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Article

Methoxychalcones and Cinnamaldehyde as Herbicidal Compounds

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ABSTRACT: Synthetic compounds inspired by natural products provide innovative herbicide leads. Here, 18 methoxychalcones, inspired by cinnamaldehyde and curcumin, were designed, synthesized, and evaluated for their herbicidal activity. Additionally, cinnamaldehyde and curcumin were also assessed. Laboratory and greenhouse bioassays evaluated the inhibition caused by the compounds on initial growth in lettuce and weeds. In laboratory settings, 4'-hydroxy-3′-methoxychalcone induced the highest inhibition for lettuce root (66%) and shoot length (53%) compared to the negative control. The compounds 4'-hydroxy-3′-methoxychalcone and cinnamaldehyde inhibited about 87% of *Urochloa decumbens* growth, making them the most effective in reducing initial weed growth. Chalcones displayed no selectivity, inhibiting the growth of monocotyledonous and eudicotyledonous species. Additionally, 4'-hydroxy-3′-methoxychalcone caused 50% injury in *Raphanus raphanistrum* under greenhouse conditions. Chalcones and cinnamaldehyde hold promise for developing herbicides. Additional studies assessing the combined effects of methoxychalcones would be valuable to determine whether their herbicidal activity can be intensified.

KEYWORDS: *weed, chalcones, injury, phytotoxic potential, herbicide*

1. INTRODUCTION

Weeds pose challenges in agriculture, resulting in considerable direct and indirect losses, more than 50% in some crops if left uncontrolled.[1−5] They compete with crops for resources, impeding growth and reducing yield potential.[6] Furthermore, weeds act as habitats and food sources for various agricultural pests, exacerbating crop damage.[1] Hence, effectively managing these undesirable species is of paramount importance.[6] Typically, weed control methods differ based on climate, environmental factors, and the types of weeds present.[6] Pesticides are crucial for crop protection, serving as effective tools for weed control and improving agricultural returns while reducing labor costs.[5] Nevertheless, the continuous application of chemical herbicides results in adverse effects on both the environment and human health, while also hastening the evolution of weed resistance.[1,4,7] There is a societal call for new approaches in weed management, emphasizing the pressing requirement for inventive, environmentally friendly, and cost-effective herbicides to address the challenge.[1−4,8−10]

The use of natural and natural-based herbicidal products is increasingly desired for weed control[9] and can be an alternative to conventional herbicides.[2] Bioherbicides are compounds derived from secondary metabolism, commonly found in plants but also present in other sources such as microbes and fungi, and they may play a role in plant stress response.[2] Because of their natural source, they typically have a brief presence in the environment and cause fewer negative impacts on other organisms within the ecosystem.[2] Utilizing bioherbicides offers a potential solution to mitigate the stress caused by conventional herbicides in crops and alleviate their associated negative impacts.[3]

Bioherbicides are relatively new options in the global markets, and the acquisition of natural raw materials is not yet highly efficient, leading to elevated extraction costs compared to synthetic alternatives.[3] Otherwise, biologically active natural compounds often inspire the development of new herbicides.[5] Additionally, creating agrochemicals from natural sources is proven effective, with 38% of crop protection compounds derived from natural products.[11]

In this context, synthetic chalcones inspired by natural analogs are a viable alternative for creating novel pesticides. Numerous compelling biological activities associated with chalcones have already been documented,[7] including insecticidal properties,[12] fungicidal effects, and nematicidal activities,[13] among others. However, the herbicidal potential of chalcones has received comparatively less attention. Chalcones, or 1,3-diaryl-2-propen-1-ones, are secondary metabolites belonging to the flavonoid group.[14,15] They play essential roles in plant growth and defense against pathogens.[7] Chalcones are characterized by the presence of two aromatic rings linked by a three-carbon α,β-unsaturated ketone.[14,15] Additionally, attaching specific functional groups to chalcones can improve their desired activities.[7] Chalcones are predominant pigments in petals, although they have also been identified in various plant parts such as heartwood, bark, leaves, fruits, and roots.[14] These compounds are components of certain wheat products, hops, strawberries, berries, tomatoes, pears, apples and citrus fruits.[7] Since plants produce chalcones in limited quantities, extracting them from natural sources is challenging.[7] Up to now, only a limited number of chalcones have been recognized as inhibitors of photosystem

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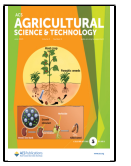

II[16] and growth inhibitors in plants.[17] Consequently, there is a vast array of unexplored chalcones awaiting development for use as herbicides in crop protection.[9] Additionally, both biosynthetic and synthetic aspects of chalcones have garnered great research interest,[7,9] further supporting their potential as bioactive compounds.

Beyond chalcones, structurally related compounds such as cinnamaldehyde and curcumin also exhibit biological activity relevant to plant growth regulation. Cinnamaldehyde, also known as 3-phenyl-2-propenal, is a plant-derived secondary metabolite.[2] Several commercial products containing cinnamon oil and cinnamaldehyde are available for purchase.[2] While cinnamaldehyde is renowned for its antimicrobial properties, its potential as a bioherbicide has received less attention[3] Curcumin is a polyphenol in *Curcuma longa*, a plant native to Asia, especially India. Curcumin is the compound responsible for the orange color of turmeric. It is recognized for its flavor and digestive properties.[18] Curcumin is slightly soluble and stable in acidic conditions but becomes unstable, soluble, and undergoes hydrolysis under neutral or alkaline conditions.[19] Its main biological activities include antimicrobial, insecticidal, antifungal, larvicidal, and antiparasitic.[20] This study aimed to assess the herbicidal activity of methoxychalcones, cinnamaldehyde, and curcumin on the initial growth of some weed species through laboratory experiments and greenhouse trials.

