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ORIGINAL RESEARCH ARTICLE

Does roundup® affect worker bees (*Apis mellifera*) that inhabit areas of high agrochemical pressure?

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ABSTRACT

Glyphosate-based herbicides (GBHs) find wide application in conventional agroecosystems due to their effective mode of action. The impact of herbicides on bees may be underestimated due to the scarcity of studies assessing various exposure routes to GBHs, including contact and ingestion of contaminated food. This study evaluated the survival, food consumption, and body weight of honey bee populations in three different locations, characterized by different degrees of exposure to GBHs. In bioassays, honey bees were exposed to diets containing sucrose solutions infused with glyphosate, with dosages ranging from 0 to 14 mg (a.i./bee). During the experiment, the honey bee population had a period of exposure (6 h) to the syrup infused with glyphosate. Mortality counts and feeder weight measurements were performed to assess the effects. A significant reduction in syrup intake was observed in all three bee populations during the Roundup® exposure phase, leading to a decrease in GBH intake. Notably, the decrease in syrup consumption emerged as the main factor contributing to the lower body weight observed among honey bees from low- and high-impact locations, persisting into the post-exposure period. Overall, the results demonstrate that honey bees in the high-impact region are more sensitive to Roundup®. However, studies using biochemical biomarkers are still necessary to unravel how glyphosate interferes with the acquisition and expenditure of energy during periods of exposure of honey bees and which physiological changes allow them to adapt to inhabited places with high agricultural pressure.

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Toxicity; oral exposure; populations of honey bees; feeding activity; glyphosate

Introduction

The honey bees of the species *Apis mellifera* play a pivotal role as pollinators and honey producers, contributing significantly to the maintenance of healthy ecosystems. However, within agricultural environments, this non-target insect is subjected to consistent exposure to a diverse range of agrochemicals utilized in the control of pest species (de Assis et al., 2022). Examining the effects of these chemicals is imperative for comprehending the negative influence that different agrochemicals exert on the populations of honey bees (Vázquez et al., 2020).

The exposure of honey bees to pesticides primarily occurs through the consumption of residues present in pollen from cultivated plants or treated weeds, leading to the contamination of subsequently produced nectar (Sanchez-Bayo & Goka, 2014), as well as their storage within beehives (Orantes-Bermejo et al., 2010). Furthermore, pesticides, alongside other agrochemicals, induce stress responses in honey bees, resulting in escalated mortality rates within bee colonies. Such effects can consequently trigger more severe consequences, including the collapse of colonies, a phenomenon known as Colony Collapse Disorder (CCD) (Tan et al., 2022; de Assis et al., 2022).

Brazil has a climate favorable to extensive agricultural production, driven by the enormous demand for food on both a national and global scale (Camargo et al., 2017). The country is, therefore, the second largest exporter of agricultural products worldwide. This food demand, however, leads to the massive use of pesticides in Brazil (IBAMA, 2020).

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More than 90% of Brazilian farmers depend on pesticides and the country is the fifth largest consumer of pesticides in the world (Brovini et al., 2021), representing around 20% of its global use (Albuquerque et al., 2016). Data from the Food and Agriculture Organization of the United Nations (FAO, 2020) indicate that the use of pesticides per agricultural area in Brazil (5.94 kg/ha) is high when compared to countries with larger agricultural areas, such as India, USA and Russia. In the region of Formoso do Araguaia, located in the state of Tocantins, Brazil, an area of intense agricultural activity, the presence of five active ingredients of pesticide formulations (azoxystrobin, fenamidone, imazethapi, tricyclazole and trifloxystrobin) was identified in the water and soil matrices. These residues represent a potential threat to biodiversity, as they can exert toxic effects on non-target species, many of which provide important ecosystem services, such as bees (Guarda et al., 2022).

