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Acute exposure to herbicide imazethapyr causes neuronal and reproductive damage to the nematode Caenorhabditis elegans

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Abstract

Imazethapyr is a broad-spectrum herbicide with high environmental permanence, belonging to the imidazolinone family, widely consumed in several crops. In previous data, imazethapyr has already been detected in natural water samples that had contact with pre- and post-harvest crops, however, its effects have not yet been sufficiently explored. The aim of our study was to investigate for the first time the direct effects of acute exposure to imazethapyr on reproductive and neuronal parameters in the free-living nematode, Caenorhabditis elegans. Animals exposed to concentrations of 1, 5, 10, 50, and 100 µg/mL did not show any significant changes in relation to body length, however, even the lowest doses of the pesticide altered the survival rate, egg laying and egg production, all parameters related to reproduction. In addition, we observed an increase in the process of cell apoptosis, however the viability of these eggs was not so significantly affected. In addition to significant impacts on reproduction, the neurotoxic effects of imazethapyr were also demonstrated with changes in swimming movements, dopamine-induced paralysis, but only at the highest concentration was it possible to visualize dopaminergic and cholinergic damage in neurons, respectively. Our data demonstrates, for the first time in C. elegans, that imazethapyr causes neurotoxicity and reproduction impairments before germline cells fertilization, which can suggest an impact to nematodes that are important to the soil quality of the crops.

1. Introduction

Globally, the escalating demand for food production has driven increased use of various pesticide classes to safeguard intensive agricultural practices. Brazil, renowned for its substantial agricultural output and crop exports, exemplifies this trend. The availability of pesticides in the Brazilian market has expanded significantly over time, while environmental impacts remain a great concern (Barizon et al., 2022; Rico et al., 2022; Silva et al., 2023). However, pesticide poisoning has been recognized as a contributing factor for mortality among native species and a potential threat to local community health (Stoyanova & Harizanova, 2019; Yancheva, Velcheva, Stoyanova, & Georgieva, 2016; Yancheva, Velcheva, Stoyanova, Iliev, et al., 2016).

In a previous study conducted by our research group, we identified several pesticides in natural samples from the Uruguay River watershed, including the herbicide Imazethapyr (IMZT). This river serves as a water source for agricultural production in southern Brazil. The introduction of such stressors can have detrimental effects on the water body. Rice farmers often use IMZT-based mixtures to control red rice. As an imidazolinone, IMZT is known for its effectiveness at low concentrations, its ability to control a wide range of weeds, and its long-lasting soil persistence. Its mechanism of action is by inhibiting the enzyme Acetolactate Synthase (ALS) in weeds. This enzyme is essential for the synthesis of amino acids valine, leucine, and isoleucine. By blocking ALS, the herbicide prevents the production of these proteins, disrupting DNA synthesis and cell growth, leading to underdeveloped plants and eventual plant death. Factors such as soil pH, moisture, and organic matter influence the persistence(Kuhn et al., 2021; Tran et al., 2019; S. Wang et al., 2024).

On the other hand, IMZT has also been reported to cause effects in non-target species, such as metabolic alterations in *Cyprinus carpio*, genotoxicity in tadpoles of the species *Rhinella arenarum* and *Hypsiboas pulchellus*. Toxicity evidence has been observed in mammalian cells, in addition to interfering with the absorption of essential vitamins, affecting the adequate formation of adipose tissue, and causing anxious behavior in rats (Carvalho et al., 2019; Pérez-Iglesias et al., 2015, 2017; Soloneski et al., 2017; Tsatsakis et al., 2019). However, there are still a few updated references regarding the toxicity of this herbicide and its putative hazard to nematodes.

The free-living nematode *Caenorhabditis elegans* has been employed in numerous studies involving potentially toxic pesticides. Its short lifespan enables rapid data acquisition, while its low maintenance requirements, transparent body, fully sequenced genome, and high genetic homology with humans (about 80%) make it an effective research tool for toxicological studies and also to identify the toxic effects of IMZT in nematodes species (Hughes & Hessel, 2024). Therefore, in this study we aimed to evaluate the toxicological effects of IMZT in *C. elegans*, using acute exposure and multiple endpoints that comprehend neuronal and reproductive systems of these worms.

2. Materials and methods

2.1 IMZT solution

The IMZT active ingredient used was obtained from Sigma/Merck PESTANAL®, analytical standard – 37923 (2-(4,5-Dihydro-4-isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)-5-ethyl-3-pyridinecarboxylic acid). 0.01g was solubilized in 100 µl of dimethylsulfoxide (DMSO) and 900 µl of ultrapure water in a microtube (10%). Ultrasonic bath equipment was used to solubilize for 2 hours, with temperature control at around 20°C. From this mother solution, a stock solution of 1 mg/mL was prepared using distilled water and kept in the freezer at -20ºC (DMSO at 1%).