2. MATERIALS AND METHODS

2.1. Curcumin, Cinnamaldehyde, and Methoxychalcones. Curcumin and cinnamaldehyde were purchased from Merck (Darmstadt, Germany). 4'-Hydroxy-3'-methoxy-chalcone (MC-01) was prepared according to Morão et al.[21] Characterization and purity of MC-01 using Nuclear Magnetic Resonance (NMR) And High-Performance Liquid Chromatography with Photodiode Array Detection (HPLC-PAD) were presented in Supporting Information. 2'-Methoxychalcone (MC-02), 3'-methoxychalcone (MC-03), 4'-methoxychalcone (MC-04), 2',4'-dimethoxychalcone (MC-05), 2',5'-dimethoxychalcone (MC-06), 3',4'-dimethoxychalcone (MC-07), 2',4',5'-trimethoxychalcone (MC-08), 3',4',5'-trimethoxychalcone (MC-09), 4-methoxychalcone (MC-10), 4-hydroxy-3-methoxychalcone (MC-11), 2-methoxychalcone (MC-12), 2,3-dimethoxychalcone (MC-13), 2,4-dimethoxychalcone (MC-14), 2,5-dimethoxychalcone (MC-15), 3,4-dimethoxychalcone (MC-16), 2,4,5-trimethoxychalcone (MC-17) and 3,4,5-trimethoxychalcone (MC-18) were prepared, characterized and their purity were described in our previous work[22] (Figure 1).

2.2. Study Species. The herbicidal activity of compounds was evaluated on *Lactuca sativa* (L.) (lettuce), two monocotyledonous weeds: *Digitaria insularis* (L.) Fedde (sourgrass) and *Urochloa decumbens* (Stapf) R.D. Webster (signalgrass), and four eudicotyledonous weeds: *Amaranthus viridis* L. (slender amaranth), *Bidens pilosa* L. (hairy beggartick), *Conyza canadensis* (L.) Cronquist (horseweed) and *Raphanus raphanistrum* L. (wild radish). These weed species were selected as they are among the most problematic in soybean, corn, cotton, vegetables, beans, wheat, citrus, vineyards, barley, oats, canola, sugar cane, pastures, general winter crops, and perennial crops. The lettuce seeds used in the experiments were from Isla (Porto Alegre, Brazil). The weed seeds were commercially obtained from Agro Cosmos (Engenheiro Coelho, Brazil).

2.3. Test Solutions. Methoxychalcones, curcumin, and cinnamaldehyde were dissolved in absolute ethanol (since they are not completely soluble in water) and thoroughly mixed to achieve a 1×10^{-3} mol L^{-1} concentration. This concentration was selected as the phytotoxicity of natural herbicides typically ranges between 10^{-2} and 10^{-3} mol L^{-1}.[23] Glyphosate, the active ingredient in Roundup herbicide for postemergence control, was also dissolved in absolute ethanol at 1×10^{-3} mol L^{-1}. Glyphosate (≥98.0%) reagent was purchased from Sigma-Aldrich (San Luis, Missouri, USA).

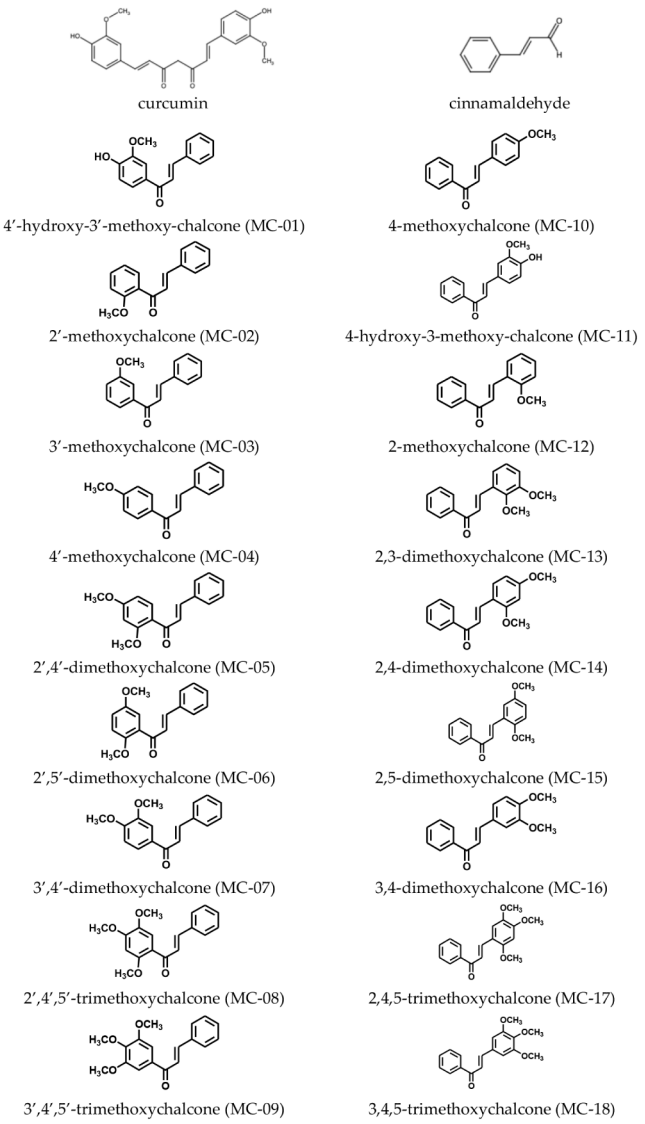

Figure 1. Chemical structures of curcumin, cinnamaldehyde, and methoxychalcones used in this study.

2.4. Initial Growth in Laboratory Protocol. *L. sativa* was used for initial growth screening due to its rapid germination and sensitivity to phytotoxic compounds.[24] The most effective chalcones, curcumin, and cinnamaldehyde were tested on weeds. The lettuce screening experiment began in April 2016, while the weed experiments started in January 2017. A sheet of Whatman number 1 filter paper was placed in 9 cm Petri dishes and moistened with 5 mL of the test solutions. Glyphosate was used as the positive control, while an equal volume of ethanol acted as the negative control. After the ethanol evaporation, 5 mL of distilled water was added to the Petri dishes of all treatments. Twenty-five seeds of each species (*L. sativa* and six weeds), previously germinated with a 1 mm radicle protrusion, were placed on the filter paper in four Petri dishes (n = 100). The dishes were then incubated at 25 ± 1 °C under a 12-h photoperiod with a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1}. At the end of the incubation period, 60 seedlings were removed from the Petri dishes (15 random seedlings per dish) and dried with filter paper before measuring root and shoot lengths. Initial growth was assessed when untreated seedlings reached approximately 5 cm, adapted from Garrido et al.[25] The effect of the study compounds on growth was calculated as the mean percentage difference compared to the negative control[25] as follows:

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$$\text{Percentage difference (\%)} = \frac{(\text{study compound} - \text{negative control})}{(\text{negative control})} \times 100 \quad (1)$$

Positive values represent stimulation, and negative values represent inhibition concerning the negative control.

