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Article

Tolerance of Cabbage Crop to Auxin Herbicides

Tolerância do Repolho aos Herbicidas Auxínicos

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ABSTRACT - The presence of weeds in cabbage cultivation areas causes a reduction in the productivity and quality of the commercial product. Given the difficulties in management and the scarcity of registered products for weed control in cabbage cultivation, this study aimed to evaluate the tolerance of the cabbage cultivars Astrus Plus to the herbicides 2,4-D and dicamba. Two experiments were performed; one under controlled conditions and another conducted in the field. The efficacy of the herbicides on the initial growth of the crop was evaluated in the first experiment, measuring the dry matter of the aerial part, the root of the plant, and evaluating the intoxication of the plants. Plant poisoning was also evaluated in the second experiment, along with crop production. No changes in the dry matter and plant poisoning were observed after the analyses performed in the controlled environment, showing that the cabbage presented tolerance to the applied herbicides. On the other hand, results from the experiments performed in the field showed that herbicide 2,4-D poisoned the cabbage causing reductions in production. Dicamba did not cause any damage in the initial phase of cultivation to this crop, with no reductions in its production. These results indicated the potential use of this herbicide in cabbage cultivation. It can be concluded from this study that the cultivar Astrus Plus is tolerant to dicamba. Under controlled conditions, 2,4-D does not cause toxicity to this cultivar, different from its effect when used in the field, where this herbicide can kill the cabbage plants.

Keywords: *Brassica oleracea* var. *capitata*, auxin mimics, weeds.

RESUMO - A presença das plantas daninhas na área de cultivo do repolho promove redução de produtividade e qualidade do produto comercial. Diante da dificuldade de manejo e escassez de produtos registrados para o controle de plantas daninhas na cultura do repolho, objetivou-se com este trabalho avaliar a tolerância do cultivar de repolho Astrus Plus ao 2,4-D e dicamba. Para isso, dois experimentos foram conduzidos: um em condições controladas e o outro em campo. No primeiro ensaio avaliou-se a ação dos herbicidas sobre o crescimento inicial da cultura, sendo mensurada a matéria seca da parte aérea e raiz e a intoxicação das plantas. No segundo, foram avaliadas as intoxicações nas plantas e os componentes de produção da cultura. O ensaio em ambiente protegido comprovou a hipótese de o repolho apresentar tolerância aos herbicidas aplicados, pois não foram observadas intoxicação de plantas e alterações na matéria seca. Todavia, no ensaio em campo, observou-se que o herbicida 2,4-D causou intoxicações e reduções nos componentes de produção que comprovam que este herbicida é severamente tóxico à cultura. No entanto, o dicamba não provocou danos à cultura na fase inicial de cultivo e não reduziu a produtividade, indicando potencial uso. Conclui-se que o cultivar de repolho Astrus Plus é tolerante ao dicamba. O 2,4-D não causa intoxicações a este cultivar em condições controladas, mas, em campo, o repolho não foi tolerante, podendo levar as plantas de repolho à morte.

Palavras-chave: *Brassica oleracea* var. *capitata*, mimetizadores de auxina, plantas daninhas.

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INTRODUCTION

Cabbage (*Brassica oleracea*) is considered the most economically important vegetable species from the Brassicaceae family (Ferreira et al., 2002; Melo and Vilela, 2007; Reis et al., 2017). Farmers report many difficulties in weed management for the cultivation of this species, mainly due to slow crop growth (Moreira et al., 2011), and planting spacing (Cavarianni, 2008; Silva et al., 2011), which favours weed growth, in addition to the small number of herbicides registered (Agrofit, 2018).

Chemical control is one of the most widely used methods within integrated weed management. The use of herbicides is often the best cost-effective option and is also important to avoid crop yield losses (Green, 2014). However, only two herbicides are registered for the cabbage crop in Brazil: *glufosinate ammonium*, that can crop damage, and trifluralin, which is more recommended for narrow leaf weed control (Powles and Yu, 2010; Agrofit, 2018).

