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Effect of sub-doses of 2,4-D choline salt on the characteristics of cotton fiber[1]

Efeito de subdoses de 2,4-D sal colina nas características da fibra do algodoeiro

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ABSTRACT - Cotton is considered to be one of the most sensitive crops to the herbicide 2,4-D, however, there are no studies related to the effects of contamination of this herbicide on the fiber quality characteristics of cotton. Thus, the objective of this study was to evaluate the effect of sub-doses of 2,4 -D choline salt formulation on the quality characteristics of cotton fiber at different phenological stages. The experimental design was based on randomized blocks, with four repetitions and treatments distributed in a 7 x 3 factorial design, with seven fractions of the mean label dose of the herbicide 2,4 -D choline salt formulation (0 (control); 0.4275; 0.855; 1.71; 3.42; 8.55; 17.1 g a.e. ha^{-1}), associated with three different phenological stages of cotton: V4, B4 and C4. The lint samples were analyzed by the HVI (High Volume Instruments) equipment to determine the main fiber quality characteristics. No sub-dose of the 2,4 -D choline salt herbicide affected the fiber quality variables. Contaminations of 2,4 -D choline salt at the V4 and B4 stages of cotton directly influence the visual effects of damage and some quality characteristics of cotton fiber, showing that contamination of the herbicide in the initial stages of plant development drastically compromises the crop. The fiber quality variables of cotton plants in the C4 stage were not influenced by the application of 2,4 -D choline salt, with no risk if accidental contamination occurs in this stage.

Key words: Synthetic auxin. *Gossypium hirsutum* L.. 2,4 -dichlorophenoxyacetic acid. High Volume Instruments.

RESUMO - O algodoeiro é considerado como uma das culturas mais sensíveis ao herbicida 2,4 -D, no entanto, não existem estudos relacionados aos efeitos de contaminações desse herbicida sob as características de qualidade da fibra do algodoeiro. Por conta disso, objetivou-se com a realização deste trabalho avaliar o efeito de subdoses de 2,4 -D formulação sal colina sobre as características de qualidade da fibra do algodoeiro em diferentes estádios fenológicos. Adotou-se o delineamento experimental em blocos casualizados, com quatro repetições e os tratamentos distribuídos em esquema fatorial 7 x 3, sendo sete frações da dose média do rótulo do herbicida 2,4 -D formulação sal colina (0 (testemunha); 0,4275; 0,855; 1,71; 3,42; 8,55; 17,1 g ea ha^{-1}), associadas a três estádios fenológicos distintos do algodoeiro: V4, B4 e C4. As amostras de pluma foram analisadas pelo equipamento HVI (High Volume Instruments) para determinação das principais características de qualidade da fibra. Nenhuma subdose do herbicida 2,4 -D sal colina afetou as variáveis de qualidade da fibra. Contaminações de 2,4 -D sal colina nos estádios V4 e B4 do algodoeiro influenciam diretamente os efeitos visuais de injúria e de algumas características de qualidade da fibra de algodão, evidenciando que contaminações do herbicida nas fases iniciais de desenvolvimento das plantas comprometem drasticamente a lavoura. As variáveis de qualidade da fibra de plantas de algodoeiro no estádio C4 não foram influenciadas pela aplicação de 2,4 -D sal colina, não havendo riscos caso ocorra contaminações acidentais neste estádio.

Palavras-chave: Auxina sintética. *Gossypium hirsutum* L.. Ácido 2,4 -diclorofenoxiacético. High Volume Instruments.

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INTRODUCTION

Cotton fiber is the most widely cultivated and most important textile fiber in the world. The growth of the area planted with cotton in Brazil resumed in the 1990 s, with a significant increase in the areas cultivated in the Central-West region. Favorable climate conditions, topography that allows for agricultural mechanization, government incentive programs and, especially, the intensive adoption of modern technologies are the main factors that drove this increase in cotton cultivation in the Brazilian Cerrado (ZONTA *et al.*, 2014).

Presenting a photosynthetic metabolism of the C3 type, with a high rate of photorespiration depending on environmental factors such as light and temperature, cotton has a high morphological and physiological complexity (TAIZ; ZEIGER, 2013). Due to this high complexity, factors related mainly to plant stress, both environmental and due to operational errors or crop management, can negatively influence the quantity and quality of cotton fiber produced (ZHAO *et al.*, 2013).