2.5. Initial Growth in Greenhouse Protocol. The greenhouse experiments began in January 2021. Herbicide efficacy against weeds was investigated using a protocol adapted from Garrido et al.[25] The species that exhibited the highest inhibition in laboratory experiments were selected for the greenhouse experiments. Seeds of weeds *B. pilosa*, *R. raphanistrum*, and *U. decumbens* were placed on Whatman number 1 filter paper in 9 cm Petri dishes, moistened with 10 mL of distilled water, and subjected to a 12-h photoperiod with a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1} at 25 ± 1 °C. Once reaching a shoot height of 2 cm, the seedlings were transplanted into plastic bags (10 cm × 20 cm) filled with soil (one per bag). Each bag received a daily irrigation of 25 mL of water and was placed in a greenhouse exposed to regular day and night lighting conditions. Plants were chosen for uniform size once they had grown four leaves, and each plant was then sprayed with 5 mL of distilled water (used as a negative control), glyphosate (used as a positive control), or the experimental solution (with 10 plants per treatment), ensuring that the leaf surface was adequately moistened. Another spray was conducted after 10 d. Assessment of plant mortality occurred 10 d after the second spray, while injury evaluation was performed 10 d after both the first and second sprayings. Mortality was calculated as the percentage of dead plants relative to the total number of plants in the treatments. Injury scores were assigned based on the degree of injury, where a score of 0% indicated no difference from the control and 100% indicated complete plant death. Injury was determined as the average of the injury scores assigned to the plants in each treatment. The dry mass assessment was conducted 10 d after the second spraying. The plants were carefully uprooted from the bags, and their roots were washed to remove any soil residue. The plant parts, including roots and shoots, were separated and dried at 105 °C for 72 h before weighing. The impact of methoxychalcones on weed foliage was monitored by counting green, wilted, yellow, dry, and total leaves: before the first and second sprays, and at the conclusion of the experiment.

2.6. Statistical Analysis. The experiment was arranged in a fully randomized design (DIC). All data were primarily tested for normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variances (Levene test, $p > 0.05$). When the data set met these criteria data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's test. Data that did not meet these criteria were subjected to the Kruskal–Wallis test, followed by Dunn's test ($p < 0.05$). To correct type I error rate in multiple comparisons and control the false discovery rate (FDR), the Benjamini-Hochberg procedure was applied to adjust p values. Data analysis was conducted using BioEstat (version 5.0) and R software (version 4.2.2).

3. RESULTS

3.1. Effects on Initial Growth in the Laboratory. Methoxychalcones inhibited the initial growth of the seedlings. Compared to the negative control, all methoxychalcones, curcumin, and cinnamaldehyde demonstrated inhibition in at least one parameter during the initial growth of lettuce in the screening process. Also, methoxychalcones and cinnamaldehyde had greater inhibition than glyphosate in at least one parameter for the initial growth of lettuce (Table 1). It is important to note that glyphosate was not applied at the commercially recommended dose and was used without adjuvants, to ensure a fair comparison of herbicidal activity.

MC-01 induced the highest inhibition in root (−66%) and shoot length (−53%). Other methoxychalcones, such as MC-13 with a −49% reduction and MC-16 with a −45% reduction, caused decreases in root length. Noteworthy shoot reductions were observed for MC-17 and MC-07 at −51 and −48%, respectively. In most instances, shoot growth was more sensitive to methoxychalcones than roots (Table 1).

Table 1. Effects of Methoxychalcones, Curcumin, and Cinnamaldehyde at 1 × 10^{-3} mol L^{-1} on Initial Growth of Lettuce[a]

Treatment	Root length growth rate (%)[b]	Shoot length growth rate (%)[b]
glyphosate	−36.0 ± 15.3*	−11.1 ± 15.5*
cinnamaldehyde	−40.9 ± 18.3*	**−37.3 ± 24.3***
curcumin	−33.1 ± 26.4*	**7.4 ± 34.2**
MC-01	**−65.8 ± 32.7***	**−52.7 ± 24.3***
MC-02	**55.1 ± 69.7***	**−34.4 ± 17.9***
MC-03	**−6.6 ± 23.2**	**−28.2 ± 10.8***
MC-04	**−1.3 ± 24.2**	**−32.8 ± 17.3***
MC-05	**1.3 ± 28.2**	**−34.0 ± 17.9***
MC-06	**−23.1 ± 25.5***	**−42.3 ± 14.5***
MC-07	**−24.0 ± 27.0***	**−48.3 ± 12.8***
MC-08	**−28.3 ± 20.6***	**−35.0 ± 15.2***
MC-09	**−13.8 ± 20.7***	**−28.9 ± 13.9***
MC-10	**15.2 ± 27.5***	**−39.0 ± 15.1***
MC-11	**−17.5 ± 22.0***	**−33.2 ± 11.6***
MC-12	**8.2 ± 28.4**	**−19.2 ± 14.7*–**
MC-13	**−49.5 ± 28.3***	**−26.0 ± 18.3***
MC-14	**9.4 ± 24.1**	**−30.7 ± 13.2***
MC-15	**12.7 ± 26.0***	**−17.4 ± 16.0***
MC-16	**−44.8 ± 9.4***	**−39.8 ± 10.6***
MC-17	**−4.0 ± 32.1**	**−50.7 ± 14.4***
MC-18	**−12.2 ± 22.8***	**−31.7 ± 17.5***

[a]Root and shoot length was assessed when the untreated seedlings reached approximately 5 cm. [b]Significant differences from the negative control (distilled water) are indicated by *, and significant differences from the positive control (glyphosate) are indicated by bold according to the one-way ANOVA test followed by Tukey's test for parametric data and Kruskal–Wallis test followed by Dunn's test for nonparametric data ($p < 0.05$); standard deviation (±); ($n = 60$ seedlings); positive values indicate stimulation, and negative values indicate inhibition concerning the negative control.