The auxin-mimetic herbicides are used in controlling *broadleaf* weeds in *fallow*, grazing, and in crops with narrow leaves such as corn, sugarcane, sorghum and wheat (Brazil, 2018). They act similarly to the natural auxins and are more effective as well as being lethal to the sensitive plants (Pazminõ et al., 2012). The auxinic herbicides are divided into five classes: phenoxycarboxylic acids (for example 2,4-dichlorophenoxyacetic acid); benzoic acids (e.g. dicamba); pyridinacids (e.g. picloram, clopyralid); quinolinecarboxylic acids (e.g. quinclorac); and pyrimidine carboxylic acid (e.g., aminocyclopyrachlor) (Christoffoleti et al., 2015). The structural variation in each herbicide molecule influences the receptor protein binding (Tan et al., 2007) and the rate of degradation within the cell. In Brazil, four substances of this group of herbicides are registered: triclopyr, picloram, dicamba and 2,4-D (Brasil, 2018).

In literature, there are few studies on the selectivity of herbicides for cabbage cultivation, and no studies reporting the use of herbicides in controlling broadleaf weed. Thus, this research aimed to evaluate the tolerance of the cabbage cultivar Astrus Plus to the herbicides 2,4-D and dicamba.

MATERIAL AND METHODS

Two experiments were performed, one in a greenhouse at the Universidade Federal Viçosa - Rio Paranaíba Campus, and the second in a field at Santo Amaro Farm, in Rio Paranaíba, MG, Brazil (Latitude - 19°11’39” S, Longitude - 46°14’37” W, elevation 1,073 m). The cabbage cultivar Astrus Plus was used in both experiments.

Experiment 1 - Greenhouse

This first experiment was performed in order to evaluate the effects of auxinic herbicides on the initial growth of cabbage plants. A completely randomized experimental design arranged in a 2 X 8 + 1 factorial scheme was performed, with the first factor consisting of two commercial products of auxinic herbicides: the 2,4-D (Campeon® 806 g ae Stockton-Agrimor do Brasil Ltda.) and dicamba (Atectra® 480 g ae BASF Corporation). The second factor was composed of eight herbicide doses and a control without the application of herbicide, with four replications. 8.0 L pots filled with Red-Yellow Latosol samples and moistened to the maximum field capacity were used to sow the culture.

Dilutions of 2,4-D and dicamba were prepared in solutions corresponding to 0; 0.5; 1.0; 2.5; 5.0; 7.5; 10; 20; and 100% of the recommended dose of the product for the transgenic soybean crop, which is 1 L ha^{-1}. These doses correspond to 0.005; 0.01; 0.025; 0.05; 0.075; 0.1; 0.2 and 1.0 L ha^{-1} respectively. The spray volume of the commercial product used was 200 L ha^{-1}.

The application of the doses was performed using a CO_2 pressurized sprayer equipped with a hydraulic double-ended spray bar from a single fan jet, model API-11002, operating at a pressure of 3 kgf cm^{-2}, positioned at 50 cm in plant height, when the cabbage plants had two pairs of true leaves.

The evaluation of the plant poisoning at 7, 14, 21 and 28 days after the herbicide application was performed according to the grading scale proposed by the Brazilian Society of Weed Science (SBCPD, 1995), where 0% corresponds to injury absence and 100% plant death.

At the end of the experiment (28 DAA), the aerial and the roots of the plants were separated by cutting the plant parts close to the substrate surface. The roots were removed from the soil and cleaned using water. These plant parts were then placed in a forced air circulation drying oven (70 ± 2 °C) *until* a *constant weight* was *achieved*. The aerial part dry mass (APDM) and the root dry mass (RDM) were determined using a 0.001 g analytical balance.

The dry matter and intoxication were submitted to analysis of variance (ANOVA) and when significant, the treatments were subjected to polynomial regression analysis ($p<0.05$).

Experiment 2 - Field Farming

This experiment was carried out to evaluate if the auxinic herbicides cause any intoxication and/or changes to the production of cabbage plants. A completely randomized experimental design arranged in a 2 x 6 factorial scheme was performed. The first factor was composed of two auxinic herbicides: the 2,4-D (Campeon® 806 g ae Stockton-Agrimor do Brasil Ltda.), and dicamba (Atectra® 480 g ae BASF Corporation). The second factor was composed of six herbicide doses (0, 0.05, 0.075, 0.1, 0.2, and 1 L ha^{-1}), and the control group without herbicide application, with four replications.

