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HORMESIS EFFECT OF 2, 4- DICHLOROPHENOXIACETIC ACID IN SOY SEEDS

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ABSTRACT

Herbicides of the auxin mimic group, such as 2,4-dichlorophenoxyacetic (2,4-D), at low dosages may show a direct relation to the growth and development of the plant, in order to evaluate the influence of the different dosages of this plant herbicide in soybean (Glycine max) crop. The seeds were treated with fungicides and insecticides and then with 2,4-dichlorophenoxyacetic dimethylamine salt and inoculated. The experimental design was completely randomized (DIC), composed of five treatments with eight replicates, using seeds without inoculant and with inoculant of Bradyrhizobium sp. Plant length evaluations were performed at 7, 14, 21 and 28 days after emergence and at 30 days after emergence for the other variables analyzed (chlorophyll content, root length, green shoot mass). In the experiments with inoculated seeds, the dose of 0.5 mL ha-1 of 2,4-dichlorophenoxyacetic acid showed inhibition of plant development. In the seeds without inoculant, the results showed that in doses from 5.0 mL ha-1 2,4-D caused damages in the development of the plants, however in lower dosages the herbicide presented hormonal action allowing the development of soybean plants.

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INTRODUCTION

Soybean (*Glycine max*) is considered the most important protein source in recent years, being the crop that produces more protein per hectare compared to any other type of crop. It offers a wide range of products and by-products such as soybean meal, which is used as a feed that supplies the market with meat, eggs, milk and derivatives, leveraging the economy such as the cosmetics, leather, food and other sectors. (ROESSING *et al*., 2005). According to the United States Department of Agriculture - USDA (2018) world soybean production currently reached about 336.699 million tons. According to the National Supply Company - CONAB (2018a), in terms of monitoring the Brazilian crop, Brazil stands out on the world stage as a major soy producer, with a 4.6% increase in 2017/2018 crop production when compared to the previous one, and with an increase of approximately 3.7% in planted area. Moreover, in Brazil, it is the main crop in area and volume of production. Also according to CONAB (2018a) Brazil occupies the second place in soy production, with 116,996 million tons. This production is strongly linked to new technologies present today as biotechnology and genetic improvement of cultivars; the expansion of new areas; the use of foliar mineral fertilizers; among other technologies (SOUZA *et al*., 2008; CONAB 2018b). Thus, it is of great importance to produce more information with research in order to increase the productivity and quality of the crops, also paying attention to the use of bioregulators that has been promisingly used. According to Castro (2006), hormonal regulators have deserved more and more attention in tropical agriculture as cultivation techniques have evolved in such a

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way that some crops in Brazil are no longer constrained by nutritional and water limitations, and are adequately protected with pesticides. Plant regulators is the term used for substances that when exogenously applied to plants play a similar role to plant hormone groups (auxins, gibberellins, cytokines, etc.). Thus, these natural or synthetic substances, when applied directly to the plant as leaves, fruits and seeds, can cause a variation in the physiological and morphological processes of plants, with the purpose of increasing production, improving quality and may facilitate harvesting. Through this, they can interfere in various processes such as germination, rooting, flowering, fruiting and senescence (CASTRO; VIERA, 2001).

Changes in tissue hormone concentration can mediate a whole range of plant developmental processes, many of which involve interactions with environmental factors (CROZIER *et al*., 2000). Auxins activate enzymes that act on constituents of the bonds between cell wall cellulose microfibrils, causing rupture and increased plasticity, facilitating the entry of water into cells and increasing their dimensions (CASTRO; VIERA, 2001). Many herbicides have been originally developed as growth regulators, and these support the hypothesis of “hormone”, which is characterized by the use of toxic substances at lower dosages that can stimulate plant development (CALABRESE; BALDWIN, 2002). The most notable example is the 2,4-dichlorophenoxyacetic product, originally developed as auxin and which at high doses has a herbicidal effect (MUSDALE; COGGINS, 1991; HALTER, 2009). In a study by Rosa *et al*. (2011), the application of increasing 2,4-dichlorophenoxyacetic subdoses promoted an increase in leaf calcium contents and did not influence the chlorophyll index and the cottonseed yield was increased by 2.72 g i.a. ha^{-1}. Cedergreen *et al*. (2009) and Silva *et al*. (2012) also observed that glyphosate underdoses promoted productivity increase in barley and bean crops Velini *et al*. (2008) observed growth stimuli of soybean (non-transgenic and susceptible to glyphosate), eucalyptus, maize, pine, maize, coffee and citrus, for glyphosate doses lower than 36 g ai ha^{-1}, applied to drift. The low cost of the product and the high availability of the active ingredient in the market favor the use of 2,4-D as a growth regulator. In this context the work analyzed the influence of the application of 2,4-dichlorophenoxyacetic in reduced doses on the formation of roots, shoot and chlorophyll content, analyzing its effect on Glycine max seeds.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse at the NBA Applied Biotechnology Research Center, belonging to the UEM - Maringá State University, Maringá-PR, located 23°23'56.5 ”south latitude 51°57'07.1” west longitude. The conduction period of the tests was from 01/05 to 12/31/2018.

