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CROP SENSITIVITY TO DICAMBA AND 2,4-D APPLIED AT COMMERCIAL AND SUBDOSE LEVELS

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ABSTRACT

The development of dicamba and 2,4-D resistant crops may result in the widespread use of these herbicides in agricultural areas, potentially affecting nearby susceptible crops. This study aimed to evaluate the sensitivity of bean, peanut, and cotton to different doses of Dicamba and 2,4-D. To do so, separate experiments were conducted for each crop and herbicide in a greenhouse, using a completely randomized design with four replications. Both herbicides were applied at different doses to plants with the second pair of true leaves. Phytotoxicity and dry biomass were evaluated. For cotton, 2,4-D showed high phytotoxicity at doses of up to 83.75 g ai ha^{-1}, with a reduction in suppressive effect observed at 20.93 g ai ha^{-1}. Dicamba exhibited a pronounced reduction in crop biomass up to a dose of 70 g ai ha^{-1}, with 94 % phytotoxicity. For beans, the evolution of symptoms using 2,4-D occurred more slowly, but the highest doses resulted in phytotoxicity of up to 95 % and a 30 % reduction in biomass at 167.5 g ai ha^{-1}, indicating a significant impact on the crop. Dicamba also had a high negative impact, with a 100 % reduction in biomass at 70 g ai ha^{-1}. The peanut crop was more tolerant to herbicides, with 2,4-D doses of 670, 335, and 167.5 g ai ha^{-1} resulting in phytotoxicities of 69.5, 37.49, and 14.37 %, respectively. Dicamba significantly reduced dry biomass at doses of up to 70 g ai ha^{-1}. The results show that, despite the differences in sensitivity of cotton, bean, and peanut to 2,4-D and Dicamba, even low doses of these herbicides applied early in development had a significant negative impact on these crops.

Keywords: *Phaseolus vulgaris, Arachis hypogaea, Gossypium hirsutum*, phytotoxicity, hormonal herbicides, contamination.

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INTRODUCTION

Synthetic compounds acting as phytohormonal “superauxins” have been effective herbicides in agriculture for over 60 years. These so-called auxin herbicides are more stable in plants than the main natural auxin, indole-3-acetic acid (IAA), and have systemic mobility and selective action. They are primarily used to control dicotyledonous weeds in cereal crops. These herbicides belong to different chemical classes, such as phenoxycarboxylic acids, benzoic acids, pyridinecarboxylic acids, carboxymethyl aromatic derivatives, and quinolinecarboxylic acids (Grossmann, 2010).

Concerns about synthetic auxin herbicides stem from increased use of 2,4-D and Dicamba in several countries, including Brazil, as a result of recent transgenic crops releases. In 2019, at least 9 million kg of Dicamba were applied to soybeans and cotton in the United States, either before sowing or during the growing season, whereas less than 5 million kg were applied to all other cropping systems and pastures (USGS, 2019).

Currently, more than 90 % of the soybeans grown in Brazil are transgenic, with tolerance to glyphosate and insect attacks. The National Biosafety Technical Commission (CTNBio), an agency responsible for approving genetically modified organisms in Brazil, released a soybean cultivar resistant to Dicamba in 2016 to increase the number of technologies available to farmers. In addition to Dicamba-tolerant soybean, CTNBio has been marketing soybean resistant to 2,4-D, glyphosate, and ammonium glufosinate since 2019 (CTNBio, 2022). While the new technology helps manage weeds, especially for glyphosate-resistant species, herbicides can cause damage to sensitive crops due to drift or residue contamination in sprayer tanks (Behrens *et al*., 2007; Riter *et al*., 2021; Tian *et al*., 2023).

2,4-D is the first modern herbicide and has been used on farms, roads, and lawns since the late 1940s (Peterson *et al*., 2016). The Brazilian Health Regulatory Agency (ANVISA), an agency linked to the Ministry of Health, has reassessed 2,4-D and will maintain it in the Brazilian market with restrictions on its application, such as preventing the same worker from preparing and applying the product when a tractor is used and setting crop-specific time intervals for worker entry into treated areas. These measures are consistent with recent reassessments of the herbicide in other countries. Currently, 2,4-D is available worldwide except in Mozambique, where its use is prohibited (ANVISA, 2019).