The experimental plots consisted of four 4 m long cabbage lines, with a 0.4 m row spacing between lines and a 0.3 m row spacing between plants, with a total of 62,000 plants per hectare. The dosage preparation and drift simulation were carried out in the same way as proposed in experiment 1.

At 7, 14, 21 and 28 days after application of the herbicide, evaluations of plant poisoning were made according to the grading scale proposed by the Brazilian Society of Weed Science (SBCPD, 1995), where 0% corresponds to injury absence and 100% plant death. At the end of the experiment, at the harvest stage, the production of the cabbage was evaluated. The cabbage heads contained in the useful area (two central rows of each plot) were collected and packed in wooden crates for sale. The heads contained in the crates were then counted and weighed to obtain the number of heads per box, average head mass and an estimation of the cabbage productivity.

The data obtained after evaluating the cabbage productivity was submitted to multivariate analysis (percentage of similarity between variables), in order to select the discriminating variables that could be represented by a single evaluation, considering ideal similarity values above 80%. This analysis was based on the absolute correlation between variables. The software used for the statistical analysis of the data was Minitab® 16.2.1. After discriminating the groups, the means were submitted to descriptive analysis and were then represented in bar graphs. The dry matter data were first submitted to analysis of variance (ANOVA) and then to regression analysis. The data from the plant poisoning obtained were analysed using the nonlinear model proposed by Seefeldt et al. (1995):

$$Y = f(x) = C + \frac{D - C}{1 + \left(\frac{X}{C_{50}}\right)^{b}}$$

where *C* and *D* correspond to the minimum and maximum level of the dose response curve respectively; *b* corresponds to the slope of the curve around C_{50}; and C_{50} is the dose response corresponding to a 50% reduction of the plant variable under study.

RESULTS AND DISCUSSION

Experiment 1

No toxicity symptoms were observed in the cabbage plants at 7, 14, 21 and 28 DAA after the application of 2,4-D or dicamba (Figure 1A and B). Differences in dry matter between the aerial parts and roots after the application of these herbicides were also not observed (Figure 2A, B, C and D). These analyses were performed three times each to confirm the

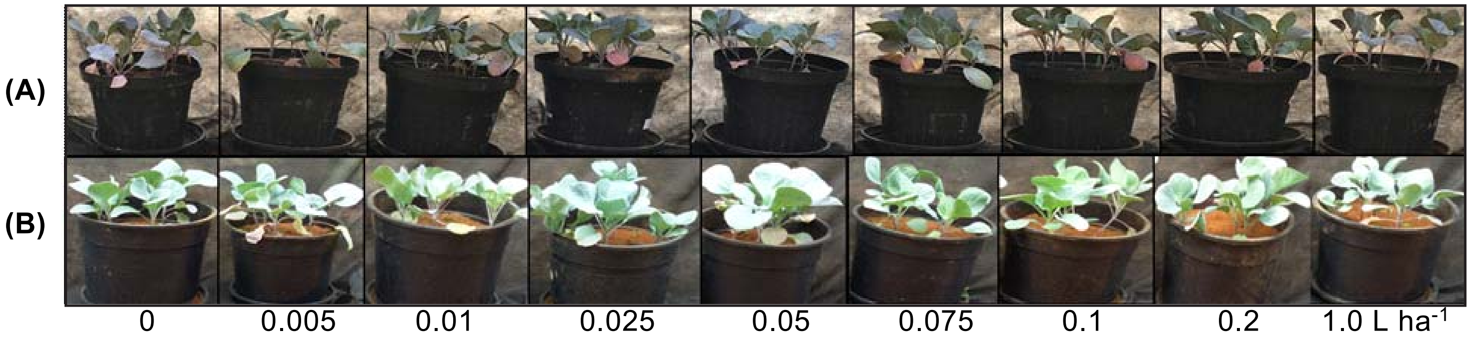

Figure 1 - Plants of cabbage cultivar Astrus Plus after the application of increasing doses of the herbicides 2,4-D (A) and dicamba (B) at 28 DAA.

