# Application timing affects the selectivity of herbicides for garlic crops

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## Abstract

Chemical control is the main weed control method in garlic crops; however, this crop is sensitive to herbicides, which can cause injuries and decrease yield depending on the products used, application timing, rate, and cultivar. In this context, the objective of this study was to evaluate the selectivity of herbicides for garlic plants (cultivar Chonan), using different application timings. A greenhouse experiment was conducted in a completely randomized design with four replications. An 8×2 factorial arrangement was used, in which the first factor consisted of a control without herbicide and seven herbicides (linuron, diuron, pendimethalin, s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon) applied according to the second factor, application timings (pre-emergence or post-emergence). Phytotoxicity, photosynthetic rate, stomata morphometry, bulb diameter, and crop yield of garlic were evaluated. Pre-emergence applications were more selective than post-emergence applications. The herbicides linuron, diuron, s-metolachlor, and oxadiazon applied at pre-emergence, and s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon applied at post-emergence were not selective for the Chonan garlic cultivar. Pendimethalin presented the highest selectivity, regardless of the application timing. Oxyfluorfen and flumioxazin applied at pre-emergence and linuron and diuron applied at post-emergence were selective for the cultivar Chonan.

Keywords: Allium sativum, chemical control, phytotoxicity, weed

#### Introduction

Weed control is important for garlic crops, as weeds compete for water, nutrients, light, and space, release allelopathic substances, serve as inoculum source for pests and diseases, and hinder the crop harvest (Lawande et al., 2009; Rahman et al., 2011, Maciel et al., 2021). According to Kumar et al., (2013), a decrease of 72.5% in garlic bulb yield was found in crops without weed control; Sahoo et al. (2018) found even higher losses, reaching up to 94.8%.

The critical period for the effects of weeds on garlic crops is, in general, after 25 days after planting, when the seed-bulb reserves run out. This control may be carried out until 120 days after emergence, which is the average time for the crop to be ready for harvest (Lucini, 2010). Weed control until harvest is necessary because the presence of these species can increase the labor and decrease the value of the product, as the bulbs can be damaged when plucked from the soil.

The long period required for adoption of control measures is because garlic plants have narrow leaves and small, semi-erect sizes (Lawande et al., 2009; Rahman et al., 2012; Sahoo et al., 2018). These characteristics result in low soil cover, making the development of weeds more intense (Lawande et al., 2009; Chopra et al., 2014). In addition, garlic plants present shallow root system and require high fertility soils and irrigation management; thus, weeds use these resources and have vigorous grow.

Garlic seedlings are planted with narrow spacing and have shallow roots. These characteristics make the mechanical control difficult due to risks of causing physical damages to plants and bulbs (Siddhu et al., 2018). Therefore, chemical control is the main weed control method (Guerra et al., 2020; Ganapathi et al., 2020; Ahirwar et al., 2021). However, garlic crops present problems of sensitivity to herbicides, which causes

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phytotoxicity; it leads many garlic growers to use sub-rates or splitting rates to solve this problem. The application of pre-emergence herbicides to emerged garlic plants at approximately 20-30 days after planting is also common; however, this practice is empirical, without an actual understanding of the effects of these herbicides on the crop, mainly regarding selectivity. In addition, the limited number of herbicides approved for garlic crops makes garlic growers to use non-approved products.

Based on in this information, the objective of the present study was to evaluate the selectivity of herbicides for garlic crops of the cultivar Chonan, using different application timings.

# **Material and Methods**

The experiment was conducted in a greenhouse at the Federal University of Santa Catarina, in the municipality of Curitibanos, state of Santa Catarina, Brazil (27°17'6"S, 50°32'5"W, and altitude of approximately 1000 m), from July 05 to November 24, 2018. The region has occurrence of low temperatures in the winter and frequent frosts. The greenhouse had controlled conditions to meet the thermal and hydrological demands of the garlic crop over its developmental cycle. The temperatures and photoperiod oscillated from 08 to 25 °C and from 10 to 12 hours, respectively, over the crop developmental cycle.

