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Research paper

EVALUATION OF RESIDUAL EFFECTS OF PESTICIDES ON FRUIT FLIES (*Drosophila melanogaster*)

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ABSTRACT

Pesticides are used worldwide to control agricultural pests, but their non-target effects on beneficial organisms remain a growing environmental concern. This study evaluates the residual effects of four commonly used pesticides on fruit flies (*Drosophila melanogaster*) as a model non-target organism. Adult flies were exposed to each pesticide at a 1-10 mmol concentration under controlled conditions, with three replicates per treatment. Acute toxicity, fecundity eclosion and enzyme activities such as catalase (CAT) and superoxide dismutase (SOD) were measured to assess physiological stress and toxicity using residual film contact method. The results showed that all pesticides induced varying levels of toxicity compared to the untreated control. Lambda-cyhalothrin with the steepest slope (3.45), was most rapid n causing mortality over time with the LC_{50} value of 4.18 mmol at 72h, 5.23 mmol at 48h and 7.45 mmol at 24h and LC_{90} of 7.05 mmol at 72h, 8.92 mmol at 48h and 10 mmol at 24h. Glyphosate was the least with LC_{50} of 5.12 mmol at 72h, 7.83 mmol at 48h and 10 mmol at 24h. Imidacloprid caused the highest reduction in the number eggs laid per female with the mean value of 11.67±4.48 while the least was paraquat (22±5.49). There was no significant difference across the treatment groups ($F = 0.825$, $df = 4$, $P > 0.539$). Imidacloprid was the most toxic against fruit flies eclosion with 100±0.00 % mortality and 0.00 ±0.00% eclosion while paraquat was the least with 81.67±10.93% mortality and 18.67±10.93% eclosion with observed significant difference across the treatment groups ($F = 7.554$, $df = 4$, $P < 0.005$). Glyphosate induced the highest oxidative stress with the SOD and catalase activities of 253.67±82.3 and 215.8±92.9 while the least was lambda-cyhalothrin (742.57±255.6 and 355.4±40.3), respectively. Therefore, there was a significant difference in the SOD activity across the treatment group ($F = 39.021$, $df = 4$, $P<0.001$) likewise catalase activities ($F = 7.641$, $df = 4$, $P<0.004$). These findings indicate that even pesticides intended for specific pests can adversely affect non-target organisms. Therefore, careful selection and use of pesticides are essential to reduced ecological risks and maintain environmental balance.

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Introduction

Widespread application of herbicides and insecticides in agriculture has resulted in their ubiquitous presence in the environment, causing inadvertent exposure for non-target organisms (Sharma et al., 2019). This is especially important for invertebrates, which are very important for keeping ecosystems stable (Chagnon et al., 2015). A vital measure in comprehending the ecological impacts of these chemicals is to perform comprehensive residual effects assessments for each pesticide individually. These effects are crucial for pinpointing specific hazards and mechanisms of action, irrespective of comparative analyses with other

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substances. Paraquat chloride is a bipyridyl herbicide that is very poisonous to both mammals and insects, its main way of working is through redox cycling, which causes a lot of oxidative stress and reactive oxygen species (ROS) (Dinis-Oliveira et al., 2008). This makes it a classic model for causing Parkinson's disease-like symptoms in lab animals like *Drosophila melanogaster* (Dinis-Oliveira et al., 2008). Glyphosate is one of the most commonly used herbicides worldwide, it works by blocking the EPSPS enzyme in the plant shikimate pathway (Van Bruggen et al., 2018). Even though this pathway doesn't exist in animals, there is more and more evidence that it could be bad for non-target species, like causing oxidative stress and possibly neurotoxicity (Van Bruggen et al., 2018).

Lambda-Cyhalothrin, a pyrethroid insecticide, is a neurotoxin that works by changing voltage-gated sodium channels, which makes neurons stay depolarized and hyperexcited for a long time (Soderlund, 2012). Its residue can harm beneficial insects, causing behavioral problems that aren't deadly to them (Pisa et al., 2017). Imidacloprid is a neonicotinoid) is an insecticide which functions as an agonist of nicotinic acetylcholine receptors (nAChRs), resulting to excessive stimulation of the insect central nervous system (Jeschke et al., 2011). There is a lot of evidence that it causes sublethal effects in pollinators, affecting things like learning and memory (Tosi et al., 2022). The model organism *Drosophila melanogaster* is particularly well-suited for this study because of its clearly defined genetics, short generation time, and the high level of conservation in its neurological and stress-response pathways compared to other animals (Jennings, 2011). Its validated behavioural assays offer precise indicators for identifying sublethal toxicity (Pandey & Nichols, 2011). This study was conducted to assess the residual effects of two herbicides (paraquat chloride and glyphosate) and two insecticides (imidacloprid and lambda-cyhalothrin), each exhibiting a distinct mode of action using the model organism, *Drosophila melanogaster*.