Dicamba has been used for weed control in corn, wheat, pasture, and turfgrass plantations since the 1960s, and its sale in Brazil began in the 2021-2022 growing season. Its use is recommended in the pre-planting of soybeans and cotton and in post-emergence applications for soybean crops with the Intacta2 Xtend™ technology, which metabolizes the herbicide into non-toxic compounds. Symptoms of auxin herbicides include inhibition of cell division, obstruction of phloem flow, epinasty (bending) of leaves, petioles, branches, and stems, changes in leaf venation and wrinkling, brittle stems, chlorosis, wilting, and necrosis of leaves, and alteration of growth and atrophy of roots. The death of susceptible plants occurs slowly, ranging from 3 to 5 weeks after application (Vidal and Merotto, 2001).

Some older formulations of 2,4-D and Dicamba were known to be highly susceptible to spray drift and post-application volatilization, which can lead to irreversible problems, as reported by Mueller *et al.* (2013), Everitt and Kelling (2009), and Marple *et al.* (2007). Even at very low doses, auxin herbicides may have collateral effects (da

Silva *et al.*, 2018), and several crops are highly sensitive, such as non-Dicamba-resistant soybean, beans, cucumber, beet, tomato, cotton, and peanut, as reported by Johnson *et al.* (2012) and de Aguiar *et al.* (2020).

To reduce the potential for external movement, legal proceedings and government regulations resulted in changes in the way these herbicides are used and in the physicochemical characteristics of their formulations (Miller and Butler-Ellis, 2000). However, 2,4-D and Dicamba were reported as first and third, respectively, on the list of herbicides that caused crop damage due to drift in the US. Farmers in 25 American states have filed more than 2700 complaints with state agricultural agencies regarding Dicamba damage to conventional soybean and other crops (AAPCO, 2017).

Under field conditions in Florida, dose-response studies have shown that peanut plants treated with 2,4-D (doses ranging from 70 to 1120 g ai ha^{-1}) and Dicamba (from 35 to 500 g ai ha^{-1}) had phytotoxicity from 0 to 35 and 20 to 78 %, respectively, as reported by Leon *et al.* (2014). Dicamba volatilization in the post-emergence application was reported as the primary exposure route, causing damage to non-target vegetation (Sciumbato *et al*., 2004). Cotton plants subjected to subdoses (0, 1/200, and 1/400 of the commercial dose (CD) of 561 g ai ha^{-1}) of 2,4-D and Dicamba at different phenological stages showed higher phytotoxicity at the 3–4 leaf stage at the time of application, and higher plant recovery was observed in Dicamba-treated plants, as reported by Marple *et al.* (2008).

Dicamba drift on ‘Ponkan’ mandarin seedlings was more toxic compared to 2,4-D, but both herbicides severely affected the physiological processes of the plants, according to Brochado *et al.* (2022). Lettuce was highly sensitive to 2,4-D drift, resulting in plant death with doses of up to 1/8 of the commercial dose, while Dicamba provided phytointoxication levels higher than 30 % for up to 1/8 CD. The tomato crop also showed high susceptibility up to subdoses of 1/32 for both herbicides (Roesler *et al.*, 2020).

Contamination with 2,4-D and Dicamba can arise when residues in sprayer tanks are not properly washed. When these residues come into contact with other herbicides, solvents, or adjuvants, they can dilute and cause contamination (Roesler *et al.,* 2020). It is crucial to distinguish plant responses resulting from particle drift, volatilization, and tank contamination in the field because the management approaches for each are fundamentally different (Riter *et al.,* 2021).

Therefore, to address the imminent problems related to the large-scale use of auxin herbicides, this study aims to evaluate the effects of different doses of Dicamba and 2,4-D on commercial cotton, bean, and peanut crops that are sensitive to these herbicides. The hypothesis is that different doses of auxin herbicides can cause initial damage to crops that are not tolerant to them.

