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Methyl Jasmonate Elicited *Helichrysum stoechas* (L.) Moench Cell Suspensions, A Promising Source of Extracts with Allelopathic Activity?

Gourguillon Lorène[1,2,5], Rustenholz Camille[3], Le Gélébart Erwan[2], Lobstein Annelise[1], and Gondet Laurence[1,4]

[1]*Université de Strasbourg, CNRS, LIT UMR 7200, Strasbourg, France*

[2]*SEPPIC-Biotech Marine, ZI BP72, F-22260, Pontrieux, France*

[3]*Université de Strasbourg, INRAE, SVQV UMR-A 1131, Colmar, France*

[4]*Université de Strasbourg, CNRS, IPHC UMR 7178, Strasbourg, France*

[5]*LVMH recherche, Innovation Matériaux Naturels et Développement Durable, Saint Jean de Braye, France*

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***Corresponding author:** Gondet Laurence, IPHC - UMR7178, ECPM Bât. R3-N0, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France

Abstract

Methanolic extracts of *Helichrysum stoechas (L.)* Moench cell suspensions were analyzed by LC-ESI-QTOF, which highlighted the predominance of 3,5-O-dicaffeoylquinic acid (3,5-diCQA). Elicitation of *H. stoechas* cells with methyl jasmonate (200 µM) led to a massive rise in 3,5-diCQA, up to 5-fold-increase compared with control, reaching the concentration of 10.2 $mg.g^{-1}$ dry weight after 14 days of culture. Previous data showed that diCQA isomers are potent allelopathic compounds, thus the methanolic extracts of control and MeJa-elicited *H. stoechas* cells were tested for their phytotoxicity. With this in mind, activity on the seed germination and seedling growth of the model plant *Lepidium sativum* were tested. Phytotoxicity of both extracts occurred in a dose-dependent manner, with a greater activity of elicited cells extract. Indeed, in the concentration range from 0.31 to 0.83 $mg.mL^{-1}$, the latter showed a significantly higher inhibition rate of *L. sativum* seedlings root growth, when compared to control cells extract. The data presented may contribute to explore new strategies towards the conception of bioherbicides from plant origin. To our knowledge, this study is the first report of the use of elicited plant cells grown *in vitro* as a raw material for the production of allelopathic metabolites.

Keywords: *Helichrysum Stoechas*; Cell Suspensions; Elicitation; Methyl Jasmonate; Dicaffeoylquinic Acid Isomers; Allelopathy; Bioherbicides

Abbreviations: diCQA: dicaffeoylquinic acid; IC_{50}: Half Maximal Inhibitory Concentration; MeJa: Methyl Jasmonate; PPFD: Photosynthetic Photon Flux Density; SA: Salicylic Acid

Introduction

Helichrysum stoechas (L.) Moench (Asteraceae), also called « immortelle » or « everlasting flower », is a common shrub in western and southern coastal vegetation in France, as well as in several countries of the Mediterranean area. In contrast to *Helichrysum italicum*, which has been extensively studied [1], only a few studies were conducted on *H. stoechas* [2]. They showed interesting biological activities of methanolic extracts from flowering aerial parts towards cosmetic [3] and pharmaceutical [4] applications, as well as in the food industry because of its antimicrobial potential and strong antioxidant capacity [5-7]. Recent data showed that powder or extract of *H. stoechas* had antifungal activity against plant pathogenic fungus *Sclerotinia sclerotiurum*, opening new perspectives in the field of agro ecology [8]. All these reports established a link between *H. stoechas* biological activity and its high phenolic content. The research of plant-based weeds biocontrol products (botanical bioherbicides) is gaining an increasing interest as an emerging method for weed control in sustainable agriculture, instead of using chemicals with negative environmental impact [9-11]. The capacity of one plant to inhibit germination and/or growth of other plants is defined as allelopathy and is based on the production of phytotoxic metabolites (allelochemicals) by the emitting plant. Based on the fact that *H. stoechas* is rich in phenolic compounds, which are crucial molecules in allelopathic activity [12,13], the phytotoxic activity of *H. stoechas* methanolic extracts was investigated to test its potential as source of allelochemicals.