Materials and Methods

Study Area

The study was conducted in *Drosophila* laboratory, Department of Biochemistry, Federal University of Lafia, Nasarawa State, Nigeria from August to October, 2025.

Insect Culture

Wild-type *Drosophila melanogaster* (Harwich strain) were maintained on a sugar–yeast medium consisting of 25 g of sugar, 25 g of yeast, 7.5 g of agar–agar, 1 g of Nipagin, and 1,000 mL of distilled water, following Markow and O'Grady (2006). The cultures were maintained under a 12-h light/12-h dark cycle at 25 °C in the Biochemistry and Drosophila Laboratory, Department of Biochemistry, Federal University of Lafia, Nasarawa State, Nigeria.

The pesticides used in the study were Imiforce (imidacloprid, 200 g/L SL), Lambat (lambda-cyhalothrin, 2.5% EC), Chase (glyphosate, 360 g/L), and Parabest (paraquat dichloride, 276 g/L, equivalent to 200 g/L of paraquat ion).

Dilution Procedure of Pesticide Using 10 mL of Distilled Water

In agricultural, public, and private settings, insecticides and herbicides are commonly used as commercial formulations containing active ingredients and other components, rather than as chemically pure active ingredients. Although manufacturers may not disclose the full composition of these formulations, commercially available products were used in this study to improve the ecological relevance of the findings. The volume of commercial formulation required to prepare the desired working concentration was calculated using the dilution equation:

$V_1 = (C_2 \times V_2)/C_1$ (NIST, 2000)

Where:

C_2 = Final Concentration

V_2 =Volume of Solvent required (ml)

C_1 = Initial Concentration

V_1 = Initial mass required (g)

Lambda-cyhalothrin

Lambda-cyhalothrin (2.5% EC; 25 mg/mL active ingredient; molecular weight = 449.85 g/mol) was diluted to obtain the required molar concentrations. A volume of 180 µL of the commercial formulation was used to prepare 1 mmol/L solution in 10 mL distilled water. Higher concentrations (2–10 mmol/L) were obtained by proportional dilution.

Imidacloprid

Imidacloprid (200 g/L SL; molecular weight = 255.66 g/mol) stock solution corresponded to 0.782 M. A volume of 12.8 µL of stock solution was diluted in 10 mL distilled water to obtain 1 mmol/L working solution. Serial proportional dilutions were used to obtain 2–10 mmol/L concentrations.

Paraquat dichloride

Paraquat dichloride (276 g/L formulation; molecular weight = 257.16 g/mol) had an equivalent stock molarity of 0.778 M. A volume of 12.9 µL stock solution was diluted in 10 mL distilled water to obtain 1 mmol/L solution, with higher concentrations prepared proportionally.

Glyphosate

Glyphosate (360 g/L; molecular weight = 228.19 g/mol) stock solution corresponded to 1.58 M. A volume of 6.3 µL of stock solution was diluted in 10 mL distilled water to prepare 1 mmol/L solution. Concentrations of 2–10 mmol/L were prepared by proportional dilution.

For all pesticides, working concentrations (1–10 mmol/L) were prepared fresh prior to each experiment by appropriate dilution of stock solutions. All solutions were thoroughly mixed before use

Residual Effects Assay

Toxicity experiments were carried out using residual contact method of Plapp et al. (1987), with little modification of 2 ml of diet, 10 ml volume glass test tube was used, test tube was washed using ethanol. 1-10 mmol. of the lambda-cyhalothrin 2.5% EC, imidacloprid 200 g/L SL, glyphosate 360 g/ltr and Paraquat chloride 276 g/SL was measured into the glass test tube and was top up with distilled water to 10ml (treatment group). A glass test tube was filled with 10ml of distilled water (control group). Treated test tubes was rolled until the chemical was coated and evaporate completely. A 2 ml of diet was added into the vials and wait till it became solid, ten (10) adult fruit flies *Drosophila melanogaster* was introduced to each test tube and the test tube was cover with form plug to prevent the flies from flying out. Three replicates were used for each concentration. Both the treated and control test tubes was placed in the same environmental condition. Mortality was recorded after 24, 48 and 72 hours, respectively. The average percentage of adult mortality was calculated for each concentration. The LC_{50} and LC_{90} was calculated according to Finney (1971) and two (2) mmol concentration was used for Fecundity test, Eclosion Assay and antioxidant stress markers in three replicates.

