Article

Identification of Allelochemicals with Differential Modes of Phytotoxicity against *Cuscuta campestris*

Antonio Moreno-Robles [1], Antonio Cala Peralta [2,3], Gabriele Soriano [2], Jesús G. Zorrilla [2,3], Marco Masi [2], Susana Vilariño-Rodríguez [4], Alessio Cimmino [2] and Mónica Fernández-Aparicio [5,*]

1 Campus de Rabanales, University of Córdoba, 14071 Córdoba, Spain
2 Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario Monte S. Angelo, Via Cintia, 80126 Naples, Italy
3 Allelopathy Group, Department of Organic Chemistry, School of Science, Institute of Biomolecules (INBIO), University of Cádiz, C/República Saharaui 7, 11510 Cádiz, Spain
4 ALGOSUR S.A., Ctra Lebrija-Trebujena km 5.5, 41740 Sevilla, Spain
5 Department of Plant Breeding, Institute for Sustainable Agriculture (IAS), CSIC, Avenida Menéndez Pidal s/n, 14004 Córdoba, Spain
* Correspondence: monica.fernandez@ias.csic.es

Abstract: *Cuscuta campestris* is a parasitic weed species with noxious effects in broadleaf crops worldwide. The control of *Cuscuta* in the majority of crops affected is limited or non-existing. We tested, for the first time, the effect of eighteen metabolites in in vitro-grown *Cuscuta* seedlings. We found that 2-benzoxazolinone, hydrocinnamic acid and pisatin caused the strongest inhibition of seedling growth. In addition to seedling growth, pisatin caused necrosis of the *Cuscuta* seedling, occurring mostly at the seedling shoot. Scopoletin and sesamol treatments caused toxicity, observed as a black staining, only at the *Cuscuta* root apices, while caffeic acid, ferulic acid and vanillic acid caused toxicity, observed as brown staining, in the root apices. The structure–activity relationships in four structural derivatives of 2-benzoxazolinone, and five structural derivatives of hydrocinnamic acid, were also studied. The identification of new herbicidal modes of action against *Cuscuta* is the first step in creating new alternatives to sustainable chemical control of parasitic weeds.

Keywords: field dodder; parasitic weeds; bioherbicides; sustainable crop protection; 2-benzoxazolinone; hydrocinnamic acid; pisatin

1. Introduction

Among all biotic stresses that negatively affect crop yields, weeds are responsible for the largest economic impact [1] and, among them, parasitic weeds are one of the most devastating and difficult to control, types of weed, due to their capacity to withdraw nutritive resources and water using connections with the crop vascular system [2,3]. Field dodders (*Cuscuta campestris* Yunck.) are obligate parasitic weeds from Convolvulaceae, that infect the stems of many broadleaf crops worldwide [4,5]. *Cuscuta* seedlings emerge from the seed coat as a thread-shaped hypocotyl without cotyledons, which use nastic movements and chemotropism, to locate the crop and coil around the crop stems [6,7]. After *Cuscuta* has coiled around the crop stem, epidermal cells at the site of attachment differentiate into disk-like meristems forming the haustorium [8,9]. The seedlings of *C. campestris* contain no, or trace amounts of, chlorophyll and, instead of a regular root system, they only form a rudimentary root of a few millimeters [6]. Therefore, they are incapacitated for autotrophic growth and, in absence of host infection, they become senescent and die in 7 to 10 days after germination. Due to its vulnerability, the pre-attached stage of *Cuscuta* development is an obvious target for the design of control strategies [10]. Once *Cuscuta* attaches to the crop stem, there are no selective and effective control methods against *Cuscuta* for the protection of the majority of crops affected. On one hand, the

intimate vascular connections between the parasitic plant and the crop plant make the available herbicides in many crops ineffective, and, on the other hand, there is a notable lack of development of resistant varieties against *Cuscuta* infection [4,5,11].

The identification of novel allelochemicals and new modes of action in known natural compounds that interfere with the necessary contact between the *Cuscuta* seedling and the host, either by stopping the parasitic seedling growth or by repelling the host attraction is an alternative solution to provide sustainable efficacy in chemical control of weeds [7,10]. Many compounds of plant and microbial origin have been identified with allelochemical activity against weeds [12,13]. Besides the direct haustorial extraction of nutrients, *Cuscuta* plants also exert strong inhibition against other plants by means of allelochemicals contained in their tissues [14,15]. However, the identification of allelochemicals with activity against *Cuscuta* itself has been poorly investigated. Previous attempts included two studies investigating phytotoxic activity of plant extracts [10,16], which led to the identification of (4Z)-lachnophyllum lactone [10] and inuloxins and α-costic acid [17] with suppressive effects against *Cuscuta*. In the only previously reported molecule screening for phytotoxicity against *Cuscuta*, dideacetyl-FC, ophiobolin A and fusicoccin were also identified as inhibitors [18]. In the present study, a library of eighteen candidate metabolites, belonging to different classes of natural compounds [19], has been screened, for the first time, to identify allelochemicals active against *Cuscuta* seedlings. In addition, some derivatives of two of the most phytotoxic compounds were also tested to carry out structure–activity relationship studies.

2. Materials and Methods

2.1. Plant Material and Chemicals

Cuscuta seeds were collected in agricultural fields of the IAS-CSIC in July 2019 from mature *Cuscuta campestris* plants parasitizing *Pisum sativum*. Dry *Cuscuta* seeds were separated from capsules by sifting with a 0.6 mm mesh-size sieve, followed by winnowing with a fan. Seeds of *Cuscuta* were stored at room temperature in the dark until use for this work in 2022.

A first screening was performed using a total of 18 compounds (Figure 1). Unless otherwise indicated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA): L-lysine (**1**, cat. no. L5501), gramine (**2**, cat. no. G10806), L-tryptophan (**3**, cat. no. PHR1176), L-phenilalanine (**4**, cat. no. P1150000), 2-benzoxazolinone (**5**, cat. no. 157058), hydrocinnamic acid (**6**, cat. no. 135232), *p*-coumaric acid (**7**, cat. no. C9008), caffeic acid (**8**, cat. no. C0625), ferulic acid (**9** cat. no. 12870-8), scopoletin (**10**, cat. no. S2500), umbelliferone (**11**, cat. no. 54826), vanillic acid (**12**, 8.41025.0050), benzoic acid (**13**, cat. no. 242381), coumalic acid (**14**, cat. no. C85409), sesamol (**15**, cat. no. 11428673) obtained from Thermo Fisher Scientific (Waltham, MA, USA), 1,4-benzoquinone (**16**, cat. no. B10358), naringenin (**17,** cat. no. L09834) obtained from Thermo Fisher Scientific and lastly pisatin (**18**) which was purified in our laboratory, using the method described by Evidente et al. [20].