Moreover, previous studies showed that the main phenolic acid in *H. stoechas* aerial parts is 3,5-O-dicaffeoylquinic acid [3,14]. Allelopathic effects of caffeic acid derivatives, including dicaffeoylquinic acids (diCQA), were already demonstrated in *Bellis perennis* [15], in *Chrysanthemum coronarium* [16] and

in the invasive plant *Tithonia diversifolia* [17,18], highlighting the relevance of studying *H. stoechas* as a source of allelopathic metabolites. This link between allelopathic and medicinal potential of plants has already been explored: 239 medicinal plant species were tested for their allelopathic activities [19]. In another study, the effects of juglone, a naphtoquinone characterized as the main allelochemical from species of genus *Juglans L.* [20], was evaluated as an effective anticancer agent in pancreatic cancer [21]. These reports give a new insight on plant resources, disclosing their multifunctional potential. However, plants grown in field are submitted to seasonal and climatic fluctuations, inducing metabolic variations [22-24] unsuitable with industrial valorization, which needs standardized plant raw material. Thus, production of biomolecules by plant cell cultures – considered as plant cell factories - appears as a powerful alternative strategy, allowing a stable plant sourcing, as well as the opportunity of using elicitors to increase the content in bioactive molecules, and is largely documented [25-30].

Therefore, our first aim was to establish *H. stoechas* cell suspensions, to avoid the problem of metabolic variability and plant sourcing, this species being protected in several French regions such as Brittany, Center and the Alps, and allelopathy was investigated directly in this renewable raw material from biotechnological origin. Moreover, we tested two hormonal elicitors - methyl jasmonate (MeJa) and salicylic acid (SA) - on *H. stoechas* cell suspensions for their potential capacity of increasing dicaffeoylquinic acids content (3,5-O-dicaffeoylquinic acid and its isomers 3,4- and 4.5-O-dicaffeoylquinic acids), with an expected enhanced allelopathic activity of *H. stoechas* elicited cells extracts. Indeed, it was already shown that MeJa and/or SA induced important increase of phenolics concentration in *in vitro* cultures of *Salvia virgate* [31], *Scutellaria lateriflora* [32], and *Salvia miltiorrhiza* [33]. Thus, allelopathic studies were performed with methanolic extracts of non-elicited and elicited *H. stoechas* cells, further tested for their effect on the model plant *Lepidium sativum* germination and growth, in order to explore the interest of a biotechnological plant approach for the research of weeds biocontrol new products.

Materials and Methods

Plant material

The protocol for callogenesis of *H. stoechas* was adapted from [34]. Leaves were washed during 3 min in ethanol 80% with 0.1% Tween 20 (polyethylene glycol sorbitan monolaureate), then sterilized during 3 min in 1% sodium hypochlorite and finally rinsed in sterile water. Explants were incubated on medium containing minerals and vitamins (25 $g.L^{-1}$ KNO_3 ; 1.34 $g.L^{-1}$ $(NH_4)_2SO_4$; 1.5 $g.L^{-1}$ $CaCl_2$, $2H_2O$; 1.32 $g.L^{-1}$ NaH_2PO_4, H_2O ; 2.5 $g.L^{-1}$ $MgSO_4$, $7H_2O$; 169 $mg.L^{-1}$ $MnSO_4$, H_2O ; 86 $mg.L^{-1}$ $ZnSO_4$, $7H_2O$; 62 $mg.L^{-1}$ H_3BO_3 ; 8.3 $mg.L^{-1}$ KI ; 2.5 $mg.L^{-1}$ Na_2MoO_4, $2H_2O$; 0.25 $mg.L^{-1}$ $CuSO_4$, $5H_2O$; 278 $mg.L^{-1}$ $FeSO_4$, $7H_2O$; 373 $mg.L^{-1}$ Na_2EDTA, $2H_2O$; 0.1 $mg.L^{-1}$ biotine ; 1 $g.L^{-1}$ myo-inositol ; 10 $mg.L^{-1}$ nicotinic acid ; 10 $mg.L^{-1}$ D-pantothenic acid, hemicalcium salt; 10 $mg.L^{-1}$ hydrochloride pyridoxine; 10 $mg.L^{-1}$ hydrochloride thiamine), supplemented with 30 $g.L^{-1}$ sucrose, 1 $mg.L^{-1}$ α-naphthaleneacetic acid (NAA), 1 $mg.L^{-1}$ 2,4-dichlorophenoxyacetic acid (2.4D), 1 $mg.L^{-1}$ 6-benzyladenine (BA). The pH was adjusted to 5.8 and media were solidified by 1% (w/v) agar before autoclaving for 15 min at 121°C. To establish cell suspension, 1 g of 1 month-old friable calli was transferred in 250 mL Erlenmeyer flask containing 100 mL of medium. The medium for suspension was the same as the medium for callogenesis without agar. Suspensions were put on a rotary shaker at 130 rpm, 20 mm range. For further subcultures, every 14 days, dilution was performed (1:4, v/v), in 250 mL Erlenmeyer flasks containing 100 mL of liquid medium. Cultures were kept in culture room at 25°C, under continuous light intensity (PPFD: 50 $\mu mol.m^{-2}.s^{-1}$).

