

## **Effects of physico-chemical and biological properties of soil on the allelopathic activity of barley (*Hordeum vulgare* L. subsp. *vulgare*) root exudates against *Bromus diandrus* Roth. and *Stelleria media* L. weeds**

I. Bouhaouel<sup>2\*</sup>, A. Gfeller<sup>a</sup>, Kh. Boudabous<sup>2</sup>, M.L. Fauconnier<sup>1</sup>, O. Slama Ayed<sup>2</sup>,  
H. Slim Amara<sup>2</sup> and P. Du Jardin  
Plant Biology Laboratory, Gembloux Agro-Bio Tech, University of Liège,  
2 Passage de Déportés, 5030 - Gembloux, Belgium.  
E-mail: imenbouhaouel@gmail.com

(Received in revised form: October 10, 2019)

### **ABSTRACT**

In greenhouse experiment, the allelopathic effects of 6-barley genotypes were assessed on the morphological features of weeds, *Bromus diandrus* Roth. and *Stelleria media* L. The inhibitory effects of root exudates depended on the barley genotype and the physico-chemical properties of the soil, and their interactions. The sandy soils with low organic matter and nutrients content showed more the allelopathic potential. A predictive model of the allelopathic activity of barley was proposed based on soil properties and tested weeds. Overall, the stepwise model showed that the content of phenolic acids was the major determinant of allelopathic activity, besides the soil chemical characteristics (electrical conductivity and carbon and sodium content). Soil microbial communities decreased the allelopathic activity of barley. Drainage and aeration might explain the slightly higher inhibitory activity in a non-autoclaved sandy substrate than a clay-loam substrate. When recommending allelopathic barley genotypes for cultivation, the environmental factors, physico-chemical properties of soil and rhizosphere microbiome might reduce or enhance their allelopathic potential.

**Key words:** Allelopathy, barley, *Bromus diandrus*, *Hordeum vulgare*, microorganisms, model, phenolic acids, soil, soil biological properties, soil physico-chemical, root exudates, *Stelleria media*, weeds.

### **INTRODUCTION**

The different non-herbicidal control methods (mechanical control etc.), ensures sustainable management of weeds and reduces the herbicide resistance development in weeds. Currently, the suppressive ability of crop plants through production of allelochemicals, or their direct use has received much attention and has been proposed as new biological strategy to control weeds in sustainable agriculture. These allelochemicals directly affects the growth and development of neighbouring plants through root exudation, or indirectly by changing the chemical and physical properties of the soil and microbial communities (11,30,66). This phenomenon, known as allelopathy may be harmful, due to autotoxicity in crop plants or by its role in plant invasiveness, or beneficial

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\*Correspondence author, <sup>1</sup>General and Organic Chemistry Laboratory, Gembloux Agro-Bio Tech, University of Liège, 2 Passage de Déportés, 5030 – Gembloux, Belgium, <sup>2</sup>Department of Agronomy and Plant Biotechnology, Genetics and Cereal Breeding Laboratory, National Agronomic Institute of Tunisia, University of Carthage, 43 Charles Nicolle Street, 1082 - Tunis Mahragene, Tunisia, <sup>a</sup>Current address: Swiss Federal Research Station Agroscope Changins Wädenswil AC, CH-1260 Nyon, Switzerland.

in weed biocontrol (19,54). The allelopathic compounds are more biodegradable and less harmful to the environment than synthetic chemical herbicides, hence, they are attractive alternatives to present herbicides which have caused development of herbicide resistance in weeds (56,65,69).

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is weed smothering crop (9,21,25), showing allelopathic potential against many weeds [chickweed (*Stellaria media* L.) (60), white mustard (*Sinapis alba* L.) (52), ryegrass (*Lolium perenne* L. and *Lolium rigidum* Gaudin) (8,16), bristly foxtail (*Setaria verticillata* L.) (24), wild mustard (*Brassica kaber* [DC.] L.C.) (59), barnyard grass (*Echinochloa crus-galli* P. Beauv.) (77), great brome (*Bromus diandrus* Roth.) (15) and others (26,77)]. The barley is effective as cover crop, smother crop or as mulch to suppress the weeds (24,60,63). This may be due to the presence of alkaloids (hordenine and gramine) and phenolic acids and their derivatives, such as scopoletin, and water-soluble allelochemicals.

Even so, the allelopathic effects of barley root exudates on weeds have been less studied compared to aqueous extracts of its residues or fresh material. The detection of allelopathic activity in living plant root tissues remains challenging due to the lack of reliable method to distinguish the chemical interference from resource competition (64). Our recent studies investigated the inhibitory effects of barley root exudates against the weed great brome (*Bromus diandrus* Roth., syn. *Bromus rigidus* Roth. subsp. *gussonii* Parl.) (15,16). In Tunisia, this species is troublesome grassy weed causing yield losses up to 80 % in heavily infested crops (71,72). Barley-great brome chemical interactions were assessed only in early growth stage (5 and 10 days old plants), using filter paper or agar medium in bioassays (15,16), and did not considered factors found in the field.

The allelopathic effects depend on two components, allelochemicals and the soil. Allelochemicals are synthesized and released by roots, and depends on various factors, (species, variety, phenological stage and biomass of the donor plant), while in natural conditions, the plant-plant chemical interactions depends on the soil (34). The chemical, physical and biological properties of soil, greatly affects the allelochemicals production (34), sorption and their fate (32). Soil pH, oxidoreductive conditions and sorbing materials significantly affects the allelochemicals concentration and activity (12,14). The impact of soil properties have been investigated on allelochemicals (23,42,75,76); but predictive model of barley allelopathic activity based on soil characteristics was not developed.