Two additional bioassays were performed to study the structure–activity relationships in four structural derivatives of 2-benzoxazolinone (**5**) and five structural derivatives of hydrocinnamic acid (**6**). All the derivatives (Figure 2) were purchased from Sigma-Aldrich: 3-(4-fluorophenyl)propionic acid (**19**, cat. no. 560502), 3-(4-chlorophenyl)propionic acid (**20**, cat. no. 656151), 3-(4-bromophenyl)propionic acid (**21**, cat. no. 595438), 3-(4-hydroxyphenyl)propionic acid (**22**, cat. no. H52406), 3-(2-hydroxyphenyl)propionic acid (**23**, cat. no. 393533), 6-hydroxy-2(3*H*)-benzoxazolinone (**24**, cat. no. 705500), 6-benzyloxy-2-benzoxazolinone (**25**, cat. no. 653462), 6-chloroacetyl-2-benzoxazolinone (**26**, cat. no. 535400), 5-bromo-2-benzoxazolinone (**27**, cat. no. 653454).
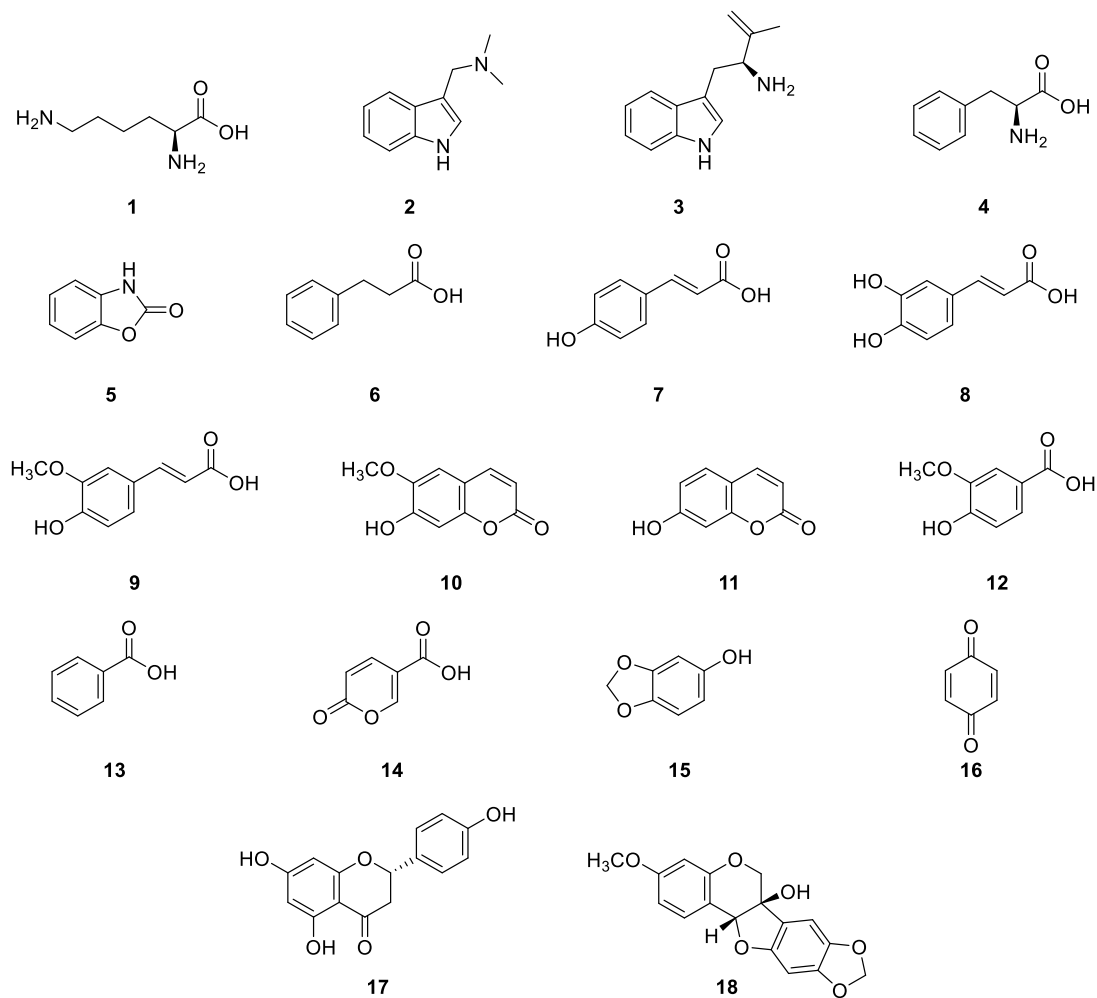
Figure 1. Chemical structures of the compounds studied in the first screening: L-lysine (**1**), gramine (**2**), L-tryptophan (**3**), L-phenilalanine (**4**), 2-benzoxazolinone (**5**), hydrocinnamic acid (**6**), *p*-coumaric acid (**7**), caffeic acid (**8**), ferulic acid (**9**), scopoletin (**10**), umbelliferone (**11**), vanillic acid (**12**), benzoic acid (**13**), coumalic acid (**14**), sesamol (**15**), 1,4-benzoquinone (**16**), naringenin (**17**) and pisatin (**18**).
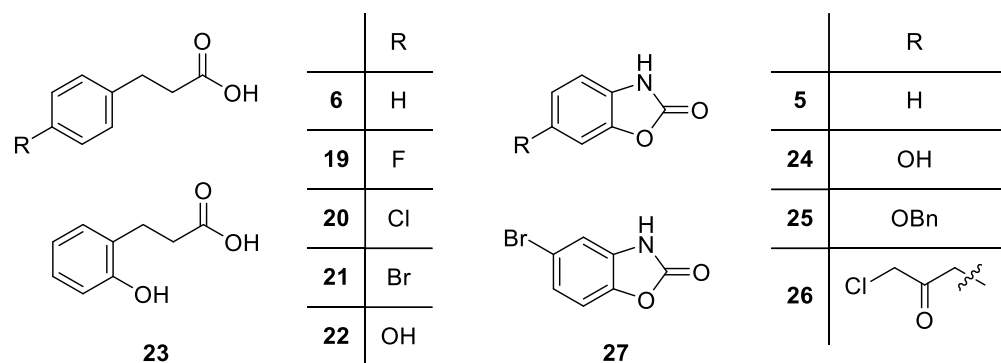

Figure 2. Chemical structures of derivatives of hydrocinnamic acid (**6**): 3-(4-fluorophenyl)propionic acid (**19**), 3-(4-chlorophenyl)propionic acid (**20**), 3-(4-bromophenyl)propionic acid (**21**), 3-(4-hydroxyphenyl)propionic acid (**22**), 3-(2-hydroxyphenyl)propionic acid (**23**), and derivatives of 2-benzoxazolinone (**5**): 6-hydroxy-2(3*H*)-benzoxazolinone (**24**), 6-benzyloxy-2-benzoxazolinone (**25**), 6-chloroacetyl-2-benzoxazolinone (**26**), 5-bromo-2-benzoxazolinone (**27**).

2.2. In Vitro Experiments for Screening of Allelopathy against Cuscuta *Seedling Growth*

A first screening of the eighteen compounds (**1–18**), described in Figure 1, was performed to identify allelopathic activity against *Cuscuta* seedlings. The thick coat of *C. campestris* seeds induces physical dormancy that allows staggered germination over time preserving the viability of its seed bank in agricultural fields [7]. To promote *Cuscuta* germination in the laboratory, *Cuscuta* seeds were scarified with sulfuric acid for 45 min to eliminate the hard coat [21]. Scarification was followed by thorough rinsing and air drying under a flow cabinet. Then, five scarified *Cuscuta* seeds were placed on 5 cm-diameter sterilized filter paper discs inside 5.5 cm-diameter Petri dishes using tweezers. Methanol solutions of each compound were diluted up to 1 mM in sterilized distilled water. This was done for all compounds, except for the amino acid compounds **1**, **3** and **4**, which were dissolved directly in distilled water. The final concentration of methanol in all treatments was 2%, including for the compounds **1**, **3** and **4**. For each treatment, triplicate aliquots of 1 mL of each treatment were applied to filter paper discs containing the seeds of *Cuscuta*. Triplicate aliquots of a treatment containing only sterile distilled water and 2% methanol was used as control. Petri dishes containing the treated *Cuscuta* seeds were sealed with parafilm, wrapped in aluminum foil and placed in the dark in a growth chamber with an average temperature of 23 °C and relative humidity of 65% for five days.

A second in vitro bioassay was performed to study the structure–activity relationship in four structural derivatives of 2-benzoxazolinone (**5**). All compounds, **5**, **24–27**, were dissolved in dimethyl sulfoxide and then diluted up to 1, 0.5, 0.25 and 0.1 mM in sterilized distilled water. Triplicate aliquots of 1 mL of each treatment were applied to filter paper discs containing five *Cuscuta* seeds scarified as described above. Triplicate aliquots of a treatment only containing sterile distilled water and 2% dimethyl sulfoxide were used as control. Petri dishes containing the treated *Cuscuta* seeds were incubated in the same conditions as already described.

A third in vitro bioassay was performed to study the structure–activity relationship in 5 structural derivatives of hydrocinnamic acid (**6**). All compounds, **6**, **19–23**, were dissolved in methanol and then diluted up to 1, 0.5 and 0.25 mM in sterilized distilled water. Triplicate aliquots of 1 mL of each treatment were applied to filter paper discs containing five *Cuscuta* seeds scarified as described above. Triplicate aliquots of a treatment containing only sterile distilled water and 2% methanol was used as control. Petri dishes containing the treated *Cuscuta* seeds were incubated in the same conditions as already described.

2.3. Calculations and Statistical Analysis

For each treatment, the length was measured in each of the five *Cuscuta* seedlings for each of the three replicate filter paper discs. Seedling growth for each treatment was calculated relative to the seedling growth of the corresponding control. In addition, note was taken of whether the root apice of each *Cuscuta* seedling had developed necrosis. The percentage of seedlings that developed a necrotic root was calculated in each triplicated disk for each treatment. *Cuscuta* seedlings were observed using a stereoscopic microscope (Leica S9i, Leica Microsystems GmbH, Wetzlar, Germany).