Elicitation

Elicitation assays were performed in three independent experiments and in triplicates for each of them.

3-days old suspensions were treated with various concentrations of elicitors, and cells were harvested after 14 days of culture. Methyl jasmonate (746398, Sigma, Saint-Quentin-Fallavier, France) or salicylic acid (S1367.0100, Duchefa Biochemie, Haarlem, Netherlands) was added at 50, 100, 200 and 400 µM or 100, 150, and 200 µM, respectively. The elicitors were sterilized thanks to filtration on sterile 0.22 µm membrane filter. Because elicitors were dissolved in ethanol, the same volume of solvent was added to the control suspension to reveal any adverse effect.

Growth measurements

The growth measurements were performed on 14 days old suspensions, by measuring the fresh weight of cells in the pellet after 10 min centrifugation (4000 rpm), then by measuring the dry weight after freeze-drying of cells.

Preparation of cell extracts

12.5 mL of methanol HPLC grade (Sigma, Saint-Quentin-Fallavier, France) were added to 500 mg of freeze-dried cells. The mixture was vortexed at 30000 rpm during 30 s. Then, samples were sonicated during 25 min (Fischer Scientific, Illkirch, France). Before being centrifuged (9000 rpm, 15 min, 20°C). The supernatants obtained were passed through a 0.2 µm membrane filter and injected in HPLC.

Determination of dicaffeoylquinic acids content

Identification of diCQAs: The liquid chromatography system was an Ultimate 3000 RSLC model (Dionex, Sunnyval, Californie, USA) with a binary pump, thermostatic column oven, automatic sample changer and DAD detector. The stationary phase was a kinetex C18 column (2,6 mm, 100 Å, 100 x 3 mm) (Phenomenex, Torrence, USA). The mobile phases were acetonitrile (solvent A)

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and water (solvent B), both with 0.05 % formic acid. The flow rate was 0.7 mL/min. The gradient used was: 0 – 5 min, A: 10%; 10min, A: 20%; 15min, A: 30%; 20 to 25 min, A: 100%. The mass spectrometry detector was a maXis Q-TOF (Bruker, Billerika, Massachusetts, USA). The softwares used were Hyster for the data acquisition and Data Analysis for the data processing (Bruker, Billerika, Massachusetts, USA). Pressure of the nebulization gas (nitrogen) was 1.2 bar. Nitrogen was also used as collision gas. Electrospray was at -4.5 kV, in negative mode. Mass spectra were recorded with a frequency of 1 Hz and extending over a range from 50 to 2500 m/z. The BPC (Base Peak Chromatogram) was used for the data processing. Major phenolic compounds of extract of control cell suspension were characterized thanks to their UV, MS and MS/MS spectra, in comparison with literature and the standard injection purchased at Biopurify Phytochemical Ltd (Chengdu, China).