Soil biota may also influence the performance of allelochemicals from the time of their release until their contact with target plant. Some microbes degrade or inactivate the allelochemicals molecules; yet, they may also improve their efficacy by producing more potent molecules from less active precursors (39). These interactions largely explain the controversies that still exist, concerning the ecological and agronomic importance of these chemical interactions between the plants and the difficulty in defining them (20).

This study aimed to determine: (i) the allelopathic activity of root exudates of different barley genotypes against two weeds, great brome (*Bromus diandrus* Roth. family Poaceae, monocot) and chickweed (*Stellaria media* L. family Caryophyllaceae, Dicot) and (ii) the influence of soil physico-chemical components (pH, organic matter, carbon, nitrogen, phosphorus and potassium content, and microflora) on the allelopathic activity of barley and (iii) to develop predictive model of allelopathic activity of barley roots based on soil properties.

## MATERIALS AND METHODS

### I. Plant materials

Test barley (*Hordeum vulgare* L. subsp. *vulgare*) genotypes used in this study were: 3-Tunisian varieties ('Manel', 'Rihane' and 'Tej'), 2-Tunisian landraces ('Ardhaoui' and 'Arbi') and one Saudi Arabian barley landrace ('Saudi'). These genotypes were chosen based on the genetic diversity and their tolerance to abiotic stress (28,38). Barley seeds were obtained from the National Agronomic Institute of Tunisia. Seeds of weed great brome (*Bromus diandrus* Roth., syn. *Bromus rigidus* Roth. subsp. *gussonii* Parl.), were collected from infested sites in the Beja region, northern Tunisia (36°42'07.0"N, 9°12'46.3"E and 36°41'00.2"N, 9°13'09.8"E). Seeds of the common chickweed (*Stellaria media* L.) were purchased from Arbiotech (Rennes, France). This species was chosen based on its higher sensitivity to barley allelochemicals (16,60).

### II. Sterilization and pre-germination

Barley and great brome seeds were surface-sterilized as previously described (15,16). After sterilization, the seeds were maintained on moist sterile filter paper and placed in dark in growth chamber (22 °C, relative humidity: 65 %). To observe the allelopathic inhibition, barley and great brome seeds were pre-germinated for 72 and 96 h, respectively. The common chickweed seeds were incubated for 7 days (light/dark: 16/8 h, 22 °C and inflorescent light of  $3.56 \pm 0.16 \times 10^3$  lux).

### III. Soil physico-chemical properties and barley allelopathic activity

To determine the allelopathic effects of barley root exudates on weed growth (*B. diandrus* and *S. media*) and the effects of soil physico-chemical characteristics on the allelopathic potential, a greenhouse experiment was done using two substrates: (i) **Sandy substrate (Substrate 1)** and (ii) **Sandy-clay-loam substrate (Substrate 2, USDA classification system)**. The latter substrate was mixture of sand and soil (50:50) taken from surface layer of crop field (0-20 cm), National Agronomic Institute of Tunisia (36°49'52.4"N, 10°11'01.0"E). The physico-chemical properties of two substrates were determined using 12-parameters [sand, loam and clay content, pH, electrical conductivity (EC), percentage of organic matter (OM %) and carbon (C), and nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) content]. The soil particle size was evaluated by sedimentation (Robinson pipette). pH (1: 2, soil: water, w / v) and EC (EC, 1: 5, soil: water, w/v) was measured with pH meter (Consort C860) and a digital conductivity meter (Cond 330i / SET, Germany), respectively. Organic matter, C, total N (Kjeldahl method), P (Olsen and molybdenum blue methods), K (flame photometer), Na (flame photometer) and Ca (extraction with ammonium acetate solution) amounts were determined as per Pauwels *et al.* (61). Five replications were used for each analysis.

Based on previous studies (15,16), the 'donor' and the 'receiver' species were grown sequentially to reduce the competition effects. Thirty sodium hypochlorite disinfected pre-germinated seeds of each barley genotypes (donor species) were sown in polypropylene square pots (13x13 cms). Pots without barley seeds were used as control. Each pot contained 800 g autoclaved sandy or sandy-clay-loam substrate. The experimental treatments consisted of 3 factors (i) barley varieties: 6 ('Manel', 'Rihane', 'Tej', 'Ardhaoui', 'Arbi' and 'Saudi'), (ii) two weeds (great brome and chickweed) and (iii) two soil substrates (sandy and sandy-clay-loam substrates). i.e.  $6 \times 2 \times 2 = 24$  Total treatment

combinations. The treatments were replicated 5-times in randomised complete block design. The experiment was done in greenhouse [26/22 °C day/night temperature, 16h light/8 h dark photoperiod, with photon flux density of about 220  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and relative humidity 60 %]. The pots were irrigated daily with autoclaved tap water. The substrate was kept at 100 % water holding capacity to minimize the competition for water. To maintain saturation levels, the amount of water absorbed was completed every day. Boxes were placed under the pots to recover the water in case of flow. After 30 days, barley plants were removed. The substrate was then sieved using 2 mm mesh to remove any remaining barley roots. Thereafter, 10-pre-germinated seeds of weed: great brome or chickweed (receiver species) were sown on the same substrate. After 30 days, the above- and belowground parts of weed plants were harvested and root length, shoot length, root dry weight and shoot dry weight were recorded. Both the root and shoot parts of plants were removed and dried in oven at 70 °C for 72 h to determine dry matter content. The allelopathic effect of barley roots on weed growth was quantified by the rate of inhibition of these morphological traits, calculated as under:

$$\text{Rate of Inhibition: } (\text{Control} - \text{Treatment}) / \text{Control} \times 100$$