The selected methoxychalcones (MC-01 and MC-16) and cinnamaldehyde inhibited the growth of all weed species relative to the negative control. These compounds often demonstrated equal or greater inhibitory effects than glyphosate across the study parameters (Table 2).

MC-16 caused a −78% reduction in root length, and −73% reduction in shoot length for the species *R. raphanistrum*. Cinnamaldehyde demonstrated the highest inhibition (−72%) of shoot length in *R. raphanistrum*, while in *U. decumbens* it led to the greatest reductions in roots (−90%). For *D. insularis* and *R. raphanistrum*, both MC-16 and cinnamaldehyde were more potent than glyphosate. Additionally, MC-01 caused the most substantial inhibition (−90%) of shoot length in *B. pilosa*, and in *U. decumbens* it resulted in the greatest reductions in roots (−89%). Furthermore, MC-01 demonstrated greater inhibitions than glyphosate on *A. viridis*, *D. insularis*, and *R. raphanistrum*. In most cases, the roots experienced a slightly greater reduction in length than the shoots when treated with methoxychalcones.

3.2. Effects on Initial Growth in a Greenhouse. Initial growth of the plants in the greenhouse experiments was impacted by methoxychalcones. Specifically, mortality was observed among *R. raphanistrum* plants, with 30% experiencing

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Table 2. Effects of Selected Methoxychalcones and Cinnamaldehyde at 1×10^{-3} mol L^{-1} on Initial Growth of Weeds[a]

Species	Treatment	Root length inhibition rate (%)[b]	Shoot length inhibition rate (%)[b]
A. viridis	glyphosate	−62.0 ± 6.67* a	−72.8 ± 11.6* a
	cinnamaldehyde	−69.4 ± 11.72* b	−66.7 ± 14.7* a
	MC-01	−78.3 ± 10.29* c	−79.5 ± 8.7* b
	MC-16	−56.6 ± 12.11* a	−38.9 ± 29.3* c
B. pilosa	glyphosate	−88.7 ± 6.98* a	−83.4 ± 16.0* a
	cinnamaldehyde	−67.2 ± 16.45* b	−42.0 ± 17.7* b
	MC-01	−80.5 ± 27.37* a	−90.0 ± 18.9* a
	MC-16	−64.3 ± 21.02* b	−40.3 ± 25.3* b
C. canadensis	glyphosate	−72.8 ± 11.42* a	−64.5 ± 15.9* a
	cinnamaldehyde	−86.0 ± 8.76* b	−55.8 ± 29.9* bc
	MC-01	−69.9 ± 20.70* a	−47.7 ± 21.8* b
	MC-16	−62.8 ± 20.63* a	−52.2 ± 16.4* cd
D. insularis	glyphosate	−55.7 ± 11.74* a	−32.6 ± 15.0* a
	cinnamaldehyde	−75.2 ± 11.04* b	−53.6 ± 15.0* b
	MC-01	−73.6 ± 8.80* b	−44.9 ± 19.0* c
	MC-16	−65.7 ± 13.78* c	−45.1 ± 17.2* c
R. raphanistrum	glyphosate	−70.8 ± 8.90* a	−51.0 ± 21.9* a
	cinnamaldehyde	−86.7 ± 15.33* b	−72.1 ± 21.2* b
	MC-01	−74.0 ± 26.56* c	−65.2 ± 29.5* b
	MC-16	−78.2 ± 7.77* c	−72.9 ± 9.3* b
U. decumbens	glyphosate	−64.1 ± 10.20* a	−63.6 ± 12.0* a
	cinnamaldehyde	−90.0 ± 11.53* b	−60.9 ± 25.2* a
	MC-01	−89.4 ± 10.67* b	−68.3 ± 17.1* a
	MC-16	−73.0 ± 10.18* c	−13.0 ± 40.9 b

[a]Root and shoot length was assessed when the untreated seedlings reached approximately 5 cm. [b]Significant differences from the negative control (distilled water) are indicated by *, and significant differences between the treatments are indicated in each column by different letter(s) according to the one-way ANOVA test followed by Tukey's test for parametric data and Kruskal–Wallis test followed by Dunn's test for nonparametric data ($p < 0.05$); standard deviation (±); ($n = 60$ seedlings); values indicate inhibition concerning the negative control.

death following treatment with MC-01 and 10% with MC-16 (Figure 2).

MC-01 was phytotoxic, causing 50% injury after the second application on *R. raphanistrum*. Similarly, MC-16 increased the injury rate, reaching 43% after the second application on *U. decumbens*. In many instances, the injury rate following the second spray exceeded that of the initial application (Figure 3).

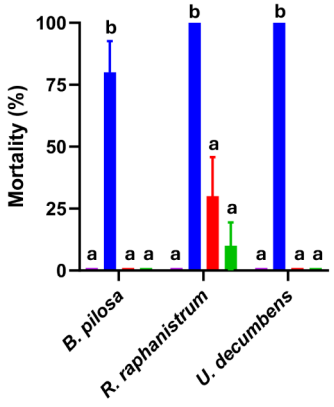

Figure 2. Seedling mortality rate resulting from the application of methoxychalcones at 1×10^{-3} mol L^{-1} in greenhouse experiments, 10 days after the second spray. Purple-filled square = negative control (distilled water), blue-filled square = glyphosate, red-filled square = MC-01, and green-filled square = MC-16. Significant differences are indicated by different letters according to the Kruskal–Wallis test followed by Dunn's test ($p < 0.05$); standard error of means (±); ($n = 10$).