Quantification of diCQA: The content in dicaffeoylquinic acid isomers was measured by HPLC-DAD. The apparatus was a 1260 model (Agilent, Santa Clara, Californie, USA) with a quaternary pump and diode array detector. The stationary phase was a kinetex C18 column (2,6 22 mm, 100 A, 100 mm x 3 mm) (Phenomenex, Torrence, USA). HPLC software was OpenLab, and the data were processed on ACDLabs. The mobile phases were acetonitrile (solvent A) and water (solvent B), both with 0.05 % formic acid. The flow rate was 0.7 mL.min^{-1}. The gradient used was: 0 -5 min, A: 10%; 7 min, A: 20%; 12min, A: 30%; 17-20 min, A: 100%. Injection volume was 1 µL. Peaks were monitored at 320 nm which is one of the lambda maxes of interest compounds. The content of each diCQA isomer was calculated thanks to an equation which was obtained from corresponding standards calibration curves. For each isomer, stock solutions were prepared at 1 mg.mL^{-1} in methanol and diluted to the appropriate concentration range for making calibration curves.

Germination bioassay: For the study of allelopathic effects, 500 µL of *H. stoechas* control and elicited (with MeJa 200 µM) cells methanolic extracts (10 mg. mL^{-1}) or increasing dilutions (4x, 6x, 8x, 12x, 16x, 32x and 64x in methanol, leading respectively to the following concentrations: 2.5, 1.67, 1.25, 0.83, 0.62, 0.31 and 0.16 mg extract. mL^{-1}) were added to Whatman paper (n°1) in 5.5 cm diameter Petri dishes. After evaporation of methanol, 1.5 mL of a 0.05% (v/v) aqueous solution of polyoxyethylene sorbitan monolaurate (Tween 20) were added, and 20 seeds of garden cress (*Lepidium sativum* -Botanic®) were placed on the paper and incubated at 25°C under dark conditions. Control was performed with 500 µL methanol. After 2 days, the germination percentage of seeds, as well as the length of the radicle and hypocotyl, were measured. Each test was performed in duplicate (n=40) for each of three independent experiments. The growth inhibition rate was defined as 100-(Treatment x 100/Control).

Statistical analysis: Three batches were produced to assess root and hypocotyl growth at germination under four treatments: control, methanol, extract of non-elicited and methyl-jasmonate elicited *H. stoechas* cells. Various dilution levels were used for the cells extracts, i.e., 4x, 6x, 8x, 12x, 16x, 32x and 64x. The root and hypocotyl lengths were measured 48h post-sowing. Root and hypocotyl lengths for each batch were standardized by subtracting their batch average and dividing the difference by their batch standard deviation. The "batch" effect was therefore corrected, which allowed a fair comparison between the three batches. The variance analysis of the "treatment" and "dilution" factors was assessed with an ANOVA. The means were further compared using Tukey's test (R software).

Results and Discussion

Methanolic extracts of control and elicited *H. stoechas* cells were first characterized for their diCQA content, before analysis of their allelopathic potential towards garden cress (*Lepidium sativum*), a model plant for allelopathy studies, by germination and growth measurements.

Identification of major compounds by LC-ESI-QTOF dereplication

Identification of the diCQA isomers, which were the major compounds, was done by comparison of the retention time and UV, MS and MS/MS spectra with those of standards. LC-ESI-QTOF analysis showed the presence of three isomers of diCQA: 3,4-, 3,5- and 4,5-diCQA (Figure 1). Retention time, UV and mass spectra were the same as the standards (Table 1). The diCQA quantities for 1g of dry cells are 2.4x10^{3}µg, 446 µg and 277 µg for 3,5- , 3,4- and 4,5-diCQA respectively (Figure 2b). Thus, most abundant diCQA in *H. stoechas* cells is 3,5-diCQA, representing around 80% of total diCQA, while 3,4- and 4,5-diCQA are in minority (12% and 8% of total diCQA respectively) (Figure 2b). These results are in accordance with those already published [3,14]: 3,5-diCQA was also the most abundant phenolic acid in a methanolic extract of *H. stoechas* aerial parts. Thus 3,5-diCQA can be considered as a marker of *H. stoechas*, regardless the nature of raw material (plants or undifferentiated cells). 3,5-diCQA is also the main compound in aerial parts of *H. obconicum* [35] and was the most anti-HIV active metabolite in *H. populifolium* aerial parts [36]; it was also detected in plants of this species propagated *in vitro* [37]. Presence of 3,5-diCQA was reported in flowers of *H. italicum* [38]. *Tithonia diversifolia*, another Asteraceae, was also characterized by a high dicaffeoylquinic acids content in a report focused on the research of metabolites responsible for antifungal as well as insecticide properties of this invasive plant [18]. 3,5-DiCQA was also detected in *Salicornia herbacea* (Amaranthaceae) extracts with strong antioxidant activity [39], as well as in artichoke cell cultures submitted to nutrient deficiency [40], and in elicited tobacco cells [41], revealing the key role of this metabolite for plant defense and adaptation mechanisms.