The experiment was conducted in a completely randomized design with 16 treatments and four replications, using an 8×2 factorial arrangement; the first factor consisted of herbicide applications (control, linuron 810 g ha<sup>-1</sup>, diuron 1000 g ha<sup>-1</sup>, pendimethalin 1600 g ha<sup>-1</sup>, s-metolachlor 1200 g ha<sup>-1</sup>, oxyfluorfen 720 g ha<sup>-1</sup>, flumioxazin 40 g ha<sup>-1</sup>, and oxadiazon 1000 g ha<sup>-1</sup>) and the second factor consisted of herbicide application timings (pre-emergence or post-emergence of the garlic crop). The commercial products used were Afalon<sup>®</sup> 450 g L<sup>-1</sup>, SC (linuron); Diuron Nortox<sup>®</sup> 500 g L<sup>-1</sup>, SC (diuron); Herbadox<sup>®</sup> 400 g L<sup>-1</sup>, EC (pendimethalin); Dual Gold<sup>®</sup> 960 g L<sup>-1</sup>, EC (s-metolachlor); Galigan<sup>®</sup> 960 g L<sup>-1</sup>, EC (oxyfluorfen); Flumyzin<sup>®</sup> 500 g kg<sup>-1</sup>, WP (flumioxazin); and Ronstar<sup>®</sup> 250 g L<sup>-1</sup>, SC (oxadiazon).

The experimental units consisted of 11-liter pots filled with a soil classified as Haplic Cambissol of clayey texture (587.5 g kg<sup>-1</sup> of clay, 187.5 g kg<sup>-1</sup> of silt, 225.0 g kg<sup>-1</sup> of sand, and 37.63 g dm<sup>-3</sup> of organic matter), which was collected at the Agricultural Experimental Farm of the Federal University of Santa Catarina. Soil fertilizers were applied using 1500 kg ha<sup>-1</sup> of the 09-33-12 NPK formulation and two nitrogen applications as topdressing, using urea as N source (45% N), with application of 45 kg ha<sup>-1</sup> of N at 28 and 60 days after planting. The cultivar Chonan was used for the experiment, which is from the noble class and has early maturation, with a cycle of approximately 125 days. The planting was carried out considering the spacing used in the field, with the planting of 3 garlic cloves per pot, to a depth of 1 to 2 cm.

The herbicide application at pre-emergence was carried out on July 05, 2018, soon after the garlic clove planting, whereas the application at post-emergence was carried out on July 26, 2018, at 21 days after planting, when the garlic plants had 3 leaves (V3 phenological stage). Both applications were carried out using a  $CO_{2-}$  pressurized backpack sprayer, with a 2-meter spray boom and 4 flat fan spray tips (110015) spaced 0.5 m apart; the work pressure used was 172 kPa, with speed of 1 m s<sup>-1</sup>, resulting in an application rate of 150 L ha<sup>-1</sup>.

The pre-emergence application was carried out between 10:45 and 11:15 a.m., under air temperature of 23.7 °C, relative air humidity of 58%, absence of wind, and wet soil. The post-emergence application was caried out between 2:00 and 2:45 p.m., under air temperature of 20.6 °C, relative air humidity of 63%, absence of wind, and wet soil.

Phytosanitary management was carried out as required for the crop, avoiding applications of products that could cause phytotoxicity to plants. Weeds that emerged in the pots were manually uprooted to avoid their interference, thus isolating the effect of herbicides on the garlic plants.

Visual evaluations of phytotoxicity were carried out to determine the effect of the treatments on the garlic plants, considering leaf damages caused by the herbicides, the level of interference on seedling emergence, and the height and developmental stage of plants. A visual scale was used for this evaluation, from 0% to 100%: zero representing the absence of symptoms and 100% representing the death of the plant. Three evaluations were carried out: at 28, 36, and 49 days after application for pre-emergence (DAA-Pre), and at 7, 15, and 28 days after application for post-emergence application (DAA-Pos).

Photosynthetic analyses were carried out at 36 and 49 DAA-Pre and 15 and 28 DAA-Pos, using an infrared gas analyzer model LI-6400 XT. The evaluations were carried out using the middle third of the third fully expanded leaf, with photosynthetically active radiation of 800 umol m<sup>2</sup> s<sup>1</sup>, collecting data of gas flow in the stomata (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), internal CO<sub>2</sub> concentration (ppm), net carbon assimilation rate (µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), transpiration (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and photosynthetic water

use efficiency (mm mol of  $H_2O$ ) during the first hours of photosynthetic activity of plants (9:00 a.m. to 12:00 p.m.).

