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Identification of Barley (*Hordeum vulgare* L. subsp. *vulgare*) Root Exudates Allelochemicals, Their Autoallelopathic Activity and Against *Bromus diandrus* Roth. Germination

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Abstract: Crops with weed suppressive root exudates or the direct use of bioherbicidal allelochemicals is a new approach in integrated weed management systems. In this context, the allelopathic activity and chemical composition of root exudates from six genotypes (modern varieties and landraces) of barley were characterized. The phenolic acids appeared to be particularly implicated in the inhibitory action of barley root exudates against *Bromus diandrus*. The amount of these compounds was higher in sandy substrate than in sandy-clay-loam substrate. Ten phenolic acids and one phenylpropanoid derivative were present, in addition to saponarin, a newly identified flavonoid in barley root exudates. Seven compounds explaining variability in the inhibitory activity of barley roots (stepwise analysis) and one compound detected only in highly allelopathic genotypes were toxic against receiver plants. Most compounds had a greater inhibitory effect on the growth of great brome than the barley genotypes. The synergistic and/or additive effect of the eight compounds appeared to be the source of the toxicity. Benzoic acid, the mixture of compounds, saponarin and salicylic acid were the most efficient compounds against the great brome and the less aggressive against barley. Overall, the results revealed the allelopathic potential of the water-soluble compounds exuded by the roots of living barley plants. These compounds included saponarin, a flavonoid not yet recognized as a barley root allelochemical.

Keywords: *Hordeum vulgare*; allelochemicals; root exudates; phenolic acids; saponarin; weed management

1. Introduction

The above- and below-ground organs of living plants emit a wide range of compounds into their environment [1,2]. These compounds are known as 'allelochemicals' when they affect, positively or negatively, the growth and development of neighboring plants or microorganisms, a phenomenon

known as 'allelopathy' [3,4]. Allelochemicals are specialized metabolites that might contribute to a plant's ecological fitness [5] and to plant-to-plant interactions within plant communities [6]. Due to the increasing number of herbicide-resistant weeds and the environmental and human health concerns about the use of synthetic herbicides, 'allelopathy' has been proposed as a new approach to the biological control of weeds, together with suitable crop management techniques. This phenomenon might be exploited by the cultivation of allelopathic species or the direct use of bioherbicidal allelochemicals. In Tunisia, great brome (*Bromus diandrus* Roth., syn. *Bromus rigidus* Roth. subsp. *gussonii* Parl.) is a ubiquitous weed in cereal crops, causing significant (20–50%) yield reduction [7]. Crop management and chemical methods using no-selective herbicide have been widely used to control this troublesome weed [8]. Some research has focused on the biological control of great brome by using pathogenic rhizobacteria [9,10]. The identification of compounds with bioherbicidal proprieties, however, has not yet been described and deserves more attention.

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is considered to be a weed-competitive species [11–13]. It is also known to have allelopathic proprieties involved in plant-plant interactions [14–16], as well as in direct [17] or indirect defenses [18] against pests and pathogens [19]. However, the plant toxins associated with allelopathic effects are still poorly characterized. Potential barley allelochemicals include alkaloids, phenolic compounds (e.g., phenolic acids, flavonoids, coumarins, tannins), cyanoglucosides and polyamines [20]. Phenolic compounds contribute to the greatest number of allelochemicals (circa 43%) in barley, particularly the phenolic acids. Many biochemical studies of barley have also revealed a rich source of flavonoids with biological activity, but little is known about the function of these compounds, particularly their allelopathic activity [20].

Until now, most research on barley allelochemicals has focused on identifying the allelochemicals from barley extracts, with only a few studies focusing on the root exudates. Some 39 non-volatile compounds have been associated with the toxicity of barley leaf, stem, root or grain extracts [20], but 12 compounds have been identified in barley root exudates [21,22], most of them alkaloids and phenolic acids. Liu and Lovett [21] identified two species-specific alkaloids from root exudates, hordenine and gramine; they were thought to account for most of the allelopathic potential of barley. The abundance of these compounds appeared to vary greatly and to be influenced by both genetic and environmental factors [23,24]. The exogenous application of hordenine and gramine on mustard plants (*Sinapis arvensis* L.) was shown to cause cell membrane damage, autophagy, an increase in the number and volume of vacuoles and damage to mitochondria, and the authors assumed they might play a significant role in barley self-defense. Later, Baghestani et al. [22] analyzed the barley root exudates for 15 common phenolic acids and one phenylpropanoid derivative. They proposed *o*-coumaric acid, vanillic acid and scopoletin as indicators of the allelopathic effectiveness of barley root exudates. This finding was based only on the abundance of these molecules in highly competitive barley genotypes; the effects of purified compounds were not assessed. It is possible, however, that the laboratory screening of crop cultivars, coupled with advanced statistical analyses of allelochemicals (e.g., correlations, Principal Component Analysis) could offer new insight on below-ground plant-to-plant interactions. All the identified allelochemicals of barley root exudates were obtained from plants cultivated in hydroponic systems [21,22], but never in soil that might affect the allelopathic activity of plant.

In this study, our objectives were to (i) assess the importance of phenolic acids, seen as a major class of allelochemicals [20], in the allelopathic potential of Tunisian barley root exudates in two types of soil substrates (sandy and sandy-clay-loam) and (ii) profile the allelochemical compounds of barley genotypes exuded in sandy soil substrate, identifying potential water-soluble compounds with bioherbicide potential.

2. Materials and Methods

2.1. Plant Materials

Seeds of six barley (*H. vulgare* L. subsp. *vulgare*) genotypes, comprising three Tunisian improved varieties ('Manel', 'Rihane' and 'Tej') and two landraces ('Ardhaoui' and 'Arbi') and one Saudi Arabian barley landrace ('Saudi'), were obtained from the National Agronomic Institute of Tunis. Currently, Rihane and Manel are the most cultivated varieties in Tunisia and cover 60% of barley area. Barley landraces are still cultivated by small farmers in Tunisian marginal environments (e.g., semi-arid and arid regions) [25]. Seeds of great brome (*B. diandrus* Roth., syn. *Bromus rigidus* Roth. subsp. *gussonii* Parl.) were collected from infested sites in the Beja region in northern Tunisia (between 36°42'07.0" N, 9°12'46.3" E and 36°41'00.2" N, 9°13'09.8" E).