Over the last years, for example, the concerns of cotton farmers have increased due to possible contamination from the application of herbicides in neighboring crops, because of the early release of cotton and soy cultivars resistant to herbicides such as 2,4 -D (BLANCHETT *et al.*, 2017; BYRD *et al.*, 2016). The 2,4 -dichlorophenoxyacetic acid (2,4 -D) can easily enter the atmosphere by volatilization, drift, leaching, or runoff due to high volatility and water solubility, resulting in potential threats to sensitive crops (NIU *et al.*, 2018).

Cotton is considered as one of the most sensitive crops to the herbicide 2,4 -D, and this herbicide can cause visual symptoms ranging from leaf malformations to severe epinasty and plant death, depending on environmental conditions, dose and especially the development stage at the time of exposure (PETERSON *et al.*, 2016; SMITH *et al.*, 2017).

However, there are no studies in the literature related to the effects of the herbicide 2,4 -D, applied alone, on the quality characteristics of cotton fiber. Nevertheless, Manuchehri *et al.* (2020) evaluated the response of cotton to applications of the herbicide mixture glyphosate + 2,4 -D choline salt and concluded that contamination rates of 0.0008%, 0.008%, 0.08% and 0.8% (equivalent to 0.0183, 0.183, 1.83 and 18.3 g a.e. ha^{-1} of the mixture) did not affect fiber quality. However, the researchers reported that a contamination rate of 8% (183 g a.e. ha^{-1} of the mixture) decreases micronaire indices, fiber length, fiber length uniformity, and fiber strength.

In general, it is known that cotton plants show higher sensitivity to the herbicide 2,4 -D when contaminations occur in the early stages of crop development (MARQUES *et al.*, 2019, 2020), since losses between 32% and 71% of productivity can be expected (EGAN; BARLOW; MORTENSEN, 2014).

Such sensitivity declines significantly in the reproductive stages, especially at the beginning of flowering, where the main effects of the herbicide reported are the fall of floral buds and the delay in opening the bolls, explained by the increased time for the physiological maturity of the bolls (BYRD *et al.*, 2016). When contamination occurs after the formation and opening of the bolls, the herbicide acts as a foliar desiccant (CONSTANTIN *et al.,* 2007), which is not recommended because it makes the leaves dry and brittle, which can directly compromise the quality of the fiber.

The objective of this study was to evaluate the effect of sub-doses of the herbicide 2,4 -D, choline salt formulation, on the fiber quality characteristics of the herbaceous cotton plant and to observe how the mode of action of the herbicide on these characteristics is altered as a function of exposure at different phenological stages.

MATERIAL AND METHODS

The experimental phase of the present research was represented by two studies conducted under field conditions, during the agricultural crops of the years 2018 and 2019, with experimental unit in cotton plantation area whose geographical coordinates are 15º00'52.2" S and 52º31'37.2" W.

The climate of the region according to Köppen's classification (1948) is the Aw type, characterized as having average temperatures above 27 ºC in the hottest months (November to February), average temperatures above 18 ºC in the coldest months (June to August) and with average annual precipitation between 1,000 and 1,500 mm distributed in two well-defined periods in terms of precipitation: a heavy rainy season between the months of October and March and a clear dry period between the months of April and September (MARCHI *et al.*, 2019).

The experimental design was based on randomized blocks, with four repetitions and the treatments were distributed in a 7 x 3 factorial design, with seven fractions of the average label dose of the herbicide 2,4 -D choline salt formulation (0 (control); 0.4275; 0.855; 1.71; 3.42; 8.55; 17.1 g a.e. ha^{-1}) that were established according to the dose of 1.5 L ha^{-1} recommended in the package insert (684 g a.e. ha^{-1}), namely: 0; 0.0625%; 0.125%; 0.25%; 0.5%; 1.25% and 2.5%; associated with three different exposure times during the phenological stage of cotton, namely: V4, B4 and C4. For the definition of phenological stages the recommendations of Marur and Ruano (2001) were followed.

Soil samples from the area were collected and sent to the laboratory for analysis. The chemical and physical characteristics of the soil were: pH in $CaCl_2$ of 5.6; 31.4 g dm^{-3} of organic matter; P not significant; V of 71.8%; K, Ca, Mg and H+Al contents of 0.41; 5.76; 1.30 and 2.9 cmolc. dm-3, respectively; 475 g dm-3 of sand, 50 g dm-3 of silt and 475 g dm-3 of clay.