#### IV. Total phenolic contents exuded by barley roots

The phenolic acids contribute the greatest number of allelochemicals in barley (49). To determine the impact of physico-chemical properties of soil on allelochemicals, total phenols content of sandy and sandy-clay-loam substrates was determined (in which the 6-barley genotypes were grown for 30 days). This analysis was done using the Folin-Ciocalteu reagent (74). To extract the allelopathically active phenolic acids, water was used as extractant. Soil extracts (1:5 soil/water, w/v) were prepared as per Zhang *et al.* (78). A blank was used with distilled water instead of filtrate. The treatments were replicated 5- times. Optical density was determined with spectrophotometer (Shimadzu, Kyoto, Japan) at 700 nm. TPC was measured as the gallic acid equivalents used as standard (0, 2, 4, 6, 8 and 10 mg l<sup>-1</sup> prepared from a stock solution of 5 g l<sup>-1</sup>).

#### V. Effects of microorganisms on the allelopathic activity of barley

Two types of substrates: **sandy substrate (Substrate 1)** and **clay-loam substrate (Substrate 3)** sampled from the surface layer of crop field (0-20 cm), National Agronomic Institute of Tunisia (36°49'52.4"N, 10°11'01.0"E). To determine the role of microorganisms on the allelopathic activity of barley root exudates, a second treatment was added using autoclaved and non-autoclaved soils. Thirty (30) pre-germinated barley 'Ardhaoui' seeds were sown pots, each containing 800 g substrate). This barley landrace (high allelopathic potential) was chosen based on present and previous studies results (15,16). The experimental set-up was similar to that mentioned above. After 30 days, the barley plants were harvested and substrate was sieved as above. Thereafter, 10- pre-germinated seeds of great brome (*B. diandrus*) were sown. The root length (RL), shoot length (SL), root dry weight (RDW) and shoot dry weight (SDW) were recorded after 30 days of growth.

#### VI. Statistical analysis

All experimental data were analyzed using the SAS package (version 9.1 for Windows; SAS Institute, Cary, NC, USA). A two-way analysis of variance (ANOVA) was

performed using the PROC GLM procedure to assess the effect of genotypes, substrates (or substrate and sterilization) and their relative interactions for all morphological traits and total phenolic content. Means were compared by least significant difference (LSD) test ( $P < 0.05$ ). Pearson correlation coefficients were determined between all measured traits and the physico-chemical characteristics of the soil (e.g. N, P, K, % OM, pH) in order to establish their mutual relationship. Multiple linear regression analysis (stepwise) using the PROC REG procedure was used to analyze the relationship of morphological data with the physico-chemical traits of the soil including the total phenolic content.

## RESULTS AND DISCUSSION

### Effects of barley root exudates on weed growth

The allelopathic activity of barley root exudates was assessed against two weed species, great brome (*B. diandrus*) and chickweed (*S. media*), grown in two types of substrate, in which barley plants were first grown. After one month of culture, allelochemicals from barley roots reduced the growth of great brome and chickweed plants (Fig. 1). This inhibitory effects of 6-barley genotypes were significant than control in sandy and sandy-clay-loam substrates, except in genotypes 'Manel' and 'Tej' (data not shown).

In both weed species, there were significant variations in the allelopathic activity of barley genotypes ( $P < 0.001$ ) and growing substrates ( $P < 0.01$ ) on weeds seedlings growth (Fig. 1). However, a non-significant difference between the two types of growing substrate was observed for shoot length ( $P = 0.208$ ) and root dry weight ( $P = 0.097$ ) of great brome plants. There were significant (genotype x substrate) interactions between the morphological variables ( $P < 0.01$ ), except for root dry weight of great brome ( $P = 0.48$ ) and chickweed ( $P = 0.96$ ) plants. This suggested that the allelopathic activity of each barley genotype depended on the type of soil.

The inhibitory effects of barley roots affected the root and shoot length of great brome and chickweed to greater extent than root and shoot biomass (Fig. 1). However, barley root exudates had similar effects on the root and shoot growth of both weed species, suggesting that aboveground and belowground organs were affected by the growth inhibitory compounds. Even so, barley allelochemicals did not act simultaneously on both parts (i.e., root and shoot) of the weed plant during developmental stages, our previous research had shown that after 5 and 10 days of growth, great brome roots were the first and most affected organs (15,16).

The inhibition rate of four variables was higher in the sandy substrate for all genotypes, compared to sandy-clay-loam substrate. The barley genotypes had variable effects on the growth of weed species (Fig. 1). In sandy substrate, the rate of inhibition of root length and shoot length for great brome was 9 to 42 % and from 10 to 36 %, respectively, while for chickweed it was 39 to 60 % and 25 to 52 %, respectively. Under these conditions, barley landraces 'Saudi', 'Arbi' and 'Ardhaoui' showed higher inhibitory effects than modern varieties, 'Manel' and 'Tej'. This was consistent with previous findings that barley or wheat landraces are low-yielding, but defend themselves better against weed species (27). However, great brome did not react in the same way to barley root exudates. Indeed, the growth of great brome (e.g. the inhibition rates of root length in sandy and sandy-clay-loam substrates by the 6-barley genotypes were 27.8 % and 20.68

%, respectively) was less affected than chickweed (e.g. the inhibition rates of root length in sandy and sandy-clay-loam substrates by the six barley genotypes were 50.92 % and 30.86 %, respectively), confirming strong allelopathic potential of cultivated species. Our results suggested that barley allelochemicals might be less effective against species in same class and family. The dicots are more sensitive to allelochemicals than monocots (5,6). Further research is needed to confirm these hypotheses, using many weed species.