2.2. Greenhouse Experiment

The barley and great brome seeds were surface-sterilized following the protocols described by Lanoue et al. [19] and Wu et al. [26], respectively. After sterilization, the seeds were pre-germinated on moist sterile filter paper and placed in darkness in a growth chamber at 22 °C for 72 h for barley and 96 h for great brome. Thirty pre-germinated barley seeds of each genotype were sown in polypropylene square pots (13 × 13 cm) that had been disinfected with sodium hypochlorite and contained 800 g of a sandy substrate (SS) or sandy-clay-loam substrate (Table S1). It is well known that soil biota may impact the performance of allelochemicals from the time of their release until their contact with the target plant [27]. The objective of this study is to highlight the initial molecules exuded by barley roots in order to valorize them as natural herbicides. Soil sterilization was adopted as a technique, frequently used for this purpose. The substrates were autoclaved three times at 120 °C and at a pressure of 1 bar for 20 min. Pots without barley seeds were used as controls. The experiment was conducted in a glasshouse at 26/22 °C day/night temperature, 16 h light/8 h dark photoperiod, with a photon flux density of about 220 µmol m^{-2} s^{-1} and a relative humidity of 60%. The statistical design was a completely randomized block with five replicates per treatment, repeated twice simultaneously in order to (i) assess the phytotoxicity of the barley root exudates on great brome growth (Experiment 1) and (ii) identify the chemical composition of the root compounds (Experiment 2). The substrate was kept at 100% of its water holding capacity to minimize competition for water. To maintain saturation levels, the amount of water absorbed was completed with autoclaved tap water and estimated by weight difference of each pot between two successive days. In addition, boxes are placed under the pots to recover the water in case of flow. After 30 days, the barley plants were removed. The substrate was then sieved using a 2 mm mesh in order to remove any remaining barley roots that might be allelopathically active [28]. For Experiment 2, the substrates were collected for chemical analysis, but for Experiment 1, they were put back into the same pots. Then, 10 pre-germinated great brome seeds were sown in the recovered substrate. After 30 days, four morphological variables were measured: root length, shoot length, root dry weight and shoot dry weight. Both the root and shoot parts of the plants were removed and put into an oven at 70 °C for 72 h in order to determine their dry matter content.

2.3. Total Phenolic Content According to Soil Type

The total phenolic content of the sandy and sandy-clay-loam substrates, in which the six barley genotypes had been grown and which were therefore assumed to contain root exudates, was determined by the Folin-Ciocalteu reagent [29]. Soil extracts (1:5 soil/water, *w/v*) were prepared by shaking them in a rotary shaker for 1 h, after which the filtrates were recovered [30]. One millimeter of each filtrate added to 1 mL of the Folin-Ciocalteu reagent (diluted to 50% with distilled water, *v/v*), followed by the addition of 1 mL of sodium carbonate (Na_2CO_3, 20%). The solutions were shaken well and kept in the dark for 30 min. A blank was prepared using distilled water instead of the filtrate and five replicates were taken for each treatment. Optical density was determined with a spectrophotometer (Shimadzu,

Kyoto, Japan) at 700 nm. The total phenolic content was measured as the gallic acid equivalents used as standard (0, 2, 4, 6, 8 and 10 mg L^{-1} prepared from a stock solution of 5 g L^{-1}).

2.4. Collection of Barley Root Exudates

For this experiment, the sandy substrate used as an inert material (e.g., minimal adsorption) was chosen for the extraction of compounds. Each sample of sandy substrate (total weight ~ 800 g) was divided into four equal parts (each about 200 g). One part of the sandy substrate was extracted with an orbital shaker (Yellowline OS 10 basic) at 300 rpm for 30 min using 100 mL of methanol (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA) with an internal standard (IS, 20 mg L^{-1}), 3,5 dimethoxybenzoic acid (Sigma-Aldrich Chemical Co., Burlington, MA, USA). The extract was then recovered by vacuum filtration with a compressor, using Whatman # 1 filter paper. The resulting volume (circa 80 mL) was made up to 100 mL. A second part of the soil sample was extracted using the same volume (100 mL) of methanol. This procedure was repeated for the remaining soil sample, but there was no compensation for methanol losses at the end of the extraction. The recovered extract (circa 80 mL) was further concentrated with a rotary evaporator (Heidolph, Laborota 4003 control, Darmstadt, Germany) at 40 °C in order to obtain a final volume of 1 mL. Finally, the extract was filtered through a 0.2 µm microfilter before analysis.

2.5. HPLC Analysis

Barley allelochemicals were identified with high performance liquid chromatography (HPLC) using Agilent 1260 Infinity Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV-DAD detector and an XDB C18 column (4.6 × 150 mm, 3.5 µm), preceded by a C18 guard column (4.6 × 12.5 mm, 5 µm), thermostatically controlled at a temperature of 30 °C. The analysis was performed with a flow rate of 1 mL min^{-1}, five UV-wavelengths (225, 245, 258, 280 and 330 nm) and a self-injection of 10 µL per sample. The mobile phase was a mixture of water/formic acid (A, 99:1, *v/v*) and acetonitrile (ACN, HPLC grade, Aldrich Chemical Co., USA)/formic acid (B, 99:1, *v/v*). A 57 min linear gradient was programmed as follows: 0–35 min, 98% (A); 35–45 min, 80% (A); 45–48 min, 75% (A); 48–49 min, 74% (A); 49–53 min, 0% (A); 53–53.5 min, 0% (A); 53.5–57 min, 100% (A).

Standards used in the HPLC analysis of the phenolic acids and the phenylpropanoid derivative (scopoletin) were purchased as high purity grade: caffeic acid, *p*-coumaric acid, *o*-coumaric acid, scopoletin, ferulic acid, benzoic acid, salicylic acid, *trans*-cinnamic acid (Sigma-Aldrich Chemical Co., Burlington, MA, USA), *p*-hydroxybenzoic acid, gentisic acid, vanillic acid, syringic acid and *m*-coumaric acid (Acros Organics, USA). The retention time (t_R) and maximum of absorbance (λ_{max}) of each standard used individually or in a mixture were recorded. Five replicates were run for each genotype. The barley root exudate compounds were identified according to the description given by Banwart et al. [31] and Robbins and Bean [32], as well as their retention times (t_R) and UV spectra. The amounts of each phenolic acid were calculated from peak areas according to the calibration curves (0.4, 1.5, 2.9, 4.5, 5, 9 mg L^{-1}).

2.6. UPLC-ESI-MS Profiling of Allelochemical Compounds

The ultra-performance liquid chromatography-mass spectrometry (UPLC-ESI-MS) technique was used to identify compounds derived from the barley root exudates. The main peak of an unknown compound was located at 25.61 min in all the chromatograms of the six barley genotypes, except for the controls (Figure S1). This compound was purified by HPLC from the sandy soil extracts. The sample was then SpeedVac-concentrated and injected into the UPLC-ESI-MS system.

The analysis was performed using an Acquity UPLC Hclass and SQ Detector. LC separation was carried out on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm) preceded by an Acquity UPLC BEH C18 guard column (2.1 × 5 mm, 1.7 µm). The mobile phase consisted of two solvents: water/formic acid (A, 99:1, *v/v*) and acetonitrile/formic acid (B, 99:1, *v/v*). A 10 min linear gradient was programmed as follow: 0–5.67 min, 100% (A); 5.67–7.29 min, 80% (A); 7.29–7.77 min, 75% (A); 7.77–7.93 min, 74%

(A); 7.93–8.58 min, 0% (A); 8.58–8.66 min, 0% (A); 8.66–10 min, 100% (A). The injection volume was 10 µL of sample solution and the flow rate was 0.5 mL min^{-1}. The ionized conditions were optimized and the following parameters were used: nitrogen as a nebulizing gas at a rate of 1000 L hr^{-1}, capillary temperature 400 °C, capillary voltage 3 kV, source temperature 130 °C and cone voltage 60V. Positive ion mass spectra were recorded over an *m/z* range of 150–800. The pattern fragmentation of the target compound was compared to that of saponarin used as standard (Extrasynthèse Genay, France).