MATERIALS AND METHODS

The experiments were carried out in a greenhouse at the Federal University of São Carlos, Brazil, using bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*), and cotton

(*Gossypium hirsutum*) as the cultivated species. The experimental design involved a completely randomized setup with four replications, and each crop was evaluated separately for two herbicides (2,4-D and Dicamba) at nine different doses, including control treatments without herbicide application. The herbicide doses tested were 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.791, and 0 % of the commercial dose for each herbicide. Each experimental unit was comprised of a 5 L pot with a single plant. Crop varieties were selected based on their importance for producers, including Netuno for bean, BS2106 GL for cotton, and Runner IAC 886 for peanut. Soil samples used in the experiment were subjected to chemical analysis (Table 1).

Table 1. Chemical analysis of Latossolo Vermelho-Escuro (Oxisol) samples used in the experiment.

Latossolo Vermelho-Escuro (Oxisol)									
P resin	OM	pH	K	Ca	Mg	H+Al	SB	CEC	V
Mg dm^{-3}	g dm^{-3}	CaCI$_2$	mmol dm^{-3}						%
15	38	5.6	5.4	53	13	26	71.4	97.4	73

To define the doses for the experiments, we consulted existing literature on simulated drift for the herbicides studied (Johnson *et al*., 2012; Kruger *et al*., 2012). Nine doses were used for each herbicide: 670 (commercial dose, CD), 335, 167.5, 83.75, 41.87, 20.93, 10.46, 5.23, and 0 g ai ha^{-1} for 2,4-D, and 560 (CD), 280, 140, 70, 35, 17.5, 8.75, 4.37, and 0 g ai ha^{-1} for Dicamba. In all three crops, plants were treated at the four-pair true leaf stage, with three tests conducted simultaneously. Spraying was carried out using a CO$_2$-pressurized knapsack sprayer at a constant pressure of 245.16 kPa, using an application boom equipped with nozzles with 110.03 fan spray tips and a spray solution volume of 200 L ha^{-1}. Relative humidity, air temperature, and wind speed during application were 70 %, 25 °C, and 4 km h^{-1}, respectively. After treatment, the plants were maintained in a greenhouse and irrigated as required.

Phytotoxicity was assessed at 5, 10, and 15 days after application (DAA) using a percentage score scale, in which 0 corresponds to no injury and 100 to plant death (ALAM, 1974). Shoot dry biomass was evaluated at 15 DAA, removing plants close to the soil surface, placing samples in paper bags, and drying them in a forced-air circulation oven at 65 °C until constant dry mass weight (about 72 h).

Dry biomass data were transformed into a percentage (%) relative to the control (without herbicide application), according to the following formula:

$$X\ (\%) = 100 - \left[\left(\frac{m\ rep\ treat\ x\ 100}{m\ \bar{x}\ \ test}\right)\right]$$

where X is the percentage reduction of the treatment, m is the mass (g), $treat$ is the treatment, $\bar{x}$ is the mean, and $test$ is the control.

The collected data (phytotoxicity and biomass reduction) underwent analysis of variance using the F-test. When significant, a regression analysis was performed to select the explanatory model of the dose-response curve based on statistical significance ($p < 0.05$) and determination coefficients (R^2) using SigmaPlot software (Systat Software Inc.).

RESULTS AND DISCUSSION

The herbicide 2,4-D presented a fast increase in phytotoxicity levels (Figures 1A and 1C), with low doses showing high phytotoxicity just five days after application. The commercial dose and half of it (670 and 335 g ai ha^{-1}) caused 90 % phytotoxicity at 15 DAA, leading to plant death. The dose of 83.75 g ai ha^{-1}, corresponding to 12.5 % of the commercial dose, resulted in a high phytotoxicity of approximately 90 %. The application of 41.87 g ai ha^{-1}, representing 6.24 % of the commercial dose, caused a progressive increase in phytotoxicity, reaching about 50 % in the last evaluation and potentially causing irreversible damage. The lowest doses of 2,4-D (20.93, 10.46, and 5.23 g ai ha^{-1}) did not show high phytotoxicity, presenting less than 10 % and causing only mild chlorosis in plants.

Cotton plants were sprayed with herbicide at the stage of four pairs of true leaves, i.e., in a juvenile phase. According to de Oliveira (2015), phytotoxicity in cotton plants caused by 2,4-D is inversely proportional to plant height, leaf number, and stem diameter. Hence, less developed plants showed more intense symptoms, which supports our results. Early application of 2,4-D in cotton crop development leads to injuries and negatively affects some cotton fiber quality characteristics, resulting in reduced productivity (de Souza, 2021).