Based on soil analysis, fertility and acidity corrections consisted of 300 kg ha^{-1} of NPK of the 09-30-10 formulation at sowing and three covering fertilizations, the first two with 160 kg ha^{-1} and 140 kg ha^{-1} of the 20-00-20 formulation and the third one with 110 kg ha^{-1} of urea. Sowing was performed with the cotton cultivar TMG 47 B2RF not tolerant to 2,4 -D choline salt.

Each experimental plot was composed of four rows, eight meters long, spaced 0.9 m apart, with the two central rows being the useful area, discounting 0.5 m at each end of the plot.

The sub-doses of herbicide were applied when at least 50% of the plants entered the phenological stage corresponding to the objective of the study, using a CO_2 pressurized backpack sprayer with a spray bar equipped with four ADIA 110015 fan spray nozzles with air induction, spaced 0.5 m apart, and calibrated at 1.8 kgf cm^{-2}, in order to obtain a droplet consumption equivalent to 150 L ha^{-1}.

The effect of treatments was evaluated by observing the visual symptoms of damage at 3, 7, 14, 21 and 30 days after application (DAA), assigning values between 0 (no damage observed) and 100% (death of plants). It should be noted that the visual evaluations of 3, 7, 14 and 21 DAA served as a parameter for the evaluation of 30 DAA.

The values obtained in the 30 DAA evaluations were adjusted to the exponential non-linear regression model using the ORIGIN 8.5.1 SR1 program. To choose the regression model, the highest value of the coefficient of determination (R^2) was considered at $p \leq 0.05$ according to the F test, respecting the biological response.

At harvest time, 40 clusters located in the middle third of the plants within the useful area of each experimental plot were randomly selected. These samples were processed, separating the seed from the lint in roller machines with five knives, by the Instituto Mato-grossense do Algodão (IMA), located in Primavera do Leste – MT.

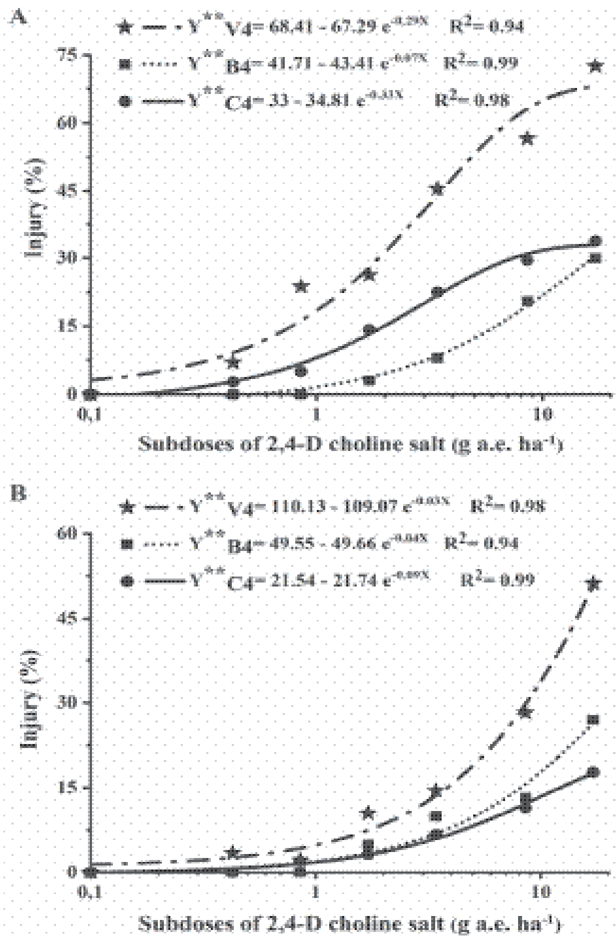
The lint samples coming from the processing were then sent to the laboratory of the Cotton Producers Cooperative (UNNICOTTON), located in Primavera do Leste – MT, for analysis of the fiber quality by the HVI (High Volume Instruments) equipment. The following fiber characteristics were determined: SCI – Spinning consistency index; UHM – Fiber length; UNF – Length uniformity; SFI – Short fiber index; STR – Fiber strength; MIC – Micronaire index; MAT – Maturity; ELG Uniformity Fiber elongation; (+b) – Yellowing degree.