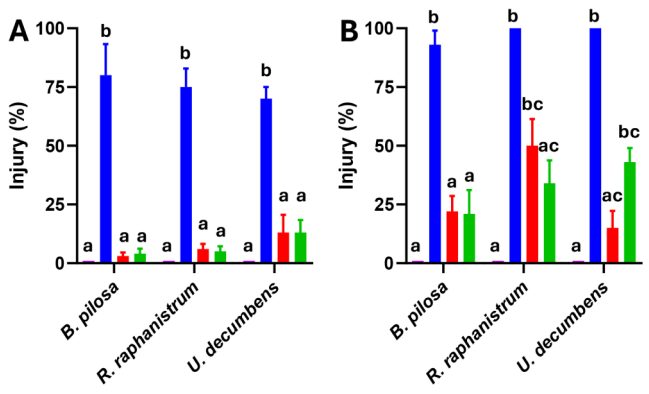

Figure 3. Seedling injury rate resulting from the application of methoxychalcones at 1×10^{-3} mol L^{-1} in greenhouse experiments. (A) 10 d after the first spray and (B) 10 d after the second spray. Purple-filled square = negative control (distilled water), blue-filled square = glyphosate, red-filled square = MC-01, and green-filled square = MC-16. Significant differences are indicated by different letters according to the Kruskal–Wallis test followed by Dunn's test ($p < 0.05$); standard error of means (±); ($n = 10$).

MC-16 caused a 56% decrease in the dry mass of *R. raphanistrum*'s shoot but only 18% in *U. decumbens* (Table 3). Chalcones decreased the number of green leaves throughout the experiment (Table 4). Specifically, MC-16 caused a 75% decrease in the number of green leaves for the weed species *R. raphanistrum* and *U. decumbens* by the end of the experiment. In the case of MC-01, there was a 50% reduction in the number of green leaves for the species *B. pilosa* and *U. decumbens* by the end of the experiment. Additionally, MC-01

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Table 3. Effects of Methoxychalcones (at 1×10^{-3} mol L^{-1}) on Weed Dry Mass, 10 Days after the Second Spray

Species	Treatment	Root (mg)[a]	Shoot (mg)[a]
B. pilosa	negative control (n = 10)	2.3 ± 0.9 (a)	7.1 ± 1.9 (a)
	glyphosate (n = 2)	1.4 ± 0.7*	6.6 ± 0.6*
	MC-01 (n = 10)	2.1 ± 0.6 (a)	8.0 ± 1.4 (b)
	MC-16 (n = 10)	2.4 ± 0.5 (a)	9.3 ± 1.4 (a)
R. raphanistrum	negative control (n = 10)	7.1 ± 6.1 (a)	58.8 ± 41.2 (a)
	glyphosate (n = 0)	-	-
	MC-01 (n = 7)	4.4 ± 1.3 (a)	33.6 ± 20.3 (a,b)
	MC-16 (n = 9)	4.5 ± 2.4 (a)	25.6 ± 15.1 (b,c)
U. decumbens	negative control (n = 10)	15.1 ± 4.4 (a)	30.8 ± 9.9 (a)
	glyphosate (n = 0)	-	-
	MC-01 (n = 10)	20.6 ± 11.9 (a)	57.3 ± 29.7 (a,b)
	MC-16 (n = 10)	12.3 ± 3.8 (a)	25.2 ± 7.9 (b,c)

[a]The negative control was distilled water, the positive control was glyphosate, MC-01 was 4'-hydroxy-3'-methoxychalcone and MC-16 was 3,4-dimethoxychalcone. Significant differences from the treatments are indicated in each column by different letter(s) according to the one-way ANOVA test followed by Tukey's test for parametric data and Kruskal–Wallis test followed by Dunn's test for non-parametric data ($p < 0.05$); standard deviation (±). *Data obtained from two replicates.

increased the number of dry leaves in *U. decumbens*, reaching a total of 2 leaves by the conclusion of the experiment. MC-16 increased the number of dry leaves in *R. raphanistrum* and *U. decumbens*, also reaching 2 leaves at the end of the experiment. Furthermore, both MC-01 and MC-16 reduced the total number of leaves for *B. pilosa* and *R. raphanistrum*, with a 50% decrease observed in the case of the former.

4. DISCUSSION

4.1. Effects on Initial Growth in the Laboratory. In the preliminary assays, lettuce was selected due to its rapid germination and growth, as well as its heightened sensitivity to compounds with phytotoxic properties. Additionally, weeds typically have low germination rates and often necessitate stimulation, such as scarification, light exposure, or heat treatments.[24]

All methoxychalcones, curcumin, and cinnamaldehyde inhibited at least one parameter in the initial growth of lettuce. Methoxychalcones caused greater inhibition than glyphosate in at least one parameter for the initial growth of lettuce. MC-01 was the most potent inhibitory molecule in root and shoot lettuce length. The lettuce growth inhibition observed in this study aligns with the findings reported by Chen et al.[26] for the same species subjected to *trans*-chalcone (1,3-diphenyl-2-propen-1-one). In general, shoots had slightly greater reductions in length than the roots when exposed to methoxychalcones in the present study.

Pádua et al.[16] assessed the herbicidal characteristics and inhibitory effects on photosystem II of 24 chalcones, including MC-04 and MC-10. Both chalcones MC-04 and MC-10 did not impact photosynthetic electron transport and, consequently, were not further assessed for herbicidal activity.[16] In the present investigation, MC-04 and MC-10 demonstrated weak inhibitory effects in lettuce total length, with reductions of only 17 and 11%, respectively.

The selected methoxychalcones and cinnamaldehyde effectively impeded the growth of all weed species. Saad et al.[4] investigated the herbicidal activity of cinnamaldehyde against *Echinochloa crus-galli*, both in laboratory and greenhouse settings. In the laboratory experiments, cinnamaldehyde was tested at a concentration of 1×10^{-3} mol L^{-1}, mirroring the concentration used in our study. Saad et al.[4] observed an 80.4% reduction in *Echinochloa crus-galli* root growth, while our research revealed higher inhibitions for three other weed species, reaching 90% in *U. decumbens*. In terms of shoot length, Saad et al.[4] recorded a 32.1% decrease in *Echinochloa crus-galli*, whereas our study reports greater inhibitions across all species, with a 72% reduction in *R. raphanistrum*, indicating its herbicidal potential. In addition, Moreno-Robles et al.[27] discovered that cinnamaldehyde was phytotoxic, nearly completely inhibiting the early growth of *Cuscuta campestris* at 1×10^{-3} mol L^{-1}. Chotsaeng et al.[6] described that cinnamaldehyde, at 0.4×10^{-3} mol L^{-1}, markedly inhibited the growth of shoots and roots in *Amaranthus tricolor*, resulting in reductions of 76 and 85%, respectively. Also, López-González et al.[2] observed that *Arabidopsis thaliana* root growth was suppressed upon exposure to cinnamaldehyde. The degree of root length reduction became increasingly noticeable with higher concentrations.[2] Thus, the reported phytotoxicity results, as well as the fact that cinnamaldehyde is part of the Food and Drug Administration list as "Generally Recognized as Safe − GRAS", which designates compounds that are safe for use and do not present potentially adverse effects on human health, indicate its use as a potential eco-friendly herbicide.