The morphometry of stomata was evaluated using samples from the middle third of the second fully expanded leaf from the garlic plant base at 36 DAA-Pre and 15 DAA-Pos. Three leaves per treatment were collected. Five replications of leaf samples were fixed in FAA 70 (37% formaldehyde + glacial acetic acid + 70% ethanol) for 48 hours and conserved in 70% ethanol. The epidermis surface print technique was used, applying colorless nail polish, with the removal of the pellicle after drying, for preparation of temporary slides and frontal view observations. The observations were carried out in a light microscope (Olympus BX53F) with digital system of capture and measurement of images (Cell Sens Standard®). The stomatal analyses consisted in determining the stomatal density and length and width of the stomatal complex and stomatal pores.

The number of plants per pot was determined at the pre-harvest of bulbs. After harvesting, the plants were taken to a barn for approximately 20 days for dehydration process and, then, they were evaluated for bulb diameter (mm) by using a digital caliper, and for bulb weight, which was converted into yield (kg ha<sup>-1</sup>).

The data obtained in the evaluations were subjected to analysis of variance by the F test at 5%

probability level. The factor application timing presented only two levels; thus, the F test was conclusive. The herbicides were compared using the Scott Knott grouping test at 5% probability level.

# **Results and Discussion**

Table 1 shows the results of phytotoxicity in garlic plants. The plants presented, in general, smaller heights due to the delay in the emergence when the herbicides were applied at pre-emergence, mainly for s-metolachlor, oxyfluorfen, and oxadiazon. The symptoms found for the post-emergence applications were leaf necrosis, mainly when using oxyfluorfen, flumioxazin, and oxadiazon. These herbicides also resulted in lower growth of garlic plants over the developmental cycle.

Ali et al. (2017) also found that garlic plants treated with s-metolachlor (1920 g ha<sup>-1</sup>) at pre-emergence present low growth, affecting the crop development and production. The low growth plants treated with s-metolachlor is due to the mechanism of action of this molecule, since it inhibits the synthesis of long-chain fat acids. This herbicide inhibits the action of elongase enzymes, responsible for elongate short lipids chains; these lipids are essential for the formation of membranes and the cell wall and their absence can result in lower growth (Matthes et al., 1998).

		28 D,	AA-Pre			36 DAA-Pre				49 DAA-Pre			
		7 DA	A-Pos			15 DAA-Pos				AA-Pos			
Herbicide	Pr	е	Pos	st	Pre	Э	Po	st	Pr	e	Post		
Control	0.00	bA	0.00	dA	0.00	bA	0.00	dA	0.00	bA	0.00	сА	
Linuron	0.00	bA	4.00	dA	0.00	bA	4.50	dA	0.00	bA	3.00	сА	
Diuron	0.00	bA	3.00	dA	0.00	bA	3.00	dA	0.00	bA	3.50	сА	
Pendimethalin	0.00	bA	4.00	dA	4.00	bA	7.00	сА	3.00	aA	4.00	сА	
S-metolachlor	9.75	aA	9.25	сА	11.00	aA	8.50	сА	7.75	aA	6.75	сА	
Oxyfluorfen	3.00	bB	63.75	aA	6.00	аB	58.75	aA	4.00	аB	41.25	aA	
Flumioxazin	0.00	bB	13.75	сА	0.00	bB	13.00	сА	0.00	bA	4.75	сА	
Oxadiazon	0.00	bB	46.25	bA	0.00	bB	41.25	bA	0.00	bB	33.75	bA	
CV (%)		39	9.58			50.90			43.88				

 Table 1. Phytotoxicity (%) in garlic plants after application of herbicides at pre-emergence or post-emergence.

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

The application of oxyfluorfen and oxadiazon at post-emergence resulted in the greatest injuries (41.25% and 33.75%, respectively), even at 28 DAA-Pos. These results show that the plants failed in totally recovering from the symptoms caused by these herbicides, in the first evaluations. However, the phytotoxicity levels were less intense when applied at pre-emergence, regardless of the evaluation time. Linuron, diuron, and pendimethalin resulted in the lowest phytotoxicity percentages, regardless of the application timing (Table 1).

The photosynthetic analyses showed that the

pre-emergence application did not affect the net carbon assimilation rate of garlic plants in any of the evaluations (Table 2). The post-emergence application (28 DAA-Pos) of oxadiazon decreased the net carbon assimilation rate in approximately 50% when compared to the control without herbicide, and the pre-emergence application of the herbicide. Thus, the phytotoxicity symptoms caused by this herbicide also affected physiological characteristics, which can affect yield components. Oxadiazon is a molecule that inhibits the protoporphyrinogen oxidase (PPO) enzyme, which acts on the oxidation of protoporphyrin IX; this reaction results in the formation of reactive oxygen species, causing peroxidation of lipids and proteins. This enzyme acts in the chlorophyll synthesis pathway, and its inhibition results in lower synthesis of this pigment (Becerril & Duke,1989), which may be connected to the low net  $\rm CO_2$  assimilation rate.

