Publication status: Preprint has been submitted for publication in journal

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Cleyton Batista de Alvarenga, Dalton Luiz Benz, Edson Aparecido dos Santos, George Deroco Martins, Paula Cristina Natalino Rinaldi, Jair Rocha do Prado, João Paulo Arantes Rodrigues da Cunha

https://doi.org/10.1590/SciELOPreprints.12512

Submitted on: 2025-07-04
Posted on: 2025-07-15 (version 1)
(YYYY-MM-DD)

Assessing the effect of using stored spray solutions in weed control efficacy by remote sensing[1]

Cleyton Batista de Alvarenga[2], Dalton Luiz Benz[3], Edson Aparecido dos Santos[3], George Deroco Martins[4], Paula Cristina Natalino Rinaldi[2], Jair Rocha do Prado[5], João Paulo Arantes Rodrigues da Cunha[3]

[1]This article was extracted from the second author's Master Dissertation.

[2]Universidade Federal de Uberlândia, Instituto de Ciências Agrárias (ICIAG), Monte Carmelo, MG, Brazil. cleytonalvarenga@ufu.br (https://orcid.org/0000-0002-0431-6171), paularinaldi@ufu.br (https://orcid.org/0000-0001-9290-9040)

[3]Universidade Federal de Uberlândia, Instituto de Ciências Agrárias (ICIAG), Uberlândia, MG, Brazil. daltonbenz@hotmail.com (https://orcid.org/0000-0002-6783-681X), edsonsantos@ufu.br (https://orcid.org/0000-0003-2316-4502), jpcunha@ufu.br (https://orcid.org/0000-0001-8872-3366)

[4]Universidade Federal de Uberlândia, Faculdade de Engenharia Civil (FECIV), Monte Carmelo, MG, Brazil. deroco@ufu.br (https://orcid.org/0000-0001-9738-7325)

[5]Universidade Federal de Uberlândia, Instituto de Matemática (IME), Uberlândia, MG, Brazil. jairrp@ufu.br (https://orcid.org/0000-0002-6165-4126)

ABSTRACT

The preparation and handling of herbicide spray mixtures, encompassing aspects like storage time and efficacy assessment methods, presents significant challenges in Brazilian agriculture. This study aimed to evaluate the impact of storage duration on the efficacy of spray mixtures containing dicamba and glyphosate, assessed through both remote sensing and conventional visual methods. The experiment was conducted in a randomized block design with four replicates, employing a split-plot scheme where plots received one of four herbicide spray mixturess, subplots eight storage periods, and sub subplots were assessed at seven distinct time points. Results indicated that glyphosate and dicamba spray mixtures maintained their herbicide efficacy even after being stored for up to 28 days. While efficacy varied across the tested rate, one specific combination (1,400 g ha^{-1} glyphosate + 624 g ha^{-1} dicamba) consistently provided the highest weed control. The peak efficacy of the glyphosate and dicamba mixture was observed at approximately fourteen days after application. Furthermore, the Normalized Difference Vegetation Index (NDVI) proved advantageous by providing earlier detection of new weed emergence compared to visual assessment.

Keywords

Tank-mixing, herbicide management, remote sensing, NDVI.

INTRODUCTION

Managing herbicide spray mixtures presents a significant challenge in agriculture[1]. Ideally, herbicide spray mixturess should be applied within 2 to 4 hours from preparation, with application time often dictated by factors such as tank size and required application rate. However, various operational constraints can extend the period during which the spray mixture remains in the tank. These include the use of pre-mixed or concentrated formulations designed for extended storage, adverse weather conditions leading to application delays[2,3], an insufficient number of sprayers relative to the cultivated area, and unforeseen mechanical issues with application equipment. Such delays can result in significant economic losses and environmental concerns due to the potential discarding of unused spray mixtures.

To enhance weed control efficacy, manage resistant weed populations at various developmental stages, and improve operational performance, farmers commonly employ tank-mixing strategies[4]. However, current product labels often provide limited or no comprehensive information regarding the stability and compatibility of these mixtures, particularly concerning the maximum permissible time between product addition to the tank and the completion of application. This lack of clear guidance is particularly critical for hormonal herbicides, where detailed information on mixing and storage time is essential[5,6].