2.2 *C. elegans* maintenance and synchronization

For this study, we used wild-type Bristol N2, in addition to other strains with GFP fluorescence to investigate possible neuronal damage, such as BY200 vtIs1(dat-1p::GFP; rol-6) (GFP bright in dopamine neuronal soma and processes) and LX929 vsIs48 [unc-17::GFP] (GFP expressed in all cholinergic neurons). We also used the strain MD701 bcIs39 [lim-7p::ced-1::GFP + lin-15(+)], which expresses a functional CED-1::GFP fusion protein in the sheath cells, allowing us to observe apoptosis. All strains were obtained from the Caenorhabditis Genetics Center (CGC). The animals were kept at 21–22°C and cultured in NGM seeded with *Escherichia coli* OP50 as a food source. To obtain animals at the same larval stage, the population was synchronized by exposing adult pregnant worms to a lysis solution (0.45 N NaOH, 2% HOCl). Worms were washed to remove the lysis solution, and eggs were maintained in M9 buffer (KH2PO4 20 mM, Na2HPO4 0.04 M, NaCl 0.08 M, and MgSO4 0.001 M). After 14 hours, the eggs hatched, releasing L1 larvae that were used for the following treatments (Brenner, 1974).

2.3 Acute exposure to IMZT

Worms were exposed to 1, 5, 10, 50, 100, and 200 µg/mL of IMZT at the first larval stage. To this date, there are no reports in the literature regarding the use of IMZT in this experimental model, therefore these concentrations were determined based on pilot assays. For the controls, an M9 buffer was used. IMZT stock solutions were prepared to be pipetted considering DMSO at 0.2% maximum in the treatments. We added approximately 1500 L1 worms in the treatments and placed the microtubes in a homogenizer for 30 minutes, which was the total period of exposure to IMZT. Afterwards, we placed the microtubes in the centrifuge at 7000 rpm for 3 minutes (20°C) and proceeded with the washes. We washed and treated the worms three times with M9 to remove IMZT from the animals by withdrawing 500 µl from the microtubes, adding 1000 µl of M9, and centrifuging after each wash to pellet the worms. At the end, we removed up to 0.5 ml from the microtubes without disturbing the pellet, then poured the contents onto identified Nematode Growth Medium (NGM) plates containing *E. coli* OP50 as a food source. After 48 hours, we proceeded to the experiments.

2.4 Survival

After 48h exposure, the live animals were counted using a grid with quadrants marked under the microscope. This assay was repeated at least three times (n = 3). Controls were considered as 100% survival, and all data were normalized as a percentage of the control.

2.5 Body length

To assess body length, worms were collected 48 hours after treatment, in 1.5 ml microtubes and washed with distilled water at least three times. Worms were transferred to slides containing a 1 mM levamisole solution to paralyze them and to obtain photographs with the aid of the ECLIPSE 50I® (NIKON®) microscope. About 10 animals were selected, and imageJ® software was used to measure the body length of the worms. This assay was repeated at least three times (n = 3).

2.6 Brood size

For brood size and egg viability, one worm from each group at the L4 stage, in duplicates, was transferred daily for 3 days to NGM plates with *E. coli* OP50, and reproduction was evaluated by counting the progeny daily until the end of the reproduction period. Each experiment was repeated at least three times. For the egg viability test, the eggs were also counted and the hatched worms were counted the next day. This assay was repeated at least three times (n = 3).

2.7 Egg Production

To determine the number of eggs produced in the uterus chamber in a determined time of the reproductive period, on the first day of the adult molt, hermaphrodites were transferred to a slide containing ten drops of the lysis solution (0.45 N NaOH, 2% HOCl). After 1 minute, the bodies of the hermaphrodite worms ruptured, the moment the body ruptures the number of eggs inside the worm was counted. This assay was repeated at least three times (n = 3).

2.8 Fluorescence of apoptotic cells

For this experiment, the strain MD701 ([lim-7p::ced-1::GFP + lin-15(+)]) was used. After 48h exposure cessation, worms were transferred to a microtube containing M9 buffer and washed at least 3 times to remove excess bacteria. The worms were placed on glass slides, and the photos were taken on an Eclipse Ti2-A fluorescence microscope (Nikon® Instruments Inc.), quantifying apoptotic cells throughout observation of the distal and proximal gonads. The microscope's magnification is used to confirm the results. This assay was repeated at least three times (n = 3).