Fecundity Assay

Procedure for Preparing an Egg Laying Plate

Agar-agar (12.5 g) was added to 250 mL of cold water and stirred to mix. The mixture was heated to boiling and stirred continuously for approximately 2 min to ensure that the agar-agar dissolved completely. Red grape juice (150 mL) was then added, and the mixture was stirred until it returned to a boil. The mixture was removed from the heat, and 25 mL of cold water was added. The solution was stirred until its temperature decreased to approximately 65 °C, after which 10.5 mL of 10% Nipagin solution was added and mixed thoroughly. The prepared medium was poured into Petri dishes and allowed to cool to room temperature, with the dishes left uncovered during cooling to allow steam to escape. Once the medium had solidified and cooled, the Petri dishes were covered and stored at 4 °C until use.

Procedure for determining Egg-laying Ability of Female Fruit Fly:

Ten (10) three-day-old female flies were transferred from the treatment test tubes into individual egg-laying chambers. A small amount of yeast paste was smeared onto the agar plates to stimulate egg laying. The experimental setup was maintained at room temperature for 24 h, after which the number of eggs laid on each plate was counted under a dissecting microscope. The egg-laying plates were then replaced with fresh plates, and egg collection continued at 24-h intervals as required by the experimental design. Fecundity was determined by dividing the total number of eggs laid by the number of female flies in each chamber, and the result was expressed as the mean number of eggs laid per female.

Developmental: Eclosion Assay

Procedure

A fresh grape plate was placed a on an egg-laying cage, female flies were transferred into the cage for 24 hour and egg was laid, the egg cages was incubated at 25˚C for 18 hours, a lid was placed on the plate and incubate, the embryos was age for 24 hours and a full plate of 1st instar (L1) larva was established, 20 L1 was transferred into each treatment test tubes, all flies that eclosed was counted, and recorded.

Oxidative stress Assay: Measuring Activity of Superoxide Dismutase and Catalase Antioxidant Enzymes

Procedure

Preparation of the fly lysate

Twenty-five (25) male *Drosophila melanogaster* flies were transferred into a microcentrifuge tube, and 0.5 mL of phosphate-buffered saline (PBS) was added. The flies were homogenized using a homogenizer, and the resulting homogenate was centrifuged at 10,000 rpm for 15 min. The supernatant was carefully collected and used for subsequent analyses of superoxide dismutase (SOD) and catalase (CAT) activities.

Determination of Superoxide Dismutase (SOD)Activity

Step1: Determination of autoxidation rate (blank)

A measurement of 2.35 ml of Tris-HCl buffer was mixed with 2.00 ml of distilled water into a 10 ml test tube, 0.15 ml pyrogallol solution was added into the test tube and vortexed immediately. An aliquot was taken from the test tube for absorbance measurement at 325 nm, another aliquot was taken again after 1 min and absorbances was measured. The difference in the absorbance between the two aliquots, A325(min^{-1}), indicate the rate of pyrogallol autoxidation.

Step2: Determination of the SOD Activity:

A measurement of 2.35 ml of Tris-HCl buffer was mixed in 10 ml test tube with 1.9 ml of distilled water, 0.1ml lysate fly tissue was added and a further 0.15 ml pyrogallol solution added to the test tube and vortexed immediately. An aliquot was taken from the test tube for absorbance measurement at 325 nm, the tissue lysate was properly diluted to give a 50% reduction in the autoxidation rate, i.e., a 325 was approximately 0.030, the SOD and catalase was measured in triplicate and the mean was determined.