Baup 5400 IPRO® soybean seeds treated with Imidacloprido 150 g L^{-1} (15% m v^{-1}), Tiodicarb 450 g L^{-1} (45% m v^{-1}) in the proportion of 0.7 L were used 100 kg^{-1} seeds as recommended by the manufacturer. Carboxin 200 g L^{-1} (20% m v^{-1}), Thiram 200 g L^{-1} (20% m v^{-1}) in 0.4 L 100 kg^{-1} seed, as recommended by the manufacturer. Two experimental conditions were carried out, one without seed inoculation and the other with seed inoculated by bacteria with strains Semia 5079 (Bradyrhizobiumjaponicum) and Semia 5019 (Bradyrhizobiumelkanii) in the order of 5 billion bacteria g^{-1} being used 200 g 100 kg- 1 seed in all treatments. Sowing was performed with 4 seeds / pots where after germination only the most vigorous seedling was left. 3.5 L pots containing substrate composed of pine bark, vermiculite, acidity concealer and macronutrients were used (CTC = 200 mmolc kg^{-1}, CRA 60% by mass). The experiments were carried out in a completely randomized experimental design (DIC), with 5 treatments and 8 replications. The seeds were treated with 806 g L^{-1}dimethylamine (2,4-D) salt, equivalent to 2,4-dichlorophenoxyacetic 670 g L^{-1} (67% m v^{-1}) at the dosages of: T0 = no 2,4-D added; T1 = 0.005; T2 = 0.05; T3 = 0.5; T4 = 5.0; T5 = 50.0 mL ha $^{-1}$. After sowing, the pots were kept in a greenhouse with periodic irrigation. Shoot size evaluations occurred at 7, 14, 21 and 28 days after emergence (DAE), verifying plant height with the aid of a millimeter ruler. Indirect chlorophyll analysis was performed after 30 DAE using the SPAD-502 chlorophyll meter (Soil-Plant Analysis Development Section, Minolta Camera CO; Osaka, Japan), and the chlorophyll meter uses light-emitting diodes in the 650 to 940 nm across the sheet. The 650 nm wavelength is close to that of the two primary wavelengths associated with chlorophyll activity (645 and 663 nm). The 940 nm wavelength serves as an internal reference to compensate for differences in leaf thickness and water content. The chlorophyll meter measures the difference in light attenuation between 650 and 940 nm as an index of color intensity or chlorophyll concentration (YADAVA, 1986). For this reading it was considered the average of three readings in the median part of the first fully expanded leaf, in each plant of the experimental unit, sufficient to establish optimal relation of N and SPAD reading in soybean plants (BOAS *et al*., 2002). The shoot green mass and root length were also determined 30 DAE, where the average mass was verified on a precision scale (BEL UMARK, 1000) and the root size was measured using a millimeter ruler. Data were subjected to analysis of variance ANOVA and means between treatments were compared by Scott Knott test at 5% significance, with the aid of the SISVAR program (FERREIRA, 2014).