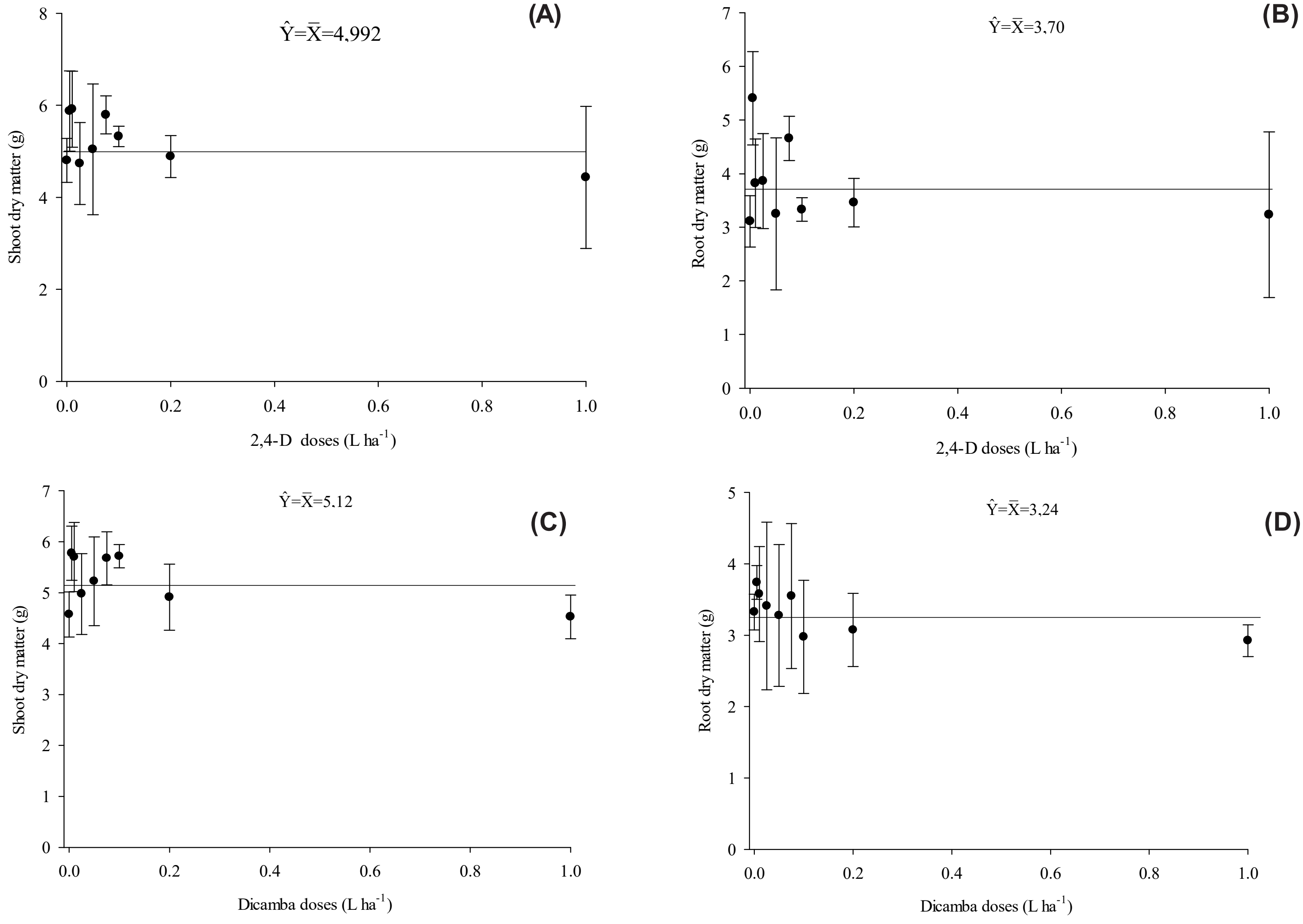

Figure 2 - Dry matter of the aerial part (APDM) and root (RDM) of the cabbage plants, after the application of different doses of 2,4-D and dicamba at 21 DAA. A) APDM/2,4-D, B) RDM/2,4-D, C) APDM/dicamba and D) RDM/dicamba. Rio Paranaíba, MG, 2018.

reproducibility of the results. From the data obtained, it can be said that the initial growth of the cabbage plants was not affected by the herbicide application. This could be the result of the cabbage being tolerant to this group of herbicides.