Glyphosate stands out as one of the most extensively utilized herbicides, constituting a notable 71.6% of the global sales for active ingredients (Benbrook, 2016). In Brazil alone, 382 million tons were sold in 2022 (Ibama & Instituto Brasileiro do Meio Ambiente, 2023). Manufacturer guidelines of Glyphosate-based herbicides (GBH) prescribe the application of 120 L/ha for soybean and corn cultivation, and 200 L/ha for irrigated rice, utilizing soil-based equipment. Glyphosate is classified as a non-toxic, non-selective, systemic, and post-emergent herbicide. Its excessive use has resulted in soil and water contamination, with residues being detected in soil, water bodies, and even in food sources (Gill et al., 2018; Silva et al., 2018; Farina et al., 2019). According to Ordinance 2914/2011 of the Brazilian Ministry of Health, the maximum allowable concentration of glyphosate for drinking water is 500 μg a.i/L (Brasil, 2011). Furthermore, contamination of honey by glyphosate residues and its derivative metabolite, aminomethylphosphonic acid (AMPA), has been identified across various nations (Rubio et al., 2014; Pareja et al., 2019; Thompson et al., 2019; de Souza et al., 2021). This compound is known to be extremely toxic to bees and can, even in sublethal doses, trigger disturbances in colony dynamics, reducing reproductive performance, weight gain, and resistance to diseases (Alburaki et al., 2017).

Glyphosate is applied in the control of various undesirable weed species by exerting its effect through the disruption of the 5-enolipruvylshikimate-3-phosphate synthase (EPSPS) enzyme's functionality. This enzyme plays a pivotal role in inhibiting plant growth, and it also impacts certain microorganisms (Gill et al., 2018). The targeted enzyme operates within the shikimic acid metabolic pathway, a pathway integral to the synthesis of phenylalanine, tyrosine, and tryptophan (Richmond, 2018; Ledoux et al., 2020; Battisti et al., 2021). Hence, the EPSPS enzyme is of paramount importance to plants, as well as fungi and selected bacteria, as its inhibition can trigger plant mortality (Mesnage et al., 2017). Glyphosate is lethal to bees and has exhibited sublethal effects (Battisti et al., 2021), recent research has shown that it might affect their survival (Faita et al., 2020; Castelli et al., 2021), intestinal microbiota composition and diversity (Dai et al., 2018; Blot et al., 2019; Motta et al., 2020), interfere with food consumption (Faita et al., 2018, Pal et al., 2022), cognitive ability (Balbuena et al., 2015), taste perception and olfactory learning (Mengoni Goñalons & Farina, 2018), and decrease both antennal activity and sleep bout frequency (Vázquez et al., 2020).

These are mostly sublethal effects that negatively impact the general health of the honey bees individually, ultimately impairing the correct functioning of their entire colonies. Therefore, glyphosate may be closely associated with the decline of certain honey bee populations and the phenomenon of colony collapse disorder (Beringer et al., 2019; Faghani & Rahimian, 2018). Despite the existing body of evidence, numerous studies conducted on honey bee species have revealed often contradictory results regarding the toxicity of glyphosate (Herbert et al., 2014; Battisti et al., 2021; Straw & Brown, 2021). This variability could potentially halt from genetic disparities arising due to an individual bee's historical exposure to contamination throughout its lifecycle (Almasri et al., 2021). Moreover, the physiological condition of individual bees can significantly influence their susceptibility to specific compounds. As Almasri et al. (2021) elucidate, the prior exposure of honey bees to contaminants, regardless of the concentration or quantity of substances, can bring concerning alterations in their susceptibility to subsequent exposure events.

Hence, the hypothesis raised in this study is that populations of the honey bee inhabiting contaminated regions with different levels of agricultural pressure will show differences in mortality rates, consumption, and body weight to pesticide exposure compared to the ones inhabiting a reference site. Thus, this study aimed to evaluate the effects of the commercial formulation Roundup® (active ingredient glyphosate) in three populations of *Apis mellifera*.