The values obtained were analyzed by the F test and the effects of the treatments were compared by the Tukey test at 5% probability, using the AgroEstat statistical program (BARBOSA; MALDONADO JÚNIOR, 2016).

RESULT AND DISCUSSION

The visual effects of damage (%) observed at 30 DAA of sub-doses of 2,4 -D choline salt on cotton crops were higher at the V4 phenological stage for all sub-doses evaluated, regardless of the year in which this experiment was conducted. An exponential and ascending behavior was observed with maximum damage values of 74% (Figure 1 A) and 55% (Figure 1 B) when 17.1 g a.e. ha^{-1} of the herbicide was applied. The main damage observed at this stage was epinasty and wrinkling of the young leaves and stem apex.

Figure 1 - Visual damage (%) observed at 30 DAA of sub-doses of the herbicide 2,4-D choline salt at different phenological stages of herbaceous cotton plants in the years 2018 (A) and 2019 (B). ** Significant ($p \leq 0.01$)



For stages B4 and C4 it was also possible to observe exponential and ascending behavior, with maximum damage values of 30 and 33%, respectively, when 17.1 g a.e. ha^{-1} of 2,4 -D choline salt was applied in the year 2018 (Figure 1A) and 17 and 23%, respectively, when the same sub-dose of the herbicide was applied in the year 2019 (Figure 1B).

The main damage observed in the B4 stage was epinasty of the branches, purplish leaf spots, thickening of the collar of the plants, death of the stem apex and loss of floral buds. For the C4 stage it was possible to observe leaf dryness, intense purplish tone of the leaves and reddish spots on the developing bolls.

It is also observed an apparent similarity in the behavior of the trend lines of the visual damage values for the years 2018 and 2019 and, because of the intense change of direction of the curves, it is evident that the sub-doses higher than 1.71 g a.e. ha^{-1} caused the most severe visual damage effects to cotton plants, regardless of the phenological stage studied.

As has been widely known since the discovery of 2,4 -D, cotton is extremely sensitive to this herbicide mainly during the vegetative stages, and from the pre-flowering stages (floral buds) this sensitivity drops due to the leaves being better developed (BYRD *et al.*, 2016). The age of the leaves can influence the uptake of the herbicide through the development and maturity of the cuticle, since developing leaves contain less thick epicuticular waxes and with variable chemical composition compared to mature leaves (PETERSON *et al.*, 2016).

However, Everitt and Keeling (2009) comment that visual estimates of early season damage often overestimate the damage caused by herbicide contamination. The indeterminate growth habit of cotton allows considerable compensation for stress, and this compensation depends on weather and other conditions that may vary, even if early season damage was consistent, and thus it is not a good indicator of effects on many different quality parameters of the final product.

Evaluating the characteristics of cotton fiber analyzed by the HVI equipment (High Volume Instruments) as a function of the application of sub-doses of 2,4 -D choline salt and the phenological stages (Table 1, 2 and 3), it is observed that no sub-dose of 2,4 -D choline salt herbicide applied at the three stages evaluated affected the variables of fiber quality, since there was no significant difference between the treatments and the control for the factor "doses" and, because of this, the results are not presented.

For the factor "phenological stage" there was a significant difference for the variables ELG and (+b) for the 2018 crop (Table 3) and MIC and ELG for the 2019 crop (Table 2 and 3), indicating that possible contamination by the herbicide 2,4 -D choline salt can affect cotton fiber quality depending on the age of the plant at the time of exposure.

For the variable spinning consistency index (SCI), no significant differences were observed among phenological stages, regardless of the year of the experiment. However, it is important to highlight that the V4 stage showed lower values than the other stages for the 2018 and 2019 harvests (Table 1).

This fact is important to understand the behavior of the other variables, since the spinning consistency index is the value determined by a multiple regression mathematical equation developed from the relationship between the most important physical properties of the fibers. It is understood that the lower the SCI value, the lower is the consistency power of the fiber, and consequently less resistant yarns will be obtained.

Evaluating the fiber length (UHM) it is observed that, regardless of the sub-doses of 2,4 -D choline salt and phenological stages, the fibers showed an average length of 30.2 mm for the 2018 crop and 30.7 mm for the 2019 crop (Table 1). Cotton fibers with lengths greater than 30 mm are considered long (VAN DER SLUIJS *et al.*, 2015). Fiber length plays a major role in the limit of the reliability index and the evenness of fiber distribution in the yarn, and has a direct effect on fiber strength. Longer fibers are preferred by the industry, because they are stronger and finer for spinning (JERONIMO *et al.*, 2014).