To date, three auxin receptors responsible for the mechanism of action of auxin mimics have been proposed: (1) auxin binding protein 1 (ABP1) (Tromas et al., 2010; Shi and Yang 2011); (2) *auxin signaling F-box protein* (TIR1/AFB); and more recently (3) S-kinase-associated protein 2 (SKP2) (Jurado et al., 2010). In addition to being found in different subcellular locations, these three auxin receptors also differ in their proposed functional roles in cell expansion, cell division, and regulation of plant development processes (Zazimalova et al., 2014).

In many cases, the selectivity of auxinic herbicides depends on the plant metabolism. Plants generally metabolize herbicides converting the original molecule into more polar products and insoluble residues (Hatzios et al., 2005). The 2,4-D metabolic pathways in sensitive and tolerant species have some similarities. Although sensitive species can sometimes metabolize 2,4-D or dicamba faster than tolerant species, the metabolites produced can be readily converted back to the original molecule. On the other hand, tolerant species usually produce non-phytotoxic and irreversible metabolites of these herbicides. The metabolites produced during the auxin metabolism in the sensitive eudicotyledons and tolerant monocotyledons are similar, but vary in the number of metabolites produced, resulting in a lower concentration of tolerant monocotyledons compared to eudicotyledons (Pillmoor and Gaunt, 1981).

Experiment 2

The toxicity evaluated at 7, 14 and 21 DAA in plants after the application of 2,4 D did not allow models adequacy in describing the behavior of intoxication of cabbage plants when the dose of herbicide was increased (Figure 3A). At 7 DAA signs of toxicity in plants was observed at the highest dose of 2,4-D (1.0 L ha^{-1}). At 14 DAA, the toxicity was observed from the dose of 0.1 L ha^{-1}, with values close to 10% and reaching over 60% at the highest dose. From 21 DAA, the doses above 0.2 L ha^{-1} caused severe symptoms of poisoning in the plant, which resulted in plant death after the application of the highest dose at 28 DAA.

The symptoms observed in cabbage plants after the application of 2,4-D included leaf curl and folded leaf margin, followed by chlorosis and necrosis of the leaves and stems of the plants, as also observed by Santos et al. (2013).

The proposed model was not adequate to describe the evaluations of intoxication in plants caused by dicamba (Figure 3B). However, mild plant poisoning was observed only at doses of 0.2 and 1.0 L ha^{-1} at 14 and 21 DAA, with values close to 20%. However, in the evaluation performed at 28 DAA, no intoxication was observed, indicating that the symptoms of poisoning observed at the initial growth did not cause enough damage to be noticeable during the last evaluation of the culture.