RESULTS

Visual symptoms of 2,4-dichlorophenoxyacetic phytoxication were observed with both inoculated and uninoculated seeds on soybean plants treated at the herbicide doses of 5.0 and 50.0 mL ha^{-1}. Some seeds did not germinate and presented deterioration aspects. The plants that emerged showed injuries and epinastia, and over the days these symptoms accentuated and they died and died. In addition to epinastia, the plants showed leaf curl. Table 1 shows at 7, 14, 21 and 28 DAE that the 0.005 and 0.05 mL ha^{-1} treatments showed no significant difference in relation to the average plant height compared to the control in the experiment with plants inoculated with Bradyrhizobium sp. . For uninoculated seeds, the results of treatments 1, 2 and 3 showed no significant difference at 5% probability in relation to the control (Table 1). These results show that 2,4-D used in the treatment of seeds at low concentrations has a hormone effect, ie, it did not have any negative interference on plant growth at doses of 0.005 mL ha^{-1} and 0.05 mL ha^{-1}. for inoculated plants and 0.005 mL ha^{-1}, 0.05 mL ha^{-1} and 0.5 mL ha^{-1} for uninoculated plants. According to the results (Table 1) for inoculated soybean seeds, treatment 3 presented statistical difference in relation to the others in all periods analyzed. The average plant size was smaller, besides presenting plants with epinastia and injuries related to the phytotoxicity of 2,4-dichlorophenoxyacetic. In the results shown in Table 2 for the inoculated seeds, regarding the variables chlorophyll content and green mass of the aerial part of the plants, the treatments 0.005 and 0.05 mL ha^{-1} did

Table 1. Mean size of plants (cm) of soybean treated with 2,4-dichlorophenoxyacetic acid, by seed treatment without inoculation and inoculation whith*Bradyrhizobium* sp. and evaluated at 7, 14, 21 and 28 days after emergence (DAE)

Treatment	Dose	Days							
	(mL ha^{-1})	7		14		21		28	
		Plants inoculated with Bradyrhizobium sp. (centimeter)							
0	0	10.69	a	14.50	a	18.69	a	25.44	a
1	0.005	10.75	a	14.74	a	19.55	a	26.08	a
2	0.05	10.06	a	13.21	a	17.99	a	24.85	a
3	0.5	6.94	b	10.34	b	13.44	b	17.05	b
4	5.0	-		-		-		-	
5	50.0	-		-		-		-	
Coefficient of variation %		18.01		11.02		13.35		15.82	
		Uninoculated plant size (centimeter)							
0	0	7.46	a	13.30	a	17.34	a	21.81	a
1	0.005	8.00	a	14.53	a	18.33	a	24.53	a
2	0.05	7.89	a	12.58	a	17.08	a	23.70	a
3	0.5	7.90	a	10.94	a	16.08	a	19.30	a
4	5.0	-		-		-		-	
5	50.0	-		-		-		-	
Coefficient of variation %		21.62		21.08		18.47		18.67	

Averages followed by equal column letters within each category do not differ significantly from each other (Scott-Knott test at 5% level). Results followed by a hyphen (-) mean that there were no data to be analyzed due to the herbicidal characteristic of 2,4-D at high doses.

Table 2. Data on chlorophyll meter (%), root length (cm) and shoot green mass (g) of soybean plants treated with 2,4-D, without inoculation and inoculation with *Bradyrhizobium* sp.

Treatment	Dose (mL ha^{-1})	Chlorophyll content (%)		Length from the root (cm)		Green Mass from Aerial Part (g)	
		Plants inoculated with Bradyrhizobium sp.					
0	0	42.46	a	71.25	a	24.35	a
1	0.005	44.39	a	80.25	a	25.23	a
2	0.05	42.51	a	66.63	a	25.53	a
3	0.5	38.72	b	74.75	a	14.48	b
4	5.0	-		-		-	
5	50.0	-		-		-	
Coefficient of variation %		7.55		19.92		21.86	
	Uninoculated Plants						
0	0	41.04	a	49.70	a	21.78	a
1	0.005	39.78	a	64.75	a	26.46	a
2	0.05	38.63	a	40.00	a	25.96	a
3	0.5	39.32	a	58.63	a	18.18	a
4	5.0	-		-		-	
5	50.0	-		-		-	
Coefficient of variation %		8.0		33.35		45.28	

Averages followed by equal column letters within each category do not differ significantly from each other (Scott-Knott test at 5% level). Results followed by a hyphen (-) mean that there were no data to be analyzed due to the herbicidal characteristic of 2,4-D at high doses.