Calculation of CLog*P* was performed using ChemOffice v20.1 (PerkinElmer, Waltham, MA, USA), using the appropriate tool in ChemDraw Professional [22].

All bioassays were performed using a completely randomized design. Percentage data were approximated to normal frequency distribution by means of angular transformation. Then, percentage data were subjected to analysis of variance (ANOVA). The significance of mean differences among treatments was evaluated by Tukey at $p < 0.05$. Statistical analysis was performed using SPSS software 27 (SPSS Inc., Chicago, IL, USA).

3. Results

Identification of inhibitors of *C. campestris* seedling growth. A first screening was performed by applying 1 mM treatments of eighteen compounds (**1–18**), described in Figure 1, on scarified *Cuscuta* seeds. Five days after treatment, inhibition of *Cuscuta* growth

was significantly affected by the compound treatment (ANOVA, $p < 0.001$). Different levels of activity were obtained, which allowed a well-defined classification to be made between highly, moderately and barely active compounds (Figure 3).
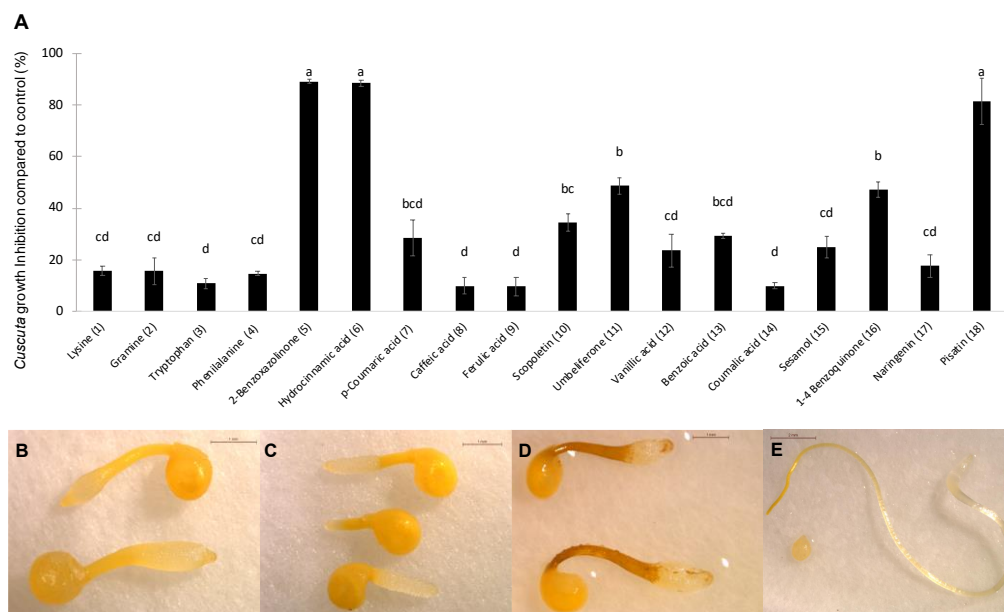

Figure 3. Inhibition of *Cuscuta* growth observed in the first screening (**A**). Illustrative photographs showing the growth inhibition observed in 1 mM treatments of (**B**) 2-benzoxazolinone (**5**), (**C**) hydrocinnamic acid (**6**) and (**D**) pisatin (**18**) in comparison with (**E**) *Cuscuta* seedlings treated with control. In (**A**), bars with different letters are significantly different (Tukey test at $p < 0.05$). Error bars represent the standard error of the mean.

Thus, the compounds 2-benzoxazolinone (**5**), hydrocinnamic acid (**6**), and pisatin (**18**) (Figure 3B–D) showed the highest inhibition activity, compared to control (Figure 3E) (respectively, $89.2 \pm 0.9\%$, $88.5 \pm 1.2\%$ and $81.6 \pm 9\%$ of inhibition). The compounds umbelliferone (**11**) and 1–4 benzoquinone (**16**) showed moderate activity with respective levels of inhibition of $48.7 \pm 3.2\%$ and $47.3 \pm 3\%$. Five compounds showed low but significant levels of growth inhibition, scopoletin (**10**), benzoic acid (**13**) and *p*-coumaric acid (**7**), sesamol (**15**) and vanillic acid (**12**), with growth inhibition ranging from $34.6 \pm 3.4\%$ in scopoletin to $23.5 \pm 6.6\%$ in vanillic acid. Negligible inhibition activity was observed with ferulic acid (**9**), caffeic acid (**8**), coumalic acid (**14**), gramine (**2**), naringenin (**17**) and also with the three amino acids tested, L-lysine (**1**), L-tryptophan (**3**) and L-phenilalanine (**4**).

Study of structure–activity relationship on the growth inhibition induced by 2-benzoxazolinone (**5**). The growth inhibition of 2-benzoxazolinone identified in the first screening (Figure 3A,B) was studied in a second in vitro bioassay in a range of concentrations from 0.1 to 1 mM, and compared to the four derivatives, 6-hydroxy-2(3*H*)-benzoxazolinone (**24**), 6-benzyloxy-2-benzoxazolinone (**25**), 6-chloroacetyl-2-benzoxazolinone (**26**), 5-bromo-2-benzoxazolinone (**27**) (Figure 2). Five days after treatment, inhibition of *Cuscuta* growth was significantly affected by the type of 2-benzoxazolinone derivative (ANOVA, $p < 0.001$), by its concentration (ANOVA, $p < 0.001$) and by the interaction (ANOVA, $p < 0.001$). The results are shown in Figure 4.

Compound **5** stood out as the most active compound of this group, with inhibition values higher than 80% both at 1 and 0.5 mM. At a concentration of 1 mM, only the compounds **27** ($79.3 \pm 6.9\%$) and **25** ($50.6 \pm 15.2\%$) showed strong activity, whereas at a concentration of 0.5 mM, compound **27** was the only derivative, among **24**–**27**, to show an interesting activity value ($45.23 \pm 5.3\%$).
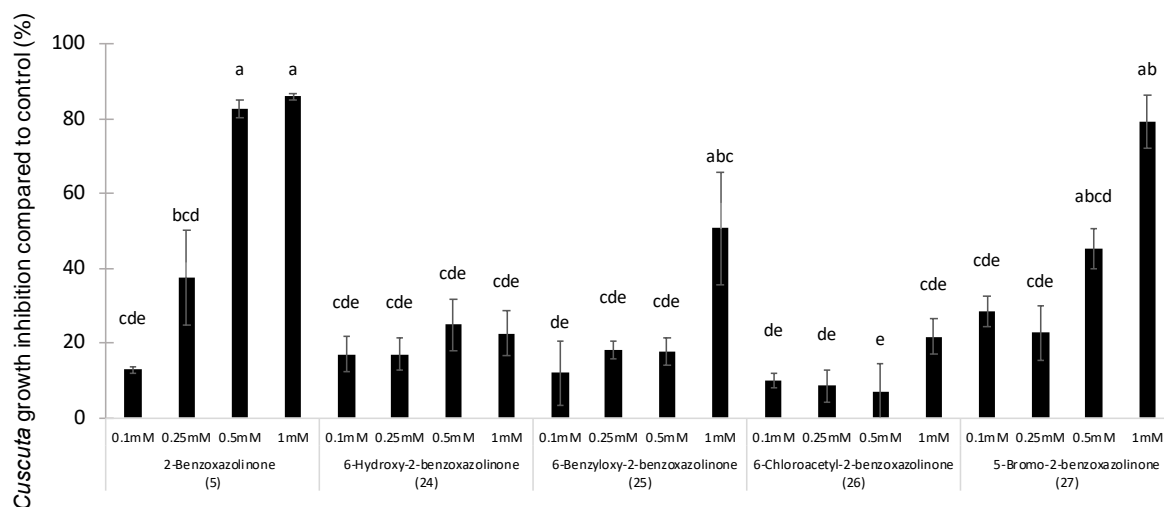
Figure 4. In vitro assessment of the *Cuscuta* growth inhibition induced by 2-benzoxazolinone (**6**) and its derivatives (**24–27**). Bars with different letters are significantly different (Tukey test at $p < 0.05$). Error bars represent the standard error of the mean.