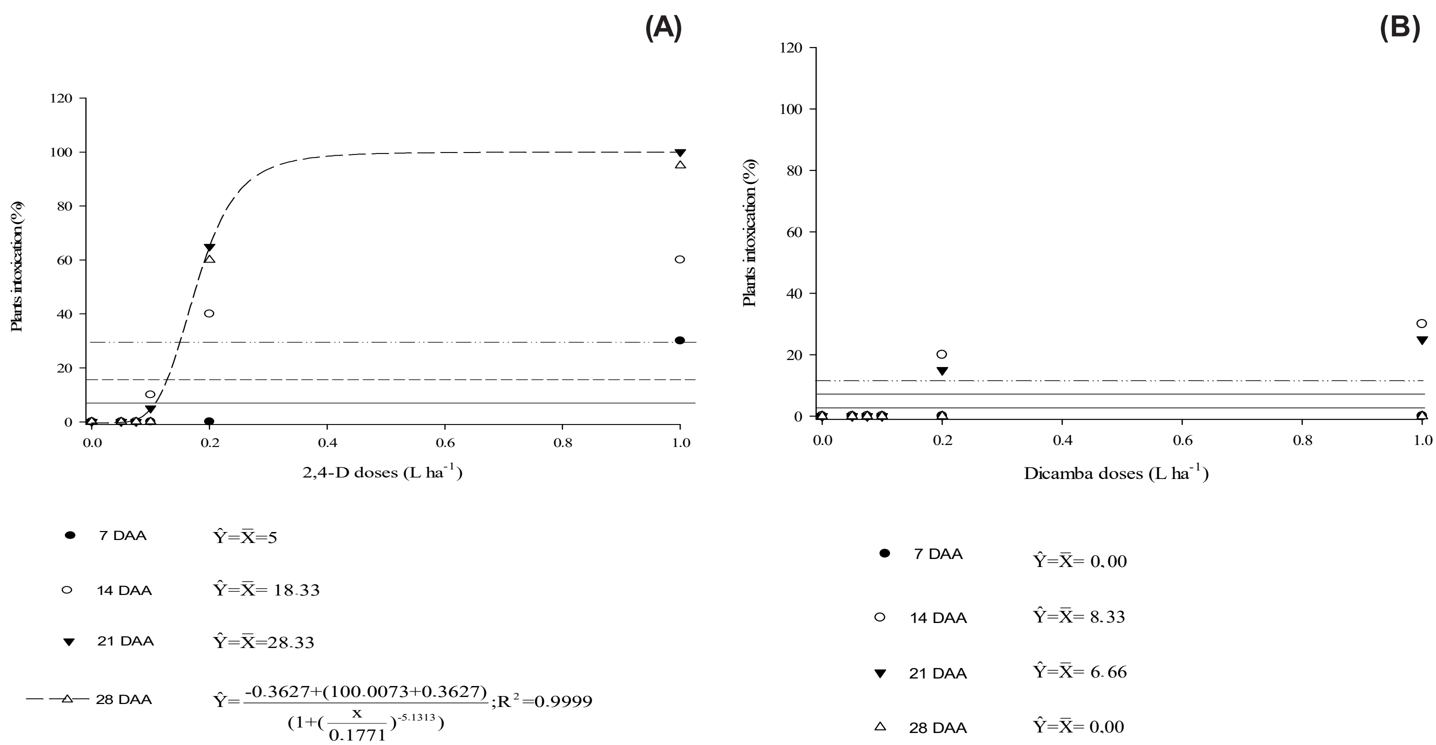

Figure 3 - Toxicity of cabbage plants at 7, 14, 21 and 28 DAA after the application of doses of 2,4-D (A) and dicamba (B). Rio Paranaíba-MG, 2018.

A similarity above 80% between the productive variables was observed when the cabbage plants were submitted to 2,4-D application (Figure 4A), indicating that the application of this herbicide caused similar responses in the evaluated variables. In this case, these variables were then represented only by the number of marketable cabbages. Regarding the dicamba application, a similarity above 80% was just observed between the number and average weight of the cabbages (Figure 4B). Two groups were presented: the first corresponds to the correlated variables, represented by the number of marketable cabbages; and the other group corresponds only to the total box weight.

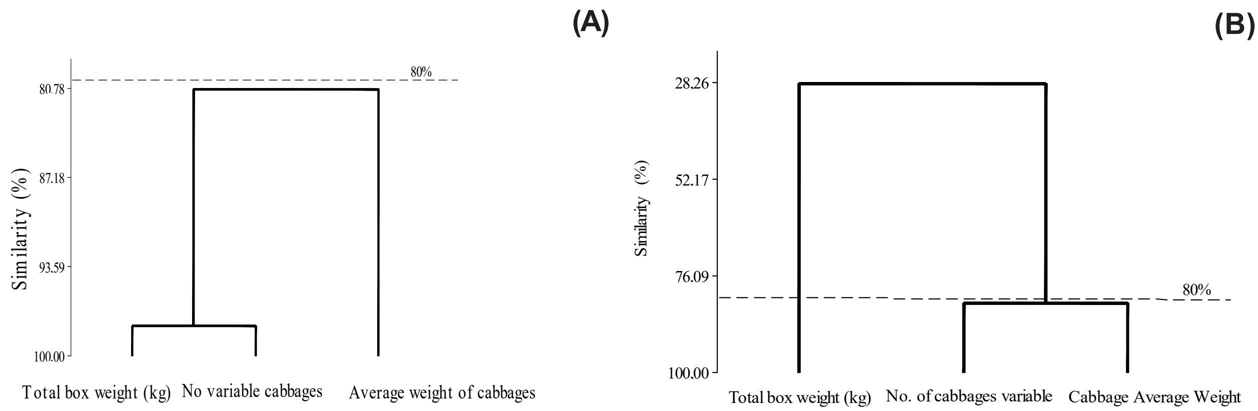

Figure 4 - Percentage of similarity between the variables total box weight, number of cabbages and average cabbage weight after the application of 2,4-D (A) and dicamba (B), according to the correlations between the variables. Rio Paranaíba-MG, 2018.