Dicamba showed 79 % phytotoxicity at 5 DAA from 70.0 g ai ha^{-1} (12.5 % CD), which progressed to 94 % at 15 DAA, causing plant death (Figures 1B and 1D). Even lower doses (17.5 g ai ha^{-1}) resulted in approximately 17.5 % phytotoxicity, with visible symptoms characteristic of auxin mimetics such as epinasty, leaf bending, and necrosis. The lowest evaluated doses of 8.75 and 4.37 g ai ha^{-1} showed no significant injuries to the plants compared to the controls.

Neto (2019) reported that cotton plants may show symptoms such as leaf burn, necrosis, epinasty, leaf bending, and reduced development after 24 hours of indirect exposure to Dicamba using volatilization chambers, indicating a high potential for yield losses due to herbicide drift. Moreover, cotton is also susceptible to other auxin-mimicking herbicides, such as aminocyclopyrachlor. In this sense, Flessner *et al.* (2012) found that crop response to aminocyclopyrachlor drift was comparable to that of 2,4-D and aminopyralid, as treated plants showed injuries and reductions in height and shoot dry biomass, demonstrating the high susceptibility of cotton to this mechanism of action.

Cotton plant dry biomass reduction (Figure 1) followed a phytotoxicity response pattern. The highest 2,4-D doses (670, 335, and 140 g ai ha^{-1}) caused a biomass reduction of over 80 % in cotton plants. Doses of 83.75 and 41.87 g ai ha^{-1}, considered low (12.5 and 6.25 % of the field dose, respectively), showed a significant reduction ranging from 23 to 50 %, respectively. Plant biomass was not significantly affected by doses below 20.93 g ai ha^{-1}, although symptoms such as epinasty and leaf wrinkling were still observed. The two highest doses of Dicamba had the greatest impact on biomass, with a reduction of over 80 % at the highest dose. Intermediate doses of 140 and 70 g ai ha^{-1} significantly reduced biomass (75.10 and 86.55 %) compared to the control. Doses lower than 35 g ai ha^{-1} did not differ from each other, ranging from 4 to 12 % reduction.