For both years of this experiment, the values obtained for the short fiber index (SFI) variable are classified in the category of short fiber with "medium" content, for being higher than 6 and lower than 9% (ARAÚJO *et al.*, 2012). It is understood that the lower this index, the better the technological quality of the fiber (ZONTA *et al.*, 2017).

Taking this fact into consideration it is noted that, even though there was no significant difference between the phenological stages, there was a tendency for the average value of the short fiber index at the V4 stage to be higher than the other stages (Table 2) in both the 2018 and 2019 crops, evidencing that possible contamination of 2,4 -D choline salt at this stage can compromise fiber quality.

Fiber strength (STR) can be considered the most responsive variable to adverse environmental conditions, since the various plant stress situations can directly compromise this qualitative variable of cotton fiber (TANG *et al.*, 2015). Fiber strength is evaluated by measuring the ability of the fiber to withstand a load until it breaks, expressed in gf tex^{-1}, representing the maximum force to break a bundle of fibers, and the minimum strength required by the textile industry is 29.5 gf tex^{-1} (GAMA *et al.*, 2017).

Table 1 - Spinning consistency index (SCI), fiber length (UHM) and uniformity of fiber length (UNF) of cotton subjected to applications of sub-doses of 2,4-D choline salt at phenological stages V4, B4 and C4 in the 2018 and 2019 crops

	2018								
	SCI			UHM (mm)			UNF (%)		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	141.2	145.2	142.8	30.2	30.2	30.2	82.9	83.4	83.2
F Doses (D)		0.28^{NS}			0.28^{NS}			0.59^{NS}	
F Stages (S)		1.62^{NS}			0.34^{NS}			1.65^{NS}	
F (D x S)		1.29^{NS}			1.32^{NS}			0.96^{NS}	
F Blocks		1.48^{NS}			2.86*			0.80^{NS}	
CV (%)		5.93			2.33			1.27	
	2019								
	SCI			UHM (mm)			UNF (%)		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	138.8	139.0	140.6	30.7	30.7	30.7	83.1	83.0	83.4
F Doses (D)		2.01^{NS}			1.66^{NS}			0.99^{NS}	
F Stages (S)		0.46^{NS}			0.22^{NS}			0.94^{NS}	
F (D x S)		1.58^{NS}			0.85^{NS}			1.12^{NS}	
F Blocks		0.34^{NS}			1.31^{NS}			0.41^{NS}	
CV (%)		5.57			1.98			1.45	

*Significant at 5% probability; NS – not significant

Table 2 - Short fiber index (SFI), fiber strength (STR) and micronaire index (MIC) of cotton fiber subjected to applications of sub-doses of 2,4-D choline salt at phenological stages V4, B4 and C4 in 2018 and 2019 crops

	2018								
	SFI (%)			STR (gf tex^{-1})			MIC (µg pol^{-1})		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	8.2	8.0	7.9	29.2	29.8	29.6	4.1	4.1	4.1
F Doses (D)		0.14^{NS}			0.75^{NS}			0.71^{NS}	
F Stages (S)		0.40^{NS}			2.12^{NS}			0.63^{NS}	
F (D x S)		1.11^{NS}			1.49^{NS}			0.77^{NS}	
F Blocks		2.13^{NS}			3.06*			0.23^{NS}	
CV (%)		14.86			3.97			5.24	
	2019								
	SFI (%)			STR (gf tex^{-1})			MIC (µg pol^{-1})		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	7.7	7.5	7.5	28.4	29.5	29.8	4.3 b	4.3 b	4.4 a
F Doses (D)		1.67^{NS}			1.83^{NS}			1.41^{NS}	
F Stages (S)		0.86^{NS}			2.67^{NS}			3.40*	
F (D x S)		1.06^{NS}			1.29^{NS}			1.08^{NS}	
F Blocks		0.83^{NS}			0.80^{NS}			0.59^{NS}	
CV (%)		10.44			3.44			3.70	

*Significant at 5% probability; NS – not significant. Means followed by the same uppercase letter in the line do not differ statistically from each other by the Tukey test ($p<0.05$)

The STR values for the B4 and C4 stages in both harvests were higher than this value. However, it should be noted that for the V4 stage it was possible to observe values of 29.2 gf tex^{-1} in the 2018 crop and 28.4 gf tex^{-1} in the 2019 crop for this same variable (Table 2). Thus, it is inferred that the application of herbicide sub-doses at this stage decreased the quality of cotton fiber, since the STR values were lower than the value desired by the industry.

