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Glyphosate residues in coffee bean: Impact of application methods and compliance with MRLs

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Abstract: **Background:** Farmers often use glyphosate for cost-effective land clearance to streamline coffee harvest processes despite recommendations against its application near the harvest period. However, as set by national and international regulatory authorities, this practice poses a high risk of exceeding the maximum residue limit (MRL) for glyphosate in coffee beans. **Objective:** In this study, glyphosate residues in green coffee beans were assessed, considering different herbicide application methods (mechanical or manual), nozzles (hooded or unhooded), application volumes, and ripening stages. **Methods:** Coffee beans were collected between 15 to 60 days before harvest, and glyphosate residues were determined by high-efficiency liquid chromatography and mass spectrometry (LC-MS/MS). **Results:** Mechanical and manual applications using a protective spray bar device, avoiding the lower third of the coffee trees, maintained glyphosate residue levels within the MRLs established by Brazilian (1.0 mg kg^{-1}) and European (0.1 mg kg^{-1}) regulatory authorities. In contrast, applying glyphosate with the TK-VS-02 nozzle (high-flow impact) without a protective device resulted in levels below the Brazilian MRL but exceeded importing countries' requirements. These residue levels persisted even when applications occurred outside the recommended rainy season but within the 15-day minimum safe re-entry interval. Applications using TK-VS-02 or AI11002 (low-flow air-induced) nozzles targeting the lower third of trees resulted in high glyphosate residue levels, surpassing national and international MRLs, even when applications were conducted 60 days before coffee harvest. **Conclusions:** These findings emphasize the importance of employing the right application technology to produce coffee that complies with the MRLs of any regulatory authority.

Keywords: Coffee Harvest; Global Coffee Platform; Hooded Nozzle; Maximum Residue Limit; Regulatory Authorities; Safe Re-entry Interval

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1. Introduction

Brazil leads global green coffee production and exports. With around 2.24 million ha of coffee comprising 79% being *Coffea arabica* and 21% being *C. canephora*, the country produced for 54.94 million bags (60 kg) in 2023, equivalent to 3.3 million tons (Companhia Nacional de Abastecimento, 2023). Coffee cultivation, largely family farming (78%), is economically viable, delivering substantial income and employment per unit area, and is suitable for mountainous topography (Volsi et al., 2019). To sustain this activity, coffee farmers continually seek methods to increase productivity and cut costs (Bravo-Monroy et al., 2016).

Weed control is essential in coffee farming, as weeds can cause losses exceeding 40% (Fontes et al., 2022). While non-chemical methods like mowing between rows are employed (Zaidan et al., 2022), herbicides, mainly glyphosate, are the primary choice for in-row weed management (Ronchi, Silva, 2018). Glyphosate, a non-selective systemic herbicide, stands out for its ability to control both broadleaf and narrowleaf species, its relatively low toxicity, and favorable cost-effectiveness (Costa et al., 2021; Duke, 2021). To maintain sustainable practices, the Global Coffee Platform of Brazil (GCP-Brazil) established a limit of three glyphosate applications annually: the first after the onset of the rainy season in October, the second in November-December, and the third in March (Global Coffee Platform Brazil, 2020).

In the Brazilian southeast, the coffee tree goes through different stages throughout the year: sprouting (August-September), flowering and fructification (October to March), hilling (April-May), and harvest (June-July) (Ronchi, Silva, 2018). Glyphosate applications, with doses between 480 to 2,880 g ae ha^{-1}, target emerged weeds between the rows and within the crop rows from spreading to fruiting. After hilling, it is advisable to apply residual pre-emergent herbicides to limit weed germination before coffee harvest (Global Coffee Platform Brazil, 2020). However, due to cost considerations, many farmers use glyphosate to clear the area and ease harvesting, deviating from the GCP-Brazil recommendations (Cunha et al., 2016). This happens because the last glyphosate application must align with the end of the rainy season (March); nevertheless, applications post-hilling fall within the safe re-entry intervals

(SRI) of 15-days established by the Brazilian National Health Surveillance Agency (Anvisa) for this herbicide (Agência Nacional de Vigilância Sanitária, 2023).