Materials and methods

Study areas

The honey bee species used in this study was collected in Brazil (state of Tocantins) from three areas with different levels of anthropogenic' impacts due

to agricultural activities: the reference site (no exposure to GBH), (11°29'20.69"S, 49°8'58.72"W); the low-impacted site (low exposure to GBH) (11°45'00"S, 49°03'11"W); and the high-impacted site (high exposure to GBH) (11°46'35.12"S, 49°42'27.01"). From each area, two beehives were collected. The climate of the study area, according to Köppen, is type Aw—Tropical with humid summer and dry period in winter, with the rainiest month being January and the driest month being August. Average annual precipitation varies around 1,500 to 2,100 mm (Alvares et al., 2013).

The reference site is in the city of Dueré, Tocantins—Brazil. The apiary from where hives were collected was established within a farm that uniquely engages in livestock farming (pasture), devoid of any significant agricultural procedures or the application of glyphosate.

The low-impacted site is in the experimental farm of the Universidad Federal do Tocantins (UFT), city of Gurupi-Tocantins. The farm was established to perform experiments with soybeans, corn, beans and vegetables. The amount of pesticides applied in this area is low and controlled according to strict use only for weed management in small areas of cultivation and research.

The high-impacted site is represented by the Rio Formoso Project, which is in the city of Formoso do Araguaia in the state of Tocantins—Brazil. It occupies an area of 27.800 hectares which has flood irrigation systems for rice cultivation in the rainy season and sub-irrigation for soybeans, corn, beans, and watermelon in the dry season (Guarda et al., 2022). In this area, Roundup® is used in significant amounts as the main herbicide to control weeds that generally compete with the crop for physical space, water, light, and nutrients.

Colonies of Africanized honey bee (hybrid European/Africanized colonies) at each study site were maintained in Langstroth model hives. Each queen bee from the 3 colonies were marked with colored queen marker pens (POSCA). Subsequently, these populated and duly identified beehives were transported to the apiary of the experimental unit.

Honey bee sampling

Foragers of Africanized honey bee' species were collected in 500 mL transparent plastic bottles at the entrance of each hive. Approximately 180 honey bees from each population were captured per experiment. The honey bees used in the experiments were 21 days old as advised by the guideline OECD 213 (OECD , 1998). Each bioassay was performed using 5 replicates per condition.

Honey bee handling and exposure to roundup®

After capture, the honey bees were anesthetized with CO_2 for eight seconds and placed in a transparent plastic pot with a capacity of 500 mL. Twenty honey bees were placed per pot that represents one treatment. Five replicates were performed per condition and at the end of the experiment, a total of 100 bees/condition were used. The jar lids used were previously perforated to facilitate gas exchange. Then, the honey bees were subjected to a 1-h fasting period in a Biochemical Oxygen Demand (BOD) incubator at $= 33 \pm 1\,^{\circ}C$; relative humidity of 70% and absence of light.

The commercial formulation containing glyphosate (370 g/L) used in the experiment was Roundup® Original DI, manufactured by Monsanto. Solutions applied in the experiments were prepared by diluting Roundup® in water to the desired concentration. All concentrations used in the experiments were calculated based on active ingredient (a.i). No solubility issues were observed. The solutions were vigorously stirred during the preparation and before use to ensure they were homogeneous.

Honey bees were orally exposed through syrup ingestion containing the Roundup® doses of 0, 0.92, 1.85, 3.70, 5.55, 7.40, 9.25 and 11.10 mg a.i/bee for the population of the reference site, and 0, 0. 92, 2.77, 4.62, 6.47, 8.32, 10.17, 12.02 and 13.87 mg a.i/bee for the low-impacted and high-impacted sites populations.

Oral lethal dose (LD_{50}) test

For the determination of Roundup® acute oral toxicity, the methodology was adapted from the OECD guideline for testing the acute oral toxicity using *Apis mellifera* (OECD, 213, 1998). The principle of this acute test is that adult worker honey bees are exposed up to six hours to a series of doses of the test substance dispersed in a sucrose solution. After the exposure period, honey bees are then fed the same diet but free of the test substance.

To carry out the oral LD_{50} test, doses of the commercial formulation Roundup® were made in a sucrose solution (50% water + 50% sugar) totaling 2 mL. A control treatment was performed by offering 2 mL sucrose solution (without Roundup®) to each replicate during the exposure period and also the subsequent 18 h period.