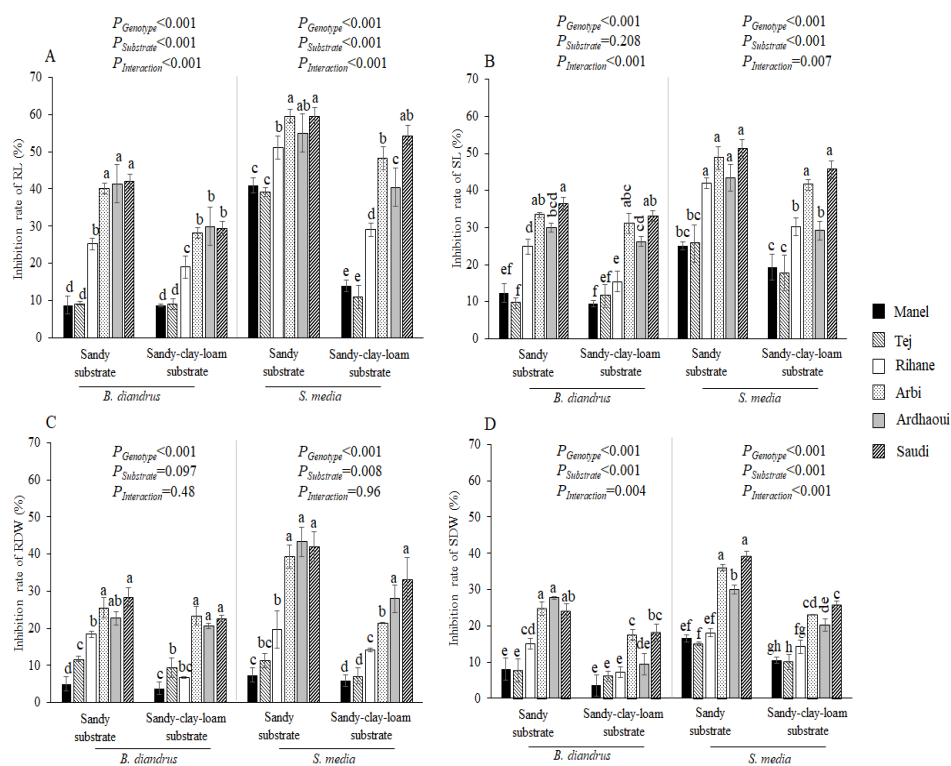


Figure 1. Inhibitory effects of preceding 6-barley genotypes grown for one month on the elongation of (A) root (B) shoot and dry weight of (C) root and (D) shoot of great brome (*B. diandrus*) and chickweed (*S. media*) grown in two substrates. For each weed, values are means of five replicates  $\pm$  SE. Data were analyzed by two-way ANOVA with barley genotype and type of substrate as sources of variation. In not-significant interactions between the factors for root dry weight parameter for both weed species, genotypic effects were separately evaluated for each substrate. Means followed by different letters are significantly different ( $P < 0.05$ ; LSD test). RL, root length; SL, shoot length; RDW, root dry weight; SDW, shoot dry weight.

### Relationship between the physico-chemical properties of soil and the allelopathic activity of barley

This study aimed to analyse the allelopathic potential of crop field soil, and to assess the impact of its physico-chemical properties on the barley-weed interactions. There were significant differences between the sand and sandy clay loam soils, texture, pH, EC, % OM, and N, P, K, C and Ca content (Table 1).

Table 1. Physico-chemical properties of growing substrates used to assess their effect on the allelopathic activity of barley.

Parameters	Substrate 1	Substrate 2	Substrate 3
Sand (%)	100±0.0a	68.51±0.0b	18.93±0.0b
Loam (%)	0±0.0a	8.08±0.0b	57.99±0.0b
Clay (%)	0±0.0a	24.61±0.0b	23.08±0.0b
Texture	Sandy substrate	Sandy-clay-loam substrate	Clay-loam substrate
pH	7.71±0.05a	8.35±0.05b	8.90±0.04b
CE (dSm <sup>-1</sup> )	0.075±0.001a	0.27±0.005b	0.316±0.005b
OM (%)	0.18±0.04a	1.29±0.14b	1.45±0.03b
C (%)	0.10±0.02a	0.75±0.08b	0.84±0.02b
N (g kg <sup>-1</sup> )	0.23±0.05a	0.57±0.12b	1.23±0.12b
P (mg kg <sup>-1</sup> )	3.25±0.25a	12.26±0.02b	17.17±0.01b
K (mg kg <sup>-1</sup> )	10±1.00a	30±0.01b	163.27±0.03b
Ca (mg kg <sup>-1</sup> )	17±2.00a	285±5.00b	317±5.00b
Na (mg kg <sup>-1</sup> )	13.5±3.5a	20±5.00a	22±4.00b

Values are means ± standard error and different letters indicate significant differences at  $P < 0.05$  according to LSD test. OM, organic matter; EC, electrical conductivity.

The soil texture significantly influenced the allelopathic activity of 6-barley genotypes. There were significant, negative correlations between the inhibition rate of root length of great brome plants and physico-chemical properties of growing substrate (% OM and N, P and K content) (Fig. 2). The same trend was also observed for shoot dry weight. However, root length and shoot dry weight were positively correlated with sand content. For chickweed, the inhibition rates of four morphological variables (i.e., root length, shoot length, root dry weight and shoot dry weight) were negatively correlated with various soil properties, though positively correlated with sand content (Fig. 2). No significant correlations were found between the inhibition rates of shoot length and root dry weight and Na content.