**Table 2.** Net carbon assimilation rate (A; µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) of garlic plants after application of herbicides at pre-emergence or post-emergence.

		36 D.A	A-Pre		49 DAA-Pre				
		15 DA	A-Pos		28 DAA-Pos				
Herbicide	Pre	;	Pos	Post			P	ost	
Control	19.52	aA	21.67	aA	16.59	aA	16.07	aA	
Linuron	17.95	aA	15.03	aA	16.90	aA	14.82	aA	
Diuron	15.38	aA	19.32	aA	17.07	aA	14.53	aA	
Pendimethalin	17.03	aA	18.87	aA	18.41	aA	15.67	aA	
S-metolachlor	16.23	aA	15.88	aA	15.72	aA	15.60	aA	
Oxyfluorfen	17.59	aA	17.85	aA	14.81	aA	17.77	aA	
Flumioxazin	17.40	aA	15.76	aA	17.22	aA	15.27	aA	
Oxadiazon	14.99	aA	16.82	aA	15.51	аB	8.95	bA	
CV (%)		18	.58		18.72				

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

Considering the results of stomatal conductance (Table 3), treatments that resulted in more intense phytotoxicity symptoms (Table 1) were the same that resulted in the lowest stomatal conductance levels. S-metolachor, oxyfluorfen, flumioxazin, and oxadiazon applied at post-emergence presented lower stomatal conductance (gs) than the control in the evaluation at 28 DAA-Pos, the two latter presented more intense decreases, 46.3% and 61.2%, respectively, also differing

from their pre-emergence application. The postemergence application of flumioxazin decreased the stomatal conductance when compared to the preemergence application, even in the first evaluation.

The internal carbon concentration of garlic plants (Table 4) decreased when flumioxazin was applied at post-emergence, compared to the pre-emergence application, regardless of the evaluation time.

**Table 3.** Stomatal conductance (gs; mol  $H_2O m^{-2} s^{-1}$ ) of garlic plants after the application of herbicides at pre-emergence or post-emergence.

		36 DA	A-Pre		49 DAA-Pre				
		15DA	A-Pos		28 DAA-Pos				
Herbicide	Pre	9	Pos	st	Pre		Pos	st	
Control	0.31	aA	0.34	aA	0.57	aA	0.54	aA	
Linuron	0.49	aA	0.36	aA	0.54	aA	0.59	aA	
Diuron	0.42	aA	0.45	aA	0.54	aA	0.66	aA	
Pendimethalin	0.40	aA	0.35	aA	0.61	aA	0.60	aA	
S-metolachlor	0.31	aA	0.32	aA	0.44	aA	0.44	bA	
Oxyfluorfen	0.45	aA	0.40	aA	0.45	aA	0.42	bA	
Flumioxazin	0.43	aA	0.17	aB	0.55	aA	0.29	сВ	
Oxadiazon	0.30	aA	0.24	aA	0.51	aA	0.21	сВ	
CV (%)		35.	89				19.23		

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

**Table 4.** Internal CO<sub>2</sub> concentration (Ci; ppm) of garlic plants after application of herbicides at pre-emergence or postemergence.

		36 DA	A-Pre						
		15 DA	A-Pos		28 DAA-Pos				
Herbicide	Pre		Pos	t	Pre		Pc	ost	
Control	288.59	aA	282.15	aA	356.11	aA	349.61	aA	
Linuron	337.17	aA	260.61	аB	359.15	aA	354.93	aA	
Diuron	338.92	aA	317.94	aA	358.91	aA	354.21	aA	
Pendimethalin	327.23	aA	314.96	aA	353.77	aA	353.86	aA	
S-metolachlor	311.01	aA	264.02	aA	341.43	aA	343.36	aA	
Oxyfluorfen	319.33	aA	305.24	aA	348.53	aA	338.01	aA	
Flumioxazin	325.45	aA	235.09	аB	352.39	aA	315.96	aB	
Oxadiazon	291.21	aA	292.78	aA	348.69	aA	341.26	aA	
CV (%)		11	.65				5.86		

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

The herbicides s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon applied at post-emergence resulted in decreases in transpiration of garlic plants (Table 5). These results are connected to the stomatal conductance, as a low stomatal conductance means

that the stomata are closed, which decreases water loss through transpiration. Thus, the herbicides that decreased the transpiration were the same that resulted in lower stomatal conductance levels (Table 3).