2.9 Swimming

Treated worms (48h) were collected and washed three times with M9 buffer to remove residual OP50 *E. coli*. Ten worms were transferred to a 10 mL beaker containing 2 mL of M9 buffer. After a 1-minute incubation period, the worms were recorded for 1 minute using a microscope with a camera stand. The ImageJ software with the worm tracker extension (Fiji) was used to analyze swimming speed and distance traveled during the video. This assay was repeated at least three times (n = 3).

2.10 Quantification of neuronal fluorescence

To quantify the fluorescence intensity of dopaminergic and cholinergic neurons using strains BY200 and LX929, respectively, we collected worms after 48h exposure with M9 buffer to transfer them to microtubes. Worms were washed three times to remove any traces of OP50 bacteria. The worms were paralyzed with a 1 mM levamisole solution. We photographed the slides of each treatment using an Eclipse Ti2-A fluorescence microscope (Nikon® Instruments Inc.), and ImageJ software was used to quantify neuronal fluorescence, the value considered for fluorescence is the average for the selected area. This assay was repeated at least three times (n = 3).

2.11 Dopamine-Induced Paralysis (SWIP)

Five treated worms from each group at the L4 stage were transferred to wells of a 24-well plate containing 250 µL of phosphate-buffered saline (PBS), pH 7.4, and allowed to habituate for 1 minute. 50 µL of dopamine at a final concentration of 50 mM was added to the well, and the latency to paralysis of each worm was recorded for 5 min, the worms were considered paralyzed in the second they showed no further movement, methodology as described in the reference article (Hardaway et al., 2014). This assay was repeated at least three times (n = 3).

2.12 Statistical analysis

All assays were performed at least 3 times, and GraphPad Prism 8 was used to generate charts and perform statistical analysis. After the normality test, One-way or Two-way ANOVA was used, and a $P < 0.05$ was considered statistically significant. Two-way ANOVA was used for egg viability. Post hoc tests were performed using the Tukey test. Values were normalized to the control, with the control set to 100%.

3. Results

3.1 IMZT affects survival in *C. elegans* but not worm body size

In Fig. 1a, we depict that exposure to IMZT caused a marked statistically significant reduction in the population size of *C. elegans* from the concentration of 10 µg/mL in relation to the control. However, even with this high mortality, exposure to IMZT did not significantly reduce the body size of surviving animals (Fig. 1b). From these results, we determined an LC50 of 44.86 µg/mL.

3.2 Exposure to IMZT causes reproductive damage in *C. elegans*

After obtaining the survival ratio, we evaluated whether exposure to IMZT would compromise the reproduction of exposed worms, as reflected in brood size (Fig. 2a). We observed a statistically significant decrease in the number of *C. elegans* progeny from the concentration of 5 µg/mL, in relation to the control. Furthermore, it is possible to observe in Fig. 2b that egg production was impaired from the lowest concentration (1 µg/mL) of IMZT.

3.3 IMZT exposure affected egg viability in *C. elegans*

The viability of the eggs produced by *C. elegans* exposed to IMZT did not show a major effect, as we observed a significant difference in the hatched-to-non-viable egg ratio only at 100 µg/mL. This demonstrates that at the highest concentration tested, not only did the exposed animals have a reduction in the number of offspring and egg production (Fig. 2a and 2b), but also that some of the eggs laid did not hatch.

3.4 Exposure to IMZT increased the number of apoptotic cells

Using the MD701 strain, apoptotic germline cell corpses can be visualized. In Fig. 4, a significant increase in the number of apoptotic cells is observed in worms exposed to IMZT at 5 µg/mL. This result is consistent with the reproductive tests, which showed that IMZT, even at acute exposure levels, has a significant negative impact on reproduction in *C. elegans*.

3.5 Exposure to IMZT reduced neuronal fluorescence intensity and affected dopamine-induced paralysis

We observed a visible decrease in dopaminergic neurons fluorescence following IMZT exposure, indicating neuronal damage in worms exposed to 50 µg/mL and 100 µg/mL (Fig. 5a). Regarding the SWIP test, worms exposed to the highest concentration took longer to paralyze in the presence of dopamine, indicating damage to the dopaminergic system (Fig. 5b). This assay is performed to assess

the integrity of the dopaminergic system. An excess of dopamine in the synaptic cleft leads to paralysis due to an overstimulation of the dopamine receptor DOP-3 found in a motor neuron. If the worms take longer to paralyze in the presence of exogenous dopamine, this may indicate damage to these neurons (Hardaway et al., 2014).