Existing scientific literature on herbicide spray mixtures storage indicates that the period between preparation and application can affect weed control efficacy, particularly when auxinic herbicides like dicamba and 2,4-D are involved[7]. However, findings are inconsistent: some studies report no significant effect of storage[8,9], while others show only weak effects[10]. Despite the widespread practice of storing spray mixtures, either prepared in advance or as unused leftovers[2], there is notably limited information in Brazil regarding the efficacy of stored herbicide spray mixturess for weed control. Storage time can also influence product efficacy and compatibility with other components[9]. Furthermore, the complexities of tank-mixing can lead to various interactions, such as antagonism between auxinic herbicides and Acetyl-CoA Carboxylase (ACCase)-inhibiting herbicides[11], as well as other diverse interactions between products[12].

Traditional visual assessment of weed control efficacy, while widely used, inherently involves subjectivity and necessitates experienced and highly skillful raters[13].

Beyond the challenge of ensuring inter-rater reliability, other critical factors must be considered, such as the adequate sample size required to estimate weed control efficacy with acceptable precision, as demonstrated by Voll et al.[14].

An alternative approach relies on remote sensing to increase precision in potentially subjective evaluations. Vegetation indices provide an effective tool for weed management. The normalized difference vegetation index (NDVI) is one of the indices most used to assess biomass and plant health[15,16,13]. Furthermore, NDVI has been shown to enhance detection capabilities in weed control applications[17]. Considering the highlighted challenges in spray mixture management and assessment methodologies, the current study aimed to rigorously assess the effect of storage duration on the efficacy of spray mixtures containing dicamba and glyphosate, utilizing both remote sensing (NDVI) and conventional visual assessment methods.

MATERIAL AND METHODS

The experiment was conducted in a study area located in geographic coordinates 18°43'23.83" S and 47°31'24.48" W, with an average altitude of 980 meters. The region has a tropical wet and dry or savanna (Aw) climate, with cold (15/16 °C) and dry winters, according to the Köppen climate classification[18]. The herbicides were applied in February 2020 between 8:00 and 9:00 a.m (local time). Weather conditions during applications were monitored using a Kestrel 4000 weather and environmental meter (Boothwyn, PA, USA). The average temperature, relative humidity, and wind speed values ranged from 26 to 29 °C, from 50 to 65%, and 4.32 to 12.6 km h^{-1}, respectively.

The experiment was conducted in a randomized block design, with a split-plot arrangement, consisting of four replicates. The main plots comprised four herbicide spray mixtures, while the subplots represented eight storage periods. Weed control efficacy was assessed at seven time points as repeated measures. For each block, a non-treated control plot was included to serve as a reference for determining herbicide efficacy. The efficacy of weed control was evaluated solely through visual assessment and Normalized Difference Vegetation Index (NDVI) measurements, without the determination of dry biomass.

The four herbicide spray mixturess were as follows: Spray mixture 1: 350 g ha^{-1} glyphosate (acid equivalent - a.e.) + 480 g ha^{-1} dicamba (acid equivalent - a.e.); Spray mixture 2: 700 g ha^{-1} glyphosate (a.e.) + 528 g ha^{-1} dicamba (a.e.); Spray mixture 3: 1,400 g ha^{-1} glyphosate (a.e.) + 624 g ha^{-1} dicamba (a.e.); and Spray mixture 4: 1,750 g ha^{-1} glyphosate (a.e.) + 720 g ha^{-1} dicamba (a.e.). The eight storage periods were in subplots (spray mixtures prepared at 28, 24, 20, 16, 12, 8, 4, and 0 days before application (DBA), and seven assessment time points (1, 3, 5, 7, 9, 14, 21, and 28 days after application (DAA) were evaluated. The experimental area of each plot was 3 × 5 meters in size (15 m^2 total area) and 1.0 m apart, with 2.5 m between blocks and 0.5-m border around each plot.

The first phase of the study in the field was a phytosociological survey[19] of weeds found in the area. Eleven species were identified in the phytosociological survey, namely *Commelina benghalensis*, *Digitaria sanguinalis*, *Euleusine indica*, *Amaranthus viridis*, *Portulaca oleracea*, *Ipomoea triloba*, *Ipomoea nill*, *Emilia fosbergii*, *Richardia brasiliensis*, *Ricinus comunis*, and *Euphorbia heterophylla*, in adult stage, near flowering.

Based on the phytosociological survey, the following herbicides were selected to prepare the pesticide spray mixtures for tank-mixing: glyphosate (62% Glycine, N-

(phosphonomethyl)-potassium salt and 50% acid equivalent) and dicamba (48% 3,6-dichloroanisic acid)[20].