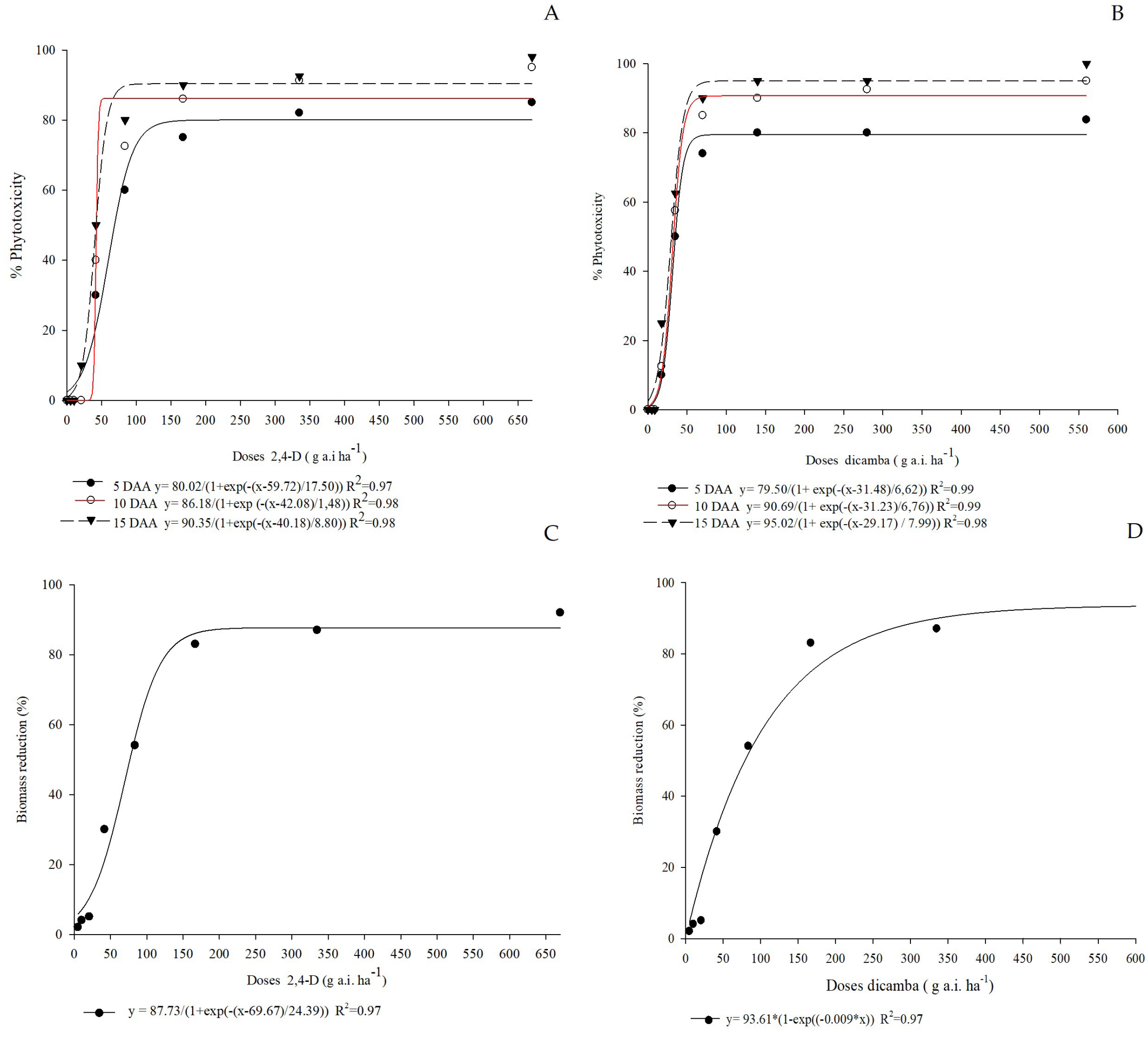

Figure 1. Phytotoxicity (%) and biomass reduction (%) on cotton plants at 5, 10, and 15 days after application (DAA). A, C: caused by 2,4-D; B, D: caused by Dicamba.

The phytotoxicity analysis of 2,4-D (Figure 2A) and Dicamba (Figure 2B), as well as biomass reduction for the bean crop (Figures 2C and 2D), show that the symptoms evolved more slowly for 2,4-D compared to the dynamics observed for cotton. The highest dose (670 g ai ha^{-1}) resulted in approximately 62 % phytotoxicity at 5 DAA, with visible symptoms of leaf yellowing and stem epinasty, progressing to 74 % at 10 DAA and 95 % at 15 DAA. Using half of the highest dose at 5, 10, and 15 DAA, the phytotoxicity reached 61.9, 72.9, and 84.2 %, respectively (Figure 2A).

Intermediate doses of 167.5 and 83.75 g ai ha^{-1} (25 and 12.5 % CD, respectively) resulted in final phytotoxicity of 55 and 35 %, respectively, indicating a high impact on the