After one month of culture, the total phenols contained in sandy and sandy-clay-loam substrates and presumed to be exuded from barley roots were determined. There were highly significant differences in phenolic contents among the barley genotypes and the growing substrates ( $P < 0.001$ ; Fig. 3). A highly significant interaction was found between the two variables, suggested that the production/secretion of phenolics by each barley genotype depended on the soil type. TPC was significantly negatively correlated with physico-chemical parameters, such as [OM (%), N, P and K content], but was positively correlated with sand content (Fig. 4). However, this variable did not correlate with pH or Na content.

The inhibitory action of barley roots was more pronounced in sandy soil (Fig. 1, 2). Although leaching of allelochemicals is higher in sandy soils (46) but there was greater production, secretion of these compounds by plants in sandy soil. This also suggested that stressed plants, for example due to a nutrient deficient in sandy soils, spend energy on defence molecules. In this context, Oleszek and Jurzysta (58) reported that saponins were better absorbed by wheat plants in light soils than in heavy soils (contains more clay). In general, minerals in clay soils associate with organic constituents to form colloids (51). This might explain the low inhibitory effects of barley compounds in sandy-clay-loam

substrate (Fig. 1, 2). The adsorption of *p*-coumaric, ferulic and benzoic acids was positively correlated with the clay content of soil (17,42). This was not the case for all phenolic acids, particularly vanillic and *p*-hydroxybenzoic acids, because the adsorption depends on the molecular structure. Overall, cinnamic acid derivatives were more adsorbed by soil than benzoic acid derivatives (23,37).

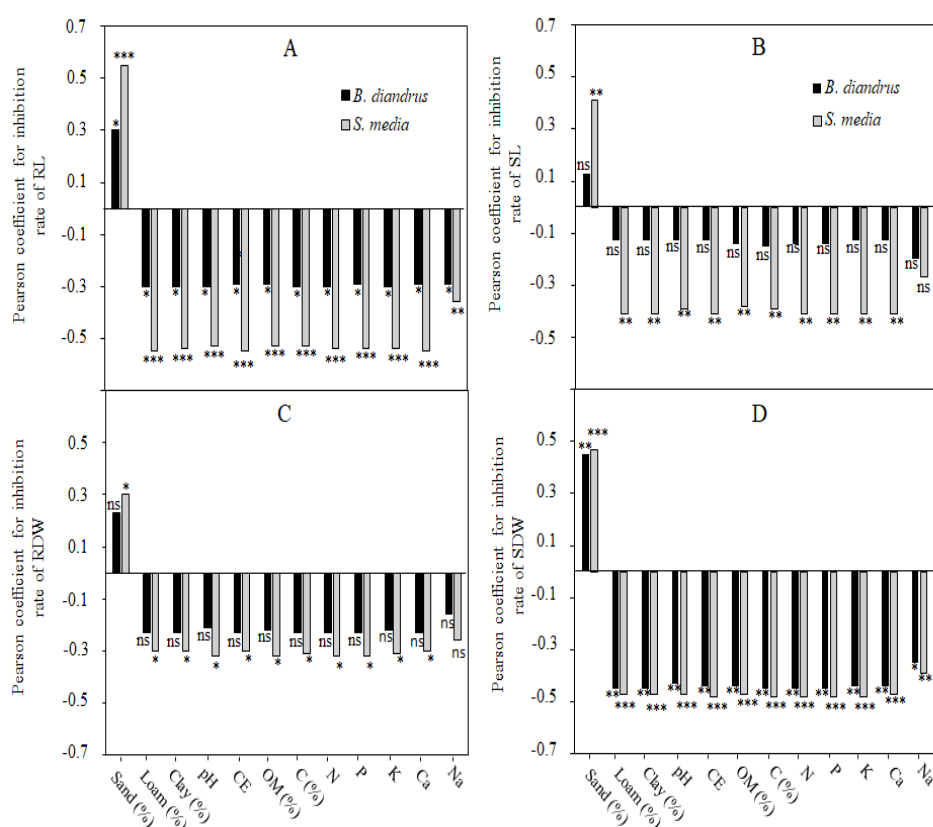


Figure 2. Pearson correlation coefficients of relationship between the inhibition rates of (A) root length, (B) shoot length, (C) root dry weight and (D) shoot dry weight of great brome (*B. diandrus*) or common chickweed (*S. media*) plants and the physico-chemical parameters of the soil. Levels of significance: ns  $P > 0.05$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . ns – not significant. RL, root length; SL, shoot length; RDW, root dry weight; SDW, shoot dry weight.

Furthermore, 87 % higher contents of OM and C content in sandy-clay-loam substrate soil than sandy substrate reduced the allelopathic effects of barley roots (Fig. 2). The OM is responsible for the production and/or adsorption of allelochemicals, including phenolic acids (Fig. 4). Dalton *et al.* (22) showed that OM is major source of irreversible adsorption of ferulic acid. However, in another study, higher toxicity of catechin was observed in OM-rich soils compared to poor soils (45).