The spray mixtures were prepared and stored in two-liter black polyethylene terephthalate (PET) bottles labeled by treatment. The bottles were black to mitigate the photodegradation effect and were kept in a dark environment (laboratory) with an average temperature of 25 °C until application. The water used to prepare the spray mixtures was collected from an artesian well. The water pH (6.3) was measured using a PHTEK portable pH meter (Curitiba, Paraná State, Brazil).

The herbicides were applied using a Herbicat CO_2-pressurized knapsack sprayer (Catanduva, São Paulo State, Brazil), equipped with a three-meter wand with six Magnojet ADIA 11002 flat-fan nozzles (Ibaiti, Paraná State, Brazil) 0.5 m apart, and the applications were timed to target late weed post-emergence. The operating pressure was 291.3 kPa, producing droplets with diameters ranging from 428 to 622 μm, classified as extremely coarse, according to the American Society of Agricultural and Biological Engineers (ASABE) S572.3 standard[21], as reported in the spray nozzle catalog. The application rate was 200 L ha^{-1} for a good droplet density, target coverage, and compatibility with a 4.56 km h^{-1} Application speed.

In the visual assessment method of plots without treatment identification, weed control efficacy was determined by averaging the scores of three raters. Weed control efficacy was scored according to the following ranges: less than 10%, no control; from 11 to 39%, poor or negligible control; from 40 to 79%, moderate control, insufficient for infestations; from 80 to 89%, good control, acceptable for infestations in the area; and from 90 to 100%, excellent control, with no effects on crops, according to the method of the Brazilian Society of Weed Science[22]. This method, along with NDVI, constituted the primary means of efficacy assessment.

NDVI was determined using images acquired with a DJI Phantom 4 PRO drone (Shenzhen, China), coupled with a MAPIR Survey 3 multispectral camera (San Diego, USA), with a built-in Global Positioning System (GPS) for Red, Green, and Near-infrared imaging[13]. The image acquisition flights were performed 1, 3, 5, 7, 9, 14, and 28 DAA[23], between 12:00 and 13:00, Brasília Time (BRT), in autonomous mode, sixty meters above the target, with 80% longitudinal overlap and side overlap and 75% lateral coverage, yielding 1.8-centimeter pixels. The NDVI of each experimental plot was calculated using the formula: NDVI = (NIR - Red) / (NIR + Red), where NIR represents the reflectance in the Near-Infrared band and Red represents the reflectance in the Red band. This calculation was performed before performing Pearson product-moment correlation with the average scores of the raters during the study period. This method, along with visual assessment, constituted the primary means of efficacy assessment.

The data were pre-processed in MAPIR Camera Control (MCC) software[24] for atmospheric correction and digital number transformation into reflectance percentage. The ortho-mosaic was assembled using Agisoft Photoscan software[25], using dense point cloud and digital elevation model (DEM) as byproducts. The final processing was performed in QGIS Development Team software[26], provided by the Open-Source Geospatial Foundation (OSGeo), to obtain the indices recorded in the images through zonal statistics for each plot.

Statistical analysis was implemented in the R software environment for statistical computing and graphics[27]. After assessing data normality using the Shapiro-Wilk test and homogeneity using the O'Neill and Mathews test, we subjected the data to analysis of variance (ANOVA) using the F test (p<0.05). When the results were significant, we compared means using the Scott-Knott test (p<0.05). A regression analysis was conducted for quantitative data and the models that best explained data variations were adjusted.

RESULTS AND DISCUSSION

The storage time of the spray mixture did not significantly reduce the final weed control efficacy, as applications yielded similar results regarding weed control. However, for visual assessments, poisoning symptoms and associated weed control scores increased in the days following the application. Notably, the storage period of the spray mixture exhibited a significant interaction with the time after application (DAA) regarding the kinetics of herbicidal effects on weed control (Figure 1). Specifically, spray mixtures stored for 12 to 24 days achieved higher scores in assessments conducted five to nine DAA.