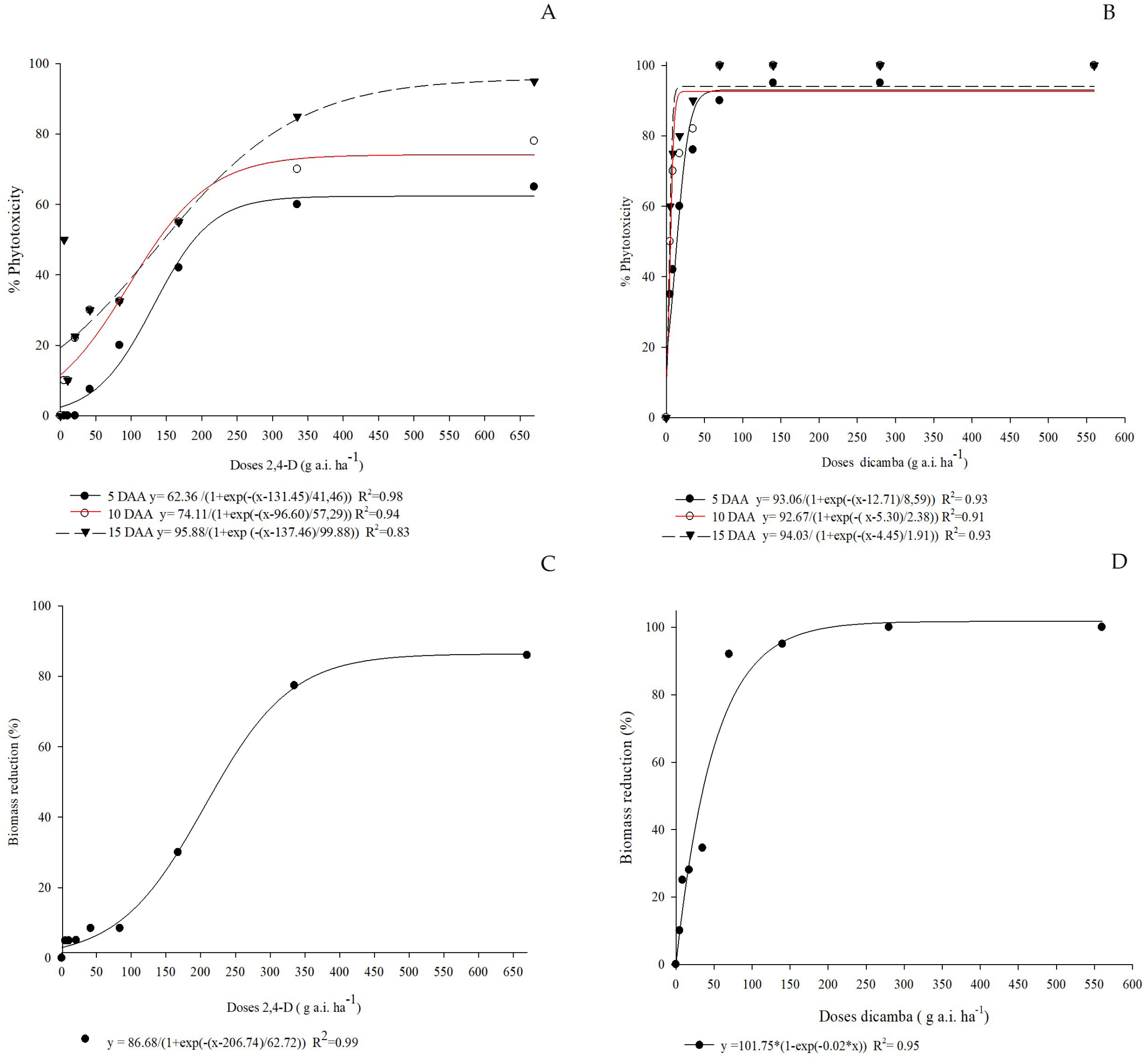

Figure 2. Phytotoxicity (%) and biomass reduction (%) on bean plants at 5, 10, and 15 days after application (DAA). A, C: caused by 2,4-D; B, D: caused by Dicamba.

crop. Although the phytotoxicity dropped as 2,4-D doses decreased, slight chlorosis was still observed in plants even with the minimum dose (5.23 g ai ha^{-1}), causing 20 % phytotoxicity. Massucato *et al.* (2021) found that bean seedlings subjected to subdoses of 2,4-D showed a reduction in germination and length, and increasing concentrations reduced emergence and increased plant injuries.

The evolution of phytotoxicity caused by Dicamba in the bean crop was observed in the first evaluation, with little or no difference between evaluation times for most doses. Phytotoxicity reached approximately 86 % in the initial evaluation (5 DAA) even at low doses (6.25 % CD). At 15 DAA, values were above 85 %, with the exception of the lowest dose of 4.37 g ai ha^{-1} (0.78 % CD), which had a phytotoxicity of 46 %. Thus, high injury rates were observed even at the lowest doses, with higher doses resulting in plant death and intermediate doses resulting in severe and irreversible damage to beans. In their study, de Aguiar *et al*. (2020) found that the dose to inhibit 50 % of bean plants was 17.75 g ai ha^{-1} at 14 DAA, indicating that the species, together with soybean, is extremely sensitive to Dicamba.