Effect of elicitors on *H. stoechas* cell suspensions growth

The elicitation during 11 days with MeJa or SA did not affect the growth of *H. stoechas* cells at any of the tested concentrations

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(Figure 2a). At the end of the culture cycle (14 days), each population showed approximately a 4-fold increase of the fresh weight when compared to the inoculum value, to reach a maximum of 450 $g.L^{-1}$ at the end of the culture cycle. The dry weight was also similar in control and elicited cells (around 15 $g.L^{-1}$ at the end of the 14-days culture cycle). These growth parameters are in accordance with those reported recently for other plant cell suspensions as *Armeria maritima* [42], *Thevetia peruviana* [43, 44], *Halodule pinifolia* [45]. Thus, concentrations of elicitors (MeJa and SA) used in our study did not show any phytotoxic effects on *H. stoechas* cells and were therefore suitable for further diCQA bio-production studies.

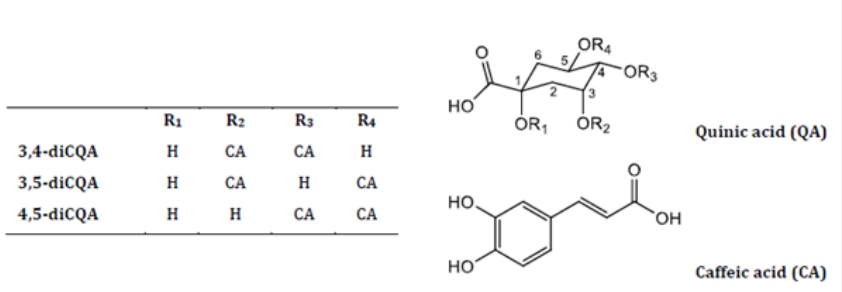
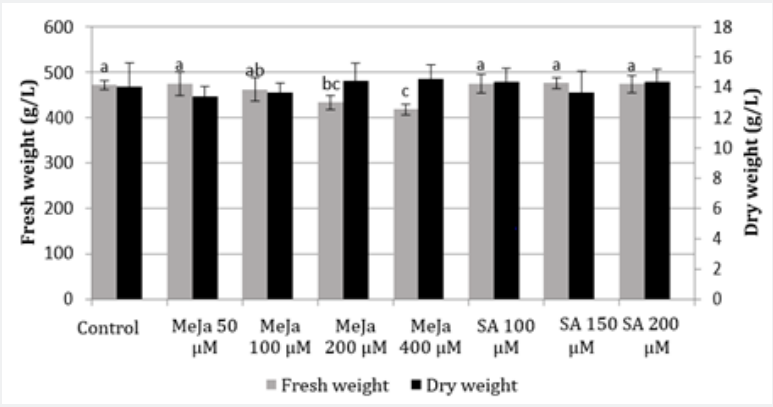
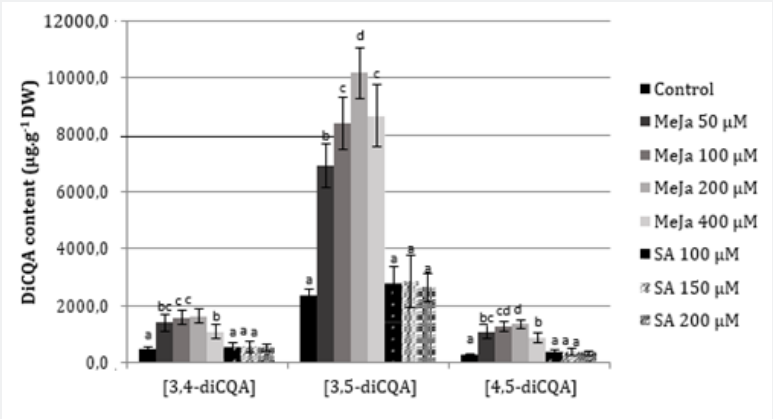


Figure 1: Structure of the markers dicaffeoylquinic acids (diCQAs) identified in *Helichrysum stoechas* cells methanolic extract. Quinic acid : R1=R2=R3=R4=H



(a)



(b)

Figure 2: Effect of methyl jasmonate and salicylic acid on growth (a) and diCQA content (b) of *H. stoechas* cell suspensions after a 14-day culture cycle.