Study of structure–activity relationship on the growth inhibition induced by hydrocinnamic acid (**6**). The growth inhibition of hydrocinnamic acid identified in the first screening (Figure 3C,D) was studied in a second in vitro bioassay in a range of concentrations from 0.25 to 1 mM, and compared to five derivatives, 3-(4-fluorophenyl)propionic acid (**19**), 3-(4-chlorophenyl)propionic acid (**20**), 3-(4-bromophenyl)propionic acid (**21**), 3-(4-hydroxyphenyl)propionic acid (**22**), 3-(2-hydroxyphenyl)propionic acid (**23**) (Figure 2). Five days after treatment, inhibition of *Cuscuta* growth was significantly affected by the type of hydrocinnamic acid derivative (ANOVA, $p < 0.001$), by its concentration (ANOVA, $p < 0.001$) and by the interaction (ANOVA, $p < 0.001$). The results are shown in Figure 5.
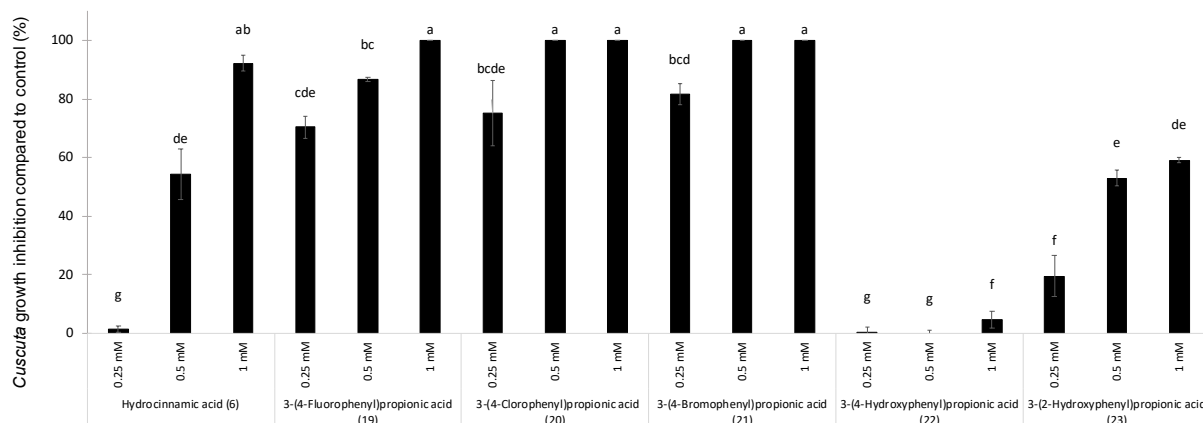

Figure 5. In vitro assessment of the *Cuscuta* growth inhibition induced by hydrocinnamic acid (**5**) and its derivatives (**19–23**). Bars with different letters are significantly different (Tukey test at $p < 0.05$).

Compounds **6** and **19–21** were highly active at 1 and 0.5 mM, with inhibition values of, or close to, 100% at 1 mM, or 0.5 mM in the case of compounds **20** and **21**. When tested at 0.25 mM, compounds **19–21** kept the activity in values ranging from 70.4 ± 3.6% to 81.6 ± 3.7%, while compound **6** was shown to be inactive. On the other hand, compound **22** was not active and compound **23** was only moderately active at the higher concentrations tested.

Identification of inductors of necrosis in *C. campestris* seedlings. Besides being inhibitors of seedling growth, the first screening was also used to identify the inductors of necrosis. No necrosis was observed in *Cuscuta* seedlings treated with control (Figure 6A,B).

The observed induction of necrosis was significantly affected by the compound treatment (ANOVA, $p < 0.001$). The different levels of activity observed allowed a classification between highly active (compounds **8, 10, 15** and **18**), moderately active (compounds **9, 12** and **17**) and not active in the remaining eleven compounds. The strong necrosis observed in all seedlings treated with 1 mM of pisatin (**18**) was observable mainly in the hypocotyl (Figures 3D and 6C). In contrast, the necrosis in the rest of the active compounds was observed in the root apices. An intense browning of *Cuscuta* root apices was observed in all seedlings treated with caffeic acid (**8**) (Figure 6D,H). Treatments with scopoletin and sesamol induced the root apices of all exposed seedlings to become black (Figure 6F,G,J,K). Ferulic acid (**9**) treatment induced intense browning of root apices in 54% of seedlings treated (Figure 6E,I). Vanillic acid (**12**) and naringenin (**17**) also induced browning of root apices in, respectively, 63.7% and 55.9% of exposed seedlings, although the browning in each seedling was less intense than the browning intensity induced by caffeic acid (**8**) and ferulic acid (**9**).
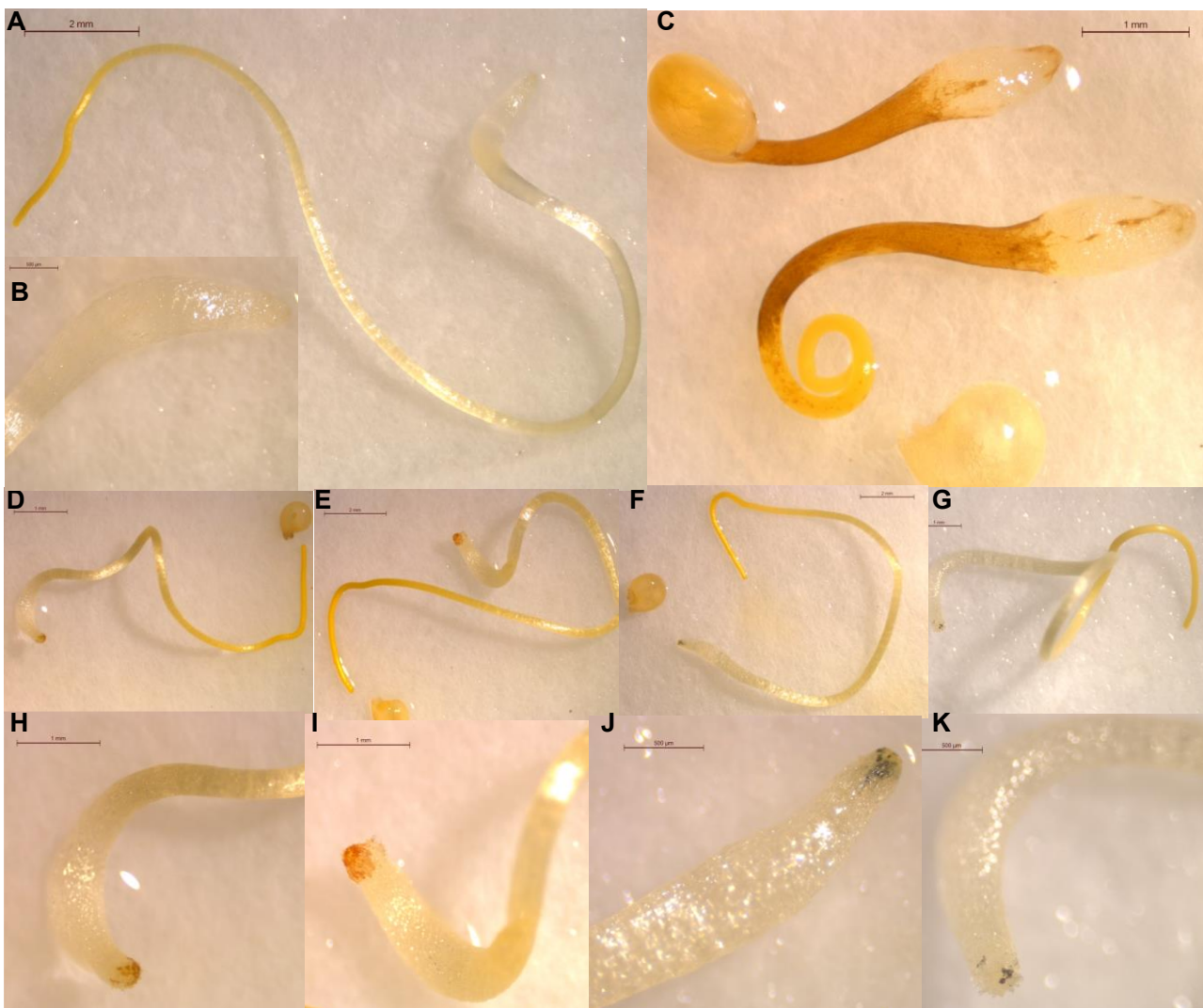

Figure 6. Illustrative photographs showing the necrosis observed in the first screening. (**A**) *Cuscuta* seedling treated with control (**B**) Detail of *Cuscuta* root apice treated with control. (**C**) necrosis in the hypocotyl of *Cuscuta* seedlings treated with pisatin (**18**), (**D–K**) necrosis in the root apice in *Cuscuta* seedlings treated with (**D,H**) caffeic acid (**8**); (**E,I**) ferulic acid (**9**); (**F,J**) scopoletin (**10**) and (**G,K**) sesamol (**15**).