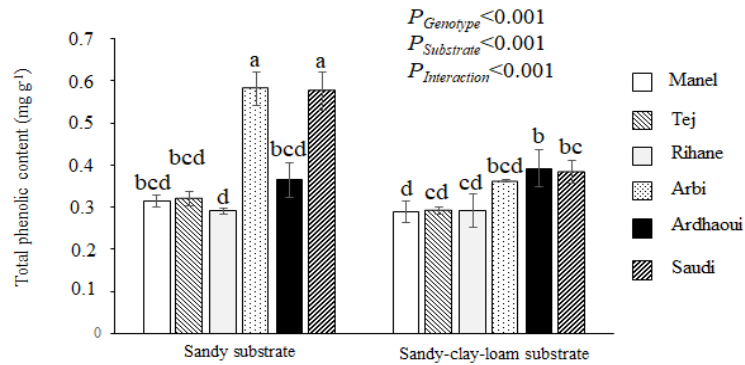


Figure 3. Total phenolic contents of 6-barley genotypes grown for 30 days in sandy and sandy-clay-loam substrates. Values are means of five replicates  $\pm$  SE. Data were analyzed by two-way ANOVA with barley genotype and type of substrate as sources of variation. Since interaction was significant between factors, all treatments were compared with each other. Means followed by different letters are significantly different ( $P < 0.05$ ; LSD test).

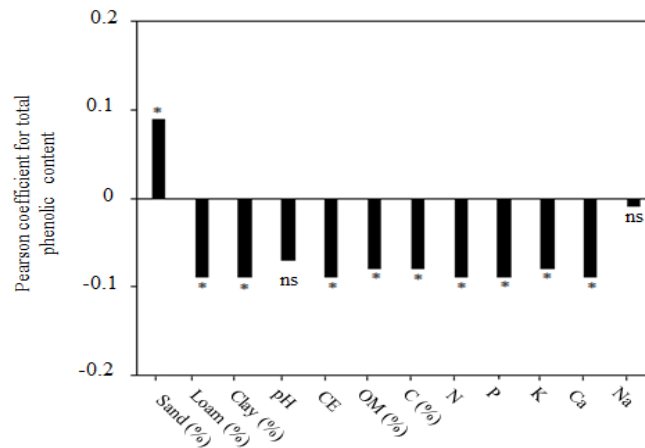


Figure 4. Pearson correlation coefficients of the relationship between the total phenolic content ( $\text{mg g}^{-1}$ ) and the physico-chemical properties of the soil. ns – not significant.

The variable allelopathic activity of barley was also attributed to the variations in the pH between the two substrates (Fig. 2). Higher pH reduced the inhibitory action of barley compounds, but had no effect on TPC in soils (Fig. 4). In conditions of this study, pH did not influence the production and/or adsorption of allelochemicals on soil particles, particularly phenolic acids. The pH affected the adsorption of vanillic and *p*-hydroxybenzoic acids, but not of veratric acid (17). However, pH affects the absorption of nutrients required for plant growth (2,31,35,68). The same trend was also observed for EC, i.e. the soil with more salt decreases the allelopathic potential.

These parameters do not affect the root exudation and sorption of allelochemicals. Phenolic acids can be strongly adsorbed to the soil, due to ligand exchange or oxidation of compounds with soil minerals (76). Soil adsorption protects the allelochemicals from microbial degradation, resulting in increased or reduced phytotoxicity (75).

Low nutrients (N, P, K and Ca) content in soil increased the allelopathic activity of donor plants (Fig. 2). Plants in sandy substrate are stressed due to nutrients deficiency, produce higher amounts of allelochemicals, including phenolic acids (Fig. 4). P deficiency plays important role in the synthesis of phenolic acids (57) and increases their roots exudation, but it is not true with all allelochemicals. Gianoli and Niemeyer (36) reported that the application of N did not affect the production of hydroxamic acid in wheat.

Besides the direct action of allelochemicals on receiver species, these compounds might help in solubilisation and release of N, P, Fe and other nutrients, thereby increasing their uptake by plants (1). Conversely, they might also make the soil nutrients deficient (e.g. nitrogen and phosphorus) that adversely affects the plant growth (44).

#### **Predictor variables of allelopathic activity of barley**

For the biological control of weeds, the influence of soil type on the allelopathic activity of barley requires the selection of genotypes for the specific environment. We did regression analysis, to assess the relative contribution of physico-chemical properties of soil in predicting the growth inhibition of weeds (Table 2). The dependent variables were root length, shoot length, root dry weight and shoot dry weight for both great brome and chickweed, for each tested barley genotypes, while, soil physico-chemical parameters (total phenols and N, P, K content), were used as independent variables (predictors).

The selected predictor variables were dependent on the donor genotype and the receiver weed, which made it challenging to build a predictive model with all soil characteristics. This was due to the complexity of allelopathy phenomenon interacting with the external environment. For example, Na content was the first predictor variable of root length in great brome using 'Rihane' as the donor genotype, while for chickweed it was OM (%) (Table 2). The same trend was also observed for the second and the third predictor variables, where they existed.

Considering the 6-barley genotypes, a specific predictive model was proposed for each morphological variable and each tested weed (Table 2). This requires the specific recognition of barley allelochemicals by the target species. For great brome and all genotypes of barley, total phenols content was chosen by the model to explain 64 %, 49 %, 67 % and 49 % variability in root length, shoot length, root dry weight and shoot dry weight, respectively. Carbon (C) was the second variable chosen by the model, but it had minor role for the differences in shoot dry weight. In chickweed, TPC alone accounted for 60 %, 62 % and 74 % genotypic variation in shoot length, root dry weight and shoot dry weight, respectively. Besides, Na had minor effects on the aboveground biomass (shoot dry weight) of this specie. However, CE was the first variable chosen by the model. TPC explained 80 % variability in root length for chickweed. Therefore, predictive modelling should consider the target species when studying allelopathic performance of crop specie. Overall, total phenols content was the first variable of all models, indicating that the amount of allelochemicals in the soil was the key factor for the allelopathic activity of barley.