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Effect of elicitor on dicaffeoylquinic acids concentration in *H. stoechas* cells

Our results showed that *H. stoechas* cells accumulate higher levels of diCQA in response to MeJa elicitation. When cell suspensions are elicited with 200 µM MeJA, 3,5-diCQA content reached 10.2 x 10^3 µg. g^{-1} DW which is 5-fold superior compared with non-elicited cells after a 14-days culture cycle. However, SA elicitation did not show any effect on diCQA content (Figure 2b). This different effect of MeJa and SA was already described for elicitation of secondary metabolites in *Rehmannia glutinosa* hairy roots [46], as well as in *Thevetia peruviana* cell suspensions [44]. In other reports, a synergistic effect between SA and MeJA on plant secondary metabolism was shown [47]. The cross talk between the SA and jasmonate metabolism is complex, either displaying antagonism or synergism pattern [48]. In both cases, activation of phenylalanine ammonia-lyase (PAL) gene expression plays a pivotal role in the capacity of elicitors to activate the phenylpropanoid pathway [49, 50].

Table 1: Compounds identified in *Helichrysum stoechas (Hs)* cells methanolic extract by LC-ESI-QTOF dereplication.

Retention time (min)	M-H- m/z	Fragments (m/z)	λmax (nm)	Compounds identified
10,63	515,1191	353,0883 [M-H-caffeic acid]-	220 - 239 - 326	3,4-diCQA
10,99		191,0559 [quinic acid]-		3,5-diCQA
11,86		179,0352 [caffeic acid]-		4,5-diCQA

The stimulating effect of MeJa on bioactive metabolites production in *Helichrysum* spp. was already described in cells of *H. kraussii* [51], and in *in vitro* propagated shoots of *H. populifolium* [37]. Other studies reported a phenolics bioproduction capacity in *H. pedunculatum* callus culture [52] in the absence of any elicitor treatment. The original aspect of the present work is that for the first time an elicitor-enhanced metabolites production in *H. stoechas* is reported. Additional studies should be carried out with *H. stoechas* cells with the aim of increasing even more their diCQA content, for example by supporting MeJa elicitation with precursor feeding [53], or by testing other elicitors such as chitosan, which is frequently used in activating phenolic metabolism in plant cell suspensions [50, 54, 55]. Thus, extracts from the cells elicited with 200 µM MeJa, with the highest diCQA content, were chosen for further investigations concerning *H. stoechas* allelopathic activity.