Identification of hydrocinnamic acid as inductor of trichomes in *C. campestris* root apices. Besides the inhibition of hypocotyl growth in seedlings treated with hydrocinnamic acid, a strong overproduction of protuberances resembling trichomes was observed in 100% of the root apices in seedlings treated with hydrocinnamic acid (**6**) (Figures 3C and 7A), and their derivatives (**19**, **20**, **21**, and **23**), except for 3-(4-hydroxyphenyl)propionic acid (**22**), which did not increase in trichome formation, in comparison with control seedlings (Figures 6B and 7B).
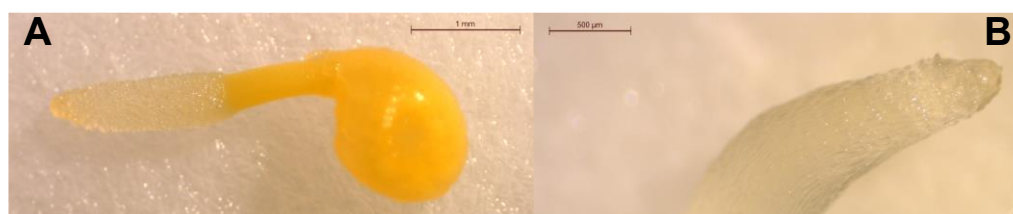

Figure 7. Increased trichome formation in *Cuscuta* root apices treated with hydrocinnamic acid (**6**) (**A**) in comparison with smother *Cuscuta* root apices treated with control (**B**).

Calculated CLog*P* values of the tested compounds **1–27**. The calculated CLog*P* values are shown in Table 1. In general, all the values were in the range of 0–3, except in two cases, the amino acids **1** and **4**, for which negative values were calculated.

Table 1. Calculated CLog*P* values for compounds **1–27**.

	CLog*P*		CLog*P*		CLog*P*		CLog*P*
1	−3.424	**8**	0.975	**15**	1.564	**22**	1.236
2	1.966	**9**	1.421	**16**	0.208	**23**	1.186
3	2.376	**10**	1.352	**17**	2.445	**24**	0.491
4	−1.556	**11**	1.623	**18**	2.420	**25**	2.845
5	1.158	**12**	1.355	**19**	2.046	**26**	0.710
6	1.903	**13**	1.885	**20**	2.616	**27**	2.021
7	1.572	**14**	0.158	**21**	2.766		

4. Discussion

The highest growth inhibition activity against *Cuscuta* seeds was obtained in the first screening for compounds **5** and **6** tested at 1 mM (Figure 3). Thus, 2-benzoxazolinone (**5**) and hydrocinnamic acid (**6**) were also tested in a range of concentrations (from 0.1 or 0.25 to 1 mM) and compared to some of their derivatives (compounds **19–27**). Compound **5** showed strong activity, also at 0.5 mM, while its derivatives (compounds **24–27**) were less active, demonstrating that the presence of a substituent on the aromatic ring of the benzoxazolinone skeleton negatively affected the phytotoxicity. Different results were obtained with compound **6**, which showed reduced activity when tested at 0.5 mM, while its *para*-substituted derivatives with halogen atoms (compounds **19–21**) were still strongly active at the lowest concentration used. Thus, in this case, it seemed that the integrity of the aromatic ring was not fundamental to imparting activity.

In the literature it is common to find synthetic strategies that use monocyclic or bicyclic aromatic compounds as starting material or intermediates to obtain products of interest for the control of parasitic weeds, mainly for emulating the structure of the widely known parasitic stimulant GR24 [23]. For this reason, the study herein presented provides valuable results of how simple aromatic compounds, such as **5** or **6**, could be equally interesting by themselves to provide a solution to the problem of parasitic weeds. For the following discussion, the experimental results were related, when possible, to the lipophilicity values calculated for this purpose and expressed numerically through CLog*P* values, depicted in Table 1.

As reported above (Table 1), CLog*P* values were positive for almost all the compounds tested, except in the case of compounds **1** and **4**, the negative CLog*P* values of which hint at a preference for the aqueous media, instead of the organic. As stated previously [24], high aqueous solubility may have a positive effect on bioactivity; however, this was not the case, since negligible activity was obtained for these compounds. A broad range of CLog*P* (higher than 0 and up to 2.445) was obtained for compounds **2**, **3**, **8**, **9**, **14** and **17**, also with negligible activity, all of them being polar compounds (with amine, hydroxyl and carboxyl groups). Regardless, compounds with medium to high activity showed CLog*P* in the range of 1.158–2.766, with only two exceptions in **16** and **24**, with moderate activity and CLog*P* values under 1, of 0.208 and 0.491, respectively.

Based on the results of bioactivity, several hints can be found regarding the structural requirements for the inhibition of *Cuscuta* growth. First, amino acids (**1**, **3** and **4**) as well as the structurally-related compound **2**, with an indole ring, were not significantly active (Figure 3). However, studies like that by Fernandez-Aparicio et al. (2017) [25] and by Kuruma et al. (2021) [26] highlight the interest of compounds, such as L-tryptophan (**3**), in the study of parasitic plants, the later study showing how some derivatives of compound **3** can regulate seed germination (inhibition or stimulation) and radicle growth of the parasitic species, *Orobanche minor*. In our study, when changing the indole ring of compounds **2** or **3** to that of a benzoxazolinone (**5**), the activity increased strongly. A similar result was obtained in a previous study testing compounds **2**, **3** and **5** on the germination of the parasitic species *Orobanche crenata* [27]. Compound **5** had a CLog*P* value of 1.158, which was at the half-way position of the other compounds; however, the other compounds with similar CLog*P* values did not exhibit the same level of activity, i.e. compounds **10**, **12**. Thus, this level of activity might be related to interaction in the active site with specific parts of the molecules. Closer structurally-related compounds to 5, namely, **24**–**27**, showed CLog*P* disparity after the addition of hydroxyl (**24**), benzyloxy (**25**), chloroacetyl (**26**) and halogen bromide (**27**). The growth inhibition activity of these derivatives dropped drastically (Figure 4). Only compound **27**, with a CLog*P* closest to that of compound **5** (2.021), preserved the activity level (around 80%) at the highest tested concentration (1 mM), while the rest of compounds, with disparate CLog*P* values, saw their bioactivities reduced to levels closer to 30%. Though this change in lipophilicity might explain the loss of bioactivity, the effect on the substitution position cannot be dismissed [24]. Derivatization of position 5 might be safer in terms of preserving the bioactivity than position 6, which might be required to be free in order to fit in the active site. Although the ClogP of compounds 24 and 25 were totally different, the activities were similar. This phenomenon could be explained by the possibility of an enzymatic oxidation in the tested organism of compound 25 to produce 24 and benzoic acid (13), which had a moderate phytotoxic effect.

In the second group, there was the group of the benzyl acids **6**–**9**. The most relevant and strong compound in this group was hydrocinnamic acid (**6**), which showed the highest activity on the initial screening (Figure 3). By comparison with compound **7**, two structural changes were carried out: the addition of a hydroxyl group in *para* to the benzyl ring and a decrease in the side chain rotability, due to adding a double bond. These changes negatively affected the activity, as was observed by the decrease from high to moderate level in compound **7**. Either by adding an extra hydroxyl (compound **8**) or a methoxy group (compound **9**) in *meta* to the propionic chain, the activity dropped again to a negligible level. It was confirmed, therefore, that the addition of hydroxyl groups in these positions negatively affected activity. It should be noted that compounds **7** and **8**, inhibitors of several plant species [27,28], showed high activity in the inhibition of the germination of *O. crenata*, whereas compound **9** was inactive [27]. Another study proved that pre-sowing seed hardening of hosts in solutions of compounds **8** and **9** reduced the induction of *Striga asiatica* (parasitic species) germination [29].