The application of the doses of 2,4-D to the cabbage plants caused a reduction in the number of marketable cabbages from the 0.20 L ha^{-1} dose (Figure 5A). At the application of the highest dose (1.0 L ha^{-1}), a drastic reduction in the values of the number of cabbages was observed, with an average below two marketable cabbages. No reductions were observed after the application of the dicamba doses, presenting an average of 13 marketable cabbages (Figure 5B). The most valued boxes for the current market were the ones containing 12 to 14 marketable cabbages, with an average mass of 1.4 to 2.0 kg per head.

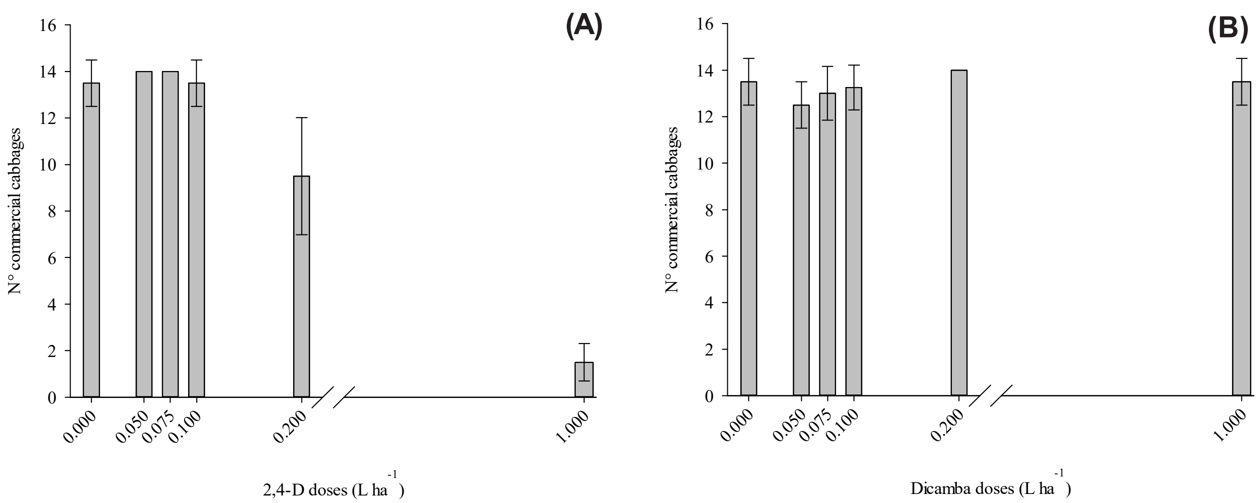

Figure 5 - Number of marketable cabbages after the application of different doses of 2,4 D (A) and dicamba (B) at 84 days after transplantation. Rio Paranaíba, MG, 2018.

The use of dicamba did not cause changes in the total weight of the cabbage box at any of the doses applied to the plant. The average weight of the cabbage boxes was 27 kg per box (Figure 6).

Similar to what was observed in the other productive variables, the average cabbage weight did not change after the application of dicamba (Figure 7). The average value of the cabbage head was 2.02 kg.