Allelopathic activity of *H. stoechas* cells methanolic extracts

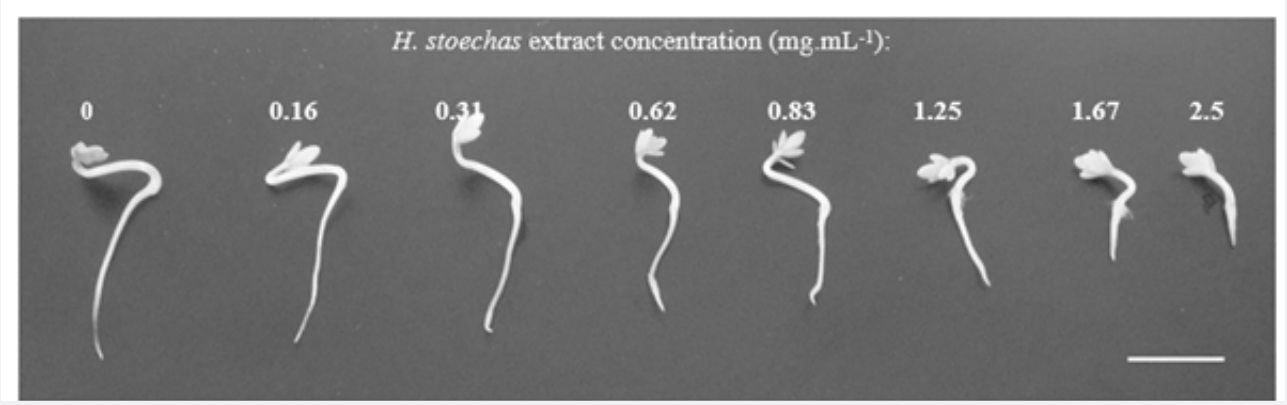

Figure 3: *Lepidium sativum* seedlings two days after sowing on increasing concentrations of non-elicited *H. stoechas* cells methanolic extract. Bar = 1 cm.
Seedlings represented above have the growth parameters (hypocotyl and root length) corresponding to the mean of the population (data shown in Table 2).

Two days after sowing, no significant effect on the germination rate of *L. sativum* was noticed, reaching in all cases 90 to 100% (data not shown). However, the cress seedlings grown on non-elicited *H. stoechas* extract concentrations of 0.16 mg.mL^{-1} and above showed a progressive inhibition of root and hypocotyl growth when compared to the control (Figure 3). The growth inhibition rate reached 78.2% for roots and 57.6% for hypocotyls when the most concentrated extract (2.5 mg.mL^{-1}) was tested (Table 2). Our results showed for the first time that *H. stoechas* cells methanolic extract possess a great allelopathic potential. The

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IC_{50} values determined from data shown in Table 2 indicate that *H. stoechas* cells extract affected root growth (IC_{50} = 1 mg.mL^{-1}) more than shoot growth (IC_{50} = 1.65 mg.mL^{-1}) of cress seedlings. Moreover, comparison of cress root growth inhibition obtained with other plant extracts tested in the same conditions showed that *H. stoechas* cells extract is a really promising candidate for developing a plant-based bioherbicide. Indeed, the IC_{50} values on cress root growth of methanolic extract of *Actinidoa deliciosa* (21.6 mg.mL^{-1} [56]), *Gleichenia japonica* (10-30 mg.mL^{-1} [57]), *Pistia stratiotes* (16.9 mg.mL^{-1} [58]) and *Lemna minor* (8.9 mg. mL^{-1} [58]) were all much higher when compared with IC_{50} value determined for *H. stoechas* extract. When this experiment was repeated with MeJa-elicited *H. stoechas* cells extract, the growth inhibition rate reached 80.2% for roots and 57.5% for hypocotyls for the most concentrated extract (2.5 mg.mL^{-1}), which was similar to phytotoxic effects of non-elicited cells extracts (Table 2). However, a stimulating effect of elicitation on phytotoxic activity on cress root growth was observable for extract concentrations in the range from 0.31 to 0.83 mg.mL^{-1} (Table 2, values in bold), with an inhibition rate being for each extract concentration 8% greater with MeJa-elicited than with non-elicited cells extracts.

Table 2: Inhibition effects of *Helichrysum stoechas (Hs)* cells extracts (non-elicited and elicited with MeJa 200 µM) on *Lepidium sativum* seedlings growth.

Extract concentration	*Lepidium sativum* seedling height[2] (mm)				Inhibition rate[3] (%)	
(mg.mL^{-1})[1]	Root	*Stats R*[4]	Hypocotyl	*Stats H*[4]	Root	Hypocotyl
Control (Methanol)	20.0 ± 5.2	a	9.2 ± 2.9	a	-	-
Non-elicited Hs cells						
0.16	17.9 ± 5.3	b	9.4 ± 2.6	a	10.4	-1.9
0.31	**15.7 ± 3.2**	c	7.4 ± 2.4	b	**21.5**	20.3
0.62	**12.9 ± 2.7**	d	6.9 ± 2.1	bc	**35.4**	24.9
0.83	**11.4 ± 2.8**	e	6.4 ± 2.1	cd	**42.8**	30.3
1.25	7.9 ± 2.4	g	5.2 ± 1.9	e	60.2	43.4
1.67	6.9 ± 2.5	gh	5.1 ± 1.7	e	65.6	44.8
2.5	4.4 ± 1.7	ij	3.9 ± 1.3	f	78.2	57.6
				IC_{50} (mg.mL^{-1}):	1	1.65
MeJa-elicited Hs cells						
0.16	17.5 ± 4.8	b	9.2 ± 2.7	a	12.3	0.7
0.31	**14.1 ± 4.0**	de	8.0 ± 2.4	a	**29.5**	13
0.62	**11.3 ± 2.9**	e	6.8 ± 1.9	bc	**43.4**	26
0.83	**9.9 ± 2.7**	f	6.3 ± 1.8	cd	**50.6**	31.7
1.25	7.6 ± 2.5	g	5.5 ± 1.7	de	61.7	40.2
1.67	5.8 ± 2.0	hi	5.2 ± 1.7	e	71.2	43.3
2.5	4 ± 1.3	j	3.9 ± 1.3	f	80 .2	57.5
				IC_{50} (mg.mL^{-1}):	0.84	2.1