Gomes et al.[28] performed a related study that assessed the phytotoxicity effects of 13 synthetic chalcones at concentrations of 0.5×10^{-3} mol L^{-1}, 1×10^{-3} mol L^{-1}, and 2×10^{-3} mol L^{-1} during the initial development of weeds. Among the tested compounds, 2-furanyl-chalcone and *trans*-chalcone were identified as the most effective, revealing growth inhibition comparable to glyphosate.[28] In the present investigation, methoxychalcones and cinnamaldehyde consistently demonstrated inhibitory effects equal to or greater than those of glyphosate across various parameters in weeds. Particularly, MC-16 inhibited root and shoot length for *R. raphanistrum*, while the cinnamaldehyde led to the greatest reduction in roots for *U. decumbens*. In *D. insularis* and *R. raphanistrum*, both MC-16 and cinnamaldehyde outperformed glyphosate regarding inhibitory effects. MC-01 induced the most potent inhibition on *U. decumbens* root length and *B. pilosa* shoot length. Furthermore, MC-01 was more potent than glyphosate for *A. viridis*, *D. insularis*, and *R. raphanistrum*.

At a 1×10^{-3} mol L^{-1} concentration, Gomes et al.[28] reported that 2-furanyl-chalcone and trans-chalcone reduced shoot growth in *U. decumbens* by 15% and 35%, respectively. In comparison, MC-01 in the present study caused a 60% reduction in shoot growth for the same species at the same concentration. Regarding root growth inhibition, Gomes et al. [28] observed reductions of 45% and 55% for 2-furanyl-chalcone and trans-chalcone, respectively, whereas MC-01 demonstrated a 90% reduction under identical conditions. These findings suggest that the incorporation of hydroxy and methoxy functional groups may greatly enhance the herbicidal activity of chalcones.

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Table 4. Impact of Methoxychalcones (at 1×10^{-3} mol L^{-1}) on the Foliage of Weeds at Three Distinct Times[a]

Treatment	Green	Wilted	Yellow	Drought	Total
Before the First Spray—Day Zero					
B. pilosa					
negative control	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
glyphosate	4 ± 0 (a)	0 ± 0 (a)	0 ± 0 (a)	0 ± 0 (a)	4 ± 0 (a)
MC-01	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-16	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
R. raphanistrum					
negative control	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
glyphosate	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-01	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-16	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
U. decumbens					
negative control	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
glyphosate	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-01	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-16	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
Before the Second Spray—Day Ten					
B. pilosa					
negative control	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
glyphosate	4 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	4 ± 0*
MC-01	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
MC-16	4 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (a,A)
R. raphanistrum					
negative control	5 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	5 ± 1 (a,A)
glyphosate	0 ± 1 (b,B)	0 ± 0 (a,A)	1 ± 1 (a,B)	0 ± 0 (a,A)	1 ± 1 (b,B)
MC-01	4 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 1 (a,A)	0 ± 0 (a,A)	4 ± 1 (a,A,B)
MC-16	4 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 1 (a,A)	0 ± 0 (a,A)	4 ± 1 (a,A)
U. decumbens					
negative control	3 ± 0 (a,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	1 ± 1 (a,B)	4 ± 1 (a,A)
glyphosate	0 ± 0 (b,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	4 ± 0 (b,B)	4 ± 0 (a,A)
MC-01	2 ± 1 (a,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	1 ± 1 (a,B)	4 ± 1 (a,A)
MC-16	3 ± 1 (a,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	2 ± 1 (a,B)	5 ± 1 (b,A,B)
End—Day Twenty					
B. pilosa					
negative control	3 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	1 ± 1 (a,A)	4 ± 0 (a,A)
glyphosate	1 ± 1*	0 ± 0*	0 ± 0*	2 ± 0*	3 ± 1*
MC-01	2 ± 1 (a,c,B)	0 ± 0 (a)	0 ± 0 (a)	0 ± 0 (a)	2 ± 1 (b,B)
MC-16	2 ± 1 (b,c,B)	0 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 1 (a,A)	2 ± 1 (b,B)
R. raphanistrum					
negative control	4 ± 1 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	5 ± 1 (a,A)
glyphosate	-	-	-	-	-
MC-01	3 ± 1 (b,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	0 ± 0 (a,A)	3 ± 1 (b,A,C)
MC-16	1 ± 0 (c,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	2 ± 1 (b,B)	3 ± 1 (b,B)
U. decumbens					
negative control	2 ± 0 (a,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	1 ± 1 (a,B)	3 ± 1 (a,A)
glyphosate	-	-	-	-	-
MC-01	2 ± 1 (a,B)	0 ± 0 (a,A)	0 ± 0 (a,A)	2 ± 1 (a,B)	4 ± 1 (a,b,A)
MC-16	1 ± 0 (b,C)	0 ± 0 (a,A)	0 ± 0 (a,A)	2 ± 1 (a,B)	3 ± 1 (a,c,A,C)

[a]The negative control was distilled water, and glyphosate the positive control. Significant differences between treatments are indicated by small letters, while differences between times are indicated by capital letters according to the one-way ANOVA test followed by Tukey's test for parametric data and the Kruskal-Wallis test followed by Dunn's test for non-parametric data ($p < 0.05$); standard error (±); ($n = 10$). *Data obtained from two replicas.