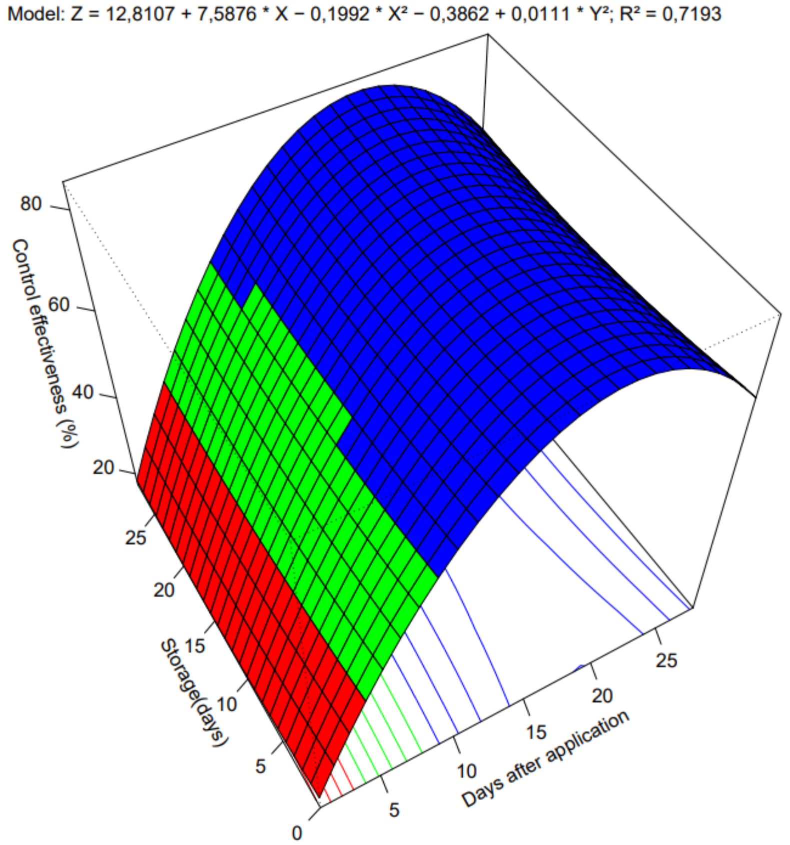

Figure 1. Visual assessment time and storage period effects on weed control efficacy

The spray mixture stored for 12 to 14 DBA provided the highest weed control efficacy (75.0%), classified as moderate control[22]. Crucially, this efficacy was not limited by the storage period but rather by factors related to the evaluation time and the late post-emergence stage of the weed plants, potentially influenced by molecular degradation over extended periods, as suggested by Stewart et al.[10]. Similar findings were reported by Eure et al.[3], who observed no storage effect on herbicide efficacy for up to 9 days. Ramos & Durigan[9] also found that storage did not affect control efficacy when working with various commercial mixtures, including glyphosate and glyphosate + 2,4-D. These results support the hypothesis that the tested spray mixtures remain viable and effective for up to 28 days after preparation.

Weed symptoms observed were typical for glyphosate and dicamba, with toxicity signs becoming clearer and more pronounced over time. The weed control curve, based on visual assessment, showed an increase in efficacy up to approximately 14 DAA, followed by a tendency to plateau at 28 DAA. Concurrently, new weed emergence began emerging in the plots by 28 DAA, influencing subsequent assessments. Castner et al.[6] also noted that dicamba can induce stronger auxin symptoms when mixed with contact herbicides compared to when applied alone.

Regarding the efficacy of spray mixtures containing a mixture of glyphosate and dicamba at different doses, the level of control varied significantly across assessment times (DAA). Spray mixture 3, consisting of 1,736 g ha^{-1} glyphosate (a.e.) + 624 g ha^{-1} dicamba (a.e.), consistently provided the highest visual weed control efficacy across the evaluation period (Table 1).

Table 1. Dose x DAA interaction scores

DAA	Spray mixture 1	Spray mixture 2	Spray mixture 3	Spray mixture 4
1	12.6a	13.8a	13.9a	13.0a
3	26.6c	28.7c	33.3a	31.1b
5	42.3c	47.9b	55.2a	53.4a
7	49.9d	55.5c	65.9a	62.2b
9	52.4c	60.5b	71.4a	69.5a
14	59.9c	68.1b	79.0a	77.7a
28	53.6c	61.8b	79.2a	77.1b
CV (%)	13.07			

p-value $_{\text{herbicide x aging}}$	0.00***
p-value $_{\text{homogeneity}}$	0.19^{ns}
p-value $_{\text{normality}}$	2.65×10^{-16}***

Means with the same letters in rows did not significantly differ from each other according to the Scott-Knott test at 5% probability. ***Significant at 0.1% probability, nsNonsignificant.

The results indicate that the optimal glyphosate and dicamba combination for weed management in the study areas, specifically spray mixture 3 (1,736 g ha^{-1} glyphosate (a.e.) + 624 g ha^{-1} dicamba (a.e.), achieved up to 79.2% visual control, which is classified as good control[22]. This solution consistently outperformed the other tested dose combinations during the study period. It is important to note that various factors, such as application time, prevailing weather conditions, and the specific weed species present[28,29], can also significantly influence the overall weed control efficacy of glyphosate and dicamba.