Regulatory authorities are deeply concerned about pesticide residue levels in food and soil (Winter, Jara, 2015). The quantity of pesticide residues in coffee beans is influenced by several factors, including application methods (e.g., spray equipment, protective devices, nozzles, pressure, and volume) and the timing concerning the crop phenological stage (Reis et al., 2015; Palma et al., 2023). For instance, when glyphosate is applied close to the harvest when fruits are ripe or dried, there is a high risk of residues increasing in coffee beans (Merhi et al., 2022) because fruits are no longer undergoing physiological maturation processes (Cassia et al., 2015). Moreover, coffee harvest coincides with a low rainfall period, hindering leaching and degradation of the herbicide, raising the possibility that glyphosate concentrations in coffee beans exceed the maximum residue limit (MRL).

Anvisa has set an MRL for glyphosate in food at 1.0 mg kg^{-1} and an acceptable daily intake (ADI) of 0.042 mg kg^{-1} day (Agência Nacional de Vigilância Sanitária, 2023), i.e., 1.0 µg g^{-1} or 1.0 µg mL^{-1} and 0.042 µg g^{-1} or 0.042 µg mL^{-1}, respectively, which are like or lower than standards in countries like the United States and Japan (Louie et al., 2021). However, coffee importers, particularly in Europe, have stricter glyphosate MRLs, with a maximum of 0.1 mg kg^{-1} (0.1 µg g^{-1} or 0.1 µg mL^{-1}) (European Food Safety Authority, 2019), which is tenfold lower than Brazil's MRL. Assessing glyphosate residues in soils and sediments is challenging due to the herbicide interactions with soil components, making extraction difficult (Valle et al., 2019; Xu et al., 2019). Furthermore, scientific studies on glyphosate residues in Brazilian green coffee beans are scarce (Pizzutti et al., 2012). In Brazil, the conventional method for detecting glyphosate residues in soil and crops employs high-performance liquid chromatography (HPLC). This method has been validated and revalidated in line with guidelines such as the Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis (Thompson et al., 2002). It can detect glyphosate concentrations as low as 0.01 µg mL^{-1}, meeting the stringent requirements imposed by green coffee importers.

The study aimed to determine glyphosate residue levels in green coffee beans, considering different herbicide application methods (mechanical or manual equipment with and without protective devices, directed at the soil and the lower third of the coffee plant), various flat fan nozzles and flow rates, and different stages from green coffee beans to dry beans of sampling time after glyphosate application.

2. Material and Methods

2.1 Experimental site

The experiments took place at the Santa Adelina farm (22°5.554' S and 48º45.7' W), situated in the municipality of Bariri, a central region of São Paulo State, at an altitude of 439 masl. The predominant soil in this area is a Red Oxisol with a clay texture, containing 13.0 g/dm^3 of organic matter and having a pH of 6.0.

At the outset of the experiments (April 2020), the coffee plantation was 22 years old, and the trees were 2.5 m tall, with a spacing of 3.2 m between rows and 0.8 m between plants. In the experimental area, three fertilizations (20-05-20, NPK) were made in October, December 2019 and March 2020, along with applying 2 tons ha^{-1} of chicken manure. Five foliar fertilizations of Wuxal N-39 (Aglukon, Dusseldorf, Germany) and two applications of 2 L ha^{-1} glyphosate in October and February were also made.

2.2 Evaluated glyphosate treatments

Nine glyphosate (Roundup Original DI, 370 g acid equivalent (ae) L^{-1}, Monsanto do Brasil Ltd.) treatments were evaluated (Table 1), using both mechanical and manual equipment (Figure 1A), with or without hood (Figure 1B), different flat flan nozzles (Figure 1C), and varying spray

Table 1 - Description of treatments (Treat.), equipment, characteristics, dose (g ae ha^{-1}), and volume (L ha^{-1}) for glyphosate residue evaluation in green coffee beans