3.6 Decrease in swimming movements in *C. elegans* exposed to IMZT

Next, we investigated the swimming movements of *C. elegans* exposed to IMZT. Here, we identified changes in the locomotion of the worms by observing the curves of the body in liquid medium, where both the movement speed (Fig. 6a) and the distance traveled (Fig. 6b) were significantly reduced compared to the control, in the highest concentrations, 50 and 100 µg/mL. Neuronal loss of cholinergic neurons was also evidenced at the highest concentration (100 µg/mL) (Fig. 6c).

3. Discussion

Our results demonstrate that IMZT does indeed cause significant reproductive damage and can affect different neuronal systems in *C. elegans*, potentially representing the same risk for other species environmentally exposed to this pesticide. Although widely used, it is peculiar that there are relatively few studies on the effects of IMZT, especially in living organisms. In the case of *C. elegans*, there are no studies exposing the nematode to the active ingredient or to any formulation containing this herbicide. In Brazil, the use of IMZT is high, given its broad spectrum and particular popularity in glyphosate-resistant crops, reaching 4,202.51 tons sold between 2018 and 2022 (CONAMA, 2005). Consequently, several studies have reported the presence of herbicide in river waters(Almeida et al., 2019; Barizon et al., 2022; Caldas et al., 2019; Folador et al., 2025; Kuhn et al., 2021; Perin et al., 2021).

To assess the toxic potential of IMZT in the *C. elegans* population, we exposed nematodes in the first larval stage L1 to a range of 1 to 200 µg/mL for only 30 minutes, acutely. However, it is worth noting that our initial tests aimed for chronic exposure of the worm to the herbicide, estimating a scenario of constant exposure (which likely occurs with other animals exposed to these contaminated environments), since in the environment, organisms can remain in contact with pesticides for long periods, given the persistence of these formulations. Our results with chronic exposure to IMZT caused total mortality of the worm population even at the lowest concentration of 1 µg/mL (Data not shown).

This suggests that, depending on the organism, even occasional exposures to IMZT, depending on the dosage and type of exposure, can have a significant impact. A study that evaluated the toxicity of IMZT found that zebrafish embryos also showed increased lethality, with an LC50 of 71.60 µg/mL after 5 days of exposure. The embryos also showed bodily malformations after exposure(Costa et al., 2022). Notably, the lowest concentration used was significantly toxic to the worms compared to untreated controls (Fig. 1a). In a previous study aimed at monitoring water quality in the Uruguay River in southern Brazil using *C. elegans* as a bioindicator, three pesticides, including IMZT, were detected. Of note, IMZT was

detected at relatively high concentrations of 26.01 µg/L of river water. Correlation and PCA analysis indicated that IMZT contributed to a reduction in nematode reproduction(Kuhn et al., 2021).

An adult hermaphrodite of *C. elegans* produces approximately 300 offspring through self-fertilization, with a reproductive cycle of 3 days. The nematode's reproductive system is a widely used parameter in toxicity studies due to the similarity of genomes and genetic functions with humans, with 1,320 and 3,118 genes related to reproduction in humans and *C. elegans*, respectively(Thapliyal & Babu, 2018a). In fact, IMZT significantly reduced Brood size at the lowest concentration tested (Fig. 2a). Egg production, in which we specifically observed the number of eggs formed in the gonad on the first day of the adult stage, suggests that the size of the offspring was reduced from the formation of eggs in the gonad, indicating that IMZT may be interfering with gamete formation. In agreement, we observed a significant increase in the number of apoptotic germ cells using the MD701 strain *([lim-7p*::*ced-1*:: GFP + *lin-15* (+)]).

The data demonstrate that acute exposure to IMZT can induce a series of apoptotic processes in the germline, consistent with the Brood size and egg production data, and indicating a direct toxic effect on the worm's reproductive system. It is worth noting that we believe the increase in apoptotic corpses was not concentration-dependent, since apoptosis may have occurred before the analysis, as we observed in a previous study(Soares et al., 2022). These effects on reproduction have been described in other species. Studies using tadpoles of *Rhinella arenarum* and *Leptodactylus latinasus* as models found that IMZT, alone and in combination with another herbicide, caused reproductive toxicity by inducing oxidative damage and direct DNA damage(Carvalho et al., 2019; Pérez-Iglesias et al., 2020). Studies also recorded the incidence of micronuclei and DNA damage when *Cyprinus carpio tadpoles* (98.5 µg/L of IMZT) were exposed for 7 days and *Hypsiboas pulchellus tadpoles* (270 µg/L) for 96 hours(Moraes et al., 2011; Pérez-Iglesias et al., 2015).