The spray mixtures exhibited moderate control from 5 DAA at different mixture concentrations. While the most effective dosage (Spray mixture 3) achieved up to 79.2% control, lower doses did not consistently reach this level of efficacy. The data obtained from the various glyphosate and dicamba doses were subjected to regression analysis to model their effect on weed development over time (Figure 2).

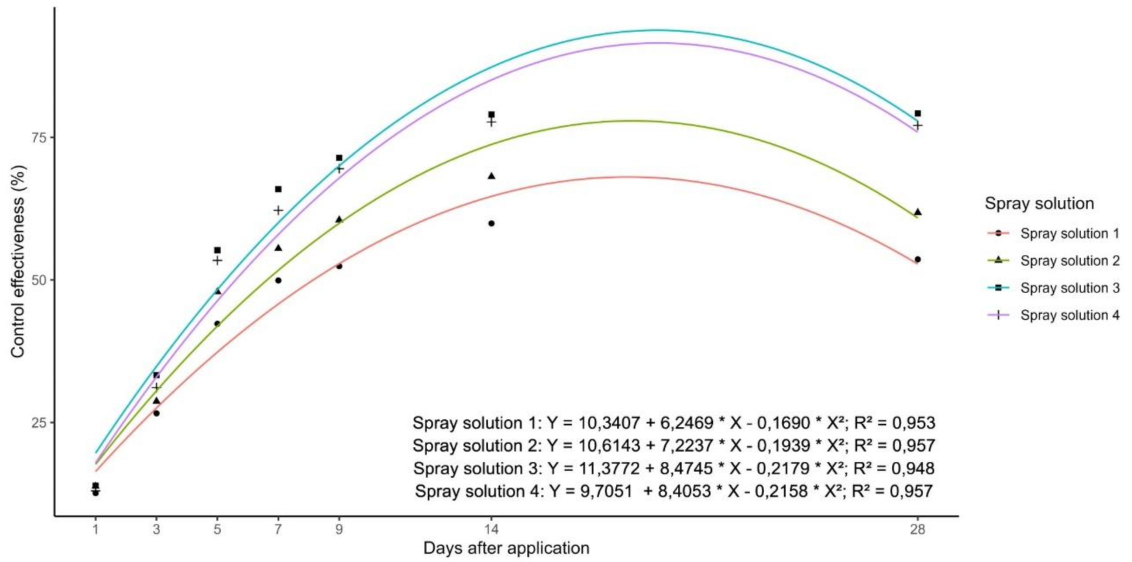

Figure 2. Effect of different herbicide spray mixturess on weed control efficacy

The observed inability to consistently reach weed control efficacy values above 80.0% is likely associated with several interacting factors, including rainfall events during the study period[29], the advanced development stage of the target weed species, and the specific weed community present in the experimental area. Importantly, the pH of the stored spray mixtures remained stable, which confirms findings by Schortgen & Patton[2] and indicates that the observed efficacy levels cannot be attributed to changes in solution pH during storage. While efficacy was observed at 14 and 28 DAA, subsequent visual assessment did not always lead to visibly increased symptoms caused by the herbicides, suggesting a plateau in effect or emergence of new weeds. Comparisons with other studies, such as Ramos & Durigan[9], who reported positive weed management with glyphosate and glyphosate + 2,4-D spray mixtures regardless of assessment time, must consider differences in doses and specific herbicide combinations. Stewart et al.[10] noted a decrease in weed control efficacy under certain conditions involving dicamba and a preemergence herbicide like atrazine, suggesting complex interactions that warrant further investigation.

Analysis of Normalized Difference Vegetation Index (NDVI) values revealed clear trends related to herbicide dosage and application time. Higher NDVI values, indicative of greater vegetation vigor, were observed with the least concentrated spray mixtures. Conversely, NDVI values generally declined as the herbicide dosage increased, reflecting improved weed control. Notably, at 1 DAA, spray mixture 4, containing 2,170 g ha^{-1} glyphosate (a.e.) + 720 g ha^{-1} dicamba (a.e.), resulted in the lowest NDVI, suggesting the earliest and most pronounced impact. By assessment times of 9 and 14 DAA, the lowest NDVI values were recorded across all treatments, indicating that the most significant herbicidal effect on weeds occurred during this period (Table 2).