2.7. Effect of Barley Allelochemicals Identified as Candidates

Eight compounds (benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin) were identified as candidate allelochemicals that can be considered as biochemical markers of the allelopathic potential of barley root exudates. These compounds were tested separately in order to determine the phytotoxic effect of each molecule, and in mixture suggesting that the inhibitory action of barley root exudates might depend on the synergistic and/or additive effect of these molecules. The eight compounds were assessed on the growth of great brome and of the 'Manel' (low allelopathic potential) and 'Ardhaoui' (high allelopathic potential) barley genotypes, chosen with reference to present and previous study results [15,33]. Briefly, 10 sterilized and pre-germinated seeds were placed on filter paper (12–15 µm) in a 90 mm diameter Petri dish moistened with 4 mL of each compound; their equimolar mixture or sterile distilled water was used as a control. Three concentrations (1×10^{-5}, 1×10^{-4} and 1×10^{-3} M) were applied for the respective compound used individually or in mixture. These concentrations were chosen based on previous studies [34–36] and on the amounts of the majority of compounds ($\sim 10^{-5}$ M) identified in the barley soil of the present study (data not shown). The experiment was arranged in completely randomized block with five replicates per treatment. After 5 days, the radicle and coleoptile lengths of the great brome and barley seedlings were recorded.

2.8. Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) using PROC MIXED of the SAS package (version 9.1 for Windows; SAS Institute, Cary, NC, USA) and the subroutine PDMIX 800.SAS in order to compare means based on Least Significant Difference (LSD) at a 5% level of probability. The rate of increase in total phenolic content of the soil samples containing barley plants compared with bare soils (Control), and the rate of inhibition of morphological traits, were calculated as [(Control − Treatment)/Control] × 100. A linear regression analysis was performed on these two variables in order to establish their mutual relationship. Multiple linear regression analysis (stepwise) using the PROC REG procedure was used to analyze the relationship between the compound amounts and the inhibition rates of the four morphological parameters of great brome.

3. Results

3.1. Total Phenolic Content According to Soil Type

The ANOVA showed highly significant differences in phenolic content among the barley genotypes (G) and growing substrates (S) (all $p < 0.001$; Table 1). There was a highly significant interaction between the two variables (G × S; $p < 0.001$). Under these conditions, the rate of increase in phenolic content was higher in the sandy substrate (94.2%) for all genotypes than in the sandy-clay-loam substrate (45.7%).

The results showed a positive correlation between the rate of increase in total phenolic content and root length ($p = 0.032$), leaf length ($p = 0.009$) and root dry weight ($p = 0.005$) inhibition in the sandy-clay-loam substrate (Figure 1). In the sandy substrate, 'Manel' (49.8%) and 'Tej' (52.4%), the least toxic genotypes against great brome, had a higher phenolic content than 'Rihane' (38.7%) (Figure 1; Tables S2 and S3). 'Arbi' (176.8%), 'Ardhaoui' (73.5%) and 'Saudi' (174.1%) varied in the rates of increase in total phenolic content, but they had similar allelopathic activity.

Table 1. Increase rate of total phenolic content (%) according to tested genotypes and type of substrate. Values represent the mean ± SE of five replicates and different letters indicate significant differences at $p \leq 0.05$ (Least Significant Difference (LSD) test). The associated df, *F* and *p*-value calculated by an analysis of variance (ANOVA) is shown for genotype (G), substrate (S) and their interaction (G × S).

Factors	Increase Rate of Total Phenolic Content (%)
Genotype (G)	
Manel	37.8 ^{a}
Tej	39.8 ^{a}
Rihane	32.6 ^{a}
Arbi	117.1 ^{b,c}
Ardhaoui	71.9 ^{a,b}
Saudi	120.6 ^{c}
df	5
F	16.4
P	<0.001
Substrate (S)	
Sandy	94.2 ^{b}
Sandy-clay-loam	45.7 ^{a}
df	1
F	35.1
P	<0.001
G × S	
df	5
F	6.66
P	<0.001

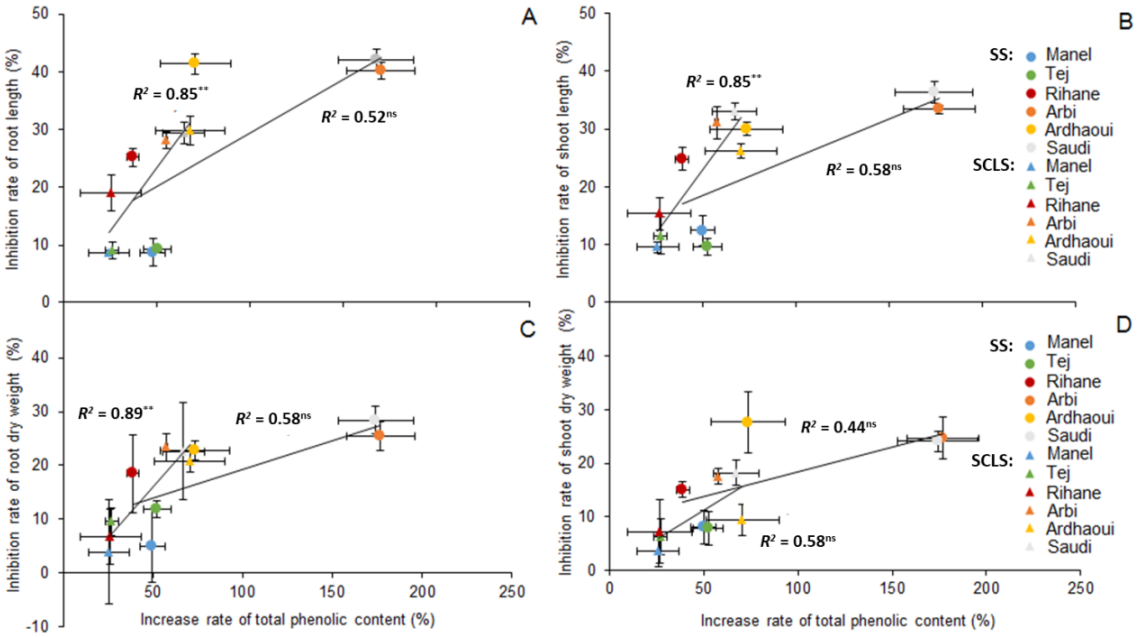

Figure 1. Relationship between the increase rate of total phenolic content (mean ± horizontal SE of five replicates) and the inhibition rate of (**A**) root and (**B**) shoot length and (**C**) root and (**D**) shoot dry weight of great brome (*B. diandrus*) plants (mean ± vertical SE of five replicates, each with 10 plants). Different symbols indicate the barley genotypes cultivated in sandy substrate (SS; circle symbols) or in sandy-clay-loam substrate (SCLS; triangle symbols). The coefficients of regression (R^2) are given and followed by the level of significance: ^{ns} $p > 0.05$, * $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. Identification of Barley Root Exudate Allelochemicals