Table 5. Transpiration (E; mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) in garlic plants after application of herbicides at pre-emergence or post-emergence.

		36 DA	A-Pre		49 DAA-Pre 28 DAA-Pos				
		15 DA	A-Pos						
Herbicide	Pre		Post		Pre		Post		
Control	4.08	aA	5.03	aA	7.57	aA	8.01	aA	
Linuron	4.83	aA	5.72	aA	6.35	aB	8.38	aA	
Diuron	4.55	aA	7.63	aA	6.65	aB	8.90	aA	
Pendimethalin	4.61	aA	6.20	aA	7.42	aA	7.97	aA	
S-metolachlor	3.71	aA	6.00	aA	5.97	aA	6.17	bA	
Oxyfluorfen	5.37	aA	8.20	aA	6.41	aA	5.87	bA	
Flumioxazin	5.69	aA	4.05	aA	7.83	aA	4.08	сВ	
Oxadiazon	4.16	aA	5.72	aA	7.37	aA	3.19	сВ	
CV (%)		37.	50				17.69		

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

The photosynthetic water use efficiency (WUE) is represented by the ratio between net carbon assimilation rate and transpiration. No difference was found for this variable in the first evaluation, regardless of the application timing and the herbicide. In the second evaluation, the highest WUE was found for oxyfluorfen, flumioxazin, and oxadiazon applied at post-emergence; this is due to the lowest transpiration values found for these herbicides, which affected the WUE (Table 6).

Table 6. Photosynthetic water use efficiency - WUE (mm mol of H<sub>2</sub>O) of garlic plants after application of herbicides at preemergence or post-emergence.

		36	DAA-Pre				49 DAA-Pre		
		15	DAA-Pos		28 DAA-Pos				
Herbicide	Pre	)	Р	ost	Pre		Pc	ost	
Control	4.99	aA	4.37	aA	2.25	aA	2.01	bA	
Linuron	3.70	aA	5.06	aA	2.70	aA	1.75	bA	
Diuron	3.40	aA	2.52	aA	2.54	aA	1.64	bA	
Pendimethalin	3.71	aA	3.07	aA	2.52	aA	1.99	bA	
S-metolachlor	4.53	aA	4.32	aA	2.72	aA	2.57	bA	
Oxyfluorfen	3.44	aA	2.29	aA	2.31	aA	3.04	aA	
Flumioxazin	3.03	aA	4.08	aA	2.19	aB	4.39	aA	
Oxadiazon	4.30	aA	3.07	aA	2.13	aA	3.11	aA	
CV (%)			46.20				31.67		

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level. DAA-Pre: days after application at pre-emergence. DAA-Pos: days after application at post-emergence.

The analysis of stomatal density of hypoestomatic leaves of garlic plants showed that the herbicides tested affected this characteristic. S-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon applied in pre-emergence decreased the values of this variable. The same result was found for the post-emergence application of diuron, oxyfluorfen, and flumioxazin. Diuron, pendimethalin, and oxyfluorfen applied at post-emergence resulted in lower stomatal density than their application at pre-emergence (Table 7).