The derivatives of compound **6** tested in the SAR study hint at new information regarding the effect of the modifications on this compound. By comparing activity shown by compound **22** with that of compound **7**, the negative effect of the hydroxyl group

in *para* position was even more evident, since compound **22** was completely inactive at the three tested concentrations (Figure 5), while compound **7** was moderately active (Figure 3). The only difference between these two compounds was in the presence of a double bond in the propionic chain, which confirmed the positive effect of the *trans* double bond present in compound **7**. The addition of a hydroxyl at *orto* position, also had a negative effect on the bioactivity (compound **23**), although the effect was less drastic than in the prior case, with moderate activities at the two highest tested concentrations. Lastly, when introducing halogens (F, Cl or Br) in the *para* position, instead of a hydroxyl group (compounds **19**, **20**, and **21**), an increase in bioactivity was observed, especially remarkable at the lowest concentrations, when compared with compound **6**. A previous study showed the inhibiting activity of plant growth of the structurally related compound, benzoic acid (**13**), increased with the addition of halogen atoms in its aromatic ring [30]. Thus, it is interesting to highlight the role of the halogen substituents in phenyl rings, as also discussed in a previous review [31], and how parameters like stability are improved by factors such as the presence of a fluorine atom in the *para*-position [31]. Indeed, the halogenation of aromatic compounds is a strategy employed for the design of agrochemicals and drugs [32,33]. By observing the bioactivity profiles in Figure 5, the bioactivity of these compounds could be ordered as: **6** < **19** < **20** < **21**, which corresponds to H < F < Cl < Br, indicating the positive effect of exchanging a hydrogen atom by, and halogen in. position **4**. Interestingly, this ranking appears to be related to the results shown by a cluster analysis obtained in a phytotoxicity study that tested triazole derivatives bearing halogenated benzyl substituents [34]. According to the CLog$P$ values (Table 1), the activity increased substantially with the lipophilicity of these compounds, showing higher activities in the cases of **20** and **21**, with close CLog$P$ (2.616 and 2.766, respectively), followed by compound **19** (2.046) and, finally, compound **6** (1.903). However, the similar CLog$P$ of compound **6**, containing H in *para*, to that of **19**, containing F in *para*, together with the similar size of both atoms, hint to other elements being responsible for the higher bioactivity of the halogenated compounds, apart from the lipophilicity, such as electronic variables.

In a third group, related to the prior, the lactones **10** and **11** could be found, which showed moderate activity (Figure 3). The activity of these compounds could be compared with their acid counterparts, **9** and **7**. The first, compound **9**, with negligible activity showed the positive effect that the cyclization into an ester (**10**) had in the activity. The same was observed when comparing **7** with **11**, although to a lesser extent. In other studies, compound **10**, a known stimulant of some *Striga* and *Orobanche* species [35,36], proved to be active for inhibiting the germination of *Orobanche cernua* and *O. crenata* [27,37]. A similar result, in which a compound showed stimulating or inhibitory germination activity depending on the parasitic species, was reported in a study with the Orobanchaceae species, where this effect was correlated to the concentration of the tested compound [38]. Regarding umbelliferone (**11**), this compound showed the ability to inhibit haustorium formation on experiments carried out on *Striga hermonthica* [39].

The smallest molecules, **12**–**16**, showed varied results (Figure 3). On the one hand, the negligible activity of compound **14** (which was found inactive for *O. crenata* germination) [27], indicated that the simplest combination of an aromatic lactone with an acid group was not enough for inhibiting growth, requiring the presence of the second aromatic ring, as in compounds **10** and **11**. On the other hand, the combination of an aromatic ring and an acid group, as in compounds **12** and **13**, was enough to cause inhibition at a moderate level. The case of vanillic acid (**12**) is of interest, since this compound stimulates the haustoria of species of the parasitic genera *Triphysaria* and *Striga* [40]. Compound **16** is a benzoquinone. This family of compounds are well known for their phytotoxicity and their structures have been used for finding new phytotoxic and herbicidal compounds able to interfere with several molecular target sites, and for their oxidative properties [41–44]. Moreover, the scaffold is relevant in the study of the induction of haustoria [45]; for example, 2,6-dimethoxy-1,4-benzoquinone, an isolated derivative from sorghum, induced haustoria in *Striga*, *Phtheirospermum*, *Triphysaria*, *Agalinis*, *Orobanche* and *Phelipanche* species [40,46].

Compound **15**, with a dioxol fragment, had a similar lipophilicity to compound **11** (1.564 and 1.623, respectively), both showing moderate activity. This compound **15** is structurally related with compound **18**, which contains this molecule as a fragment and showed a strong inhibition of growth, hinting at this fragment as being one of the most important parts of the molecule regarding its activity.

Lastly, the flavonoid **17** showed negligible inhibitory activity on *Cuscuta*. This result was in agreement with a previous study, that showed how compound **17** did not inhibit seed germination of *O. cernua* [37]. However, it should be noted that compound **17** could act differently on different parasitic weeds, since another study found it as a potent inhibitor of *O. crenata* [47]. Indeed, other flavonoids such as quercetin, have been described as inhibitors of parasitic weed growth [48].

Among the tested compounds, seven structurally diverse compounds showed significant necrosis, with strong (**8**, **10**, **15** and **18**) and moderate (**9**, **12** and **17**) effects (Figure 6). CLog*P* values for the compounds showing necrosis were in the range of 0.975–2.445. Whether a certain CLog*P* value, relating to a certain degree of lipophilicity, is needed for causing necrosis in the cellular tissue is unclear. All the compounds showing necrotic effects presented at least an acid hydroxyl group in the aromatic ring, which might be, in part, responsible for this effect. An excess of phenolic acids is considered a cause of necrosis in plants, which could be related to boron deficiency [49]. In this regard, pro-oxidant properties have been described for common phenolic acids like *p*-coumaric acid (**7**), caffeic acid (**8**) or ferulic acid (**9**) [50]. Thus, our results were consistent with this background and expand knowledge about necrosis that the tested and related compounds induce in plants, a topic for which few references are available in the literature. Previous studies proved that caffeic acid (**8**) and its derivative, chlorogenic acid, can act as necrosis-inducing compounds in sunflower or potato tubers [49,51], and necrotic effects were observed in *O. crenata* by the action of scopoletin (**10**) [27].

Cases of necrosis similar to that observed for naringenin (**17**) have been reported for compounds such as (*S*)-6-hydroxymellein in *Lepidium sativum* [52]. (-)-Catechin, compound, with a closer structure to that of compound **17**, elicited necrosis from meristematic and the central elongation zone cells of roots of *Centaurea diffusa* and *Arabidopsis thaliana* [53]. These molecules share a benzene-1,3-diol skeleton, a fragment with phytotoxic potential, and which might be key to the future search for new molecules to control *Cuscuta*. Thus, it is interesting to note the potential of some flavonoids to act as pro-oxidant compounds, like chrysin or apigenin, with structures closely related to that of compound **17** [54]. Of interest for the study or development of new necrotic-inducing compounds, a recent study on the herbicidal activity of cuminaldehyde on onions (*Allium cepa* L.) [55] showed how the aldehyde function could provide phytotoxic derivatives of monocyclic aromatic compounds structurally related to those herein reported.

From the structural point of view, it can be remarked that compounds **8** and **10**, with strong necrotic effects, shared structural similitudes to compound **9**, with moderate necrotic effect. This significant difference might be related to the replacement of one of the hydroxyl groups in compound **8** by a methoxy group (compound **9**), which provoked some decrease of the necrotic effects. However, the cyclization of compound **9** originated the structure of compound **10** and an increase of the necrotic activity. Comparison of the necrosis generated by compounds **9** and **12**, both moderately active, showed the low influence of the unsaturation of the chain containing the carboxylic function in this regard.

Sesamol (**15**) showed strong necrotic effects, which is in agreement with the pro-oxidant properties described for this compound and of interest in the pharmaceutical field [56]. The benzodioxol fragment, present in both compounds **15** and **18**, was clearly a key for the necrotic effect of **18**, which, together with the presence of an acid hydroxyl and the rest of the molecule, containing a methoxybenzyl system similar to necrotic compound 10, might explain the strong necrosis induced by this compound. These results confirmed the high potential of pisatin (**18**) as a phytotoxic compound for *Cuscuta*, since, among the three most phytotoxic compounds in the screening (the other ones being **5** and **6**), it

was the only one that induced strong necrosis in the tissues. A previous study showed the inhibitory activity of compound **18** in the growth of cress (*L sativum* L.) and lettuce (*Lactuca sativa* L.) [57].