The honey bees from all the treatment conditions, except control, were fed with a solution containing Roundup® in the first 6 h of the experiment (exposure period of 6 h). Following the 6-h exposure period, the feeder was replaced with one containing a 2 mL sucrose solution exclusively, maintaining this until 24 h (constituting the 18-h post-exposure

period). During the 0, 6, and 24-h time points, both the initial and final weights of the feeder were accurately recorded. This data enabled the quantification of ingested volume throughout both intervals and facilitated the determination of glyphosate dosage per individual honey bee during the exposure phase. For these computations, the average count of viable honey bees in each period and across each replication of all conditions was considered. At the intervals of 6 and 24 h, mortality rates were assessed, and the acquired results were subjected to analysis, yielding the computation of the LD_{50} at the 24-h mark.

In another bioassay, individuals from each honey bee population were exposed to two conditions: to a control and to a concentration corresponding to the estimated LD_{50} found for each population. After 24 h of exposure, the honey bees that survived the LD_{50} and the control ones were anesthetized in cold for 8 s for subsequent body weight measurement.

Statistical analysis

The estimated LD_{50} – 24h for Roundup in honey bee of the species *A. mellifera* was determined by dose-response analysis using a four-parameter logistic curve using the GraphPad Prism software version 9.0 for Windows (GraphPad Software, La Jolla, California, USA). The consumption effects of Roundup® exposure were determined using a one-way analysis of variance (ANOVA) followed by Dunnett's posthoc test to identify significant differences between controls and treatments. Before ANOVA, the Kolmogorov-Smirnov and Bartlett tests were used to assess the normality and homogeneity of variance of the data, respectively. When the data did not meet the assumptions of normality, an analysis of variance was performed using the chi test. For the normal data the Tukey test was used.

Results

During the initial six-hour phase of the experiment we observed that regardless of the site population (comprising honey bees from reference, low-impacted, and high-impacted locations), the honey bees exhibited a consistent reduction in syrup consumption even when exposed to the minimal glyphosate concentration available (0.92 mg a.i. per bee). This reduction was statistically significant across all populations: for reference site honey bees ($F_{(7,32)} = 1.092$, $p < 0.001$), low-impacted site honey bees ($F_{(8,36)} = 2.106$, $p < 0.001$), and high-impacted site honey bees ($F_{(8,24)} = 4.695$, $p = 0.001$) (Figure 1(a)). During the post-exposure period (from 6 to 24 h), where honey bees were fed exclusively with the sucrose solution, we observed an increased in the amount of ingested syrup, regardless of the population. However, within the same population, the amount of ingested syrup differed between treatments of Roundup®. Honey bees from the reference site that were exposed to doses of 9.25 and 11.10 mg a.i/bee ($F_{(7,31)} = 2.461$, $p = 0.000$), from the site of low-impacted that were exposed to doses 8.32 and 13.87 mg a.i/bee ($F_{(8.24)} = 3.221$, $p = 0.012$), and those from the site of high-impacted that were exposed to doses 10.17 and 13.87 mg a.i/bee ($F_{(8.26)} = 1.173$, $p = 0.039$) ingested significantly higher volumes of syrup during the 18 h post-exposure period when compared to honey bee orally exposed to the other doses of Roundup® within the same population (Figure 1(b)).

Considering the total volume of ingested syrup between populations, we observed that between the reference site ($F_{(7,\ 26)} = 1.425$, $p = 0.237$) and the high-impacted site ($F_{(8,\ 27)} = 1.822$, $p = 0.116$) there was no significant difference in the total volume of syrup ingested by the honey bees from both locations (Figure 1(c)).

However, honey bees from the low-impacted population consumed different amounts of syrup regarding the treatments they were exposed to ($F_{(8,\ 29)} = 3.119$, $p = 0.011$) at the end of the 24 h period of the acute bioassay (Figure 1(c)).