Table 2. Multiple linear regressions (stepwise) explaining the inhibition rate of four morphological traits of great brome (*B. diandrus*) or common chickweed (*S. media*) for each tested barley genotype and the whole set of six genotypes as a dependent variable, and all the physico-chemical traits of the soil including the total phenolic content as independent variables (predictors).

Dependent variables	Genotypes	Predictors for <i>B. diandrus</i>	$R^2$	Predictors for <i>S. media</i>	$R^2$
<b>RL</b>	Manel	-	-	P	0.95***
	Tej	-	-	CE	0.93***
				CE, OM	0.98***
	Rihane	Na	0.72**	OM	0.91***
	Arbi	C	0.62*	P	0.69**
				P, C	0.95***
	Ardhaoui	pH	0.79**	C	0.73**
				C, pH	0.94***
				C, pH, TPC	0.99***
	Saudi	TPC	0.87***	-	-
<b>All genotypes</b>	<b>TPC</b>	<b>0.42***</b>	<b>CE</b>	<b>0.31***</b>	
<b>Final stepwise model</b>	<b>RL = -3.70 + 75.1 TPC</b>		<b>CE, TPC</b>	<b>0.46***</b>	
			<b>RL = 31.57 + 60.1 TPC - 0.07 CE</b>		
<b>SL</b>	Manel	-	-	-	-
	Tej	-	-	-	-
	Rihane	TPC	0.71**	CE	0.77**
	Arbi	-	-	Na	0.80**
	Ardhaoui	-	-	Sand	0.64*
	Saudi	-	-	-	-
	<b>All genotypes</b>	<b>TPC</b>	<b>0.24***</b>	<b>TPC</b>	<b>0.36***</b>
	<b>Final stepwise model</b>	<b>SL = 3.77 + 52.0 TPC</b>		<b>SL = 10.44 + 66.0 TPC</b>	
<b>RDW</b>	Manel	-	-	TPC	0.73**
	Tej	-	-	-	-
	Rihane	P	0.99***	-	-
	Arbi	-	-	pH	0.79**
	Ardhaoui	-	-	MO	0.66*
	Saudi	TPC	0.73**	-	-
	<b>All genotypes</b>	<b>TPC</b>	<b>0.47***</b>	<b>TPC</b>	<b>0.39***</b>
	<b>Final stepwise model</b>	<b>RDW = -3.57 + 54.0 TPC</b>		<b>RDW = -6.69 + 80.29 TPC</b>	
<b>SDW</b>	Manel	-	-	C	0.89***
	Tej	-	-	Na	0.63*
	Rihane	P	0.73**	N	0.65**
		P, AP	0.91**		
	Arbi	Na	0.76**	K	0.98***
				K, P	0.99***
	Ardhaoui	CE	0.87***	CE	0.79**
	Saudi	AP	0.60*	P	0.93***
	<b>All</b>	<b>TPC</b>	<b>0.36***</b>	<b>TPC</b>	<b>0.61***</b>
		<b>TPC, C</b>	<b>0.44***</b>	<b>TPC, Na</b>	<b>0.68***</b>
	<b>Final stepwise model</b>	<b>SDW = 2.41 + 39.9 TPC - 7.45 C</b>		<b>SDW = 7.95 + 61.95 TPC - 0.56 Na</b>	

RL, root length; SL, shoot length; RDW, root dry weight; SDW, shoot dry weight. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

### Effects of microorganisms on the allelopathic activity of barley

To assess the effects of microbial communities on the intensity of allelopathic effects, soil was sterilized, a technique used for this purpose (40). The physico-chemical characteristics of the substrates are given in Table 1.

After one month of culture, the growth of great brome plants receiving the barley root exudates was significantly reduced than control (data not shown). This inhibitory effect was strongly dependent on the type of substrate (i.e. sandy or clay-loam) and sterilization (i.e. autoclaved or non-autoclaved substrate; Fig. 5). However, the interactions were non-significant between the two factors (i.e., type of substrate and sterilization).

The inhibitory activity of barley roots was slightly higher for root length and shoot length of great brome than for root dry weight and shoot dry weight (Fig. 5). On the other hand, the inhibition rate of four morphological variables was higher in sandy substrate than in clay-loam substrate. This effect was more pronounced in autoclaved substrate than non-autoclaved substrate. For example, the inhibition of root length for great brome plants grown in sandy and clay-loam autoclaved substrates were 35.3 % and 23.7 %, respectively and for non-autoclaved soil 24.5 % and 15.4 %, respectively. However, this difference was not significant for shoot dry weight of great brome, when compared with autoclaved and non-autoclaved clay-loam substrates. These results suggested that microorganisms in the tested soils interfered with barley allelochemicals and decreased their allelopathic potential. Likewise, the allelopathic effects of leachate, root exudates and even the application of some purified allelochemicals from donor species decreases in non-sterile soil (10,41,48,79). Lankau (50) reported that the inhibitory activity of garlic mustard (*Alliaria petiolata* [M.Bieb.] Cavara & Grande) on sycamore (*Platanus occidentalis* L.) can be detected only in sterile soil. Soil biota reduces the allelopathic potential, by degrading the allelochemicals, as demonstrated for benzoxazinones (18,33,55), flavonoids (e.g. catechin and quercetin) (3,62) and some phenolics (13). The sterilization itself influences some chemical characteristics of the soil (e.g. OM content; 41), which influences the allelopathic activity. Notably, some allelochemicals from root exudates may suppress the nitrification in presence of microorganisms, by inhibiting the activity of vital enzymes (ammonium mono-oxygenase and hydroxylamine oxidoreductase). This inhibition of biological nitrification (73) might increase the N recovery and nitrogen use efficiency for donor and/or receiver species. Another possible reason may be competition of soil microorganisms with weed seedlings for nutrients, which causes nutrients deficiency in plants (53).