Determination of Catalase activity

The spectrophotometer was set to a wavelength of 240 nm. To prepare the blank, 3.0 mL of freshly prepared hydrogen peroxide solution (0.036%, w/w) was added to a clean quartz cuvette, and the absorbance was zeroed. The solution was then discarded, and the cuvette was gently wiped dry. Subsequently, 2.95 mL of freshly prepared hydrogen peroxide solution (0.036%, w/w) was transferred into a quartz cuvette, followed by the addition of 0.05 mL of fly lysate. The contents were mixed by gentle inversion, and the cuvette was immediately placed in the spectrophotometer. The change in absorbance at 240 nm was recorded over 180 s to determine catalase activity.

Data Analysis

All assays were performed in triplicates. Statistical analysis was carried out using SPSS Version 27. Probit regression analysis was used for LC_{50} and LC_{90} estimation. Data was presented as mean ± standard error of the mean (SEM). Two way-ANOVA was used to compare the mortality rate among groups of treatments. Duncan's Multiple Range Test (DMRT) was used to indicate the group that is different from others. P-value of < 0.05 was considered statistically significant.

Results

Lethal concentration (LC_{50} and LC_{90}) of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin at 24h, 48h, and72h of exposure on adult fruit flies (*Drosophila melanogaster*)

As shown in Table 1, the LC_{50} and LC_{90} values of all four pesticides generally decreased with increasing exposure duration, indicating that lower concentrations were required to produce 50% and 90% mortality, respectively, at longer exposure periods. Lambda-cyhalothrin was the most potent pesticide based on its LC_{50} values, which decreased from 7.45 mmol at 24 h to 5.23 mmol at 48 h and 4.18 mmol at 72 h. Its corresponding LC_{90} values were 10.00, 8.92, and 7.05 mmol, respectively. At 72 h, lambda-cyhalothrin also had the steepest reported concentration–mortality slope (3.45 ± 0.32), suggesting a comparatively rapid increase in mortality with increasing concentration.

Imidacloprid was the next most potent pesticide based on its LC_{50} values, which declined from 8.91 mmol at 24 h to 6.52 mmol at 48 h and 4.52 mmol at 72 h. Its LC_{90} values were 10.00, 10.00, and 8.27 mmol at 24, 48, and 72 h, respectively. Paraquat had LC_{50} values of 8.52, 6.21, and 4.82 mmol at 24, 48, and 72 h, respectively, with corresponding LC_{90} values of 10.00, 10.00, and 9.15 mmol. Its reported slope was 2.89 ± 0.42. Glyphosate was the least potent pesticide based on its LC_{50} values, which decreased from 10.00 mmol at 24 h to 7.83 mmol at 48 h and 5.12 mmol at 72 h. Its LC_{90} values were 10.00, 10.00, and 9.50 mmol, respectively, while its reported slope was the lowest (2.45 ± 0.36). Overall, the results indicate that lambda-cyhalothrin had the greatest toxicity and glyphosate the lowest toxicity under the experimental conditions, based primarily on the estimated LC_{50} values.

Table 1: Time-dependent Acute Toxicity of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin Against Fruit Flies *Drosophila melanogaster*

Pesticide	Time	LC_{50} (mmol)	95% Cl (LC_{50})	LC_{90} (mmol)	95%CI (LC_{90})	Slope ± SE	χ^2	P	N= 330
Paraquat	24h	8.52	7.1-10.8	10	-	2.34±0.45	1.23	0.87	
	48h	6.21	5.2-7.5	10	-	2.67±0.38	0.89	0.92	
	72h	4.82	3.9-5.8	9.5	7.8-12.1	2.89±0.42	1.05	0.92	
Glyphosate	24h	10	-	10	-	1.89±0.51	2.34	0.67	
	48h	7.83	6.5-9.8	10	-	2.12±0.39	1.78	0,78	
	72h	5.12	4.2-6.1	9.15	8.2-13.5	2.45±0.36	1.56	0.82	
Lambda-cyhalothrin	24h	7.45	6.3-9.1	10	-	2.78±0.41	0.95	0.91	
	48h	5.23	4.4-6.3	8.92	7.6-11.4	3.12±0.35	0.82	0.93	
	72h	4.18	3.6-4.8	7.05	6.3-8.2	3.45±0.32	0.76	0.94	
Imidacloprid	24h	8.91	7.4-11.2	10	-	2.23±0.43	1.34	0.85	
	48h	6.52	5.4-7.9	10	-	2.56±0.40	1.12	0.89	
	72h	4.52	3.8-5.3	8.27	7.1-10.2	2.98±0.38	0.98	0.91	

Residual Effect of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin Exposure on the Fecundity of Fruit Flies

All pesticide reduced the mean number of eggs laid compared to the control. The most toxic pesticide was imidacloprid (11.67±4.49) followed by lambda-cyhalothrin (11.00±5.13), glyphosate (15.67±5.18) and paraquat (22.67±5.49). Thus, there was no significant difference across the mean number of eggs laid in relation to pesticides used (df = 4, F = 0.825 P = 0.539, Table 2).