The amount of glyphosate ingested by the honey bees differed significantly for the three populations at the study sites ($F_{(2,207)} = 4.553$, $p < 0.010$) increasing depending on the concentration, as expected (Figure 2).

In the control condition of the three populations, no honey bee mortality was observed during the 24-h test period (Figure 3). The estimated median lethal dose (LD_{50} – 24h) of Roundup® ingested by honey bees from the reference site was 54.86 µg a.i/bee (95% CI: 51.02 to 58.45, $R^2 = 0.987$), 59.48 µg a.i/bee (95% CI: 54.53 to 64.53, $R^2 = 0.992$) for the low-impacted site and 51.76 µg a.i/bee (95% CI: 48.15 to 55.35, $R^2 = 0.969$) estimated for the honey bees from the high-impacted site.

There was a significant difference in the consumption of glyphosate syrup among honey bees of each control group and in the consumptions of the ones exposed to all doses during the exposure period (first 6 h of feeding), and also the post-exposure (18 h of feeding), and in the amount of glyphosate ingested, for bees from the reference site, low-impacted and high-impacted site.

In another bioassay, individuals from each honey bee population were exposed to a concentration corresponding to the LD_{50} found for each population. After 24 h, the honey bees were weighed. The weight of the control honey bees from the three populations did not differ ($F_{(2,27)} = 1.000$, $p = 0.219$)

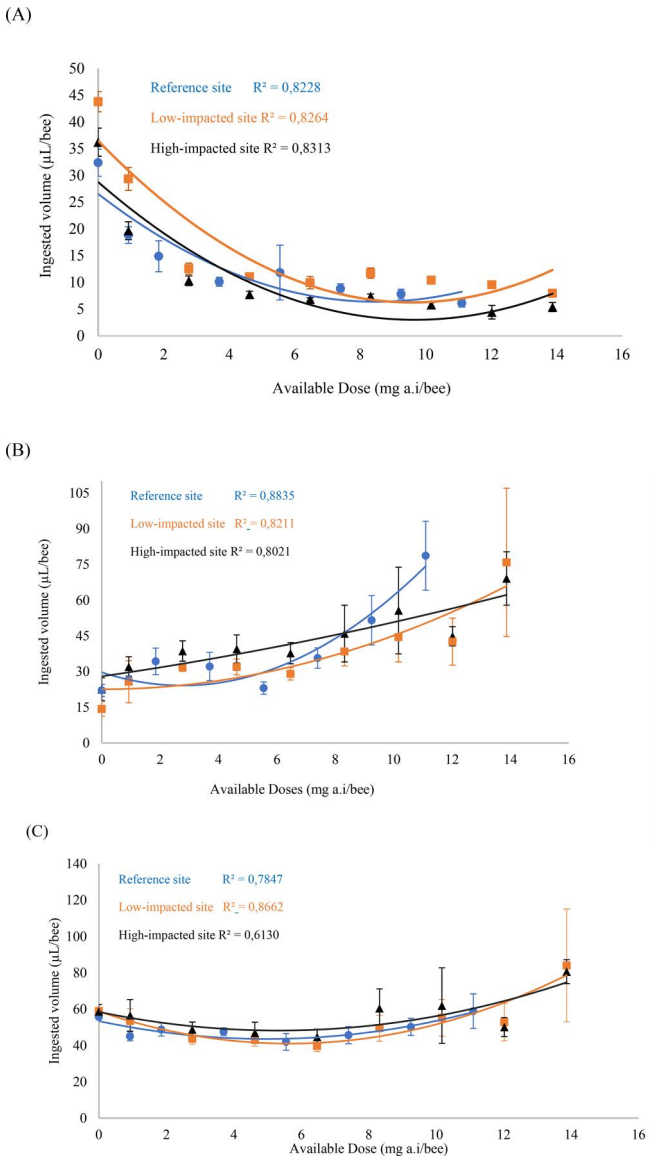

Figure 1. Volume of syrup (µL/bee) ingested by the honey bees during the exposure period of 6 h (A), post-exposure period of 18 h (B) and the total period of 24h (C; total volume), from the populations of the reference site (blue circle), low-impacted site (orange square), and high-impacted site (black triangle) exposed to the concentrations of 0 – 14 mg a.i/bee. Values represent the mean (± standard error of the mean) of five replicates per treatment containing 20 honey bees each.