This study also showed that microbial interference in plant allelopathy depended on the soil type (Fig. 5). The growth (root and shoot length, and root and shoot dry) of receiver plants, when compared with autoclaved and non-autoclaved soils, was slightly higher in sandy substrate (10.7 %, 7.6 %, 6.4 % and 4.6 % than in sandy clay loam (8.3 %, 6.9 %, 5.1 % and 2.6 % for the root and shoot length, and root and shoot dry matter, respectively). The microorganisms are protected by the clay particles [protects from desiccation, heat and pH fluctuations, promotes microbial activity (4)]. Besides, clay loam soil had higher nutrients (e.g. N, P and K) and OM content and pH (Table 1) these stimulate the microbial activity (43,47). The changes in the concentration of two allelochemicals of *Eupatorium adenophorum* (Spreng.), 9-Oxo-10,11-dehydroageraphorone and 9b-Hydroxyageraphorone, were very less in sandy soil than natural soils from different habitats of this specie. In addition, Oleszek and Jurzysta (58) reported that

incubation of alfalfa roots in four soil types decreased its toxicity to wheat seedlings and fungus, *Trichoderma viride* Pers. This decrease occurred quickly in heavier soils than in sandy soils, due to the hydrolysis of glycosides by soil microorganisms.

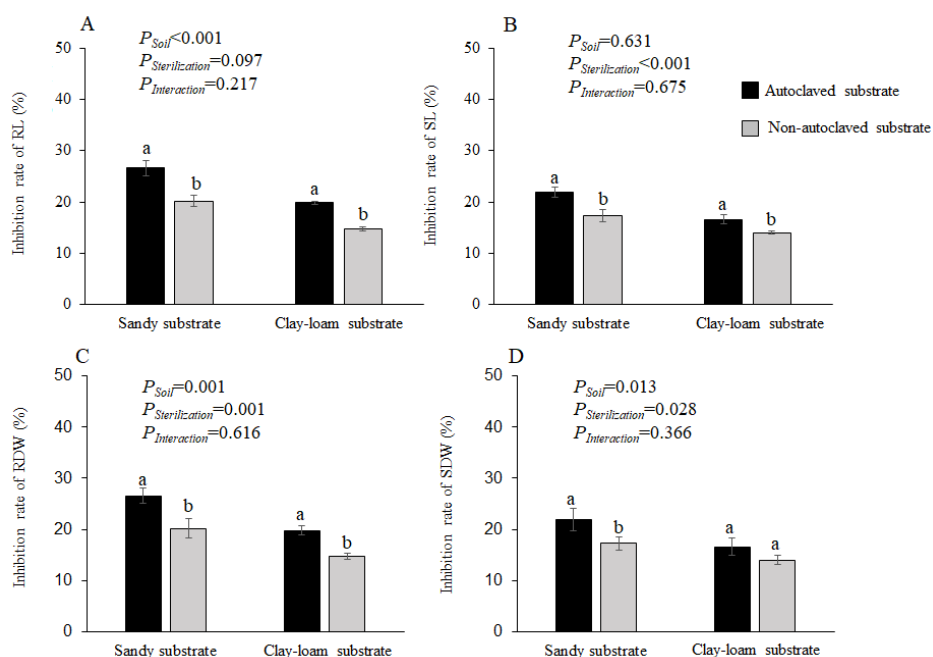


Figure 5. Inhibition rates of (A) root and (B) shoot length, (C) root and (D) shoot dry weight of great brome (*B. diandrus*) plants, grown in autoclaved and non-autoclaved sandy and clay-loam substrates in which the 'Ardhaoui' barley landrace was previously grown for one month before being removed. Values are means of five replicates  $\pm$  SE. Data were analyzed by two-way ANOVA with type of substrate and treatment of soil as sources of variation. Since interaction was not significant between factors for all morphological variables, the effect of treatment soil was separately evaluated for each type of substrate. Means followed by different letters are significantly different ( $P < 0.05$ ; LSD test). RL, root length; SL, shoot length; RDW, root dry weight; SDW, shoot dry weight.

Microbial and chemical degradation of phytotoxic compounds are less in sandy soils (70). Under the conditions in current study, the small difference in the allelochemicals growth inhibition by microorganisms in two soils types might be explained by the quality of aeration in sandy soils, which increases the action of aerobic microorganisms and thereby degrades the phytotoxins (67).

## CONCLUSIONS

This study showed that allelopathy is complex process, which depended on both intrinsic factors (donor and receiver species) and extrinsic factors (soil type and soil biota), which affected the production and secretion of allelochemicals by barley genotypes and their release and fate in soil. Overall, barley landraces showed higher inhibitory effects than modern varieties. Great brome and chickweed did not react in similar manner to

barley root exudates. Barley plants grown in sandy soils under stressed conditions (low organic matter and nutrient content), were more allelopathic. This is the first report giving a predictive model of allelopathic activity for barley based on soil properties and the tested weeds. Results showed that total phenolics content in soil could be an appropriate measure in predicting the allelopathic performance of barley roots. Soil biota decreased the allelopathic activity of barley and it depended on the soil type. The weed biocontrol by allelopathy depended on soil type and the choice of barley genotypes.

## ACKNOWLEDGEMENTS

During this work, I. Bouhaouel was the recipient of a Ph.D. fellowship of the Erasmus Mundus Averroès Partnerships Action of the European Commission. The financial support of internal grants from Gembloux Agro-Bio Tech throughout this work is acknowledged.

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