Based on their UV spectra and retention time, 10 phenolic acids and one phenylpropanoid derivative (scopoletin) were identified from the root exudates of the six barley genotypes (Table 2) in addition to an unknown major compound eluting at t_R = 25.61 min (λ_{max} = 335 nm) and accumulating differentially in the various extracts (Figure S1). This compound was purified by HPLC from these extracts and the pure fraction was further analyzed by UPLC-ESI-MS in order to identify this main compound. Chromatograms obtained upon analysis in the ESI negative and positive modes revealed a single pure peak eluting at 4.48 min with *m/z* 593.22 and *m/z* 617.41 corresponding respectively to the [M-H]- (Figure 2A,B) and $[M+Na]^+$ ions species (Figure 2C,D). Based on this molecular mass of 594.4, the compound was tentatively identified as saponarin ($C_{27}H_{30}O_{15}$) which was further confirmed based on the similar t_R at 4.48 min and λ_{max} 335 nm, identical molecular ions species and identical fragmentation pattern compared to the commercial standard analyzed under the same conditions.

Table 2. Retention time (t_R) and maximum of absorbance (λ_{max}) of barley root exudate compounds, followed by the analysis of variance (ANOVA) for the six genotypes.

Compounds.	t_R (min)	λ_{max} (nm)	df	*F*	*P*
p-hydroxybenzoic acid	13.85	258	5	12.1	<0.001
Gentisic acid	14.24	330	5	84.4	<0.001
Vanillic acid	17.42	258	5	8.6	<0.001
Caffeic acid	18.37	330	ND	ND	ND
Syringic acid	19.47	258	5	23.8	<0.001
p-coumaric acid	24.61	330	5	7.1	0.001
Saponarin	25.61	335	5	17.8	<0.001
Scopoletin	27.33	330	5	11.0	<0.001
Ferulic acid	28.14	330	5	0.7	0.644
m-coumaric acid	30.04	330	ND	ND	ND
Benzoic acid	31.23	258	5	47.4	<0.001
Salicylic acid	33.69	245	5	200.8	<0.001
o-coumaric acid	34.88	330	5	68.4	<0.001
IS	44.23	258	–	–	–
trans-cinnamic acid	46.68	258	5	22.2	<0.001

IS: internal standard; ND: not detected; –; not determined.

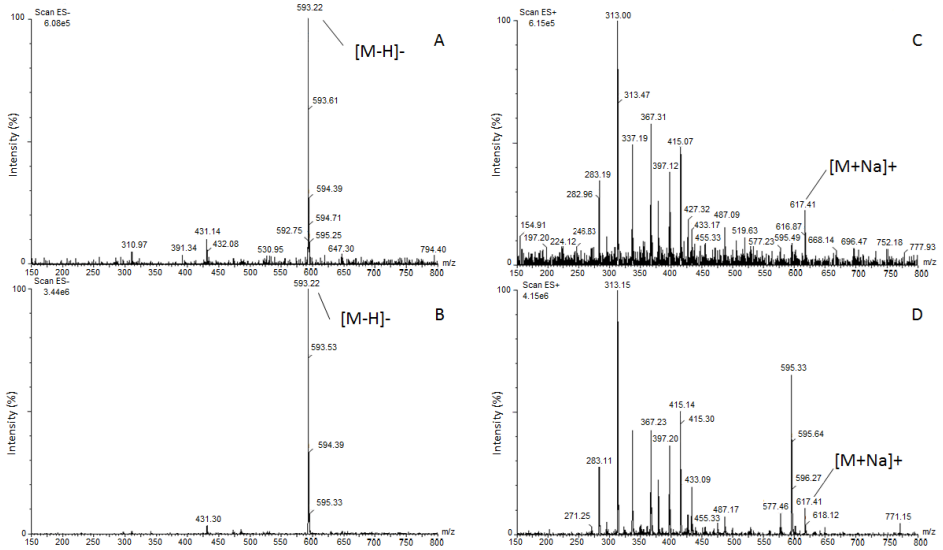

Figure 2. ESI-mass spectrometry (MS) full scan spectra of saponarin obtained in negative $[M - H]^-$ and positive $[M + Na]^+$ ion modes from sandy extracts pre-cultivated by (**A**,**C**) 'Saudi' genotype and (**B**,**D**) standard, respectively.

The barley genotypes differed significantly in their ability to exude the identified compounds (Table 2 and Table S2). Stepwise regression was performed to find out the major allelochemicals, which significantly associated with the inhibitory activity of barley roots (Table 3). The dependent variables were the inhibition rate of root and shoot length, root dry and shoot dry weight of great brome plants, while the amounts of 12 compounds identified from barley soil were used as independent variables (predictors). Overall, benzoic and *o*-coumaric acids and saponarine were chosen as predictive variables of the inhibition rate of root length to explain 72.4% of the genotypic variability. For the inhibition rate of leaf length, benzoic acid was also the first independent variable chosen by the model followed by *p*-hydroxybenzoic, *o*-coumaric, vanillic and salicylic acids. Otherwise, benzoic acid, *o*-coumaric acid, saponarin and vanillic acid were the predictive variables of the inhibition rate of root dry weight (73.5% of the variability), while the benzoic acid, *o*-coumaric acid and scopoletin were the predictive ones of the inhibition rate of shoot dry weight (88.3% of the variability). All of these compounds (i.e., benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin) might be a candidate allelochemicals of barley. Gentisic acid detected only in the highly allelopathic genotypes ('Arbi', 'Ardhaoui' and 'Saudi'), however, could also be allelochemicals of interest specific to these barley landraces.