Smailagić et al.[29] reported a 75% inhibition of *Arabidopsis thaliana* root growth when treated with phloretin at 1×10^{-3} mol L^{-1}, besides inhibiting the development of lateral roots. In our current study, methoxychalcones caused root length reductions in weeds ranging from 57 to 90% at the same concentration. Research by Pádua et al.[10] demonstrated that treating weeds with fluorinated chalcones at 0.1×10^{-3} mol L^{-1} led to a reduction in initial growth. Specifically, for *A. viridis*, the most inhibitory fluorinated chalcone decreased shoot length by 40%.[10] In our study, MC-01 at 1×10^{-3} mol L^{-1} resulted in a 78% reduction in root length and an 80% reduction in the shoot length of *A. viridis*.

It is well-known that the introduction of fluorine into molecules can modify their chemical properties, often

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enhancing biological activity due to increased lipophilicity, membrane permeability, and stability. However, this relationship between fluorination and toxicity is not always linear, as other structural and mechanistic factors influence the bioactivity of these compounds. In this study, methoxylated chalcones demonstrated greater inhibitory effects on *A. viridis* growth than fluorinated chalcones,[10] however, in a concentration 10 times higher.

Furthermore, while fluorination can enhance bioactivity, it may also lead to increased environmental persistence and bioaccumulation risks. In this context, methoxychalcones may offer a favorable balance between strong herbicidal activity and potentially lower environmental impact, making them promising candidates for further herbicide development.

In many cases, the roots suffered a slightly greater reduction in length than the shoots when subjected to methoxychalcones and cinnamaldehyde. Díaz-Tielas et al.[30] noted that *trans*-chalcone at a concentration of 0.4×10^{-3} mol L^{-1} adversely affected the initial growth of weed roots, with a 67% reduction observed for *Plantago lanceolata*. At 2.1×10^{-5} mol L^{-1}, Díaz-Tielas et al.[31] demonstrated that the same *trans*-chalcone inhibited *Arabidopsis thaliana* root growth by 50%. Overall, the studies by Pádua et al.[10] also noted a greater reduction in the roots compared to the shoots of weed plants treated with fluorinated chalcones. In this current investigation, the greatest inhibition (89%) of root length was induced by chalcone (MC-01) at 1×10^{-3} mol L^{-1} in *U. decumbens*. Conversely, in Chen et al.[26] study, which assessed the phytotoxic activity of a chalcone at 0.1×10^{-3} mol L^{-1} across 20 species, the inhibitory effect was more pronounced on the shoots than on the roots for 12 of the 20 species analyzed. Gomes et al.[28] identified disparities, where the most sensitive part of the eudicotyledonous plant *Sesamum indicum* exhibited higher inhibition in the shoots, while the monocotyledonous *U. decumbens* displayed greater inhibition in the roots, aligning with the findings of the current study. Our results on cinnamaldehyde also align with those of Saad et al. [4] who noted a more substantial decrease in root length compared to shoot length.

Interestingly, MC-01, MC-16, and cinnamaldehyde were less active on lettuce than on weeds. These data suggest that screening with cultivated species may eliminate compounds that could have satisfactory results in weed control. Various factors, including species variation, herbicide characteristics (including their selectivity, absorption rate, transport, degradation, and resistance), and metabolic pathway differences may contribute to these differences. Díaz-Tielas et al.,[30] in their study of *trans*-chalcone, also reported low inhibition for lettuce but strong inhibitory effects on weeds such as *Amaranthus retroflexus*, *Echinochloa crus-galli*, and *Avena fatua*. Therefore, studies exploring herbicidal activity should consider this aspect before dismissing the phytotoxic potential of a substance.

Considering the structure–activity relationship, it can be deduced that the most active methoxychalcones (MC-01, MC-13, and MC-16) in roots of lettuce are those with methoxyl substituents at position 3 of the A or B rings, besides presenting greater reduction than glyphosate. Upon further examination of ring B, it was noted that all methoxychalcones substituted at position 3 exhibited total inhibition equal to or surpassing that of glyphosate in lettuce. The highest inhibition of lettuce shoot growth is directly associated with substituents at position 4 of the A or B rings of methoxychalcones (MC-01, MC-07, and MC-17). The observation of both chemical structures leads to the conclusion that the presence of substituents at positions 3 and 4 is fundamental for herbicidal activity.

The MC-01, featuring a hydroxyl substituent at position 4 of ring A, demonstrated exceptional activity. A previous study from our group evaluated 15 hydroxychalcones under the same conditions described in the present work.[32] Four hydroxychalcones exhibited strong herbicidal activity, highlighting the importance of the hydroxyl group.[32] This suggests that the presence of the hydroxyl group is more crucial than methoxyl for the herbicidal activity of chalcones. Another noteworthy structural features of MC-01 is the unsubstituted B ring. The B ring and unsaturated carbons are also present in the cinnamaldehyde which may contribute to its herbicidal activity. This suggests that this part of the structure is crucial for the herbicidal effectiveness of 4'-hydroxy-3'-methoxychalcone (MC-01). Research by Moreno-Robles et al.[27] demonstrated that the inhibitory effect of cinnamaldehyde on the growth of *Cuscuta campestris* is due to the presence of the aldehyde group, in comparison with hydrocinnamic acid and its analogs.

Other research has also reported that the biological activity of chalcone structures is influenced by the presence, number, and position of substituent groups. These factors may play a pivotal role in determining the potency and selectivity of chalcones in both *in vitro* and *in plant* testing, as noted in some studies.[32−35] Comprehending the structure–activity relationship offers insights into the specific structural elements that contribute to their efficacy, facilitating the development of herbicides that are more efficient and selective. Chalcones used in this study lacked selectivity in the laboratory, impeding the growth of both monocotyledonous and eudicotyledonous species, making them a potential herbicide with a broad spectrum of action.

