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Abstract

Ailanthone (Ail) is the most phytotoxic quassinoid in plant extracts of *Ailanthus altissima* (Mill.) Swingle, an invasive tree of Simaroubaceae with allelopathic activity. Ail has raised attention as a potential biological herbicide in weed management to reduce the impact on the environment and human health. However, high costs for its extraction and purification, and low persistence in the soil have been considered so far limits for its development as herbicide for open field applications. In this study we explored its phytotoxic activity and persistence, through five experiments, to evaluate its potential for the weed management in the horticulture sector and in urban green areas, where lower herbicide amounts are needed. Ail inhibition activity on

germination and growth was evaluated on two model species (garden cress - *Lepidium sativum* L. - and radish - *Raphanus sativus* L.). Firstly, the dose-response curve between Ail concentration and index of germination was calculated; Ail persistence along 30 days was also assessed. Afterwards, Ail bioactivity and persistence were evaluated in a non-sterile urban soil and a horticultural substrate. Ail inhibited by 80 to 90 % the plant growth already at low doses (7.5 mg L^{-1}) in paper and soil, while higher concentrations (≥ 30 mg L^{-1}) were necessary in the cultivation substrate to obtain similar results. Regarding the phytotoxic persistence, the two species were similarly inhibited at the first evaluation (10 days after treatment) both in paper and cultivation substrate, whereas on the longer period (20 and 30 days after treatment), radish was more affected, with growth inhibition higher than 45 % until 30 days. Results of these experiments implement the knowledge on Ail phytotoxic activity, envisioning its potential use as a biological solution for weed management in urban areas and protected cultivation environments.

Keywords: allelopathy; degradation kinetics; phytotoxicity; quassinoids; seed germination; weed management

Abbreviations

Ail: ailanthone

DAT: days after treatment

IGe: index of germination

IGr: index of growth

1. Introduction

Researches to improve the control of weeds are mainly related to agroecosystems (Duke et al., 2000; Kohli et al., 1997; Narwal, 1994; Westwood et al., 2018). This issue, however, is a particularly serious problem in many other productive sectors, such as in horticultural nurseries for the production of vegetable crops or ornamental plants (Altland et al., 2003; Case et al., 2005; Stewart et al., 2017). In this industry, weeds can negatively affect the growth of cultivated species, but also the marketability of the final product, which must be weed-free (Altland et al., 2003; Case et al., 2005; Stewart et al., 2017). Weed control is a challenge in urban environment as well, albeit the undesirability of these plants is attributable to factors not related to plant production, for instance the negative aesthetic effect, the damage to walls and hard surfaces, the reduced visibility on the streets or the diffusion of allergenic pollen. The combination of different control techniques is necessarily required in urban areas, where chemical measures may represent a risk for the population and should be replaced with alternative methods (Benvenuti, 2004; Monaco et al., 2002; Rask and Kristoffersen, 2007), as required also by current legislation in the EU (e.g. Regulation (EU) No 1107/2009 and Directive 2009/128/CE). Studies on weed control in the abovementioned two sectors, i.e. urban areas and horticulture, are thus topical and mandatory in the attempt to answer legislation and both horticulturists and citizen needs.

A promising perspective on weed management is the development of herbicides based on natural compounds to cope with the dangerous exposure to humans and the environmental concern on synthetic products (Kohli et al., 1997; Westwood et al., 2018). Among natural compounds, plant by-products have gained increasing attention for their benefits over synthetic compounds for an eco-friendly control of weeds (Benvenuti, 2004; Bhowmik and Inderjit, 2003; Kohli et al., 1997; Narwal, 1994), since they are perceived as safer for the environment and human health (Abbas and Duke, 1995). However, few of them have been developed into commercial herbicides, namely triketones, cinmethylin, bialaphos and glufosinate (Cutler and Cutler, 1999; Duke et al., 2000).