Treat.	Equipment application and characteristics	Dose	Volume
T1	Tractor sprayer with protected bar (PH 200) equipped with four nozzles, two TK-VS-03 and two 8003	1,850	320
T2	Backpack sprayer with protected TK-VS-02 nozzle (hooded)	1,850	497
T3	Backpack sprayer with an unhooded TK-VS-02 nozzle	1,850	497
T4	Backpack sprayer with an unhooded AI11002 nozzle	1,850	180
T5	Backpack sprayer with a protected TK-VS-02 nozzle (hooded) at 15 days before harvest, directed towards the ground to avoid reaching the lower third	1,850	497
T6	Backpack sprayer with an unhooded TK-VS-02 nozzle at 15 days before harvest, directed towards the ground to avoid reaching the lower third	1,850	497
T7	Backpack sprayer with an unhooded TK-VS-02 nozzle directed to the lower third of the coffee trees	1,850	497
T8	Backpack sprayer with an unhooded AI11002 nozzle at 1-X the field dose directed to the lower third of the coffee trees	1,850	180
T9	Backpack sprayer with an unhooded AI11002 nozzle at 2.5-X the field dose directed to the lower third of the coffee trees	4,625	180

volumes from 180 to 497 L ha^{-1}, either reaching or avoiding the lower third of the coffee plants (Figure 1D). The treatments were distributed in plots measuring 16 m in width (5 rows) and 50 m in length in a completely randomized design scheme, with herbicide application on both sides of the three central rows.

The pressure employed for all treatments was 2.8 bar (40 psi). However, the application speed differed between treatments, with the TK-VS-02 nozzle being applied at 2 km h^{-1} and the AI11002 nozzle at 5 km h^{-1}. The application height for T1 (mechanical application with a tractor sprayer) was 30–40 cm. For treatments T2 to T6, the height ranged from 40 to 50 cm, on the space between the tree skirts and the ground, avoiding contact with the lower third of coffee trees. For treatments targeting the lower third (T7 to T9), the height ranged from 50 to 70 cm, simulating real scenarios where unintentional mistakes or inattention of the applicator can occur. All treatments were carried out during the hilling phase, which falls outside the rainy season and the recommended period for glyphosate applications in coffee plantations (Global Coffee Platform Brazil, 2020). Most treatments were applied on April 2, 2020, except for T7 and T8, conducted on May 17, 2020.

2.3 Coffee been sampling

Coffee bean samplings were conducted at 15, 30, 45, and 60 days after treatment (DAT) for T1–T4 and T7–T9, resulting in four collections for these treatments, except for T9, where 60 DAT samples were not feasible. Coffee samples from T1–T4 were collected along the center row at different heights of coffee trees. For T7–T9, samples were separately collected from the lower-, middle-, and upper-third of coffee trees. In the case of T7, samples were also collected from the soil, and for T9, samples collected at 0 DAT were taken solely from the lower third. T5 and T6 had a single sampling at 15 DAT along the center row at different heights of trees.

Additionally, untreated samples were collected from each treatment as controls before treatment. Four replicates (200-mL plastic containers filled with coffee beans) per treatment were collected on each sampling date. The ripening of the coffee beans varied from intermediate green, lead green, garnet green, red (ripe) and to dry coffee (Figure 1E), depending on the sampling date up to 60 days before harvest (DBH). The samples were stored in a thermal box with ice during transportation (±2 h) before storing them at −18 °C.