The micronaire values (MIC), independent of the sub-doses of 2,4-D choline salt and the phenological stage of cotton, can be considered as average and good quality for the industry. The micronaire index is important for commercialization of fiber quality, and it is suggested that this index be between 3.5 and 5.0 for the best incorporation of dyes during finishing (KLJUN *et al.*, 2014).

The values of fiber maturity (MAT) did not differ according to the phenological stages of cotton submitted to applications of 2,4 -D choline salt in both years of this experiment (Table 3). Fiber maturation is an important parameter for the textile industry, since immature fibers have lower dye absorption capacity, making the fabric non-uniform. Fibers that present values greater than 80% maturity are considered mature (LEE; FANG, 2015).

The fiber elongation (ELG) allows to evaluate the elastic behavior of the textile material subjected to tensile stress, providing an idea about the expected reliability. The fiber elongation values observed in this study can be classified as regular (6.0 to 6.9%) (KAZAMA *et al.*, 2016). However, it is highlighted that there was a statistical difference between phenological stages in the 2018 and 2019 crops (Table 3), evidencing that applications of the herbicide 2,4 -D choline salt applied at the V4 and B4 stages compromise fiber elongation.

In this study, it was possible to observe variation between the stages of cotton submitted to the application of sub-doses of 2,4 -D choline salt as to the yellowing degree (+b) in the 2018 crop. Therefore, it is evident that contamination of the herbicide in the V4 and B4 stages influence the color and consequently the quality of the fiber, since the values were higher than the C4 stage. The yellowing index (+b) indicates the fiber color, which should be as white as possible, with higher values tending to yellow and lower values tending to white. Thus, a higher +b index may imply a slightly yellowish fiber, considered to be of inferior quality (SILVA *et al.*, 2016).

Table 3 - Maturity (MAT), fiber elongation (ELG) and degree of fiber yellowing (+b) of cotton subjected to applications of sub-doses of 2,4-D choline salt at phenological stages V4, B4 and C4 in 2018 and 2019 crops

	2018								
	MAT (%)			ELG (%)			+b		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	85.7	85.8	85.7	6.3 ab	6.2 b	6.5 a	6.3 ab	6.4 a	6.1 b
F Doses (D)		0.84^{NS}			0.90^{NS}			1.47^{NS}	
F Stages (S)		0.23^{NS}			3.36*			5.40*	
F (D x S)		0.43^{NS}			0.97^{NS}			1.80^{NS}	
F Blocks		0.30^{NS}			0.31^{NS}			1.95^{NS}	
CV (%)		0.69			6.98			5.25	
	2019								
	MAT (%)			ELG (%)			+b		
Stage	V4	B4	C4	V4	B4	C4	V4	B4	C4
Mean	86.2	86.1	86.3	6.3 b	6.3 b	6.4 a	8.9	9.1	8.8
F Doses (D)		0.89^{NS}			1.35^{NS}			0.94^{NS}	
F Stages (S)		1.63^{NS}			3.42*			2.80^{NS}	
F (D x S)		1.11^{NS}			0.95^{NS}			1.39^{NS}	
F Blocks		0.63^{NS}			2.80*			2.39^{NS}	
CV (%)		0.60			2.59			4.49	

*Significant at 5% probability; NS – not significant. Means followed by the same uppercase letter in the line do not differ statistically from each other by the Tukey test ($p<0.05$)

CONCLUSIONS

1. No sub-dose of the herbicide 2,4 -D choline salt applied at the three phenological stages evaluated affected the fiber quality variables;
2. Applications of sub-doses of 2,4 -D choline salt at the V4 and B4 stages of cotton directly influence the visual effects of damage and some quality characteristics of cotton fiber, showing that contamination of the herbicide in the early stages of plant development drastically compromises the crop;
3. The fiber quality variables of cotton plants in the C4 stage were not influenced by the application of sub-doses of 2,4 -D choline salt, proving that the advanced age of the plants directly influences the effects of the herbicide, with no risk if accidental contamination occurs at this stage.

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