The impact of Dicamba on bean biomass (Figure 2D) clearly showed two groups of doses: 280 to 560 g ai ha^{-1} resulted in a total reduction in plant biomass, while 4.37 to 35.0 g ai ha^{-1} reduced plant biomass by 15 to 50 %. Thus, doses above 35 g ai ha^{-1} can be considered highly problematic for the crop. The two highest doses of 2,4-D reduced bean dry biomass by 85.99 and 77.37 %, respectively, making them the most harmful to the crop. The dose of 140 g ai ha^{-1} (25 % CD) caused a reduction of 23 %, while the other doses showed biomass reductions ranging from 0 to 10 %. Hence, the biomass reduction data shows that Dicamba has a higher negative impact on the crop when applied at this stage of development.

The results of phytotoxicity and biomass reduction caused by 2,4-D (Figures 3A and 3C) and Dicamba (Figures 3B and 3D) in the peanut crop show that phytotoxicity symptoms increased with the course of the evaluations, with epinasty in the stems, especially at doses above 83.75 g ai ha^{-1}, but with no high suppressive effect as in the other crops. The evaluation conducted at 5 DAA showed phytotoxicities of approximately 34.9, 10.3, and 2.57 % for doses of 670, 335, and 167.5 g ai ha^{-1}, respectively. These values evolved to 69.5, 37.49, and 14.37 % at 15 DAA, respectively. Doses lower than 20.93 g ai ha^{-1} resulted in mild chlorosis, which culminated in phytotoxicity values below 5 %. Similarly, Leon *et al.* (2014) observed that peanut plants sprayed with 2,4-D showed low phytotoxicity. On the other hand, Faircloth and Prostko (2010) reported that 2,4-D applied 75 days after peanut planting affected crop yield due to phytotoxicity.

Peanut plants sprayed with doses of 560, 280, 140, and 70 g ai ha^{-1} of the herbicide Dicamba showed phytotoxicity ranging from 44.9 to 48.2 % at 5 DAA, with symptoms of leaf and stem epinasty. The phytotoxicity reached values ranging from 72 to 83.6 % at these same doses at 15 DAA. Therefore, the variation between the percentages from 100 to 12.5 % CD was low. Doses of 35, 17.5, and 8.75 g ai ha^{-1} showed lower values, ranging from 10.8 to 35.4 %.

According to Blanchett *et al.* (2015), peanut plants become more sensitive as they approach the reproductive stage (initial flowering), making them more susceptible to herbicide damage at advanced stages of development. There is a direct relationship between the dose of a product and the sensitivity of the plants, reflecting the damage, which can lead to a reduction in productivity. In this study, the applications took place when the peanut plants were at the beginning of their development, thus showing greater tolerance to underdosing. Leon *et al.* (2014) also observed that Dicamba doses of 70, 140, 280, 560, and 1120 ai ha^{-1} resulted in phytotoxicity ranging from 20 to 78 % in the peanut crop, reducing its productivity by 65 %.

Results on the reductions in shoot dry biomass of peanut plants treated with 2,4-D and Dicamba (Figure 3) show that the highest dose of Dicamba led to a reduction of 59.15 % in plant dry biomass, while the dose of 70 g ai ha^{-1} showed a 24 % reduction,