Table 3. Multiple linear regressions (stepwise) explaining inhibition rate (%) of four morphological parameters of great brome (*B. diandrus*) variation across genotypic groups as a dependent variables, and the concentration (μg g^{-1} soil) of barley root exudate compounds as independent. Levels of significance are as follows: ^{ns} $p > 0.05$; * $p \leq 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Dependent Variables	Variable Chosen	R^2
Inhibition rate of root length	Benzoic acid	0.88 ***
	Benzoic acid, *o*-coumaric acid	0.93 ***
	Benzoic acid, *o*-coumaric acid, Saponarin	0.97 ***
Inhibition rate of shoot length	Benzoic acid	
	Benzoic acid, *p*-hydroxybenzoic acid	0.71 ***
	Benzoic acid, *p*-hydroxybenzoic acid, *o*-coumaric acid	0.74 ***
	Benzoic acid, *p*-hydroxybenzoic acid, *o*-coumaric acid, Vanillic acid	0.79 ***
	Benzoic acid, *p*-hydroxybenzoic acid, *o*-coumaric acid, Vanillic acid, Salicylic acid	0.83 ***
		0.86 ***
Inhibition rate of root dry weight	Benzoic acid	0.90 ***
	Benzoic acid, *o*-coumaric acid	0.92 ***
	Benzoic acid, *o*-coumaric acid, Saponarin	0.95 ***
	Benzoic acid, *o*-coumaric acid, Saponarin, Vanillic acid	0.96 ***
Inhibition rate of shoot dry weight	Benzoic acid	0.70 ***
	Benzoic acid, *o*-coumaric acid	0.83 ***
	Benzoic acid, *o*-coumaric acid, Scopoletin	0.86 ***

3.3. Effect of Allelochemicals Identified as Candidates

The ANOVA (Table 4) showed highly significant differences ($p < 0.001$) in root and coleoptile growth inhibition among the tested molecules (benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin), applied concentrations (10^{-5}, 10^{-4} and 10^{-3} M) and receiver species (great brome, 'Manel', 'Ardhaoui'). A significant triple interaction compound × concentration × species was obtained for radicle growth.

Table 4. Analysis of variance (ANOVA) for the inhibition rates of radicle and coleoptile length of receiver-allelochemical seedlings with different treatments.

Factors	Inhibition Rate of Radicle Length (%)			Inhibition Rate of Coleoptile Length (%)		
	df	*F*	*P*	Df	*F*	*P*
Compound (Comp.)	8	138.3	<0.001	8	58.8	<0.001
Concentration (Conc.)	2	1004.6	<0.001	2	366.1	<0.001
Species (Sp.)	2	17.8	<0.001	2	62.3	<0.001
Comp. × Conc.	16	61.9	<0.001	16	31.9	<0.001
Comp. × Sp.	16	7.3	<0.001	16	3.1	<0.001
Sp. × Conc.	4	9.4	<0.001	4	5.5	<0.001
Comp. × Conc. × Sp.	34	1.9	0.001	34	1.3	0.133

In most cases, the growth of weed and barley seedlings after five days of exposure to eight compounds with medium (10^{-4} M) and high (10^{-3} M) concentrations was significantly different from that of the control seedlings (Tables 5 and 6). This inhibitory effect was less marked with the low concentration (10^{-5} M). Inhibition in the receiver species proved to be dose-dependent, and a stimulatory effect was observed with low concentrations (10^{-5} M) of benzoic, *p*-hydroxybenzoic and gentisic acids or a mixture of compounds. The level of inhibition among the tested compounds against receiver species varied. Scopoletin, saponarin and salicylic acid were the most toxic compounds against radicle and coleoptile growth in great brome, 'Manel' and 'Ardhaoui'. Mixtures had a greater toxic effect than individual compounds using medium and high concentrations (10^{-4} and 10^{-3} M), apart from 10^{-4} M scopoletin, which was more inhibitory than the- mixture on coleoptile growth in great brome and 'Ardhaoui'.

In addition, receiver species responded differentially to the inhibitory effect of the compounds, with great brome being more sensitive than 'Manel' and 'Ardhaoui, apart from the inhibitory action of gentisic acid and vanillic on root growth and of vanillic acid on coleoptile growth. Interestingly, benzoic acid was the allelochemical of barley, which showed both high toxicity against great brome and the lowest toxicity against barley genotypes. This compound was followed by the mixture of compounds, saponarin and salicylic acid. Comparing the two barley genotypes, 'Manel' was more sensitive than 'Ardhaoui' for most of the tested compounds.

4. Discussion

4.1. Variation in Total Phenolic Content According to Soil Type

Phenolic acids were among the compounds exuded by barley roots after 30 days of growth. The amount of phenolic acids was higher in sandy substrate than in sandy-clay-loam substrate (Table 1), indicating that environmental context might affect the production, secretion and/or diffusion mechanisms of these compounds by the different barley genotypes (G × S; $p < 0.001$). Previous reports have shown that the synthesis of several phenolic compounds [37] or accumulation of hordatine in wild barley leaves (*Hordeum spontaneum* C. Koch) [38] depended on the physicochemical proprieties of the soil. The sorption of some phenolic compounds (e.g., ferulic, *p*-coumaric and veratric acids), however, was positively correlated with the clay content of the soil, percentage of organic matter, pH, etc. [39–42]. No significant correlations were obtained between the increase rate of total phenolic content and the inhibition rate of morphological parameters of great brome in the sandy substrate, as opposed to the sandy-clay-loam substrate (Figure 1). The inhibitory action of barley root exudates seems to depend partly on total phenolic acids, as our experiments showed, but some individual compounds, including hordenine [21,23], a well-known barley allelochemical of roots, could also be involved. A second hypothesis, that the allelopathic potential of barley might be associated with phenolic acid composition rather than the total concentration of these compounds, was proposed by Oueslati et al. [43] in their study of the autotoxicity of Tunisian barley residues.

4.2. Allelochemicals of Barley Root Exudates

The allelochemicals were identified in sterile soils in order to highlight their initial forms exuded by barley roots. In fact, soil biota are able to reduce or enhance the allelopathic potential, possibly by degrading allelochemicals [27]. The secretion of phenolic acids and one phenylpropanoid derivative (scopoletin) by the roots of the six barley genotypes differed quantitatively and qualitatively (Table 2). Similar differences in the allelochemical content of residues of various plant organs, living plants and flour have been described previously [23,43,44], with the conclusion that the production of these compounds is controlled by genetic factors in barley. In order to assess the relative contribution of the identified compounds in growth inhibition of weeds, we performed a regression analysis according to the stepwise procedure (Table 3). In particular, concentrations of benzoic, *o*-coumaric, vanillic, salicylic and *p*-hydroxybenzoic acids and scopoletin were significantly correlated with the inhibitory action of barley root exudates (Table 3) and are candidates as biochemical markers of the allelopathic potential of Tunisian barley root exudates. The gentisic acid did not exhibit a significant correlation, but was found only in the highly allelopathic genotypes ('Arbi', 'Ardhaoui' and 'Saudi). This acid might also be allelochemical of interest, but limited to a few genotypes. Overall, these results are in partial agreement with those from previous research on barley allelopathy. For example, Oueslati et al. [43] also showed that *p*-hydroxybenzoic acid was associated with the autotoxicity of Tunisian barley residues, in addition to syringic and *p*-coumaric acids, but these two latter compounds were not linked to the allelopathic potential of barley root exudates in our study. Baghestani et al. [22] reported similar findings for vanillic acid and scopoletin, showing them to be responsible for barley root exudate toxicity, in addition to *o*-coumaric and *p*-coumaric acids. In our study, the latter compound was not linked to the toxicity of Tunisian barley roots. Baghestani et al. [22] also reported that the *p*-hydroxybenzoic and benzoic acids did not discriminate between high and low allelopathic-potential barley cultivars. In our study, caffeic and *m*-coumaric acids were not detected, although they were present in other barley germplasm [22,45]. It would appear, therefore, that phenolic acid profiles and their involvement in allelopathic reactions might depend on genotype, organ and/or experimental conditions.