Considering these effects on brood size, egg production, and germ cell apoptosis, we then evaluated whether gonadal toxicity induced by IMZT would affect the viability of eggs produced by *C. elegans* (Fig. 3). Notably, only the highest concentration of IMZT (100 µg/mL) generated a significantly lower number of L1 larvae in relation to the number of eggs laid, demonstrating a percentage of infertile eggs. At the lower concentrations, IMZT did not affect the viability of the laid eggs. Our hypothesis is that IMZT directly affects mitosis and meiosis in some germline cells, which may undergo apoptosis. The remaining oocytes complete the cell division process and are fertilized, laid and hatched, which explains why we did not observe lower egg viability but found reduced brood size in relation to untreated worms.

After the reproductive analyses, we set out to investigate whether IMZT toxicity could cause neuronal damage in *C. elegans*, given that there are several studies (including those using *C. elegans* as a model) that indicate a relationship between pesticides contamination and the aggravation of neurodegenerative diseases and other damage to the neuronal system. Furthermore, studies have shown that affected neuronal signaling can also negatively influence reproductive behavior(I. Lee et al., 2024; Y. Lee et al., 2023; Lo et al., 2024; Parrales-Macias et al., 2023; Thosapornvichai et al., 2022; B. Wang et al., 2025; G. Wang et al., 2024).

Exposure of IMZT at concentrations of 50 µg/mL, 100 µg/mL to the BY200 strain, which expresses GFP under the dopamine transporter promoter in dopaminergic neurons, caused a significant loss of neuronal fluorescence intensity compared to the control group (Fig. 5a). Consequently, the latency time to dopamine-induced paralysis was significantly different from the control (100 µg/mL; Fig. 5b). *C. elegans* modulates its locomotion rate in response to the environment, such as the search for food or the presence of threats; this deceleration or reduction in speed is modulated by dopaminergic neurons related to motor neurons. These results may indicate that IMZT likely damages dopaminergic neurons or affects the dopamine receptor DOP-3 (in the SWIP assay) and the dopamine transporter DAT-1 (as observed by neuronal fluorescence in the BY200 cell line), which impairs the activity of these neurons(McDonald et al., 2007; Sawin et al., 2000).

The viability of cholinergic neurons using the LX929cell lines (Fig. 5c), demonstrated that exposure to 100 µg/mL induced neuronal damage. Corroborating these data, we observed that all concentrations from 5 µg/mL to 100 µg/mL significantly decreased both movement speed and distance traveled compared to untreated animals (Fig. 6). Cholinergic neurons (observed in the neuronal fluorescence of LX929) form dyadic synapses with GABAergic neurons, which, in turn, synapse with the muscles of the lateral body wall, allowing muscle contraction. The contraction and relaxation of muscles along this neuronal chain drives basal locomotion in *C. elegans*. Damage to this system results in loss of worm mobility. The exposure to IMZT, which damages these neurons, leads to the observed loss of locomotion. Although this potential neuronal damage is less pronounced than during reproduction, we hypothesize that it may be related to worm formation. During growth, a large proportion of neurons are still forming; IMZT could interfere with the worm's neuronal development before adulthood(Carvalho et al., 2019).

We believe that swimming movements were impaired at lower concentrations of IMZT, since herbicide can influence behavior and body movement, but not enough to render neurons unviable. Studies have shown that IMZT also increases acetylcholinesterase (AChE) activity in the brain and muscles, which may also interfere with swimming movements(Pérez-Iglesias et al., 2015; Thapliyal & Babu, 2018b).

IMZT acts in the plants by inhibiting plant acetolactate synthase (ALS), which is the first common enzyme of the branched-chain amino acid (BCAA) pathway, thus blocking the synthesis of three essential amino acids: leucine, isoleucine, and valine. It is not well known whether this herbicide could act on the other enzymes of this pathway in other species. Taking into account this possibility and analyzing which enzymes in *C. elegans* could have a homology to this pathway, we used BCAT-1, present in the *C. elegans* and in humans, as a possible molecular target for IMZT. It has been reported that reduced BCAT-1 expression decreases BCAA metabolism, resulting in low-quality oocytes in *C. elegans (*Lesnik et al., 2024). In addition to reproduction, interference with BCAT-1 in *C. elegans* causes progressive motor dysfunction and accelerates the degeneration of dopaminergic neurons, suggesting a correlation between defective BCAA metabolism and neurodegenerative diseases (Mor et al., 2020; Nong et al., 2022; Porri et al., 2024).