Table 2. Variation of the normalized difference vegetation index (NDVI) as a function of assessment time of different herbicide concentrations of spray mixture

DAA	Spray mixture 1	Spray mixture 2	Spray mixture 3	Spray mixture 4
1	0.45b	0.45b	0.45b	0.44a
3	0.41b	0.41b	0.40a	0.39a
5	0.45b	0.44b	0.42a	0.42a
7	0.37b	0.35b	0.33a	0.33a
9	0.27d	0.25c	0.22b	0.20a
14	0.27c	0.24b	0.20a	0.19a
28	0.36c	0.33b	0.28a	0.28a

CV (%)	5.32
p-value $_{\text{herbicide x aging}}$	0.0000***
p-value $_{\text{homogeneity}}$	0.99^{ns}
p-value $_{\text{normality}}$	1.46×10^{-16}***

Means with the same letters in rows did not significantly differ from each other according to the Scott-Knott test at 5% probability. ***Significant at 0.1% probability, ^{ns}Nonsignificant.

The largest differences in NDVI, reflecting the maximum impact of herbicide application, were recorded between spray mixtures 1 (lowest dose) and 4 (highest dose) at 9 DAA (35.0% difference) and 14 DAA (42.1% difference), respectively. After 14 DAA, NDVI values across all treatments showed a linear increase up to 28 DAA (Figure 3), which is consistent with the emergence of new weed flushes or recovery of some sub-lethally injured plants.

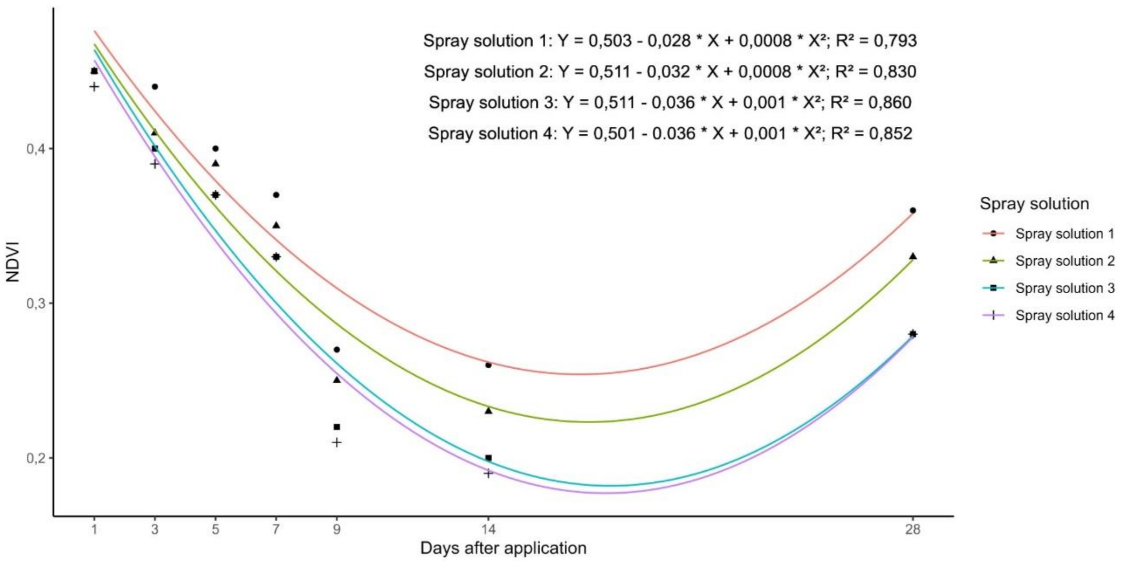

Figure 3. Variation of the normalized difference vegetation index as a function of time after application of the herbicide spray mixtures.

Weed control efficacy, whether assessed visually or via NDVI, generally peaked between 9 and 14 DAA, a period likely influenced by the specific herbicide doses and their respective modes of action. This timeframe proved to be crucial for evaluating the efficacy of different spray mixtures. While visual assessment and NDVI both captured treatment effects, NDVI demonstrated a greater sensitivity to early changes. New weeds began emerging in the plots after 14 DAA. This emergence had a delayed impact on visual assessment, as predominant leaf symptoms continued to mask new growth. In contrast, NDVI already exhibited a clear increase, indicating its capacity for earlier detection of vegetation recovery or new emergence. The strong negative Pearson product-moment correlation of -0.77 between visual assessment scores and NDVI values further indicates that lower NDVI readings correspond to higher visual control scores, reflecting the reduction in green biomass due to herbicide symptoms[13].