This study reported, for the first time, the presence of the flavonoid saponarin in barley root exudates, which was significantly correlated with their ability to inhibit the growth of great brome (Table 3). This compound had earlier been found in barley leaves and was considered to be the major flavone glucoside accumulated during the development of first leaves [46–48], as well as being present in roots, but only after treatment with herbicides [49]. It has also been found in other plant species (e.g., cucumber, Barbados aloe, passion flower) [50–52] and mosses [53] and is known for its antioxidant [54,55], hypoglycemic [56], hepatoprotective [57], anti-inflammatory [58], antibacterial [59], UV-protective [60] and phytotoxic activity against several cultivated species [53,61]. As far as we know, however, the effectiveness of this bioactive compound against weeds has not been described.

4.3. Effect of Allelochemicals Identified as Candidates

The effect of eight purified compounds (benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin) on the growth of great brome, 'Manel' and 'Ardhaoui' showed inhibitory action (Tables 5 and 6), but there were also stimulatory effects at low concentrations (10^{-5} M). This response was more pronounced for radicle growth than coleoptile growth, as reported in previous studies [62–64]. Roots might be the primary target of allelochemicals because they are in direct contact with external solutions from the soil or with artificial growing media. The higher susceptibility of roots might explain the highly significant triple interaction compound × concentration × species for roots compared with coleoptile growth (Table 4).

In our study, scopoletin, saponarin and salicylic acid were the most toxic compounds for radicle and coleoptile growth in receiver species, particularly great brome (Tables 5 and 6). Earlier reports confirm the efficiency of these compounds in the allelopathic inhibitory action of plants and their usefulness as potential bioherbicides. Scopoletin, already known as a natural inhibitor of growth, is thought to be induced in cultivated plant species by stress conditions [65–67] and has been assessed for its antifungal,

insecticide [68] and herbicidal [69] activities. Saponarin has been reported to be the most active flavonoid in inhibiting wheat [61] and radish growth [53]. Salicylic acid, a well-known plant growth regulator, has been shown to enhance the weed-suppression ability of rice [70]. Interestingly, benzoic acid, the mixture of compounds, saponarin and salicylic acid were particularly inhibitory against great brome and the lowest aggressive against barley suggesting their effectiveness in the biological control of this weed. Benzoic acid showed an inhibitory effects on a range of plant physiological processes, including water conductivity and the absorption of nutrients by the roots [71,72]. This compound has also been used as a commercial post-emergence herbicide named 'Dicamba' [73].

In our study, *o*-coumaric, vanillic, *p*-hydroxybenzoic and gentisic acids showed lower toxicity than scopoletin, saponarin and salicylic acid (Tables 5 and 6). These results differed from those reported for other plant species [62,74,75]. For example, the exogenous application of 1.44 mM *p*-hydroxybenzoic acid reduced yield components and final grain yield in spring barley [62]. This investigation revealed also that two barley allelochemicals, gentisic acid and vanillic acid, were more toxic against barley-itself than great brome. Overall, the results of this study showed that the water-soluble compounds of barley that determine its allelopathic potential seem to depend on several factors: donor genotype, applied concentration, receiver species (Table 4) and measured traits.

The mixtures of allelochemical compounds (benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin) were more effective growth inhibitors than each individual compound (in medium and high concentrations of 10^{-4} and 10^{-3} M; Tables 5 and 6). Similar findings were reported by Liu and Lovett [21] and Hura et al. [76], suggesting an additive and/or synergistic effect of these compounds in inhibiting the growth of receiver plants. Antagonistic effects have also been reported, depending primarily on the tested compounds and secondly on their concentrations [77].

Table 5. Root length of great brome (*B. diandrus*) and barley seedlings after 5 days of exposure to eight compounds identified from the barley root exudates, followed by the inhibition rate (%) of this parameter (in italic). Values represent the mean ± SE of five replicates, each with 10 seedlings and different letters indicate significant differences at $p \leq 0.05$ (LSD test) within each compound.