Higher plants produce several secondary metabolites to compete with or defend from surrounding species, namely terpenoids, tannins, saponins, flavonoids, and lactones (Ferreira and Aquila, 2000). In particular, the release of chemicals into the environment by one plant to affect another plant is called allelopathy and often results in the germination and growth inhibition of the target plant (Rice, 1984). Mergen (1959) firstly observed that extracts from *Ailanthus altissima* (Mill.) Swingle, a medium-sized tree of the Simaroubaceae family, caused phytotoxicity to other species. This plant is native to China, Japan, Vietnam, and Taiwan and was introduced in Europe as ornamental plant (Sladonja et al., 2015). It has become invasive in all continents, colonising a wide range of habitats, with allelopathy having contributed to its competitiveness and invasiveness, together with its abundant production of seeds and root suckers, a fast growth and high tolerance to pollutants (Benvenuti, 2004; Gómez-Aparicio and Canham, 2008; Kowarik and Säumel, 2007; Sladonja et al., 2015). Plant extracts and essential oils from different organs of *A. altissima* contain alkaloids, terpenoids, steroids, flavonoids, phenolic derivatives, and quassinoids (Albouchi et al., 2013; El Ayeb-Zakhama et al., 2014; Ni et al., 2019), that are sesquiterpene lactones abundantly present in all organs of Simaroubaceae plants. The first quassinoid identified in *A. altissima* was ailanthone (Ail) (Heisey, 1996; Kowarik and Säumel, 2007; Sladonja et al., 2015) that is used to treat ascariasis, diarrhea, spermatorrhea, bleeding and gastrointestinal diseases and has also recently showed antiproliferative activity (Daga et al., 2019). As far as concerns herbicidal activity, Ail showed phytotoxic activity on monocots and dicots both in pre- and post-emergence, causing a strong germination and growth inhibition (De Feo et al., 2003; Heisey, 1996, 1999). Despite showing the potential for the development as a natural-product herbicide, Ail is not commercially used (Bhowmik and Inderjit, 2003), since its application in large agrosystems appears limited by various constraints. In particular, Ail separation and purification costs are remarkably high and the molecule seems not stable and persistent in soil, even at high concentrations (Heisey, 1996, 1999). However, the development of Ail as a natural herbicide could be addressed to protected or limited

environment applications rather than to the open field, where large amount of herbicide are often required and multiple environmental factors can lead to a quick breakdown of the product (Ashton, 1982).

This study aimed to provide new insights in Ail herbicidal activity, persistence and kinetics and to assess its application perspectives for weed control in urban green areas and horticulture. To this aim, the phytotoxic activity was, evaluated on two indicator species, i.e. garden cress (*Lepidium sativum* L.) and radish (*Raphanus sativus* L.), chosen for their different sensitiveness to toxins and their rapid growth (De Feo et al., 2003; Heisey, 1990, 1996, 1999; Heisey and Heisey, 2003; Molinaro et al., 2016). Trials, performed at first on filter paper, were then carried out both on soil from urban environment and on a horticulture cultivation substrate in the attempt of reproducing the substrate conditions of two application sectors of interest. The duration of phytotoxic activity in these substrates and Ail degradation kinetics were also examined to understand stability and persistence of this natural herbicide.

2. Material and methods

Four experiments, summarised in Table 1, were conducted in the DISAFA facilities (45°03′58.5″ Lat. N; 7°35′29.1″ Long. E) during 2016-2017. Ailanthone was purchased from Herbest (Baoji Herbest Bio-Tech Co., Ltd. Baoji, China). The phytotoxic activity of Ail (*Experiments 1* to *4*) was assessed through bioassays in controlled laboratory conditions on garden cress (*Lepidium sativum* L. 'Inglese') and radish (*Raphanus sativus* L. 'Tondo Rosso BIO'), purchased from Fratelli Ingegnoli Spa (Milano, Italy). Treatments were provided by means of aqueous solutions and deionised water was used as control.

2.1. *Experiment 1.* Ailanthone dose-response curve in filter paper

The phytotoxicity of Ail was evaluated by assessing its influence on germination and on root length of garden cress and radish. These parameters were determined in 9 cm diameter Petri dishes (Supplementary Figure S1) by placing randomly ten seeds on filter paper disk (Whatman No. 1, Maidstone, UK). Then, 5 mL of seven aqueous solutions of Ail (1, 1.5, 2, 2.5, 5, 7.5 and 10 mg L^{-1}) were added. Dishes were maintained in a growth chamber, at 25°C, in dark conditions. The number of germinated seeds (n) in each dish and their root (r) length (mm) were measured 96 hours after the treatment (ISTA, 2011) in treated (t) and control (c) seeds. These data were used to calculate the Index of Germination (IGe) with Eq. (1) (Molinaro et al. 2016):

$$IGe\% = \frac{n_{(t)} \times r_{(t)}}{n_{(c)} \times r_{(c)}} \times 100 \quad (1)$$