Table 7. Morphometry of stomata of garlic leaves after application of herbicides at pre-emergence or post-emergence.
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		Stomata										
	Stomatal	Density	(stomata r	nm⁻²)		Length	n (mm)			Width (	mm)	
Herbicide	Pre		Post	-	Pre Pc		Pos	st Pr		e Po		ost
Control	211.43	aA	210.53	aA	22.98	aA	20.23	aA	9.70	aA	9.10	aA
Linuron	211.05	aA	210.28	aA	23.23	aA	19.70	аB	9.57	aA	7.87	bB
Diuron	231.11	aA	163.52	bB	19.83	aA	19.57	aA	9.09	bA	8.49	aA
Pendimethalin	222.80	aA	186.64	аB	21.47	aA	20.38	aA	8.50	bA	8.45	aA
S-metolachlor	203.51	bA	190.22	aA	20.55	aA	20.01	aA	8.35	bA	7.56	bA
Oxyfluorfen	189.08	bA	155.99	bB	20.14	aA	19.95	aA	8.37	bA	8.21	bA
Flumioxazin	192.91	bA	177.71	bA	22.20	aA	19.89	aA	8.93	bA	9.24	aA
Oxadiazon	181.15	bA	192.27	aA	21.58	aA	17.99	аB	10.45	aA	7.52	bB
CV(%)		11.	82			10.	.88			10.0	8	
		Stomatal pores										
		Length (mm)							Width	(mm)		
Herbicide		Pre			Post F			Pr	e		Post	
Control	13.24	ļ	aA	1	3.06	aA	2.	90	bA	3.1	4	aA
Linuron	14.01		aA	1	3.17	aA	3.	44	aA	2.4	4	bB
Diuron	12.90	)	aA	1	2.80	aA	3.	07	bA	3.5	2	aA
Pendimethalin	13.38	3	aA	1	2.22	aA	3.	66	aA	3.0	19	aA
S-metolachlor	13.97	,	aA	1	2.41	aA	3.	24	bA	2.6	1	bA
Oxyfluorfen	13.55	5	aA	1	3.55	aA	3.	10	bA	3.0	1	aA
Flumioxazin	14.89	)	aA	1	3.02	aA	3.	54	aA	2.7	5	bB
Oxadiazon	13.16		aA	1	1.58	aA	3.	94	aA	2.0	3	bB
CV(%)			12.0	)9				16.7	79			

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level.

The herbicides applied at pre-emergence or post-emergence did not affect the length of stomata. The application of linuron and oxadiazon at post-emergence resulted in shorter lengths than their application at preemergence. All herbicides applied at pre-emergence decreased the width of stomata, except for linuron and oxadiazon, which presented similar results to that of the control without herbicide. Linuron, s-metolachlor, oxyfluorfen, and oxadiazon applied at post-emergence decreased the width of stomata of garlic plants (Table 7).

The herbicides evaluated did not affect the length of stomatal pores, regardless of the application timing. However, this result was not found for width; diuron, s-metolachlor, and oxyfluorfen applied at preemergence, and linuron, s-metolachlor, flumioxazin, and oxadiazon applied at post-emergence resulted in smaller stomatal pore width (Table 7).

Most treatments that decreased the density

or width of stomata or stomatal pores (s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon applied at postemergence) also resulted in lower stomatal conductance and lower transpiration (Tables 4 and 6). Decreases in stomatal density and dimensions of stomata are connected to decreases in transpiration and increases in water use efficiency (Drake et al., 2013).

Table 8 shows the results of bulb diameter and yield. Most herbicides (linuron, diuron, s-metolachlor, oxyfluorfen, and oxadiazon) decreased the bulb diameter when applied at pre-emergence. Only flumioxazin and oxadiazon affected this variable when the herbicides were applied at post-emergence, resulting in smaller diameters when compared to their application at preemergence. This denotes that the choice of herbicide for the crop and the application timing affect the bulb diameter. **Table 8.** Diameter of bulbs (mm) and yield (kg ha<sup>-1</sup>) of garlic plants after application of herbicides at pre-emergence or postemergence.

		Diamet	er (mm)		Yield (kg ha-1)					
Herbicide	Pre		Post		Pre		Post			
Control	38.40	aA	39.26	aA	8575.00	aA	8019.69	aA		
Linuron	31.80	bA	36.33	aA	5460.94	bA	6599.68	aA		
Diuron	34.61	bA	36.04	aA	6916.56	bA	7272.19	aA		
Pendimethalin	40.00	aA	36.17	aA	8937.50	aA	7594.06	aA		
S-metolachlor	30.86	bA	36.21	aA	6771.56	bA	5844.06	bA		
Oxyfluorfen	37.90	bA	35.94	aA	8967.19	aA	5795.00	bB		
Flumioxazin	40.33	aA	31.08	bB	9832.81	aA	4256.88	сВ		
Oxadiazon	34.78	bA	27.34	bB	5465.94	bA	3880.00	сА		
CV(%)		2.	23		6.43					

Means followed by the same uppercase letter in the rows are not different from each other by the F test at 5% probability level. Means followed by the same letter lowercase in the columns are not different from each other by the Scott Knott test at 5% probability level.