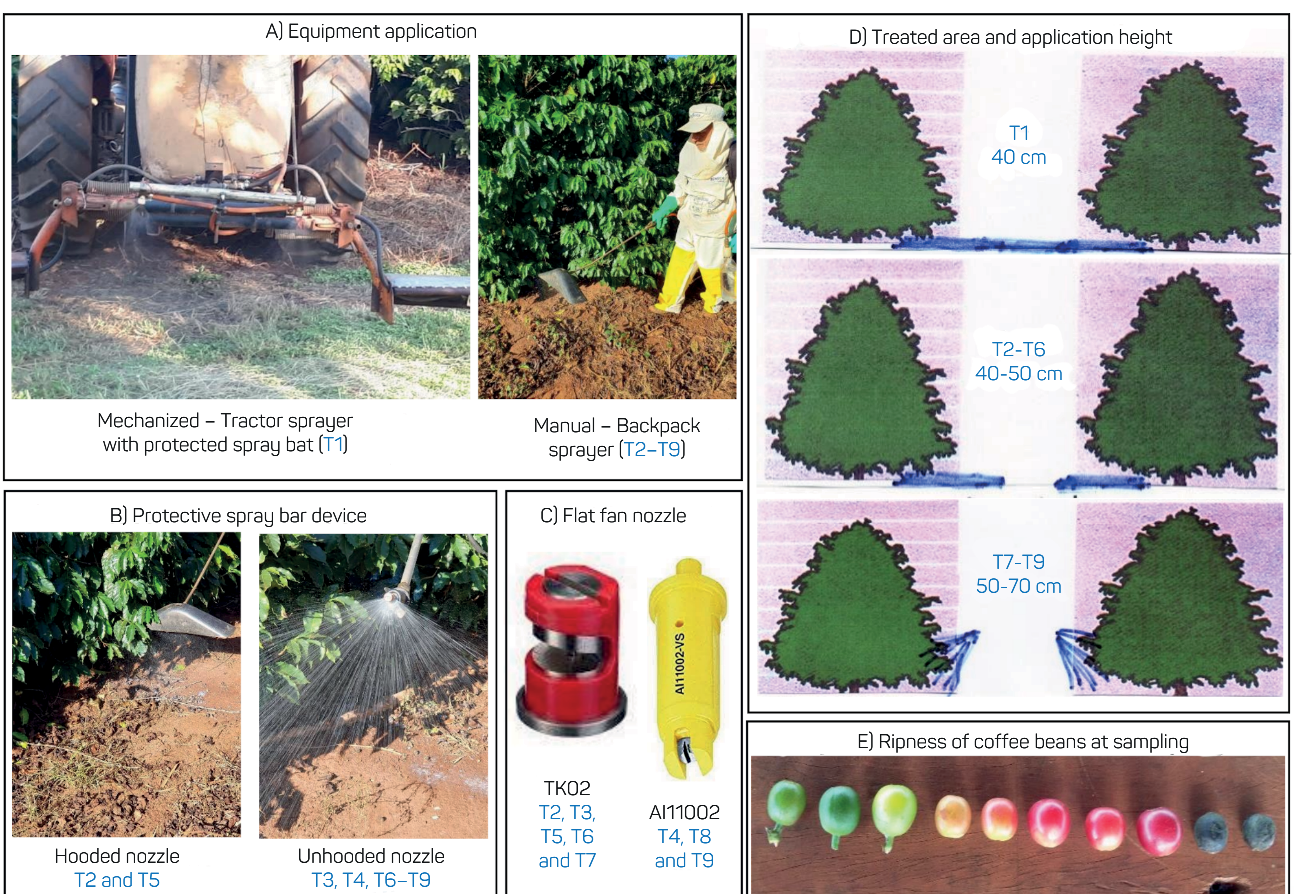

Figure 1 - Main characteristics of glyphosate applications in a coffee plantation located in the Santa Adelina farm, central region of the State of São Paulo, Brazil, for the evaluating residues in coffee beans

2.4 Sample preparation and chromatographic analysis

The samples underwent maceration in a homogenizer with dry ice to facilitate grinding. Subsequently, the samples were freeze-dried in a lyophilizer (Christ, alpha 2-4 LD Plus) at −70 °C. Quantities of 100 mg from each freeze-dried sample were weighed and placed in 5-mL tubes, where 2.5 mL of acidified water (pH 2.5) was added. The extraction of glyphosate was performed in a homogenizer with ceramic spheres (4 spheres tube^{-1}) for 4 min. Afterward, the samples were centrifuged at 4,000 g for 10 min at 20 °C. A 100 µL aliquot of the supernatant was diluted tenfold in water to prevent the contamination of the spectrometer interface by the compounds in the extract.

The extract was filtered through 0.2 µM pore filters and transferred into amber vials for subsequent glyphosate quantification. To correct detected levels for variations in coffee sample maturation stages, 10 ng mL^{-1} of an analytical standard of stable isotope-labeled glyphosate was added to each sample, considering the extraction coefficient obtained from the labeled standard. Glyphosate quantification was performed using high-efficiency liquid chromatography and mass spectrometry (LC-MS/MS) on a liquid chromatograph (Prominence UFLC System, Shimadzu Crop., Kyoto, Japan) coupled with a mass-spectrometer (3200 Q-TRAP, AB Sciex LLC, Framingham, MA, USA), following the chromatographic conditions outlined by Gomes et al. (2015). The mass-spectrometer had a resolution that enabled precise compound detection within a 0.01-mass unit, allowing the use of stable isotope-labeled standards without affecting compound readings. Glyphosate content was determined in micrograms per gram of dry plant tissue (µg g^{-1}) based on sample herbicide concentrations.