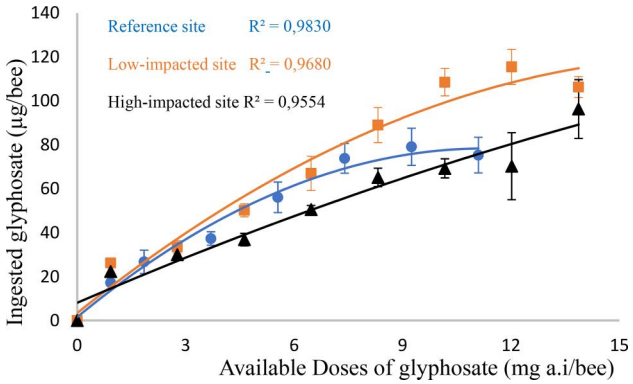

Figure 2. Quantity of Roundup® ingested (µg a.i/bee) by the honey bees from the populations of reference site (blue circle), low-impacted site (orange square) and high-impacted site (black triangle) exposed to the concentrations of 0 – 14 mg a.i/bee. Values represent the mean (± standard error of the mean) of five replicates per condition containing 20 honey bees each.

significantly (Figure 4). However, the total weight of honey bees from the low ($t = 2.934$, $p = 0.009$) and high-impacted ($t = 7.105$, $p = 0.000$) sites exposed to the respective LD_{50} was significantly lower when compared to the control (Figure 4). A significant difference was observed in the weight of honey bees

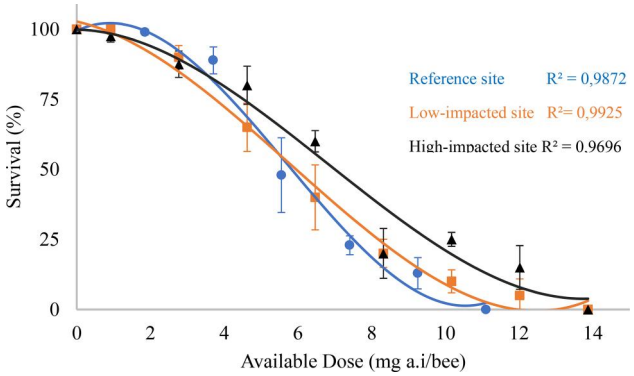

Figure 3. Survival curve (%) of the honey bees from the reference site (blue circle), low-impacted site (orange square) and high-impacted site (black triangle) at 24 h after exposure to the concentrations of 0 – 14 mg a.i/bee. Values represent the mean (± standard error of the mean) of five replicates per condition containing 20 bees each.

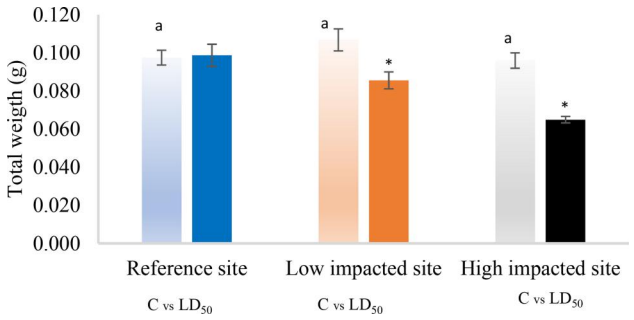

Figure 4. Total weight (g) of honey bees from the reference site (blue bar), low-impacted site (orange bar) and high-impacted site (black bar) populations exposed to control and the respective LD_{50} estimated for each population – 24 h (54.86, 59.48, and 51.76 µg a.i/bee), respectively. [a]n equal letter does not differ significantly from each other for total weight. * shows significant differences compared to the respective control ($p < 0.05$). Values represent the mean (± standard error of the mean) per condition containing 10 bees each.

exposed to LD_{50} when compared between populations ($F_{(2,27)} = 2.693$, $p = 0.000$).