Table 2: Residual Effect of Paraquat, Glyphosate, Imidacloprid and lambda-cyhalothrin Exposure on the Fecundity of Fruit Flies

Treatments	Number of Females	Number of Egg Lay in 24h
Control	5.00±1.15	18.00±6.03
Paraquat	7.00±1.16	22.67±5.49
Glyphosate	5.00±0.58	15.67±5.18
Lambda-Cyhalothrin	4.33±1.20	11.00±5.13
Imidacloprid	3.67±0.88	11.67±4.49
Df	4	4
F	1.489	0.825
P-value	0.277	0.539
LOS	Ns	Ns

M±S.E, *= significant at p<0.05, Ns = Not significance; LOS = Level of Significance

Residual Effect of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin Exposure on the Eclosion of Fruit Flies

The eclosion assay was evaluated on the fruit fly, all the pesticides were confirmed toxic to the flies compared to untreated group (56.67%). Imidacloprid demonstrate the highest toxicity with (0.0%) eclosion followed by lambda cyhalothrin (3.33%), glyphosate (16.67%) and the least was paraquat (18.33%). Therefore, there was a high significances difference in the percentage eclosion of *D. melanogaster* in relation to different pesticide used (F= 7.554, df = 4, p = 0.005, Table 3).

Table 3: Effect of Sublethal Exposure of Paraquat, Glyphosate, Imidacloprid and lambda-cyhalothrin on the Eclosion of Fruit Flies

Treatments	Number of Larvae	Emerged Adult	% Mortality	% Eclosion
Control	20.00±0.00	11.33±2.02^{b}	43.33±10.14^{a}	56.67±10.14^{b}
Paraquat	20.00±0.00	3.67±2.19^{a}	81.67±10.93^{b}	18.33±10.93^{a}
Glyphosate	20.00±0.00	3.33±2.03^{a}	83.33±10.14^{b}	16.67±10.14^{a}
Lambda-Cyhalothrin	20.00±0.00	0.67±0.67^{a}	96.67±3.33^{b}	3.33±3.33^{a}
Imidacloprid	20.00±0.00	0.00±0.00^{a}	100±0.00^{b}	0.00±0.00^{a}
Df	4	4	4	4
F		7.554	7.554	7.554
P-value		0.005	0.005	0.005
LOS		*	*	*

M±S.E, *= significant at p < 0.05, LOS = Level of Significance

NB: Values within the same column with no superscript in common differed significantly according to (DMRT).

Residual Effect of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin on Fruit Flies Enzymes Activities (Superoxide dismutase (SOD) and Catalase Assay Results)

The SOD result showed that glyphosate demonstrate the slowest rate (253.67Abs/min) followed by imidacloprid (360.97 67Abs/min), paraquat (361.97 67Abs/min) and lambda-cyhalothrin (742.5 Abs/min). Therefore, there was high significance difference in the SOD activities in relation to the pesticide used (F = 39.021, df= 4, p < 0.001, Table 4). While for catalase activities, glyphosate indicate the lowest rate (215Umol/min), followed by imidacloprid (216.2 Umol/min), paraquat (291.8 Umol/min) and lambda-cyhalothrin (355.4 Umol/min). Therefore, there was a significance difference in the catalase activities relation to different pesticide used (df = 4, F=7.641, p = 0.004, Table 4).

Table 4: Residual Effect of Paraquat, Glyphosate, Imidacloprid and Lambda-cyhalothrin on Fruit Flies Enzymes Activities (Superoxide dismutase (SOD) and Catalase Assay Results)

Treatments	Superoxide dismutase	Catalase
Control	2625±232.8^{a}	594.4±68.7^{a}
Paraquat	361.97±38.6^{a}	291.7±26.7^{a}
Glyphosate	253.67±82.3^{a}	215.8±92.9^{a}
Lambda-Cyhalothrin	742.57±255.6^{a}	355.4±40.3^{a}
Imidacloprid	360.97±10.7^{b}	216.2±18.1^{b}
Df	4	4
F	39.021	7.641
P-value	<0.001	0.004
LOS	*	*

M±S.E, *= significant at p<0.05, LOS = Level of Significance

NB: Values within the same column with no superscript in common differed significantly according to (DMRT).