3. Results

Glyphosate residues detected in coffee beans varied depending on the type of application, nozzle used, spray volume delivered, and sampling time. For T1 (mechanized application with protected bar), glyphosate residues reached maximum levels of 0.07 µg g^{-1} at 30 DAT and minimums of 0.03 µg g^{-1} at 60 DAT (Figure 2).

In treatments using the TK-VS-02 nozzle, glyphosate residue was influenced by the hood's presence and the spray jet's direction. In T2 (hooded nozzle), the maximum residues (0.62 µg g^{-1}) were measured at 45 DAT, and the minimum (0.27 µg g^{-1}) at 30 DAT. Conversely, residues increased to 5.9 µg g^{-1} at 15 DAT in T3 (unhooded nozzle), but from 45 DAT onward, they remained below 0.6 µg g^{-1}. This increase was also observed in T6 and T5, treatments applied under the same conditions as T3 and T2. However, these applications, which directed the spray jet towards the ground, avoiding reaching the lower third, were evaluated only once because the application was carried out 15 DBH. Residues were tenfold higher (0.45 µg g^{-1}) in samples collected from trees and soil of T6 (unhooded nozzle) compared to samples from T5 (hooded nozzle). When the spray jet was directed to the lower third with an unhooded TK-VS-02 nozzle (T7), residues in samples collected in this third ranged from 4.8 µg g^{-1} (60 DAT) to 8.9 µg g^{-1} (30 DAT). Samples of the middle third exhibited the maximum residue level of 20.2 µg g^{-1} for this treatment at 15 DAT, which decreased to 9.1 µg g^{-1} at 60 DAT. In the upper third, the residue levels were 1.5 µg g^{-1} at 15 DAT and 0 at 60 DAT (Figure 2).

In treatments using an unhooded AI11002 nozzle, which targeted the lower third of coffee trees, glyphosate residue was highly and influenced by the sampling method and the herbicide application rate. In T4, residues of 10.1 µg g^{-1} at 15 DAT and 3.9 µg g^{-1} at 60 DAT were detected in samples collected at different tree heights. In T8 and T9, where samples were collected separately from each third and received 1-X and 2.5-X the field dose, respectively, the highest residue concentrations (up to 33 µg g^{-1}) were observed in samples from the lower third at 15 DAT in both treatments. In T8, the residues remained above 11 µg g^{-1} at 45 and 60 DAT, whereas in T9, they reached 5.9 µg g^{-1} at 45 DAT. Samples from the middle third of T8 showed maximum residues of 3.9 µg g^{-1} at different evaluation periods, which decreased to 0.15 µg g^{-1} at 60 DAT, and no residues were found in the samples from the upper third. For T9, samples of the middle third exhibited residues up to 22 µg g^{-1} at 30 DAT and 5.9 µg g^{-1} at 45 DAT, while samples from the upper third had residues from 7.4 µg g^{-1} at 30 DAT to 4.4 µg g^{-1} at 45 DAT (Figure 2).

4. Discussion

The lowest glyphosate residue level in coffee beans was detected in applications that employed a protective spray bar device, whether with mechanical or manual equipment (T1, T2, and T5). Mechanized application ensured that glyphosate residues remained with the MRLs established by Brazilian (1.0 µg g^{-1}) (Agência Nacional de Vigilância Sanitária, 2023) and international (0.1 µg g^{-1}) regulatory authorities (European Commission, 2017). Nevertheless, mechanizing coffee farming operations can be challenging in areas with steep slopes (Santana et al., 2022), making manual pesticide applications a common practice among growers.

