The control group exhibited the highest locomotor activity, indicating normal spontaneous movement and motor coordination. In contrast, flies exposed to GBH showed a progressive reduction in locomotor performance with increasing herbicide concentration.

The 5% GBH-treated group showed a slight decrease in locomotor activity compared with the control group; however, the reduction was not statistically significant. A further decline in movement was observed in the 10% treated group, indicating increased impairment of locomotor behaviour. The 20% GBH-treated group exhibited the lowest locomotor activity among all experimental groups, demonstrating maximum impairment of spontaneous movement.

The overall distribution pattern of locomotor activity showed a concentration-dependent decline in movement ability following GBH exposure. These findings indicate that increasing glyphosate exposure negatively affects locomotor behaviour and may result in altered neuromuscular performance in *Drosophila melanogaster*.

2. Statistical Analysis of Locomotor Activity

The statistical significance of differences in locomotor activity among control and GBH-treated groups was analysed using one-way analysis of variance (ANOVA). The analysis revealed a highly significant effect of GBH exposure on locomotor activity ($p < 0.001$), indicating significant variation among the experimental groups.

Table 3: One-way ANOVA analysis of locomotor activity in control and GBH-treated groups

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	181.917	3	60.639	20.424	<0.001
Within Groups	166.267	56	2.969	–	–
Total	348.183	59	–	–	–

The statistical analysis demonstrated a significant difference in locomotor activity among the experimental groups. The observed reduction in movement activity with increasing GBH concentration indicates a concentration-dependent impairment of locomotor behaviour in *Drosophila melanogaster*.

Discussion

Locomotor activity is a sensitive behavioural parameter used to evaluate motor coordination and neurological alterations in *Drosophila melanogaster*. In the present study, GBH exposure resulted in a concentration-dependent reduction in locomotor activity. Control flies showed maximum movement performance, whereas GBH-treated groups exhibited reduced locomotor activity, with the highest impairment observed at the 20% concentration.

The observed decline in locomotion suggests that prolonged exposure to GBH may interfere with normal neuromuscular function. Similar findings have been reported in *Drosophila*, where GBH exposure induced physiological stress responses and altered antioxidant defence mechanisms (de Aguiar *et al*., 2016) [2]. These cellular disturbances may contribute to impaired motor performance and behavioural alterations.

The significant reduction in locomotor activity at higher concentrations indicates a dose-dependent toxic response. Previous studies have also reported adverse effects of glyphosate formulations on *Drosophila* physiology and organismal fitness (Strilbytska *et al*., 2022) [9]. Thus, the present findings suggest that GBH exposure negatively affects locomotor behaviour and highlights the potential neurobehavioral toxicity of GBH.

Conclusions

The present study demonstrates that exposure to GBH causes a concentration-dependent decline in locomotor activity of *Drosophila melanogaster*. While low-level exposure produced comparatively minor changes, higher herbicide concentrations resulted in significant impairment of movement performance. These findings indicate that glyphosate-based herbicide exposure can adversely affect behavioural responses and motor coordination in *Drosophila*. The study further supports the usefulness of *Drosophila melanogaster* as an effective model organism for evaluating pesticide-induced behavioural toxicity and provides insight into the potential effects of GBH on non-target organisms.

Acknowledgment

I extend my sincere gratitude to Dr. Sumeet Ranjan from the Central Laboratory at PWC for his technical support and access to laboratory facilities. This research was conducted without external funding, with all aspects self-supported in pursuit of scientific contribution.

References

1. Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI, *et al*. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: Involvement of glutamate excitotoxicity. Toxicology,2014:320:34-45. https://doi.org/10.1016/j.tox.2014.03.001
2. de Aguiar LM, Figueira FH, Gottschalk MS, da Rosa CE. Glyphosate-based herbicide exposure causes antioxidant defence responses in the fruit fly *Drosophila melanogaster*. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,2016:185-186:94-101. https://doi.org/10.1016/j.cbpc.2016.03.006
3. Duke SO, Powles SB. Glyphosate: A once-in-a-century herbicide. Pest Management Science,2008:64(4):319-325. https://doi.org/10.1002/ps.1518
4. Martin JR. A portrait of locomotor behaviour in *Drosophila melanogaster*. Behavioural Processes,2004:67(2):207-219. https://doi.org/10.1016/j.beproc.2004.04.003
5. Mesnage R, Defarge N, Spiroux de Vendômois J, Séralini GE. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food and Chemical Toxicology,2015:84:133-153. https://doi.org/10.1016/j.fct.2015.08.012
6. Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacological Reviews,2011:63(2):411-436. https://doi.org/10.1124/pr.110.003293
7. Piper MDW, Blanc E, Leitão-Gonçalves R, Yang M, He X, Linford NJ, *et al*. A holidic medium for *Drosophila melanogaster*. Nature

Methods,2014:11:100-105.
https://doi.org/10.1038/nmeth.2731

8. Rand MD. Drosophotoxicology: The growing potential for *Drosophila* in neurotoxicology. Neurotoxicology,2010:31(5):577-586. https://doi.org/10.1016/j.neuro.2010.04.002
9. Strilbytska OM, Semaniuk UV, Strutynska TR, Burdyliuk NI, Tsiumpala S, Bubalo V, *et al*. Herbicide Roundup shows toxic effects in nontarget organism *Drosophila*. Archives of Insect Biochemistry and Physiology,2022:110(4):e21893. https://doi.org/10.1002/arch.21893
10. Talyn BC, Lemon RL, Badoella MC, Melchiorre DM, Villalobos MJ, Elias RC, *et al*. Roundup®, but not Roundup Ready® corn, increases mortality of *Drosophila melanogaster*. Toxics,2019:7(3):38. https://doi.org/10.3390/toxics7030038
11. Van Bruggen AHC, He MM, Shin K, Mai V, Jeong KC, Finckh MR, *et al*. Environmental and health effects of the herbicide glyphosate. Science of the Total Environment,2018:616-617:255-268. https://doi.org/10.1016/j.scitotenv.2017.10.309