We then used the Molecular Docking tool to simulate the interactions between IMZT and human and C. elegans BCAT-1 (*Supplementary material*). We have found that IMZT has affinity with the crystal structure of the hBCAT-1 protein, interacting with amino acid residues TYR 193 and GLN 244 (*Fig. S1 and S2*). These are the same target residues identified for newly developed antagonists of BCAT-1/BCAT-2 enzymes in humans(Günther et al., 2022). We also carried out a sequence and structure alignment of hBCAT-1 and CeBCAT-1 (Fig. S3 and S4) to assess the level of similarity and homology between the sequences and the residues to which IMZT binds. The alignment showed more than 40% similarity between the analyzed sequences, exceeding the accepted homology threshold. This suggests a probable structural and functional conservation between these proteins, indicating that IMZT could interact negatively with BCAT-1 in the same manner as ALS in plants. However, although the *in silico* methodology demonstrates a possible interaction, we cannot confirm this interaction based solely on molecular docking, making further biological studies necessary to strongly confirm this and its biological outcomes.

4. Conclusion

Acute exposure to IMZT, even at low concentrations, demonstrated significant toxicity in *C. elegans*. These results, along with the significant reduction in brood size and the number of eggs formed, indicate direct damage to the worm's reproductive system. The induction of apoptosis in germline cells confirms IMZT's impact on gamete formation. Despite a decrease in offspring, without the same reduction in egg viability, we suggest that the pesticide interferes with mitosis and meiosis in germline cells, impairing oocyte production before fertilization. In addition to reproductive impacts, IMZT exposure caused neuronal damage, as evidenced by reduced fluorescence intensity and significant impairment of worm locomotion.

For a future perspective, we have found, *in silico*, that human and *C. elegans* BCAT-1 enzyme is inhibited by IMZT, which opens new and promising avenues for understanding the toxicological effects of herbicides on non-target organisms. In summary, this study demonstrates that exposure to IMZT, even for a short exposure period, has a high toxic potential in *C. elegans*. Our results are a warning about the environmental and human health risks associated with the widespread use of pesticides.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Eugênia Carla Kuhn, Jullia Dalbianco Godoy de Oliveira, Daniela Teixeira Rodrigues, Maria Eduarda Oliveira De Souza and Daiana Silva Ávila. The first draft of the manuscript was written by Eugênia Carla Kuhn and Jullia Dalbianco Godoy de Oliveira, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

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Figures

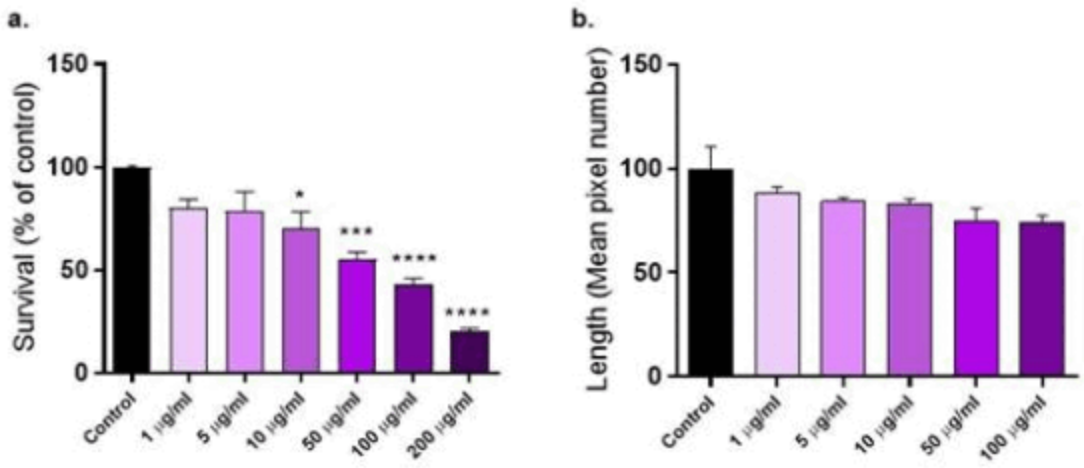


Figure 1

a. Survival assays after an acute exposure of IMZT at 1- 200µg/mL in *Caenorhabditis elegans*, and **b.** Body size. The results are expressed as mean ± S.E.M (n=3); One-Way ANOVA was used for value analysis and Tukey post-hoc test. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$)