Coffee bean samples from T2 (manual application using a hooded TK-VS-02 nozzle) exhibited glyphosate residues that met Anvisa's MRL but exceeded the MRLs established by European legislation (European Commission, 2017). Similar results were observed in T6 (samples collected from trees and soil) treated at 15 DBH without a hood but directed at the soil to avoid contact with the lower third of trees. Conversely, when the hood was used (T5), residues complied with Anvisa and European legislation MRLs. This demonstrates that when glyphosate applications are carried out without reaching the trees, even when

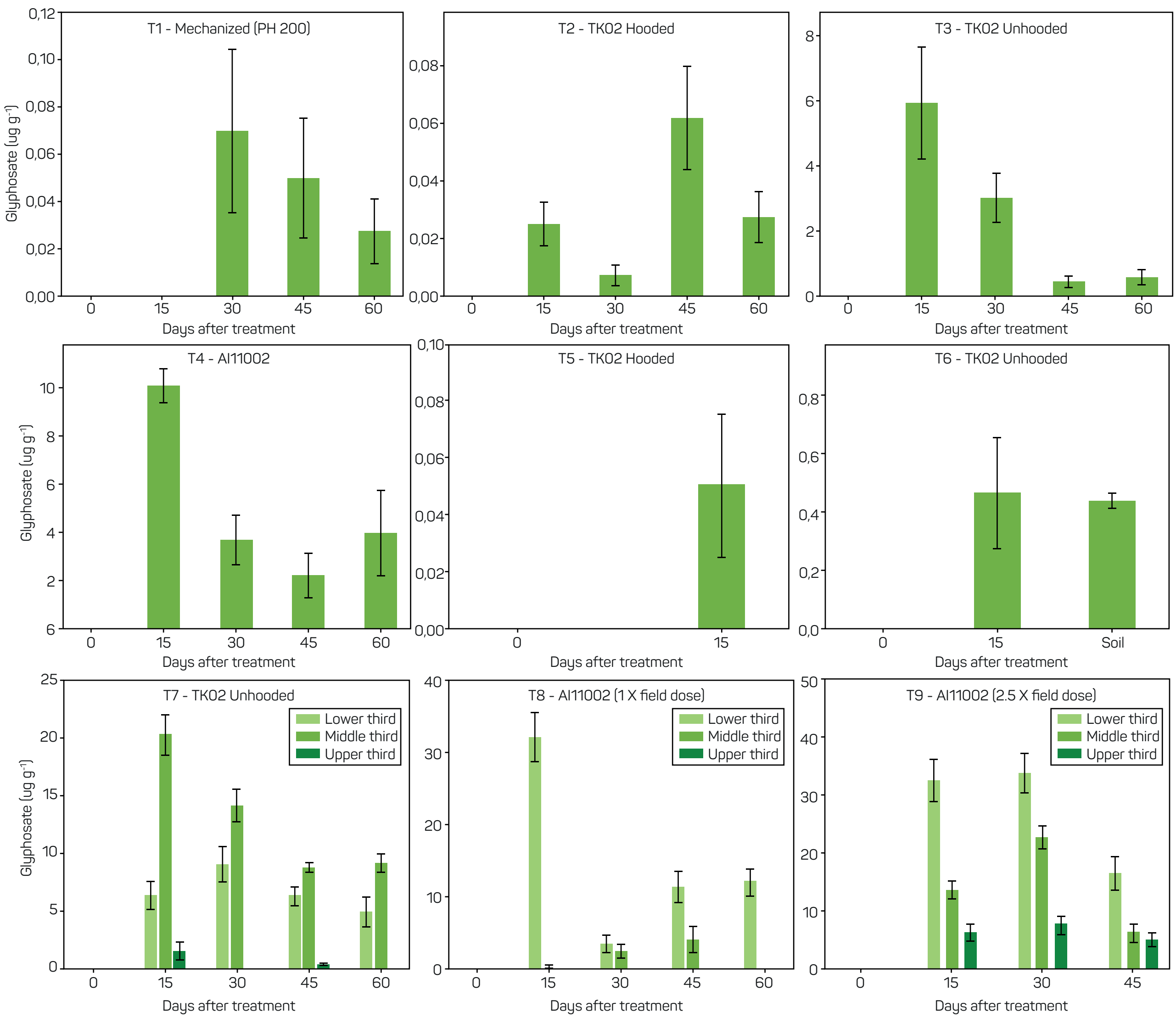

Figure 2 - Glyphosate residues (μg glyphosate g^{-1} dry mass) in coffee beans quantified by liquid chromatography and mass spectrometry (LC-MS/MS). The coffee samples were collected at different intervals after the applying glyphosate with different equipment's, flat fan nozzles, with no protective device on the application bar, volumes and doses of herbicide