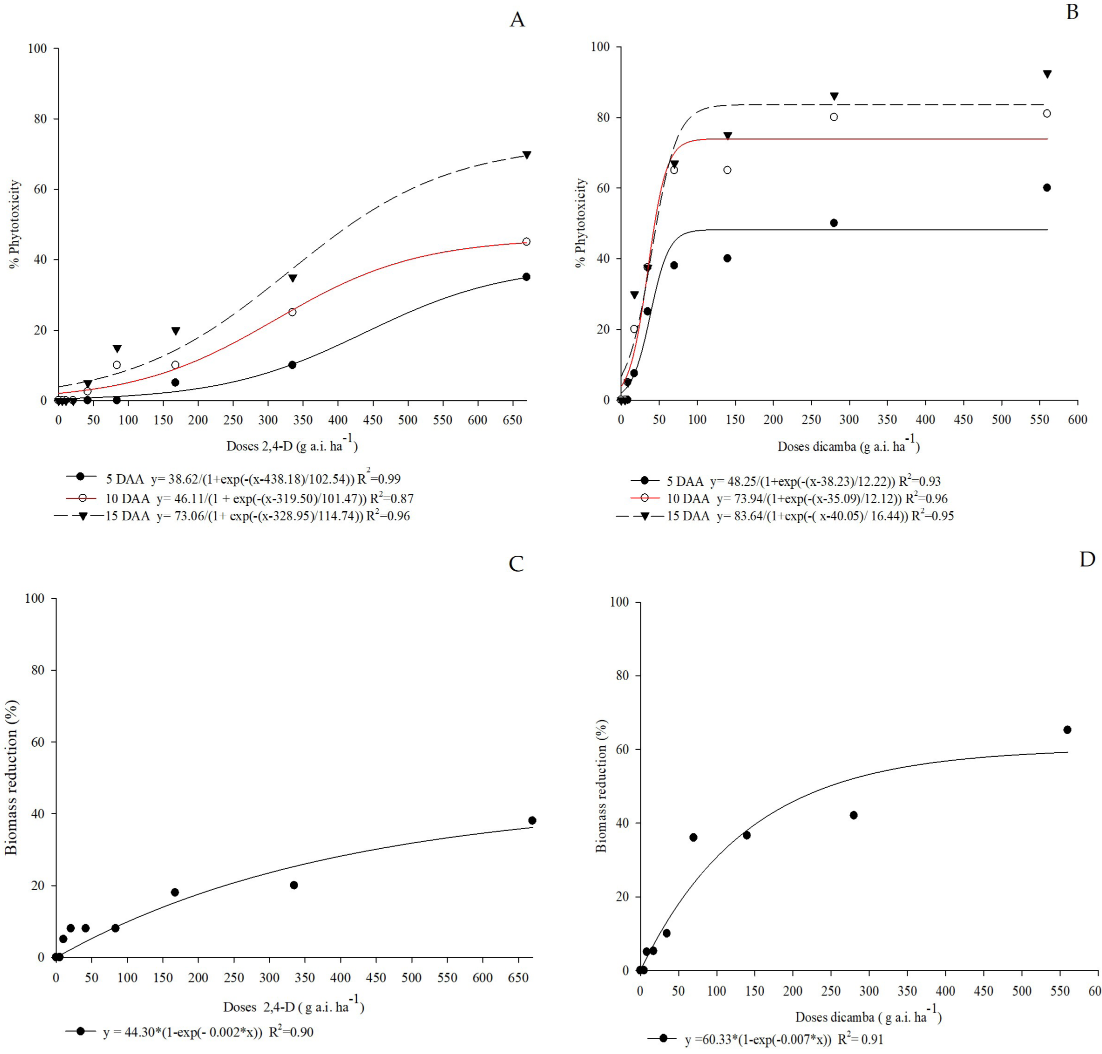

Figure 3. Phytotoxicity (%) and biomass reduction (%) on peanut plants at 5, 10, and 15 days after application (DAA). A, C: caused by 2,4-D; B, D: caused by Dicamba.

which is half of the commercial dose. The lowest doses had a smaller impact on plant biomass (11.2 % reduction) at the dose of 35 g ai ha^{-1} and no reduction at 4.37 g ai ha^{-1}. The highest reductions in dry biomass of peanut plants treated with 2,4-D were observed at doses of 670, 335, and 167.5 g ai ha^{-1}, with values ranging from 36.5 to 15 % reduction. The other doses reduced relative biomass by less than 10 %, showing a higher tolerance of plants.

It is important to highlight that hormonal herbicides require special care when applied, as they have a high potential for drift. Some municipalities even establish normative instructions for the use of these herbicides. A safety border area must be adopted between the application area and sensitive crops, in addition to observing the meteorological conditions at the time of application, the risks of thermal inversion, and other precautions to prevent potential risks of drift and volatility.

CONCLUSIONS

Low doses of the herbicides 2,4-D and Dicamba can affect cotton, beans, and peanuts at an early stage of development and therefore have a significant negative impact on these crops. Among the species evaluated, the peanut crop required higher doses of herbicides to cause phytotoxicity. These herbicides should be avoided near areas with these crops.

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