2.2. *Experiment 2.* Phytotoxic activity persistence in filter paper

Phytotoxicity dynamics of 1.5 and 7.5 mg L^{-1} of Ail on germination, root length and hypocotyl length of garden cress and radish were evaluated on filter paper in 100 mL plastic flasks to allow seedling growth. Five seeds were placed randomly on the filter paper and 1.7 mL of treatment or deionised water were added. Flasks were maintained in a growth chamber at 25°C, with a 12h-light photoperiod. In order to evaluate the persistence of Ail effects, the seeds and the obtained seedlings were removed and new seeds were positioned every 10 days after treatment (DAT), till 30 DAT. Deionised water only (without Ail) was added to all flasks to prevent dryness. The number of germinated seeds (n), their root (r) and hypocotyl (h) length (mm) were measured in each evaluation (10, 20, and 30 DAT) (ISTA, 2011) in treated (t) and control (c) plants. These data were used to calculate the Index of Growth (IGr%) with Eq. (2):

$$IGr\% = \frac{n_{(t)} \times r_{(t)} \times h_{(t)}}{n_{(c)} \times r_{(c)} \times h_{(c)}} \times 100 \quad (2)$$

2.3. *Experiment 3.* Phytotoxic activity of ailanthone in cultivation substrate and non-sterile soil

The response of garden cress and radish to Ail was also determined in a cultivation substrate suitable for containerized production of ornamentals (Floradur® B Seed, Floragard Vertriebs-GmbH) and in non-sterile soil (Table 2) sampled in the DISAFA Campus (Grugliasco, TO, Italy). One-hundred millilitres plastic flasks (Supplementary Figure S2) were filled with 20 g of substrate or soil and moistened with 5 mL of deionised water. Five seeds were then placed randomly in each flask and 1.7 mL of treatment (7.5, 30, 60, and 90 mg L^{-1} of Ail) were added. Flasks were maintained in a growth chamber, at 25°C, in dark conditions and the parameters to calculate Eq. (1) were recorded 96 hours after the treatment.

2.4. *Experiment 4.* Phytotoxic activity persistence in cultivation substrate and non-sterile soil

Phytotoxicity dynamics of 30, 60 and 90 mg L^{-1} of Ail was assessed in cultivation substrate and non-sterile soil (Table 2), with the same experimental conditions used with filter paper (*Experiment 2*). Data were collected at 10, 20 and 30 DAT to calculate Eq. (2).

2.5. *Experiment 5.* Degradation kinetics of ailanthone in cultivation substrate and non-sterile soil

2.5.1. Extraction method

The method was developed by spiking non-sterile soil and cultivation substrate samples with known amounts of an Ail aqueous solution. Different extraction solvents were tested, the best one being methanol/water (10:90, v/v) in the following conditions: suspensions of 20 g soil or substrate in 50 mL methanol/water (10:90, v/v) were stirred on a mechanical shaker (shaker mod. M102-OS, MPM Instruments, Milan, Italy) at 100 rpm for 30 min. The extraction was repeated twice with 25 mL of the same solution. The reunited extracts were filtered through a Whatman no. 1 filters (Whatman, Maidstone, UK). A 10 mL aliquot of the extract was eluted on a BAKERBOND™ SPE C18 (1 g, 6 mL) column (J.T.Baker® Avantor Performance Materials, Center Valley, PA, USA) at a

rate of about 1–2 drops/second. At last, the analyte was eluted with 5 mL of liquid chromatography–mass spectrometry (LC/MS) grade methanol, filtered with a syringe filter in PP (0.45 μm), then diluted (1:5, V/V) with LC/MS grade water and analysed by LC-MS/MS, according to the method described below.

2.5.2. LC-MS/MS determination

LC-MS/MS analysis was carried out on a Varian 310 triple quadrupole mass spectrometer (Agilent, Milan, Italy) equipped with an electrospray ionization ESI source, a 212 LC pump, a ProStar 410 AutoSampler and dedicated software. LC separation was performed on a Pursuit 5 C18 column, 5 μm particle size, 50 × 2.1 mm (Agilent, Milan, Italy). The mobile phase consisted of water (A) and methanol (B), both containing 0.1% (v/v) acetic acid. The gradient was 90 to 10 % A in 3 min with a flow rate of 0.2 mL/min. ESI conditions used in negative polarization were: needle potential – 4000 V, shield -450 V, capillary – 54 V. Gas conditions were set with 25 psi of N_2 as nebulizing gas and 25 psi at 250°C N_2 as drying gas. The respective ion transitions were *m/z* 375 → 300.7 (collision energy 13 V) and 375 → 150.6 (collision energy 24 V). The *m/z* 150.6 was used for quantification.