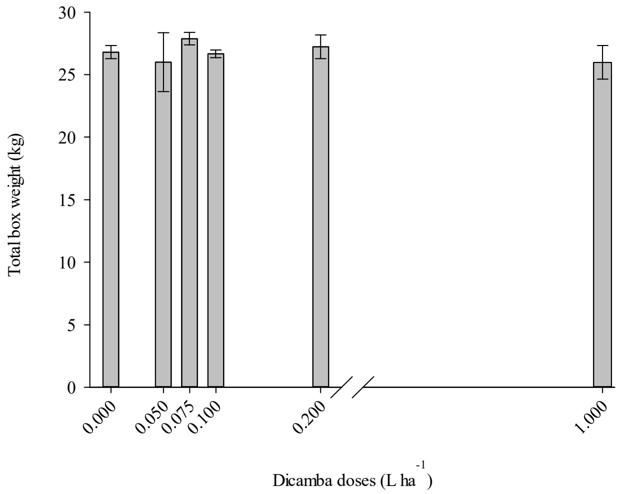

Figure 6 - Total weight of the cabbage box (kg) after the application of different doses of dicamba at 84 days after transplantation. Rio Paranaíba, MG, 2018.

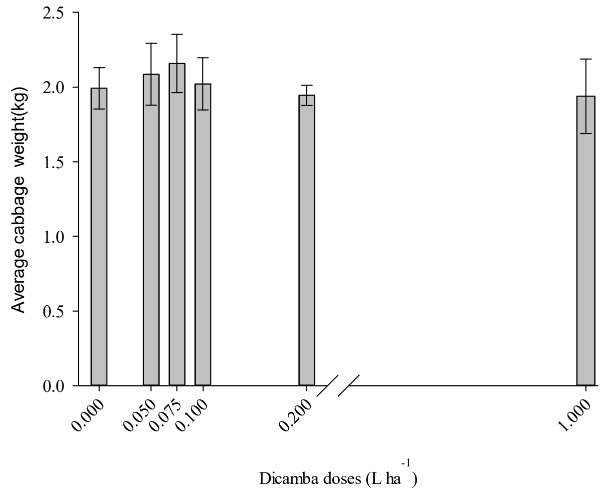

Figure 7 - Average cabbage weight (kg) after the application of different doses of dicamba at 84 days after transplantation. Rio Paranaíba, MG, 2018.

In response to an auxinic herbicide, sensitive plants develop abnormalities such as *leaf epinasty,* leaf abscission, inhibition of the root growth and the aereal part of the plant (Kelley and Riechers, 2007; Grossmann, 2009). In general, the effects of auxin herbicides on the plants can be divided into three consecutive plant phases: stimulation of abnormal growth and gene expression; growth inhibition and physiological responses such as stomatal closure and finally, senescence and cell death (Grossmann, 2009). During the stimulation phase, the auxinic herbicides stimulate an increase in the production of ethylene and the biosynthesis of abscisic acid (ABA) (Hansen and Grossmann, 2000; Kraft et al., 2007; Grossmann et al., 2001). The increased levels of ABA inhibits plant growth by closing the stomata, which limits the assimilation of carbon dioxide and leads to the accumulation of hydrogen peroxide in herbicide-treated plants (second phase effects) (Kraft et al. 2007). This accumulation of reactive oxygen species is probably an important factor to the tissue damage and cell death associated with herbicide treatment (third phase effects) (Grossmann, 2009).

The plant can be tolerant to an auxinic herbicide belonging to a specific chemical group and not be tolerant to another auxinic herbicide from another chemical group (Patton et al., 2018). This was observed in the present study, since the cabbage plants (cultivar Astrus Plus) presented tolerance to dicamba doses, but were sensitive to the 2,4-D doses.

According to Jugulam et al. (2015) and Mithila and Hall (2005), there are some wild mustard (*Brassica kaber*) biotypes resistant to the herbicide dicamba, which is a plant belonging to the same family of cabbage. However, these authors reported that these biotypes have acquired resistance by the selection pressure, after years applying auxinic herbicides in the area. There are no studies in the literature reporting on plants with natural tolerance to auxinic herbicides in this family, as observed in this current study. It is also worth mentioining that the presence of auxin in the cabbage leaves poses a serious risk to human health (Anderson et al., 2004). Thus, further research should be performed in order to evaluate the presence of these compounds as a product of interest after its application.

Therefore, the cabbage cultivar Astrus Plus is tolerant to dicamba doses up to 1.0 L ha^{-1}. The herbicide 2,4-D is not toxic to this cultivar under controlled conditions, but when applied in the field, it intoxicates the plants, causing a reduction in productivity, which can lead to death.

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