4.2. Effects on Initial Growth in a Greenhouse. Seedling growth bioassays serve as crucial initial assessments for determining compounds' phytotoxic and herbicidal potential derived from or inspired by natural products. Nevertheless, greenhouse trials are indispensable for comprehending the herbicidal effectiveness of these compounds under conditions more similar to fields, providing insights for potential commercial applications.[4] Greenhouse experiments used the two most potent chalcones on the three most sensitive weed species identified in our previous experiments. In the initial stages of greenhouse growth, the impact of methoxychalcones on plants was relatively moderate. Mortality rates reached 30% for *R. raphanistrum* plants treated with MC-01 and 10% with MC-16. Several factors could account for this, including that mature plants are usually less sensitive to herbicides, and foliar absorption of the compounds is less effective than root absorption.

Injury to *R. raphanistrum* increased to 50% after the second application of MC-01, while *U. decumbens* had a lower response, with 43% injury after the second spray of MC-16. Moreover, in most cases, the level of injury following the second spray surpassed that observed after the initial application. Various factors may contribute to this observation, including the cumulative impact of herbicides, delayed plant response, plant sensitivity, metabolism and breakdown processes, interaction with environmental factors, and the persistence of herbicides.[36]

MC-16 induced a notable decrease of 56% in the dry mass of the aboveground portion of *R. raphanistrum*. Conversely, in *U. decumbens*, it resulted in a minor reduction of 18%. Various

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factors, including plant species sensitivity, the mode of action of herbicide, and metabolic differences, among others, could contribute to the varying degrees of dry mass reduction observed in response to herbicide application.

Chalcones reduced the number of green leaves throughout the experiment, particularly with a noteworthy 75% decrease observed in plants treated with MC-16. Concurrently, chalcones induced an increase in the number of dry leaves. Additionally, both MC-01 and MC-16 led to an overall decrease in the total leaf number for *B. pilosa* and *R. raphanistrum*, reaching a 50% reduction in the case of the former species. This reduction in the number of green leaves is intricately connected to weed survival. Green leaves are vital for photosynthesis, energy generation, and plant growth. The herbicidal impact causing a decrease in green leaves disrupts the ability of plants to produce energy, weakening the weed and rendering it more susceptible to stress, diminishing its competitiveness in the environment. This compromised ability to photosynthesize results in an overall decline in the weed's health and survival, leading to reduced energy reserves, hindered growth, and heightened vulnerability to environmental pressures. Weeds with a diminished number of green leaves face challenges in resource competition and may struggle to outcompete neighboring plants.[37] Hence, the correlation between the reduction in green leaves and weed survival underscores the herbicidal impact on the physiological processes of plants, with potential long-term consequences for their persistence and thriving in their habitat.

Examining the molecular structures of the study chalcones, we observed that they lack halogenated elements such as chlorine and fluorine. Herbicides containing these elements typically have increased stability and persistence in the environment.[38,39] The presence of halogens, including chlorine and fluorine, often contributes to the herbicide's resilience against chemical and biological degradation processes, potentially leading to extended half-lives.[38,39] In contrast, chalcones with methoxyl groups remain biodegradable but may degrade more slowly than their hydroxylated analogs. The rate and extent of biodegradation depend on factors such as the number and position of methoxyl groups and environmental conditions, including the presence of suitable microorganisms. For instance, a study demonstrated that hydroxy-substituted chalcones exhibited strong antioxidant activity, which is often associated with higher reactivity and potentially faster biodegradation.[40] In contrast, methoxyl-substituted chalcones had potent antioxidant activity, suggesting that methoxy substitution does not necessarily impede biodegradation.[41] Therefore, while methoxychalcones may have a relatively short half-life, their environmental persistence could be influenced by their specific substitution patterns and surrounding ecological factors. This characteristic offers important advantages regarding environmental safety, agriculture, and human health. Benefits include rapid degradation, which minimizes environmental contamination and its impact on nontarget organisms, greater flexibility for subsequent planting, a lower risk of developing resistant weeds, reduced food residues, and decreased human exposure to these substances. However, it may be necessary to adjust the application frequency to ensure effectiveness, depending on climatic conditions and the specific crop.

In conclusion, 4'-Hydroxy-3'-methoxychalcone, 3,4-dimethoxychalcone and cinnamaldehyde have potential as herbicides on both monocotyledonous and eudicotyledonous weeds. The present study has highlighted their herbicidal activity, which appears to be influenced by their structural properties, including the presence of methoxy groups. These findings suggest that methoxychalcones may offer an alternative to traditional herbicides, with a potentially lower environmental impact. Further studies to evaluate the combined effects of these methoxychalcones could provide valuable insights into optimizing their herbicidal efficacy, potentially enhancing their activity through synergistic interactions. Additionally, investigating their biodegradation and environmental persistence is crucial for understanding their long-term impact on ecosystems. Future research should also explore the mechanisms of action of these compounds at the molecular level to identify specific targets in plant systems. Overall, the promising results presented in this study warrant further exploration of methoxychalcones as viable candidates for future herbicide development.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.5c00056.

^{1}H NMR spectrum, ^{13}C NMR spectrum, UV−vis spectrum, and HPLC-PAD chromatogram of compound MC-01 (PDF)

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Notes

The authors declare no competing financial interest.

There are patents resulting from the work reported in this manuscript. A provisional application was filed in the United States under number 63/688403 and entitled "Chalcone-Related Herbicides." A provisional application was also filed in Brazil under number BR 10 2024 026838 5 and entitled "Herbicida de Chalconas."

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■ ABBREVIATIONS

MC-01, 4'-hydroxy-3'-methoxy-chalcone; MC-02, 2'-methoxychalcone; MC-03, 3'-methoxychalcone; MC-04, 4'-methoxychalcone; MC-05, 2',4'-dimethoxychalcone; MC-06, 2',5'-dimethoxychalcone; MC-07, 3',4'-dimethoxychalcone; MC-08, 2',4',5'-trimethoxychalcone; MC-09, 3',4',5'-trimethoxychalcone; MC-10, 4-methoxychalcone; MC-11, 4-hydroxy-3-methoxy-chalcone; MC-12, 2-methoxychalcone; MC-13, 2,3-dimethoxychalcone; MC-14, 2,4-dimethoxychalcone; MC-15, 2,5-dimethoxychalcone; MC-16, 3,4-dimethoxychalcone; MC-17, 2,4,5-trimethoxychalcone; MC-18, 3,4,5-trimethoxychalcone

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