Discussion

Acute toxicity tests are commonly used to assess the effects of pesticides on organisms and are of great importance in bee ecotoxicology (Aksakal, 2020). Our results confirmed that glyphosate was relatively more toxic to the bees in the three tested populations, which differs from the results obtained by Luo et al. (2021) who in their study obtained an LD_{50} of 309 µg i.a./bee and Chen et al. (2022) an LD_{50} of 1773.06 µg a.i/bee, which suggests the low toxicity of glyphosate for bees. However, the results obtained for LD_{50} in this work and those previously reported in the literature seem to increase the variability already found, reporting sublethal effects of the herbicide at the individual level of the honey bees, as recently highlighted by other authors (Herbert et al., 2014; Zhu et al., 2017; Almasri et al., 2020; Odemer et al., 2020; Berg et al., 2018; El Agrebi et al., 2020). Such effects are a current concern as they can reduce bee reproduction, immunity, cognition and general physiological functioning, leading to sub-optimal bee performance and population decline (Chmiel et al., 2020).

Initially, our findings demonstrate that while the LD_{50} values across the three examined sites did not exhibit statistically significant differences, honey bees originating from the high-impact site displayed enhanced responsiveness to Roundup® exposure. This increased responsiveness is evident in their tendency to engage in elevated post-exposure syrup consumption as a potential compensatory mechanism for the encountered stress. This observed behavior aligns with both our working hypothesis and the conjecture put forth by Almasri et al. (2021), which suggests that the susceptibility of honey bee can undergo modulation as a result of preceding exposure to contaminants. This modulation is conceivably attributed to a shift in the physiological condition of organisms historically subjected to such exposures (Almasri et al., 2020). Similarly, related trends have been reported in the context of other insect species. For instance, Chironomid larvae cultivated within laboratory settings for more than six generations have demonstrated a greater LD_{50} for certain metals compared to their counterparts from reference sites (Pedrosa et al., 2017).

As elucidated by these researchers, the distinguished tolerance can be attributed to elevated concentrations of the non-enzymatic antioxidant metallothionein, coupled with intensified aerobic energy

production (Pedrosa et al., 2017). Consequently, a pertinent possibility for future investigations would involve exploring the detoxification and antioxidant capacities within the three honey bee populations, alongside a comprehensive exploration of their metabolic profiles within both control and exposed organisms. A notable observation relating to the three populations under study is related to the distinct volumes of syrup consumed by control honey bees across the study sites. This discrepancy seems to serve as an indicator of varied energy requirements and basal metabolic rates, with the order of magnitude being low-impacted site > reference site > high-impacted site.

Another significant observation emerging from this study pertains to the 6-h experimental interval, wherein the volume of syrup consumed by honey bees from the three distinct populations exhibited a discernible reduction correlating with increasing concentrations of glyphosate exposure. This observation carries significant implications. Essentially, it dictates the consideration that the actual concentration of glyphosate orally ingested per bee is considerably lower than the available dosage. This adjustment in assessment results in a recalibration of LD_{50} values from the perspective of micrograms of ingested glyphosate per bee.

A critical aspect accentuated by this observation is the intrinsic importance of the volume ingested during the exposure period, as it intersects with the nutritional well-being of honey bee and the energy requisites for sustaining homeostasis. This dimension potentially introduces a variable that could further influence the volume of syrup ingested during the subsequent post-exposure period.

Some adjuvants present in the formulation of many pesticides have been shown to be effective taste repellents for bees when infused in a sugar solution (Atkins et al., 1975). As in the study by Larson et al. (2021) where they showed that forager bees approached melon flowers and weeds treated with DEET and piperidine but left before encountering the flowers. Bees exhibit tarsal taste not only for sweet and saline solutions, but also taste for bitter substances (de Brito Sanchez et al., 2014), such as DEET and piperidine analogues. Previous studies demonstrate that DEET activates the taste receptors of bitter-tuned insects (Sanford et al., 2013), while picaridin, a piperidine analogue, has been shown to elicit a response in taste receptors of bitter-sensitive insects (Sparks & Dickens, 2016).