	Concentrations	Benzoic Acid	*o*-coumaric Acid	Saponarin	Vanillic Acid	Salicylic Acid	Scopoletin	*p*-hydroxybenzoic Acid	Gentisic Acid	Mixture
Brome	Control	9.48 ± 0.54 ^{c}	9.48 ± 0.54 ^{c}	9.48 ± 0.54 ^{c}	9.48 ± 0.54 ^{b}	9.48 ± 0.54 ^{c}	9.48 ± 0.54 ^{c}	9.48 ± 0.54 ^{ab}	9.48 ± 0.54 ^{bc}	9.48 ± 0.54 ^{c}
	10^{-5} M	9.19 ± 0.48 ^{bc} *(3.1)*	8.06 ± 0.45 ^{b} *(14.9)*	9.88 ± 0.28 ^{c} *(−4.2)*	9.13 ± 0.32 ^{ab} *(3.8)*	7.77 ± 0.36 ^{b} *(18.1)*	9.06 ± 0.41 ^{c} *(4.4)*	10.16 ± 0.39 ^{b} *(−7.2)*	10.74 ± 0.40 ^{c} *(−10.2)*	9.24 ± 0.27 ^{c} *(2.5)*
	10^{-4} M	8.19 ± 0.33 ^{b} *(13.6)*	7.13 ± 0.40 ^{b} *(24.8)*	8.16 ± 0.25 ^{b} *(13.9)*	8.97 ± 0.45 ^{ab} *(5.4)*	7.04 ± 0.45 ^{b} *(25.7)*	4.55 ± 0.26 ^{b} *(58.4)*	9.05 ± 0.40 ^{a} *(4.6)*	8.56 ± 0.29 ^{ab} *(9.7)*	3.51 ± 0.19 ^{b} *(62.9)*
	10^{-3} M	2.17 ± 0.33 ^{a} *(77.1)*	5.81 ± 0.39 ^{a} *(38.7)*	1.83 ± 0.11 ^{a} *(80.7)*	8.03 ± 0.26 ^{a} *(15.3)*	2.89 ± 0.22 ^{a} *(69.5)*	1.34 ± 0.23 ^{a} *(85.6)*	8.42 ± 0.38 ^{a} *(11.2)*	7.84 ± 0.49 ^{a} *(17.3)*	0.54 ± 0.09 ^{a} *(94.3)*
Average		*(31.62)*	*(26.13)*	*(30.13)*	*(8.16)*	*(37.76)*	*(49.46)*	*(2.86)*	*(5.6)*	*(53.23)*
Manel	Control	11.07 ± 0.46 ^{c}	11.07 ± 0.46 ^{c}	11.07 ± 0.46 ^{b}	11.07 ± 0.46 ^{b}	11.07 ± 0.46 ^{d}	11.07 ± 0.46 ^{c}	11.07 ± 0.46 ^{ab}	11.07 ± 0.46 ^{b}	11.07 ± 0.46 ^{c}
	10^{-5} M	11.74 ± 0.54 ^{c} *(−6.1)*	9.35 ± 0.41 ^{b} *(15.6)*	12.03 ± 0.24 ^{c} *(−8.7)*	8.93 ± 0.32 ^{a} *(19.7)*	9.13 ± 0.41 ^{c} *(17.5)*	10.52 ± 0.43 ^{c} *(4.9)*	11.97 ± 0.36 ^{b} *(−8.2)*	11.19 ± 0.41 ^{b} *(−1.1)*	12.24 ± 0.34 ^{d} *(−10.6)*
	10^{-4} M	9.65 ± 0.26 ^{b} *(12.9)*	8.37 ± 0.62 ^{b} *(24.4)*	10.34 ± 0.21 ^{b} *(6.6)*	8.68 ± 0.34 ^{a} *(21.7)*	7.72 ± 0.39 ^{b} *(30.3)*	5.36 ± 0.38 ^{b} *(51.6)*	10.70 ± 0.23 ^{a} *(3.3)*	9.40 ± 0.34 ^{a} *(15.1)*	5.87 ± 0.16 ^{b} *(46.9)*
	10^{-3} M	6.58 ± 0.34 ^{a} *(40.6)*	6.95 ± 0.40 ^{a} *(37.2)*	3.03 ± 0.22 ^{a} *(72.6)*	8.29 ± 0.34 ^{a} *(25.1)*	5.44 ± 0.30 ^{a} *(50.9)*	2.65 ± 0.29 ^{a} *(76.1)*	9.88 ± 0.60 ^{a} *(10.8)*	9.38 ± 0.46 ^{a} *(15.3)*	1.58 ± 0.10 ^{a} *(85.7)*
Average		*(15.8)*	*(25.73)*	*(23.5)*	*(22.16)*	*(32.9)*	*(44.2)*	*(1.96)*	*(9.76)*	*(40.66)*
Ardhaoui	Control	12.24 ± 0.62 ^{b}	12.24 ± 0.62 ^{b}	12.24 ± 0.62 ^{b}	12.24 ± 0.62 ^{b}	12.24 ± 0.62 ^{c}	12.24 ± 0.62 ^{d}	12.24 ± 0.62 ^{a}	12.24 ± 0.62 ^{b}	12.24 ± 0.62 ^{c}
	10^{-5} M	12.14 ± 0.31 ^{b} *(0.9)*	10.93 ± 0.49 ^{b} *(10.8)*	13.57 ± 0.68 ^{b} *(−10.9)*	10.15 ± 0.63 ^{a} *(17.2)*	10.77 ± 0.43 ^{b} *(12.0)*	10.45 ± 0.53 ^{c} *(14.7)*	12.63 ± 0.43 ^{a} *(−4.2)*	10.29 ± 0.35 ^{a} *(15.9)*	12.87 ± 0.73 ^{c} *(−5.1)*
	10^{-4} M	11.89 ± 0.40 ^{b} *(2.9)*	10.66 ± 0.46 ^{b} *(12.9)*	12.18 ± 0.80 ^{b} *(0.43)*	9.87 ± 0.42 ^{a} *(19.4)*	9.93 ± 0.48 ^{b} *(18.9)*	6.87 ± 0.43 ^{b} *(43.9)*	11.80 ± 0.35 ^{a} *(3.7)*	10.24 ± 0.65 ^{a} *(16.4)*	5.90 ± 0.22 ^{b} *(51.7)*
	10^{-3} M	8.71 ± 0.39 ^{a} *(28.9)*	7.72 ± 0.64 ^{a} *(36.9)*	3.49 ± 0.18 ^{a} *(71.5)*	9.49 ± 0.36 ^{a} *(22.6)*	6.85 ± 0.37 ^{a} *(44.0)*	3.59 ± 0.25 ^{a} *(70.7)*	11.64 ± 0.31 ^{a} *(4.9)*	9.71 ± 0.46 ^{a} *(20.7)*	2.24 ± 0.11 ^{a} *(81.7)*
Average		*(10.9)*	*(20.2)*	*(20.34)*	*(19.73)*	*(24.96)*	*(43.1)*	*(1.46)*	*(17.66)*	*(42.76)*

Table 6. Coleoptile length of great brome (*B. diandrus*) and barley seedlings after 5 days of exposure to eight compounds present in the barley root exudates, followed by the inhibition rate (%) of this parameter (in italic). Values represent the mean ± SE of five replicates, each with 10 seedlings and different letters indicate significant differences at $p \leq 0.05$ (LSD test) within each compound or each species.