DISCUSSION

The result of this study reported that out of the four pesticides tested, imidacloprid was the most highly toxic causing the dose dependent mortality, followed by lambda cyhalothrin and the least were glyphosate and paraquat. The high mortality effect of imidacloprid, may be due to their specificity. This finding aligned with Charpentier et al. (2014) who reported imidacloprid exposure in *D. melano*gaster produces dose-dependent responses, with lethal concentrations causing rapid mortality and Helen et al. (2020) also reported that Even sublethal doses causes significant consequences. The least toxic effect of paraquat may be due to its mode of action and the period of the exposure, this agreed with Souza et al. (2025) who reported that paraquat exposure results in a markedly reduced climbing ability in a time-dependent after 10 days of feeding. Lambda-cyhalothrin indicate the highest potency with the LC_{50} value of 3.45 mmol across all time, the high potency of lambda-cyhalothrin while glyphosate was less potent with the LC_{50} value of 5.12 mmol which may be due to it mode of action. This finding agreed with Doaa et al. (2022) who reported that lambda-cyhalothrin was the superior toxicant with LC_{50} of 0.65 and 1.008. Similarly, Mai et al. (2022) also report lambda-cyhalothrin as the most toxic compound with the LC_{50} value of 0.5 ppm. On the other hand, Richardson et al. (2022) reported that, Paraquat is explicitly toxic to animals through oxidative stress, with a well-established LC_{50} range of 1-10 mM in dietary assays. Pure glyphosate has low acute toxicity (LC_{50} > 10,000 mg/L).

All the tested pesticide significantly affects the egg laying ability of *Drosophila melanogaster*. The most toxic compound on the *Drosophila* fecundity was imidacloprid causing a reduction in the mean number of eggs laid followed by lambda cyhalothrin, glyphosate and paraquat. The significant toxicity of imidacloprid on the egg laying ability of fruit flies may be due to its potency (Bai et al., 1991) This finding agreed with Silva et al. (2013) who recorded that effect of chronic exposure to imidacloprid led to reduction in the egg-laying ability of the fruit flies. The least toxic compound was paraquat which may be because it was made for targeting plants. Imidacloprid caused the highest significant reduction the mean number of emerge adults followed be lambda-cyhalothrin, glyphosate and paraquat. This result is in line with Martelli et al. (2020) documented Chronic exposure to imidacloprid has profound consequences for development and reproduction in *D. melanogaster*. However, Oliveira et al. (2022) demonstrate that young flies showed tolerance with no alteration observed in locomotion or survival parameters after been exposed to paraquat.

Furthermore, data in Table 4 demonstrated the oxidative stress in *Drosophila*. The result of this research indicate that all the pesticides induced oxidative stress, the most induced oxidative stress compound was imidacloprid followed by glyphosate, paraquat and lambda-cyhalothrin. The result of this finding aligned with Martelli et al. (2020) who recorded that prolonged exposure to even low doses of imidacloprid triggers oxidative stress responses. de Aguiar et al. (2016) documented increased expression of genes encoding key antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and several components of the glutathione system following glyphosate exposure. Souza et al. (2024) who also reported that the primary mechanism underlying paraquat toxicity involves the generation of reactive oxygen species (ROS), leading to extensive oxidative stress.

CONCLUSION

This study conclusively demonstrates that all the pesticide present serious risks to nontarget organisms such as *Drosophila melanogaster*. While imidacloprid and lambda-cyhalothrin (insecticides) are potent acute neurotoxin, glyphosate and paraquat (herbicide) cause significant damage, inducing oxidative stress.

Recommendations

The underlisted are recommendations arising from this research:

i. Mandate testing of commercial formulation, not just active ingredients: environmental risk assessment for pesticide should include testing of the final commercial product
ii. Chronic sublethal endpoint such as locomotive assay should be incorporate
iii. The future research should use both residual contact and food incorporation methods
iv. Deepen mechanistic investigation into sublethal toxicity transgenerational and population level effect should be incorporate develop and adopt bee safe and insect friendly formulation.

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