Up to 5 DAA, NDVI remained relatively high (above 0.42) across all treatments, indicating initial vegetation vigor. Between 7 and 9 DAA, a notable decrease in NDVI was observed, particularly with spray mixtures 3 and 4. For instance, spray mixture 4 led to a maximum 56.8% decrease in NDVI between 5 and 14 DAA, highlighting its rapid effect. However, by 28 DAA, NDVI increased across all treatments. This increase in NDVI at later stages, consistent with the visual scores, was primarily due to the emergence of new weed flushes in the plots. This emergence was visually perceived by the raters later, typically between 14 and 28 DAA, while NDVI exhibited an earlier response.

The temporal variation of NDVI values at 1, 3, 5, 7, 9, 14, and 28 DAA proved instrumental in inferring changes in herbicide activity over time, thereby providing a rapid and objective means of differentiating plant health in the plots, as confirmed by statistical analysis. As noted by Marino[15], NDVI serves as an effective early warning tool for assessing one of the primary limiting factors of crop productivity – weeds – which can be monitored efficiently using time series analysis of this index (Figure 4).

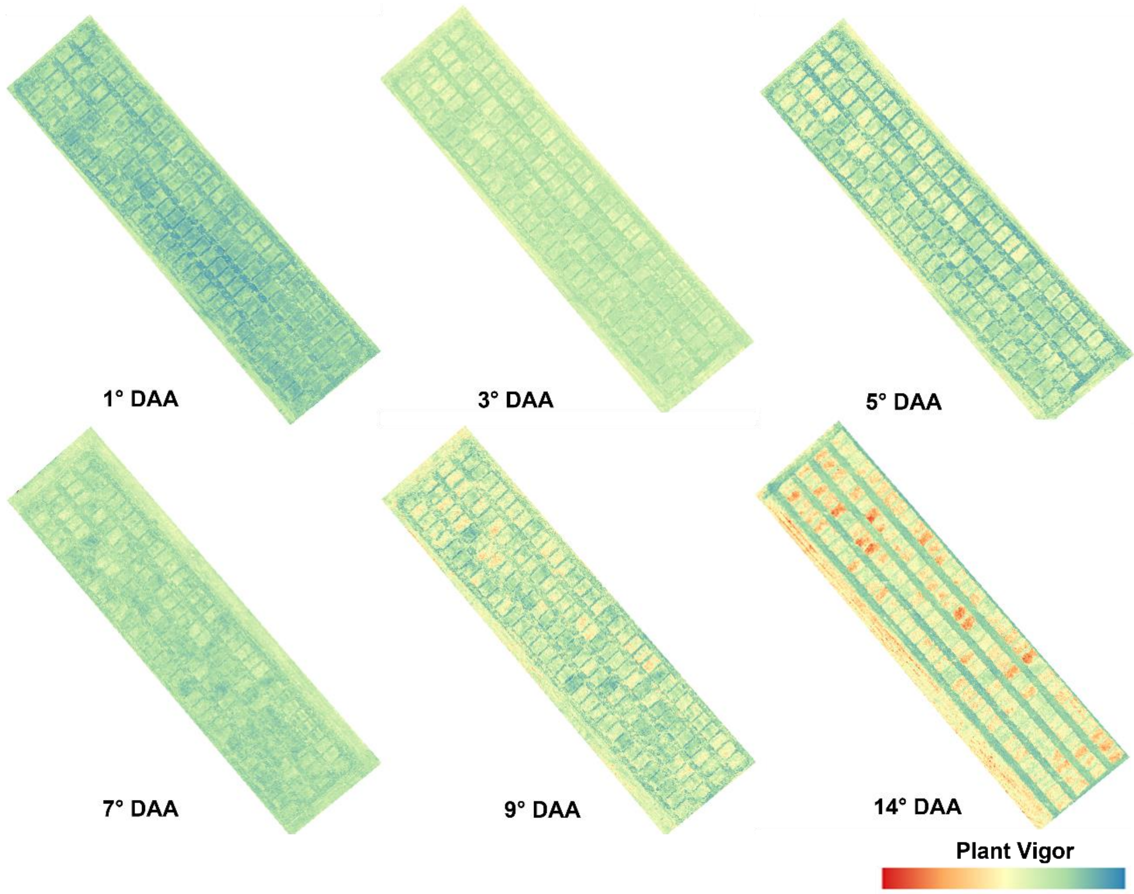

Figure 4. Variation of plant vigor in plots based on NDVI as a function of time after herbicide spray mixtures application.