performed outside the recommended period but observing the minimum safe re-entry intervals, residue levels can remain below the strict MRLs established by national and international regulatory authorities (Yeung et al., 2017). Therefore, correctly selecting, using, and maintaining pesticide application equipment conserves money and chemicals and contributes to environmental protection (Perry et al., 2002; Ozkan, 2017) and human health (Carrère et al., 2018).

When comparing the TK-VS-02 (high-flow impact) or AI11002 (low-flow air-induced) nozzles and volume applied (T3 vs. T4), the glyphosate residues detected in coffee beans were prohibitive for consumption internal and external, even when applications were made 60 DBH. Despite efforts to prevent contact with the lower third of trees in these treatments, herbicide droplets occasionally drifted onto the leaves and fruits of this section, demonstrating that glyphosate applications without a protective device are unsuitable in coffee cultivation concerning national and international MRLs. GCP-Brazil recommends the use of air-induced anti-drift nozzles to prevent coffee contamination from glyphosate drift (Global Coffee Platform Brazil, 2020). However, using these nozzles is insufficient to reduce coffee bean contamination if a protective device is not employed. Despite the reduced risk of drift, the drops produced by anti-drift nozzles that reach leaves and fruits are more concentrated compared to conventional nozzles (Wang et al., 2023), which increases residue levels, as observed in T4 (unhooded AI11002 nozzle) compared to T3 (unhooded TK-VS-02 nozzle).

GCP-Brazil recommends avoiding herbicide application in the lower third (Global Coffee Platform Brazil, 2020). However, the bushy morphology of coffee trees, with branches close to the ground that hang down due to the weight of fruits, makes it challenging to prevent the spray from reaching the plant. Additionally, in dense plantations with inadequate pruning, maneuvering between the rows and conducting agricultural tasks becomes difficult (Mesquita et al., 2016). All applications that reached the lower third exhibited glyphosate residue levels exceeding the MRL permitted by Anvisa and international standards, regardless of whether they were made at 60 DBH. Furthermore, high glyphosate doses (2.5X the recommended field dose) increased residue levels even after 45 DAT, corroborating herbicide translocation to the middle and upper third of trees. Therefore, to produce coffee within MRL standards, it is advisable to use the correct application technology, preferably opting for localized weed-targeted applications instead of broad applications, while also considering reducing the application speed (Global Coffee Platform Brazil, 2020).

5. Conclusions

Glyphosate residue levels in coffee beans were negligible, adhering to the MRLs set by both national and international regulatory authorities when correctly applying doses of up to 1,850 g ae ha^{-1}, using both mechanical and manual equipment. The application involved a protective spray bar device to ensure that the herbicide does not directly contact the lower third of coffee trees. This condition holds even when glyphosate is applied outside the recommended rainy season to weed control and streamline coffee harvest processes if the minimum safe re-entry intervals are respected to.

In contrast, applications carried out without the use of a protection device, using conventional (TK-VS-02, high-flow impact) or anti-drift (AI11002, low-flow air-induced) nozzles but reaching the lower third of the trees, resulting in high levels of glyphosate residues in coffee beans. Under these conditions, despite the gradual decrease in residue levels over time, glyphosate concentrations exceed the MRLs permitted by Anvisa and international standards, even when applications are made 60 DBH.

Author's contributions

All authors have read and agreed to the published version of the manuscript. LLF, and EDV: conceptualization. LLF, CAC, JDR, and EOO: investigation. LLF, CAC, JDR, and EOO: collection and analysis of the data. LLF, RAC, EDV, and CAC: validation. LLF, RAC, EDV, and CAC: writing–original draft. RAC: writing–review and editing. LLF, CAC, and EDV: resources, supervision and funding acquisition.

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