not present significant differences in relation to the control, showing that the herbicide applied in low amounts and in isolation does not influence the formation and degradation of chlorophyll as well as the green matter of the shoot. In contrast, treatment 3 (0.5 mL ha^{-1}) had a negative response caused by the herbicidal action of 2,4-D. Regarding the root length of the inoculated plants also did not differ significantly from the control. In seeds without inoculation, no significant results were verified regarding chlorophyll content, root length and green mass of the shoot (Table 2). Thus, it can be verified in the present work that in the 0.5 mL ha^{-1} dosage, despite presenting severe damage to the shoot development, it did not cause root interference in relation to the experiment with inoculated seeds. At doses higher than 5.0 mL ha^{-1}, 2,4-dichlorophenoxyacetic prevented seed germination, thus presenting the herbicide action under both conditions. On the other hand, at dosages less than 0.05 mL ha^{-1}, 2,4-dichlorophenoxyacetic did not interfere with plant development in relation to control. Under natural cultivation conditions, damage caused by drift or in soils contaminated with 2,4-dichlorophenoxyacetic acid may be limiting in yield and development. Detailed studies on the hormonal action of 2,4-dichlorophenoxyacetic may be of great importance for the verification of the hormonal action on the root system of soybean plants, as well as its development. Thus, studies on the action of 2,4-dichlorophenoxyacetic acid applied in a unique way are important to verify its hormone effect and to measure the interference levels on the crop exposed to the herbicide as the right dosage for this response. It is difficult to be established.

DISCUSSION

According to Raven *et al*. (2014), plant hormones are able to regulate plant growth and development. In this work the data showed that 2,4-dichlorophenoxyacetic acid, a low-dose synthetic auxin, had no herbicidal role, indicating a condition of hormones, in which under situations of low stress there would be an organic adaptation that would mobilize protection and resistance systems. to their damage (SERRA, 2011). However, at the highest concentrations used, 2,4-D caused injuries and totally inhibited the germination and consequently development of soybean plants. The action of this herbicide is highly selective and systemic, where once absorbed, it increases biosynthesis and ethylene production causing uncontrolled cell division and vascular tissue damage (PPDB, 2013). According to Yamashita *et al*. (2013) 2,4-dichlorophenoxyacetic symptomatology may vary in plants from mild leaf epinastia, followed by deformation to plant death. Hormones, as well as enzymes, DNA and vitamins have the property of exerting extremely morphophysiological effects, even when present in low concentrations (CASTRO; VIEIRA, 2001). Taiz; Zeiger (2013) concluded that the level of endogenous auxin in the elongation region of a normal healthy plant is close to optimal for growth, as sprinkling of the plant with exogenous auxin causes only a modest and brief growth spur. Such sprinkling may even inhibit growth in the case of dark-growing seedlings, which are more sensitive to supra-optimal auxin concentrations than plants that grow in light. Several studies show that increasing doses of bioregulators, as well as synthetic auxins, have a limit on the promoter effect. Overcoming a certain limit, negative physiological effects on plant growth and development occur, probably due to hormonal imbalance (ALBRECHT *et al.*, 2012). Tavares *et al.* (2017) evaluating the effect of simulated drift through 2,4-D underdoses on pequi plants found that in relation to leaf area there was a significant positive effect promoted by herbicide drift. At dosages corresponding to 0.5% of the commercial dose, a hormone effect was observed, representing a 20.7% increase in this variable in relation to the control. The authors also state that the variables: leaf area, specific leaf area and leaf area ratio of the plants present hormone at reduced doses of 2,4-D and also realized that in higher doses, the herbicide may promote reduced plant growth. being related to the reduction of the variables related to the leaves and consequently to the total dry mass of the plants.

Some authors explain that its action is due to the fact that 2,4-dichlorophenoxyacetic acid is not degraded in plants as readily as natural auxins, and the consequent high artificial level of auxin-like compounds certainly contributes to lethal effects (RAVEN *et al.*, 2014).

Conclusion

The application of 2,4-dichlorophenoxyacetic did not promote morphophysiological changes when administered up to 0.05 mL ha^{-1}. This dose can be considered borderline between the plant adaptability characterized by hormone effect and the herbicidal action of 2,4-D in the treatment of inoculated and uninoculated soybean seeds. In dosages greater than or equal to 5.0 mL ha^{-1}, severe phytotoxic effect was observed with total germination inhibition, showing a variation of the effect in response to the applied 2,4-dichlorophenoxyacetic acid.

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