Previous related research has unveiled that honey bees belonging to our focal species exhibit a reduced sensitivity to sucrose when fed with glyphosate-tainted nutrition. This alteration in sensitivity has been linked to associative memory deterioration, mitochondrial perturbations, and compromised ATP production, thereby jeopardizing their overall viability (Faita et al., 2018; Faita et al., 2020; Motta et al., 2020). Furthermore, literature reports indicate that glyphosate elicits modifications in honey bee behavior and food absorption rates (Sandrock et al., 2014; Balbuena et al., 2015). Interestingly, contrasting perspectives exist within the scientific discourse, with certain studies positing that glyphosate does not impair honey bee feeding activity during toxicity evaluations, but rather this impact could be potentially attributed to adjuvants present within herbicide formulations (Zhu et al., 2017; Almasri et al., 2021).

Studies also show that the total volume of syrup ingested by honey bees during toxicity tests is not affected by glyphosate, which contrasts with our findings (Blot et al., 2019; Zhu et al., 2017). Current results show that after glyphosate exposure, honey bee that had contact with the highest available doses consumed more syrup during the post-exposure period, suggesting compensation for stress and hunger during the exposure period. de Assis et al. (2022) underline that to characterize oral exposure, it is important to verify the food consumption of honey bees in experiments with the concentration of pesticides used. With this, it is possible to understand the exact amount of pesticide consumed by organisms and calculate the dose to which each species was exposed, allowing comparisons between bee species with different food consumption, as performed in this study.

Differences in feeding behaviors across various glyphosate dosage levels have been previously documented by other studies (Boily et al., 2013; Helmer et al., 2015; Mengoni Goñalos & Farina, 2018; Blot et al., 2019; Almasri et al., 2020). This alignment with prior investigations is consistent with broader empirical trends that indicate the multifaceted impacts of glyphosate (and other pesticides) on honey bees, manifesting through diverse mechanisms. These encompass deleterious effects on gut microbiota composition (Dai et al., 2018), disruption of foraging behaviors (Pinheiro et al., 2019), alterations in floral visitation patterns (Tschoeke et al., 2019), modifications in maternal behaviors alongside impacts on maternal brain and microbiome (Dechartres et al., 2019), compromised survival rates (Faita et al., 2020), impaired royal jelly production (Chaves et al., 2021), and impaired olfactory learning and memory capabilities (Luo et al., 2021).

In view of the above, it is also observed that glyphosate causes a reduction in the total body weight of honey bees after exposure to the LD_{50} for each population. According to Zhu et al. (2017), the body weight of *Apis mellifera* honey bees decreased when exposed to imidacloprid conjugated with other

herbicides and pesticides including Roundup®, as they ingested less sugar solution when it contained any level of these product residues. In the same study, the authors examined various enzymatic activities and found that most of the pesticide treatments increased the aerobic production of energy and detoxification processes allowing the organism to survive. However, further studies are needed to unravel how energetic metabolism and detoxification are altered by glyphosate and which physiological mechanisms are important for the tolerance in honey bees of an impacted area.

Conclusions

Roundup® affects survival, syrup intake during both exposure and post-exposure period, and body weight of honey bees. The honey bee population from the high-impacted site exhibited greater sensitivity to Roundup®, presenting a lower food intake during the exposure period, amount of glyphosate and LD_{50} compared to those from the reference and low-impacted site. However, honey bees from the low and high-impacted sites exposed to Roundup® presented a decrease in body weight, suggesting a potential reallocation of energy resources towards detoxification processes, which was not observed in the honey bees from the reference site that maintained their body weight.

To deepen our comprehension of the results presented in this paper, future research endeavors will focus on molecular and biochemical tools, to facilitate an in-depth exploration of the underlying mechanisms triggered by glyphosate exposure.

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