According to the classification of commercial bulbs of the Brazilian Agricultural Research Corporation (EMBRAPA) (MAPA, 1999), garlic bulbs with diameters smaller than 32 mm are considered non-commercial (or industry bulbs). Thus, the bulbs obtained in the treatments with flumioxazin and oxadiazon applied at post-emergence would be classified as non-commercial. Considering the herbicides applied at pre-emergence, only linuron resulted in non-commercial bulbs, presenting garlic bulbs with mean diameter of 31.8 mm. The disadvantage of the production of low-diameter bulbs is that they are intended for industry and have lower marketing value.

The yield results (Table 8) showed that the applications at pre-emergence were, in general, more selective than those at post-emergence. The herbicides linuron, oxadiazon, diuron, and s-metolachlor decreased the yield when used at pre-emergence; the first two presented the highest decreases, 36.33% and 36.27%, respectively.

The treatments pendimethalin, oxyfluorfen, and flumioxazin did not affect the yield of bulbs when applied at pre-emergence. Kumar et al. (2013) found that s-metolachlor applied at pre-emergence significantly decreased the garlic yield when compared to pendimethalin and the control (manual weeding).

The more selective herbicides for application at post-emergence for the Chonan garlic cultivar were linuron, diuron, and pendimethalin, which resulted in yields between 6,599.68 and 7,594.19 kg ha<sup>-1</sup>. The yields found for the treatments with s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon were lower than that of the control, presenting decreases of 27.13%, 27.74%, 46.93%, and 51.61%, respectively. These herbicides presented the lowest stomatal conductance in the photosynthetic analysis (Table 4) and smaller width of stomata and stomatal pores (except for flumioxazin) (Table 7); and oxyfluorfen and oxadiazon also resulted in the highest phytotoxicity percentages (Table 1). A high stomatal conductance results in better leaf gas exchanges, decreasing the canopy temperature and improving  $CO_2$  flow (Sharma-Natu & Ghilyal, 2005). Fisher et al. (1998) evaluated 41 selections of wheat and found that stomatal conductance is among photosynthetic characteristics with higher correlation with yield.

Aghabeigi & Khodadadi (2017) found that the application of oxyfluorfen (480 g ha<sup>-1</sup>) when garlic plants have 2-3 leaves decreases the yield in 27.75%, which is a similar result to that found in the present work (27.74%), even though the authors used a 44.4% lower rate than that used in the present work. Thus, decreases in herbicide rate to that level is not an alternative to improve the selectivity of oxyfluorfen to garlic plants. Different results were found for oxadiazon; Aghabeigi and Khodadadi (2017) found a decrease of 8% in garlic yield using a rate of 360 g ha<sup>-1</sup>, which differs from that found in the present work (51.61% decrease) when using a rate of 1000 g ha<sup>-1</sup>.

The overall results of the present work show that pendimethalin, among the herbicides evaluated, is the most selective for the garlic crop, regardless of the application timing. This herbicide is approved for garlic crops only for pre-emergence application, however, it showed satisfactory results when applied at post-emergence and was the best treatment tested. According to Guerra et al. (2020) and Hassanein et al. (2012), this herbicide is a great alternative for weed control in garlic crops. In addition, it presents long residual period (Mallik et al., 2017). The application of oxyfluorfen and flumioxazin at pre-emergence, and linuron and diuron at post-emergence were also selective to the cultivar Chonan.

Mohite et al. (2015) and Patel et al. (2020) evaluated the effect of herbicides on garlic bulb production and found that pendimethalin applied at pre-emergence resulted in higher yield than oxyfluorfen. Kumar et al. (2013) also found higher garlic bulb yield with application of pendimethalin when compared to trifluralin, oxyfluorfen and s-metolachlor. Rahman et al. (2012) and Ali et al. (2017) also showed that the pendimethalin was the best treatment for weed control and selectivity for garlic crops.

## Conclusions

The applications of herbicides at pre-emergence of garlic crops of the cultivar Chonan were more selective than those at post-emergence.

The herbicides linuron, diuron, s-metolachlor, and oxadiazon applied at pre-emergence, and s-metolachlor, oxyfluorfen, flumioxazin, and oxadiazon applied at postemergence were not selective for the Chonan garlic cultivar, considering photosynthetic, morphological, and production parameters.

Pendimethalin was the only selective herbicide regardless of the application timing.

Oxyfluorfen and flumioxazin applied at preemergence were selective for the cultivar Chonan.

Linuron and diuron applied at post-emergence were selective for the cultivar Chonan.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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