	Concentrations	Benzoic Acid	*o*-coumaric Acid	Saponarin	Vanillic Acid	Salicylic Acid	Scopoletin	*p*-hydroxybenzoic Acid	Gentisic Acid	Mixture	Average
Brome	Control	6.95 ± 0.30 ^{b}	6.95 ± 0.30 ^{a}	6.95 ± 0.30 ^{c}	6.95 ± 0.30 ^{b}	6.95 ± 0.30 ^{c}	6.95 ± 0.30 ^{c}	6.95 ± 0.30 ^{c}	6.95 ± 0.30 ^{bc}	6.95 ± 0.30 ^{d}	
	10^{-5} M	6.59 ± 0.21 ^{b} *(5.2)*	7.03 ± 0.36 ^{a} *(−1.2)*	6.15 ± 0.29 ^{b} *(11.4)*	6.61 ± 0.21 ^{ab} *(4.9)*	6.54 ± 0.22 ^{bc} *(5.9)*	6.90 ± 0.19 ^{c} *(0.7)*	6.39 ± 0.29 ^{bc} *(8.1)*	7.27 ± 0.24 ^{c} *(−4.6)*	6.14 ± 0.35 ^{c} *(11.6)*	
	10^{-4} M	6.09 ± 0.19 ^{b} *(12.4)*	6.97 ± 0.29 ^{a} *(−0.2)*	5.88 ± 0.27 ^{b} *(15.4)*	6.47 ± 0.20 ^{ab} *(6.9)*	6.12 ± 0.24 ^{b} *(11.9)*	4.06 ± 0.31 ^{b} *(41.6)*	5.77 ± 0.36 ^{ab} *(17.1)*	6.15 ± 0.32 ^{ab} *(11.5)*	5.03 ± 0.16 ^{b} *(27.5)*	
	10^{-3} M	3.98 ± 0.42 ^{b} *(42.7)*	6.22 ± 0.21 ^{a} *(10.5)*	2.44 ± 0.19 ^{a} *(64.9)*	5.91 ± 0.30 ^{a} *(14.9)*	4.30 ± 0.34 ^{a} *(38.1)*	2.45 ± 0.28 ^{a} *(64.8)*	5.44 ± 0.24 ^{a} *(21.7)*	5.82 ± 0.28 ^{a} *(16.3)*	2.12 ± 0.17 ^{a} *(69.4)*	
Average		*(20.1)*	*(3.03)*	*(30.56)*	*(8.9)*	*(18.63)*	*(35.7)*	*(15.63)*	*(7.73)*	*(36.17)*	
Manel	Control	9.26 ± 0.26 ^{b}	9.26 ± 0.26 ^{a}	9.26 ± 0.26 ^{b}	9.26 ± 0.26 ^{b}	9.26 ± 0.26 ^{b}	9.26 ± 0.26 ^{c}	9.26 ± 0.26 ^{a}	9.26 ± 0.26 ^{b}	9.26 ± 0.26 ^{c}	
	10^{-5} M	9.57 ± 0.42 ^{b} *(−3.3)*	8.83 ± 0.27 ^{a} *(4.6)*	9.45 ± 0.31 ^{b} *(−2.0)*	8.04 ± 0.31 ^{a} *(13.2)*	9.04 ± 0.26 ^{b} *(2.4)*	8.95 ± 0.30 ^{c} *(3.3)*	9.09 ± 0.31 ^{a} *(1.8)*	8.83 ± 0.27 ^{ab} *(4.7)*	9.49 ± 0.31 ^{c} *(−2.5)*	
	10^{-4} M	9.49 ± 0.28 ^{b} *(−2.4)*	8.73 ± 0.24 ^{a} *(5.7)*	9.06 ± 0.29 ^{b} *(2.1)*	7.96 ± 0.29 ^{a} *(14.0)*	8.77 ± 0.36 ^{ab} *(5.3)*	7.35 ± 0.29 ^{b} *(20.6)*	8.79 ± 0.26 ^{a} *(5.1)*	8.42 ± 0.21 ^{ab} *(9.1)*	7.17 ± 0.23 ^{b} *(22.5)*	
	10^{-3} M	8.12 ± 0.28 ^{a} *(13.7)*	8.64 ± 0.44 ^{a} *(6.7)*	3.72 ± 0.30 ^{a} *(59.8)*	7.52 ± 0.23 ^{a} *(18.8)*	8.21 ± 0.23 ^{a} *(11.3)*	3.79 ± 0.40 ^{a} *(59.1)*	8.58 ± 0.47 ^{a} *(7.3)*	8.01 ± 0.55 ^{a} *(13.5)*	3.39 ± 0.20 ^{a} *(63.4)*	
Average		*(2.66)*	*(5.66)*	*(19.96)*	*(15.33)*	*(6.33)*	*(27.66)*	*(4.73)*	*(9.1)*	*(27.8)*	
Ardhaoui	Control	9.80 ± 0.29 ^{a}	9.80 ± 0.29 ^{ab}	9.80 ± 0.29 ^{b}	9.80 ± 0.29 ^{a}	9.80 ± 0.29 ^{b}	9.80 ± 0.29 ^{c}	9.80 ± 0.29 ^{a}	9.80 ± 0.29 ^{a}	9.80 ± 0.29 ^{c}	
	10^{-5} M	9.97 ± 0.35 ^{a} *(−1.6)*	9.67 ± 0.23 ^{ab} *(1.4)*	10.08 ± 0.44 ^{b} *(−2.9)*	9.86 ± 0.27 ^{a} *(−0.5)*	9.56 ± 0.21 ^{b} *(2.5)*	9.44 ± 0.26 ^{c} *(3.7)*	9.69 ± 0.27 ^{a} *(1.1)*	9.97 ± 0.15 ^{a} *(−1.7)*	10.16 ± 0.47 ^{c} *(−3.7)*	
	10^{-4} M	9.73 ± 0.26 ^{a} *(0.8)*	10.31 ± 0.33 ^{b} *(1.9)*	9.94 ± 0.47 ^{b} *(−1.42)*	9.66 ± 0.29 ^{a} *(1.5)*	9.19 ± 0.40 ^{b} *(6.3)*	8.07 ± 0.34 ^{b} *(17.8)*	9.54 ± 0.36 ^{a} *(2.7)*	9.61 ± 0.34 ^{a} *(2.0)*	8.34 ± 0.51 ^{b} *(14.8)*	
	10^{-3} M	9.49 ± 0.44 ^{a} *(3.2)*	9.47 ± 0.26 ^{a} *(9.9)*	5.22 ± 0.34 ^{a} *(46.7)*	9.29 ± 0.23 ^{a} *(5.3)*	8.24 ± 0.36 ^{a} *(16.0)*	5.01 ± 0.26 ^{a} *(48.9)*	9.12 ± 0.38 ^{a} *(7.0)*	9.41 ± 0.35 ^{a} *(4.1)*	4.56 ± 0.21 ^{a} *(53.4)*	
Average		*(0.8)*	*(4.4)*	*(14.13)*	*(2.1)*	*(8.26)*	*(23.46)*	*(3.6)*	*(1.46)*	*(21.5)*	
Average		(7.8)	(4.4)	(21.5)	(8.8)	(11.1)	(28.9)	(8.00)	(6.10)	(28.5)	

In the present study, we aimed to identify allelochemicals that combine both a good allelopathic potential against the enemies (i.e., great brome) and a low autotoxic effect against barley. Considering their effect on weed and barley, benzoic acid, the mixture of eight compounds, as well saponarin and salicylic acid could, therefore, be considered for weed control, but should be used with great caution. In general, the inhibitory effect of compounds used individually or in mixture was more pronounced with great brome, followed by 'Manel' and 'Ardhaoui', even though they all belong to the same family (Poaceae). Root exudates can carry information about the genetic drift [78] and can distinguish themselves from non-self species. It is also possible that the development of allelopathic potential is coupled with an ability to tolerate these toxic compounds by the donor plant, but further work is needed to confirm this hypothesis, using a large number of genotypes. In this context, the choice of the genotype sequence in monoculture systems commonly used in Tunisia would appear to be relevant.

5. Conclusions

Barley roots emit water-soluble molecules which depend on the genotype. The allelopathic potential of barley seems to depend in part to phenolic acids and might implicate other compounds. In particular, benzoic acid, the mixture of eight molecules (benzoic, *o*-coumaric, vanillic, salicylic, *p*-hydroxybenzoic and gentisic acids, scopoletin and saponarin), saponarin (a flavonoid newly identified in barley) and salicylic acid were the most efficient compounds against the great brome followed by 'Manel' and 'Ardhaoui' and might be considered in the biological control against weeds.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/345/s1, Table S1: Physico-chemical proprieties of the growing substrates. Values are means ± SE. of four replicates and different letters indicate significant differences at $p \leq 0.05$ (LSD test). Table S2. Inhibition rate of root and shoot length, and root and shoot dry weight of great brome (*B. diandrus*) grown in sandy and sandy-clay-loam substrates in which the six barley genotypes had previously developed for one month before being removed. Values are means of five replicates ± SE and different letters indicate significant differences at $p \leq 0.05$ (LSD test). Table S3. Total phenolic acid content (mg/g soil) according to tested genotypes and type of substrate. Values represent the mean ± SE of five replicates and different letters indicate significant differences at $p \leq 0.05$ (LSD test). Figure S1: Chromatograms of sandy soil extracts of the control and of those pre-cultivated by the six barley genotypes detected at 330 nm.

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