2.5.3. Degradation studies

Sub samples of freshly collected soil and substrate were supplemented with 1.7 mL of an aqueous solution of Ail (30 mg L^{-1}) in order to obtain a 2.5 mg kg^{-1} soil concentration. The flasks were incubated in the same conditions described in *Experiment 3*. Evaporation of water was periodically (4-5 days) compensated by addition of deionized water. The quantification of Ail was performed 0, 1, 2, 3, 7, 11 and 18 DAT by determination of its concentration in three independent flasks.

2.6. Statistical analyses

Dishes in *Experiment 1* had three replicates (30 seeds in total), while flasks of *Experiment 2*, *3* and *4* had six replicates (30 seeds in total) per treatment, arranged in a completely randomized design. All the studies were conducted in triplicate. Arcsine transformation was performed on percentage data prior to analysis. All presented values are means of untransformed data. Data were tested for the homogeneity of variance (Levene test), then mean comparison and one-way ANOVA were performed to analyse the phytotoxic effect of Ail on model species and means were separated according to Tukey post-hoc test ($p < 0.05$). The analyses were performed with SPSS software (SPSS Inc., version 25, Chicago, Illinois). To define the dose-response curve, for each species a separate regression analysis was performed between Ail concentration (independent variable) and the index of germination IGe (dependent variable) fitting the following three parameters log-logistic model, Eq. (3):

$$\mathrm{IGe} = \frac{\mathrm{d}}{1+\exp[\mathrm{b}(\log(\mathrm{x})-\log(\mathrm{e}))]} \quad (3)$$

where *IGe* is the index of germination expressed as a percentage of the control, *b* and *d* are the curve parameters, with *b* being the relative slope at the point of inflection *e* and *d* the upper limit, while *x* is the Ail concentration (in mg L^{-1}). The regression analysis was performed using the function *drm* of the add-on package *drc* of the *R* software (RCoreTeam, 2017). The effective concentration required to reduce by 10%, 50% and 90% the IGe index (ED_{10}, ED_{50}, ED_{90}) was calculated using the function *ED* of the package *drc*. The function *EDcomp* of the package *drc* was used to calculate the ratio between the ED values of the two species (ED ratio) and to test the significance of differences of ED_{10}, ED_{50}, and ED_{90} between the two species. Differences were considered significant when the confidence interval of ED ratio at $p \leq 0.05$ did not included one.

3. Results

3.1. *Experiment 1*

The dose-response curve showed that at increasing rates of Ail the Index of germination (IGe%) gradually reduced (Figure 1). In particular, at lower Ail concentrations, garden cress was more sensitive compared to radish; for example, at 1.5 mg L^{-1} of Ail the average IGe values were 32 and 45 for garden cress and radish, respectively (Figure 1). The higher sensitiveness of garden cress was also demonstrated by its ED_{10} (Ail concentration able to reduce the index of germination of 10%), which was significantly lower compared to that of radish (Table 3). However, at higher rates the behaviour was opposite as radish showed a strong reduction of IGe, with values of about 1 or less at concentrations above 7.5 mg L^{-1}, while for garden cress the recorded values were about 10 or higher at the same concentrations. The ED_{50} and ED_{90} for index of germination were not significantly different between the two tested species due to data variability.

3.2. *Experiment 2*

Generally, Ail was more effective at the highest concentration (7.5 mg L^{-1}) in both species (Figure 2), because significant differences in their IGr were recorded in each evaluation. The concentration of 1.5 mg L^{-1} reduced the IGr of both species by 80% compared with control at 10 DAT, but its efficacy was gradually lost in the following evaluations. Conversely, the herbicidal effect of the highest concentration strongly persisted until 20 DAT in garden cress (Figure 2a), and 30 DAT in radish (Figure 2b), with a growth reduction of about 95% compared with control in both evaluations.

3.3. *Experiment 3*

The lowest concentration tested (7.5 mg L^{-1}) was not markedly effective on both species in cultivation substrate (Figure 3a), while the growth was reduced by more than 95% in soil compared with control (Figure 3b). The herbicidal activity was very strong at higher concentrations (30, 60, and 90 mg L^{-1}) in both substrates, with no significant differences between species.

3.4. *Experiment 4*

Moulds started to develop after the first measurement (10 DAT) in non-sterile soil in growth chamber conditions, therefore data refer solely to the cultivation substrate. Although little stimulation effects were observed in garden cress at 30 DAT, data were not significantly higher than control. A growth inhibition was instead mostly observed and garden cress (Figure 4a) appeared less sensitive than radish (Figure 4b), especially at 20 and 30 DAT. Every Ail treatments significantly affected seedlings compared with control. Moreover, 60 and 90 mg L^{-1} had stronger herbicidal activity than 30 mg L^{-1} at 10 and 30 DAT in radish, which displayed an IGr lower than 10% (10 DAT) and 60% (30 DAT), respectively.