The findings of this study confirm that spray mixtures containing glyphosate and dicamba mixtures, prepared with the tested water quality, retained their efficacy over the evaluated storage periods. This information holds significant practical value for weed management, offering farmers increased flexibility in application scheduling. This flexibility can lead to a reduction in herbicide waste disposal in inappropriate sites, thereby mitigating potential soil and water contamination[1]. The ability to store spray mixtures ensures that applications proceed only when warranted by confirmed product efficacy, avoiding futile efforts.

Discarding unused spray mixtures carries substantial environmental and economic implications[10]. Furthermore, dicamba residues can be particularly challenging to remove from application equipment, even with standard tank decontamination procedures. Schortgen & Patton[2] also found no adverse storage effects on premixed glyphosate + dicamba spray mixtures held for up to 72 hours. However, it is crucial to emphasize that spray mixture storage should not become a routine daily strategy for farming operations, except perhaps in systems specifically designed for ready-to-use spray mixtures. This practice is primarily beneficial for addressing unforeseen circumstances such as mechanical problems or adverse weather conditions that necessitate temporary application delays. It is also important to consider that environmental factors such as storage temperature, luminosity, and the volume of water added to the spray mixture can potentially influence its long-term application efficacy. Additionally, changes in the pH of the spray mixture during storage may affect the efficacy of glyphosate and dicamba mixtures, as reported by Devkota & Johnson[30].

While visual assessment of weed control efficacy can provide precise and reliable results when performed by experienced and skillful evaluators, it inherently carries a degree of subjectivity influenced by varying levels of knowledge and experience among raters. The Normalized Difference Vegetation Index (NDVI) offers a promising, less subjective alternative for evaluating herbicide efficacy. However, implementing remote sensing techniques effectively requires sophisticated equipment for image acquisition, coupled with precise calibration of flight parameters, including height, time of day, drone speed, pixel size, and image overlap[1].

Beyond objectivity, remote sensing methods like NDVI prove to be valuable for evaluating weed control efficacy by enhancing applicator safety. By minimizing direct contact with treated areas during post-application assessments, the risk of human exposure to chemical contaminants is significantly reduced, representing a crucial benefit for agricultural practices.

CONCLUSION

This study concludes that spray mixtures comprising glyphosate and dicamba spray mixtures effectively maintained their herbicidal efficacy for weed control even after being stored for periods up to 28 days. A consistent peak in weed control efficacy was observed at approximately 14 days after application (DAA) within the experimental area. Among the tested concentrations, the spray mixture combining 1,400 g ha-1 glyphosate (a.e.) and 624 g ha^{-1} dicamba (a.e.) demonstrated superior performance, consistently achieving the highest weed control efficacy as determined by both visual assessment and remote sensing techniques, particularly maintaining efficacy towards the latter stages of the evaluation period. Furthermore, the Normalized Difference Vegetation Index (NDVI) proved to be a valuable tool, enabling the earlier detection of new weed flushes compared to conventional visual methods, thus offering a more objective and sensitive alternative for assessing herbicide efficacy.

AUTHOR CONTRIBUTIONS

Conceptualization: Cleyton Batista de Alvarenga; Paula Cristina Natalino Rinaldi; João Paulo Arantes Rodrigues da Cunha

Data curation: Cleyton Batista de Alvarenga; Dalton Luiz Benz

Formal analysis: Cleyton Batista de Alvarenga; George Deroco Martins; Edson Aparecido dos Santos; Jair Rocha do Prado

Methodology: Cleyton Batista de Alvarenga; Paula Cristina Natalino Rinaldi; João Paulo Arantes Rodrigues da Cunha; George Deroco Martins; Edson Aparecido dos Santos; Jair Rocha do Prado; Dalton Luiz Benz

Project administration: Cleyton Batista de Alvarenga

Software: George Deroco Martins; Jair Rocha do Prado; Dalton Luiz Benz

Validation: Cleyton Batista de Alvarenga; George Deroco Martins; Edson Aparecido dos Santos; Jair Rocha do Prado; Dalton Luiz Benz

Visualization: Cleyton Batista de Alvarenga

Writing – original draft: Cleyton Batista de Alvarenga; Paula Cristina Natalino Rinaldi; João Paulo Arantes Rodrigues da Cunha; George Deroco Martins; Edson Aparecido dos Santos; Jair Rocha do Prado; Dalton Luiz Benz

Writin – review & editing: Cleyton Batista de Alvarenga; Paula Cristina Natalino Rinaldi; João Paulo Arantes Rodrigues da Cunha

DATA AVAILABILITY STATEMENT

All data supporting the results of this study are contained within this article.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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