5. Conclusions

The identification of novel structures and modes of action of natural products that are able to interfere with the necessary contact between *Cuscuta* seedling and the host is an important starting point for the future. Thus, in this study, eighteen metabolites were tested in vitro, for the first time, against *Cuscuta* seedling growth. Among them, 2-benzoxazolinone (**5**) and hydrocinnamic acid (**6**) caused stronger inhibition of seedling growth, while pisatin (**18**) also caused necrosis of the *Cuscuta* seedling. Some derivatives of compounds **5** and **6** were also tested to carry out a structure–activity relationship study. The results showed that the presence of a substituent on the aromatic ring of the benzoxazolinone skeleton negatively affected the phytotoxicity, while the presence of halogen atoms in *para*-substituted derivatives of compound 6 (compounds **19–21**) was an important factor in increasing its activity. Furthermore, the strong activity shown at the lowest concentration used by compounds **19–21** allowed us to consider them as suitable candidates for the control of *Cuscuta*. However, analyses on their ecotoxicological profiles are needed before conducting further studies for their formulation and tests in greenhouse and field trials.

References

1. Pimentel, D.; Zuniga, R.; Morrison, D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* **2005**, *52*, 273–288. [CrossRef]
2. Parker, C.; Riches, C.R. *Parasitic Weeds of the World: Biology and Control*; CAB International: Wallingford, UK, 1993.
3. Fernández-Aparicio, M.; Flores, F.; Rubiales, D. The effect of *Orobanche crenata* infection severity in faba bean, field pea and grass pea productivity. *Front. Plant Sci.* **2016**, *7*, 1049. [CrossRef]
4. Goldwasser, Y.; Miryamchik, H.; Sibony, M.; Rubin, B. Detection of resistant chickpea (*Cicer arietinum*) genotypes to *Cuscuta campestris* (field dodder). *Weed Res.* **2012**, *52*, 122–130. [CrossRef]

5.	Córdoba, E.M.; Fernández-Aparicio, M.; González-Verdejo, C.I.; López-Grau, C.; Muñoz-Muñoz, M.V.; Nadal, S. Search for Resistant Genotypes to *Cuscuta campestris* Infection in two legume species, *Vicia sativa* and *Vicia ervilia*. *Plants* **2021**, *10*, 738. [CrossRef]

6.	Kaštier, P.; Krasylenko, Y.A.; Martincová, M.; Panteris, E.; Šamaj, J.; Blehová, A. Cytoskeleton in the parasitic plant *Cuscuta* during germination and pre-haustorium formation. *Front. Plant Sci.* **2018**, *9*, 794. [CrossRef]

7.	Fernández-Aparicio, M.; Delavault, P.; Timko, M.P. Management of infection by parasitic weeds: A Review. *Plants* **2020**, *9*, 1184. [CrossRef]

8.	Haidar, M.A.; Orr, G.L.; Westra, P. Effects of light and mechanical stimulation on coiling and pre-haustoria formation in *Cuscuta* spp. *Weed Res.* **1997**, *37*, 219–228. [CrossRef]

9.	Vaughn, K.C. Attachment of the parasitic weed dodder to the host. *Protoplasma* **2002**, *219*, 227–237. [CrossRef]

10.	Fernández-Aparicio, M.; Soriano, G.; Masi, M.; Carretero, P.; Vilariño-Rodríguez, S.; Cimmino, A. (4Z)-Lachnophyllum lactone, an acetylenic furanone from *Conyza bonariensis*, identified for the first time with allelopathic activity against *Cuscuta campestris*. *Agriculture* **2022**, *12*, 790. [CrossRef]

11.	Nadler-Hassar, T.; Shaner, D.L.; Nissen, S.; Westra, P.; Rubin, B. Are herbicide-resistant crops the answer to controlling *Cuscuta*? *Pest Manag. Sci.* **2009**, *65*, 811–816. [CrossRef]

12.	Dayan, F.E.; Duke, S.O. Natural compounds as next-generation herbicides. *Plant Physiol.* **2014**, *166*, 1090–1105. [CrossRef] [PubMed]

13.	Westwood, J.H.; Charudattan, R.; Duke, S.O.; Fennimore, S.A.; Marrone, P.; Slaughter, D.C.; Swanton, C.; Zollinger, R. Weed management in 2050: Perspectives on the future of weed science. *Weed Sci.* **2018**, *66*, 275–285. [CrossRef]

14.	Khanh, T.D.; Cong, L.C.; Xuan, T.D.; Lee, S.J.; Kong, D.S.; Chung, I.M. Weed-suppressing potential of dodder (*Cuscuta hygrophilae*) and its phytotoxic constituents. *Weed Sci.* **2008**, *56*, 119–127. [CrossRef]

15.	Azimi, A.A.; Hashemloian, B.D. Allelopathy and anti-mitotic effects of *Cuscuta campestris* and *Cuscuta monogyna* extracts on plant cell division. *J. Med. Plants By-Prod.* **2017**, *6*, 131–138.

16.	Zermane, N.; Vurro, M.; Boari, A.; Avolio, F.; Andolfi, A.; Evidente, A. Towards broomrape and field dodder management using natural metabolites from plants. In Proceedings of the Abstract of 11th World Congress on Parasitic Plants, Martina Franca, Italy, 7–12 June 2011; p. 82.

17.	Andolfi, A.; Zermane, N.; Cimmino, A.; Avolio, F.; Boari, A.; Vurro, M.; Evidente, A. Inuloxins A–D, phytotoxic bi-and tri-cyclic sesquiterpene lactones produced by Inula viscosa: Potential for broomrapes and field dodder management. *Phytochemistry* **2013**, *86*, 112–120. [CrossRef]

18.	Vurro, M.; Boari, A.; Evidente, A.; Andolfi, A.; Zermane, N. Natural metabolites for parasitic weed management. *Pest Manag. Sci.* **2009**, *65*, 566–571. [CrossRef]

19.	Dewick, P.M. *Medicinal Natural Products—A Biosynthetic Approach*; Wiley and Sons Ltd.: Chicester, UK, 2009.

20.	Evidente, A.; Cimmino, A.; Fernandez-Aparicio, M.; Andolfi, A.; Rubiales, D.; Motta, A. Polyphenols, including the new peapolyphenols A–C, from pea root exudates stimulate *Orobanche foetida* seed germination. *J. Agric. Food Chem.* **2010**, *58*, 2902–2907. [CrossRef]

21.	Gaertner, E.E. Studies of seed germination, seed identification, and host relationship in Dodders, *Cuscuta* spp. *Mem. Cornell Agric. Exp. Stn.* **1950**, *294*, 1–56.

22.	Cala, A.; Zorrilla, J.G.; Rial, C.; Molinillo, J.M.G.; Varela, R.M.; Macías, F.A. Easy access to alkoxy, amino, carbamoyl, hydroxy, and thiol derivatives of sesquiterpene lactones and evaluation of their bioactivity on parasitic weeds. *J. Agric. Food Chem.* **2019**, *67*, 10764–10773. [CrossRef]

23.	Zorrilla, J.G.; Rial, C.; Varela, R.M.; Molinillo, J.M.; Macías, F.A. Strategies for the synthesis of canonical, non-canonical and analogues of strigolactones, and evaluation of their parasitic weed germination activity. *Phytochem. Rev.* **2022**, *21*, 1627–1659. [CrossRef]

24.	Soriano, G.; Siciliano, A.; Fernández-Aparicio, M.; Cala Peralta, A.; Masi, M.; Moreno-Robles, A.; Guida, M.; Cimmino, A. Iridoid glycosides isolated from *Bellardia trixago* identified as inhibitors of *Orobanche cumana* radicle growth. *Toxins* **2022**, *14*, 559. [CrossRef] [PubMed]

25.	Fernández-Aparicio, M.; Bernard, A.; Falchetto, L.; Marget, P.; Chauvel, B.; Steinberg, C.; Morris, C.E.; Gibot-Leclerc, S.; Boari, A.; Vurro, M.; et al. Investigation of amino acids as herbicides for control of *Orobanche minor* parasitism in red clover. *Front. Plant Sci.* **2017**, *8*, 842. [CrossRef]

26.	Kuruma, M.; Suzuki, T.; Seto, Y. Tryptophan derivatives regulate the seed germination and radicle growth of a root parasitic plant, Orobanche minor. *Bioorg. Med. Chem. Lett.* **2021**, *43*, 128085. [CrossRef]

27.	Fernández-Aparicio, M.; Cimmino, A.; Evidente, A.; Rubiales, D. Inhibition of *Orobanche crenata* seed germination and radicle growth by allelochemicals identified in cereals. *J. Agric. Food Chem.* **2013**, *61*, 9797–9803. [CrossRef] [PubMed]

28.	Zanardo, D.I.L.; Lima, R.B.; Ferrarese, M.D.L.L.; Bubna, G.A.; Ferrarese-Filho, O. Soybean root growth inhibition and lignification induced by *p*-coumaric acid. *Environ. Exp. Bot.* **2009**, *66*, 25–30. [CrossRef]

29.	Bharathalakshmi; Jayachandra. Presowing hardening of the host with phenolic acids reduces induction of seed germination in the root parasite *Striga asiatica*. *Int. J. Pest Manag.* **1980**, *26*, 309–312.