The values of *Lepidium sativum* seedlings root and hypocotyl height are mean ±SE. Three independent batches were measured and statistically analyzed[4]. Values in bold are statistically different in non-elicited and MeJa-elicited cells extracts.

1: the different extract concentrations were obtained after successive dilutions (respectively 1/64, 1/32, 1/16, 1/12, 1/8, 1/6 and 1/4) from the crude extract (10 mg.mL^{-1})

2: measured 48h after sowing with 500 µl of control and elicited *H. stoechas* cells methanolic extract

3: inhibition rate (%) = 100-(Treatment x 100/Control)

4: Root (R) and hypocotyl (H) lengths for each batch were standardized by subtracting their batch average and dividing the difference by their batch standard deviation. The "batch" effect was therefore corrected, which allowed a fair comparison between the three batches. The analysis of variance of the "treatment" and "dilution" factors was assessed with an ANOVA. The means were further compared using Tukey's test (R software).

Seedling height data followed by the same letters are not significantly different at the level of 5%.

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The comparison of the IC_{50} values of MeJa-elicited *H. stoechas* cells extract on cress root growth (0.84 $mg.mL^{-1}$) with the one obtained with non-elicited *H. stoechas* cells (1 $mg.mL^{-1}$) revealed a significant increase of its phytotoxic activity, as a consequence of the MeJa treatment. Elicitation with MeJa thus appears to be a potent mean for ensuring a gearing effect on allelopathic activity of *in vitro* cultured *H. stoechas* cells. A few studies described recently the interest of biotechnological plant raw material for crop protection in an agro-ecological context. For example, extracts of callus and cell suspension cultures of *Eysenhardtia polystachya* showed inhibition of phytopathogenic fungi mycelial growth [59]. Extracts of *Lantana camara* callus were investigated for their phytotoxic effects by inhibiting germination and seedlings growth of *Brassica campestris*, *Ipomoea aquatica*, *Sorghum bicolor L.* and *Zea mays L.* [60]. Hairy roots of *Chenopodium murale* could also constitute a promising natural resource in the management of weeds [61]. The first report of allelopathic effects of *in vitro* cultures described an experiment where soybean (*Glycine max Merr*.) calli were co-cultured with rice (*Oryza sativa* L.) calli, with an allelopathic evidence of growth inhibition on soybean calli, due to volatile compounds produced by rice calli [62]. To our knowledge, data shown in the present work represents the first description of a complementarity between elicitation, generally used for enhancing the production of bioactive metabolites in plant cultures for medical or cosmetic applications, and the allelopathic potential of plant cell suspensions, a new promising source of allelochemicals for bioherbicide development.

Conclusion

Methanolic extracts of *H. stoechas* cell suspensions represent an interesting line of research for the possible detection of weeds biocontrol products, their 3,5-diCQA content being correlated with their phytotoxic activity, measured herein by cress seedlings growth inhibition. Moreover, elicitation of these cells with MeJa led to a slight but significant enhancement of their phytotoxic activity and consequently of their allelopathic potential. These results put a new insight on the pivotal role of diCQA isomers in the adaptation of plants towards their environment. Moreover, they contribute to the optimization of plant biotechnology methods as a tool for future investigations towards the development of plant-based bioherbicides.

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Conflict of interest

The authors declare no conflict of interest.

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