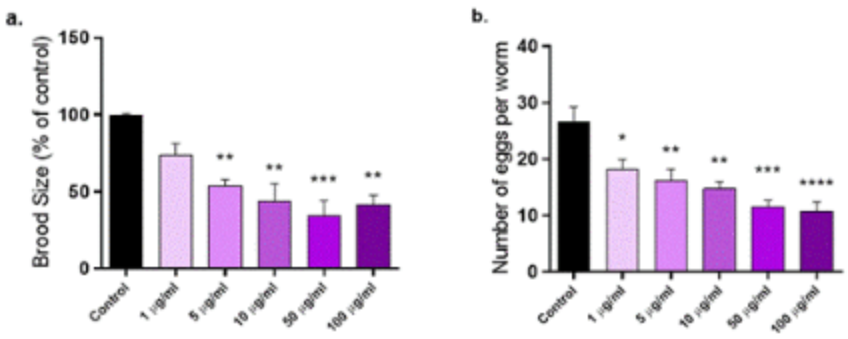


Figure 2

Reproductive parameters in *Caenorhabditis elegans* after acute exposure to IMZT **a.**Brood Size (n=3) **b.** Egg production (n=4). The results are expressed as mean ± S.E.M. One-way ANOVA was used for statistical analysis, and the Tukey post hoc test was used for pairwise comparisons. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P< 0.001$; **** = $P < 0.0001$)

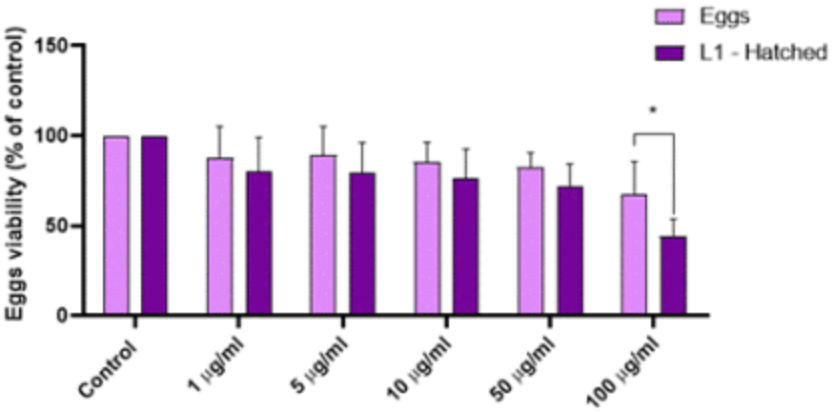


Figure 3

Ratio of non-viable eggs to hatched eggs (L1 stage) after an acute exposure to IMZT at concentrations of 0 (Control) - 100µg/mL in *Caenorhabditis elegans*. The results are expressed as mean ± S.E.M. Two-way ANOVA was used for data analysis, followed by a post hoc test. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$)

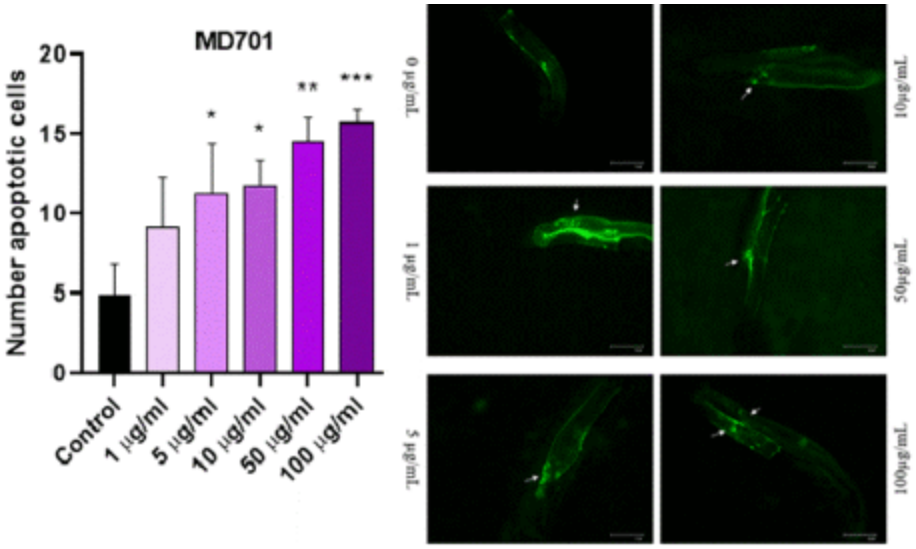


Figure 4

Increase in apoptotic cells after an acute exposure to IMZT at concentrations of 0 (Control) - 100µg/mL in *Caenorhabditis elegans*, using strain MD701 and representative photos of the GFP. The results are expressed as mean ± S.E.M (n=3); One-Way ANOVA was used for statistical analysis, and Tukey post-hoc test was used for multiple comparisons. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$)