30.	Huffman, C.W.; Godar, E.M.; Torgeson, D.C. Inhibition of plant growth by halogenated benzoic acids. *J. Agric. Food Chem.* **1967**, *15*, 976–981. [CrossRef]

31. Jeschke, P. The unique role of halogen substituents in the design of modern agrochemicals. *Pest Manag. Sci.* **2010**, *66*, 10–27. [CrossRef] [PubMed]

32. Macias, F.A.; Chinchilla, N.; Arroyo, E.; Molinillo, J.M.G.; Marin, D.; Varela, R.M. Combined strategy for phytotoxicity enhancement of benzoxazinones. *J. Agric. Food Chem.* **2010**, *58*, 2047–2053. [CrossRef] [PubMed]

33. Mejías, F.J.; Durán, A.G.; Zorrilla, J.G.; Varela, R.M.; Molinillo, J.M.G.; Valdivia, M.M.; Macías, F.A. Acyl derivatives of eudesmanolides to boost their bioactivity: An explanation of behavior in the cell membrane using a molecular dynamics approach. *ChemMedChem* **2021**, *16*, 1297–1307. [CrossRef]

34. Borgati, T.F.; Alves, R.B.; Teixeira, R.R.; Freitas, R.P.D.; Perdigão, T.G.; Silva, S.F.D.; dos Santos, A.A.; Bastidas, A.D.J.O. Synthesis and phytotoxic activity of 1, 2, 3-triazole derivatives. *J. Braz. Chem. Soc.* **2013**, *24*, 953–961. [CrossRef]

35. Yoneyama, K.; Takeuchi, Y.; Ogasawara, M.; Konnai, M.; Sugimoto, Y.; Sassa, T. Cotylenins and fusicoccins stimulate seed germination of *Striga hermonthica* (Del.) Benth and *Orobanche minor* Smith. *J. Agric. Food Chem.* **1998**, *46*, 1583–1586. [CrossRef]

36. Bar Nun, N.; Mayer, A.M. Smoke chemicals and coumarin promote the germination of the parasitic weed *Orobanche aegyptiaca*. *Isr. J. Plant Sci.* **2005**, *53*, 97–101. [CrossRef]

37. Serghini, K.; de Luque, A.P.; Castejón-Muñoz, M.; García-Torres, L.; Jorrín, J.V. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: Induced synthesis and excretion of 7-hydroxylated simple coumarins. *J. Exp. Bot.* **2001**, *52*, 2227–2234. [CrossRef]

38. Zorrilla, J.G.; Cala, A.; Rial, C.R.; Mejías, F.J.; Molinillo, J.M.; Varela, R.M.; Macías, F.A. Synthesis of active strigolactone analogues based on eudesmane-and guaiane-type sesquiterpene lactones. *J. Agric. Food Chem.* **2020**, *68*, 9636–9645. [CrossRef]

39. Wada, S.; Cui, S.; Yoshida, S. Reactive oxygen species (ROS) generation is indispensable for haustorium formation of the root parasitic plant *Striga hermonthica*. *Front. Plant Sci.* **2019**, *10*, 328. [CrossRef]

40. Cui, S.; Wada, S.; Tobimatsu, Y.; Takeda, Y.; Saucet, S.B.; Takano, T.; Yoshida, S. Host lignin composition affects haustorium induction in the parasitic plants *Phtheirospermum japonicum* and *Striga hermonthica*. *New Phytol.* **2018**, *218*, 710–723. [CrossRef]

41. Lima, L.S.; Barbosa, L.C.D.A.; de Alvarenga, E.S.; Demuner, A.J.; da Silva, A.A. Synthesis and phytotoxicity evaluation of substituted para-benzoquinones. *Aust. J. Chem.* **2003**, *56*, 625–630. [CrossRef]

42. González-Ibarra, M.; Farfán, N.; Trejo, C.; Uribe, S.; Lotina-Hennsen, B. Selective herbicide activity of 2, 5-di (benzylamine)-p-benzoquinone against the monocot weed *Echinochloa crusgalli*. An in vivo analysis of photosynthesis and growth. *J. Agric. Food Chem.* **2005**, *53*, 3415–3420. [CrossRef]

43. Dayan, F.E.; Howell, J.L.; Weidenhamer, J.D. Dynamic root exudation of sorgoleone and its in planta mechanism of action. *J. Exp. Bot.* **2009**, *60*, 2107–2117. [CrossRef]

44. Zorrilla, J.G.; Rial, C.; Varela, R.M.; Molinillo, J.M.; Macías, F.A. Facile synthesis of anhydrojudaicin and 11, 13-dehydroanhydrojudaicin, two eudesmanolide-skeleton lactones with potential allelopathic activity. *Phytochem. Lett.* **2019**, *31*, 229–236. [CrossRef]

45. Yoder, J.I. Host-plant recognition by parasitic Scrophulariaceae. *Curr. Opin. Plant Biol.* **2001**, *4*, 359–365. [CrossRef]

46. Fernández-Aparicio, M.; Masi, M.; Cimmino, A.; Evidente, A. Effects of benzoquinones on radicles of *Orobanche* and *Phelipanche* species. *Plants* **2021**, *10*, 746. [CrossRef] [PubMed]

47. Mabrouk, Y.; Simier, P.; Arfaoui, A.; Sifi, B.; Delavault, P.; Zourgui, L.; Belhadj, O. Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with *Orobanche crenata*. *J. Phytopathol.* **2007**, *155*, 728–734. [CrossRef]

48. Fernández-Aparicio, M.; Masi, M.; Cimmino, A.; Vilariño, S.; Evidente, A. Allelopathic effect of quercetin, a flavonoid from *Fagopyrum esculentum* roots in the radicle growth of *Phelipanche ramosa*: Quercetin natural and semisynthetic analogues were used for a structure-activity relationship investigation. *Plants* **2021**, *10*, 543. [CrossRef] [PubMed]

49. Dear, J.; Aronoff, S. Relative kinetics of chlorogenic and caffeic acids during the onset of boron deficiency in sunflower. *Plant Physiol.* **1965**, *40*, 458. [CrossRef] [PubMed]

50. Yordi, E.G.; Pérez, E.M.; Matos, M.J.; Villares, E.U. Antioxidant and pro-oxidant effects of polyphenolic compounds and structure-activity relationship evidence. *Nutr. Well-Being Health* **2012**, *2*, 23–48.

51. Dinkel, D.H. Chlorogenic acid associated with physiological internal necrosis of potato tubers. *Am. Potato J.* **1963**, *40*, 149–153. [CrossRef]

52. Krumsri, R.; Iwasaki, A.; Suenaga, K.; Kato-Noguch, H. Phytotoxic effects of *Senna garrettiana* and identification of phytotoxic substances for the development of bioherbicides. *Agriculture* **2022**, *12*, 1338. [CrossRef]

53. Bais, H.P.; Vepachedu, R.; Gilroy, S.; Callaway, R.M.; Vivanco, J.M. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* **2003**, *301*, 1377–1380. [CrossRef] [PubMed]

54. Cos, P.; Calomme, M.; Pieters, L.; Vlietinck, A.J.; Berghe, D.V. Structure-activity relationship of flavonoids as antioxidant and pro-oxidant compounds. *Stud. Nat. Prod. Chem.* **2000**, *22*, 307–341.

55. Sunohara, Y.; Nakano, K.; Matsuyama, S.; Oka, T.; Matsumoto, H. Cuminaldehyde, a cumin seed volatile component, induces growth inhibition, overproduction of reactive oxygen species and cell cycle arrest in onion roots. *Sci. Hortic.* **2021**, *289*, 110493. [CrossRef]

56. Khamphio, M.; Barusrux, S.; Weerapreeyakul, N. Sesamol induces mitochondrial apoptosis pathway in HCT116 human colon cancer cells via pro-oxidant effect. *Life Sci.* **2016**, *158*, 46–56. [CrossRef] [PubMed]

57. Kato-Noguchi, H. Isolation and identification of an allelopathic substance in *Pisum sativum. Phytochemistry* **2003**, *62*, 1141–1144. [CrossRef]