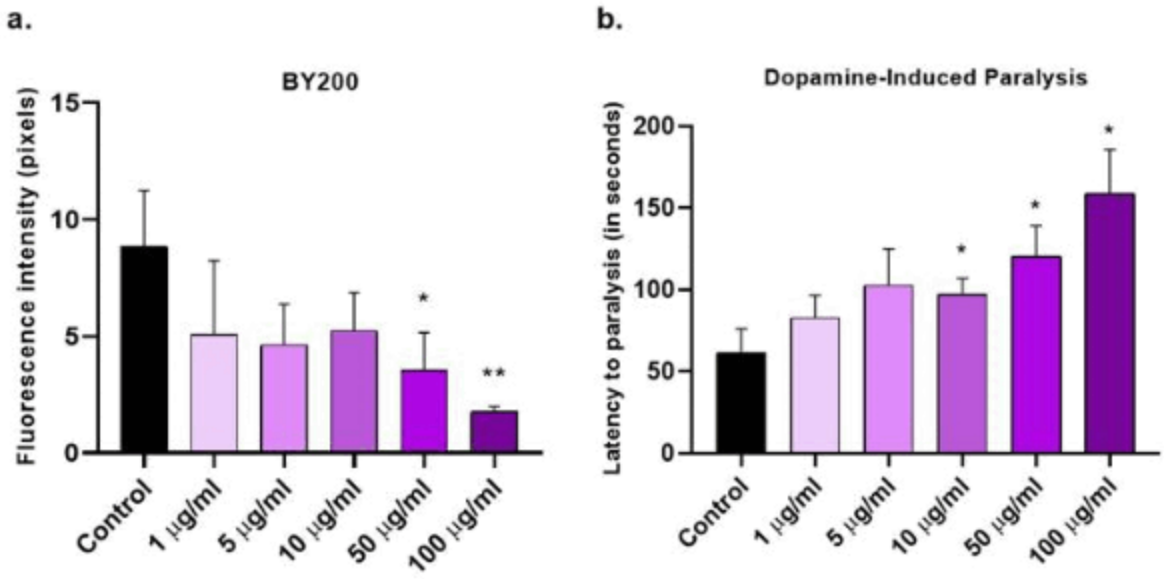


Figure 5

Damage to dopaminergic neurons after an acute exposure of IMZT to concentrations of 0 (Control) - 100µg/mL in *Caenorhabditis elegans*, **a.** Fluorescence of strain BY200, GFP-tagged dopamine neuronal soma and processes, and **b.**Latency time for dopamine-induced paralysis, in seconds**.** The results are expressed as mean ± S.E.M (n=3); One-Way ANOVA was used for statistical analysis, and Tukey post-hoc

test was used for multiple comparisons. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$)

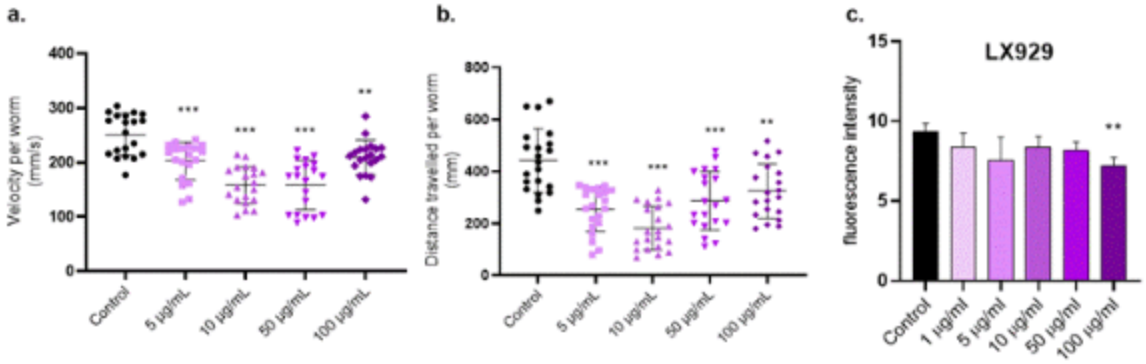


Figure 6

Swimming movement analysis in *Caenorhabditis elegans* after an acute exposure to IMZT, **a.** Velocity (n=30), **b.** Distance (n=30) and **c.** Strain LX929, GFP expressed in all cholinergic neurons. The results are expressed as mean ± S.E.M. One-way ANOVA was used for statistical analysis, and the Tukey post hoc test was used for pairwise comparisons. * Represents statistical significance level (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$). (mm/s) = micrometers per second

Supplementary Files

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