3.5. *Experiment 5*

Notwithstanding different polarity extraction solutions were tested at different pH, the highest recovery from the spiked non-sterile soil, obtained with the procedure described above, was 65.6 ± 5.9 %. In the case of the spiked cultivation substrate, the recovery dropped to 46.0± 5.8 %. The degradation rate of Ail, expressed in µg of Ail recovered at each sampling time, is illustrated in Figure 5. The degradation curves were fitted by an exponential equation ($R^2 > 0.97$), indicating that the degradation followed a first order kinetics. The calculated half-life times (DT_{50}) were 1.5 and 1.4 days for soil and substrate respectively, while the DT_{90} values were 7.0 days in the soil and 6.1 days in the substrate.

4. Discussion

Data obtained in this study deepen the knowledge on the quassinoid Ail derived from *A. altissima*, which already proved to have numerous biological activities, not only herbicidal but also antiproliferative, antiplasmodial, antituberculosis, antimicrobial, insecticidal, antioxidant, anti-

268 inflammatory, and algaecide (Albouchi et al., 2013; De Feo et al., 2005; El Ayeb-Zakhama et al.,
269 2014; Gu et al., 2014; Lü and He, 2010; Meng et al., 2015; Okunade et al., 2003; Rahman et al.,
270 2009). In controlled conditions, Ail was extremely efficient on indicator species in filter paper,
271 suggesting that it is an already active compound and does not need to be modified to reach its active
272 form (Soltys et al., 2013). Very low concentrations (2.5-10 mg L^{-1}) were sufficient to depress by
273 more than 80% the IGe of garden cress and radish in Petri dishes in 96 hours under controlled
274 conditions (Figure 1Figure 1), confirming previous results of Heisey (1996) on garden cress at
275 similar concentrations (2 mg L^{-1}). On the whole, garden cress had a lower ED_{50} and ED_{90} compared
276 to radish. At increasing Ail doses, a different behaviour between species was observed. A lower
277 amount of Ail was needed to reduce the growth of radish and this was mainly due to a strong total
278 germination decrease in this species, while in garden cress the germination was only partially
279 affected (Demasi et al., Data in brief). Conversely, the root length was markedly inhibited in both
280 species at increasing Ail concentrations. If seedlings were let to grow in paper, radish displayed a
281 high sensitivity (Figure 2) to a very low amount of Ail (1.5 mg L^{-1}) and for 10 days more than cress.
282 Again, data showed that germination of cress was only partially affected, while root and hypocotyl
283 growth were mostly inhibited (Demasi et al., Data in brief).

284 Most of the information on herbicide activity and degradation derive from field soil trials, and
285 are often assumed to be the same in other substrates. However, horticulture and urban green areas
286 use soilless substrates which consist almost entirely of organic matter (Stewart et al., 2017). The
287 effectiveness of Ail can be deeply influenced by the presence of an organic substrate (Heisey,
288 1996). In this study, the amount of 7.5 mg L^{-1} of Ail, which was very effective on filter paper (IGe
289 about 2.5%) and soil (IGe about 5%), was almost ineffective in cultivation substrate (IGe about
290 75%). This evidence was fundamental to assess the suitable amount of Ail (i.e. $\geq$ 30 mg L^{-1}) to
291 obtain herbicidal efficacy in substrate on garden cress (IGe lower than 20%) and radish (IGe lower
292 than 10%). When bioassays were conducted in cultivation substrate, radish confirmed its higher

293 sensitiveness than cress to Ail, even at 20 and 30 DAT (Figure 4), even if both species showed a
294 slight reduction of germination (Demasi et al., Data in brief). These data confirm that radish is more
295 prone to be affected by *A. altissima* allelochemicals compared to other species (De Feo et al., 2003).
296 Natural compounds can inhibit the germination or growth of plants through different modes of
297 action (Macías et al., 2003), thus the diverse responses of one species to toxic compounds can be
298 possibly due to the different mechanisms of inhibition of the active ingredient. However, in this
299 regard little is known about ailanthone (Duke et al., 2000) but, similarly to other quassinoids, can be
300 envisaged that Ail might act as mitosis inhibitor (Dayan et al., 1999).
301 In literature, the only studies on Ail toxicity duration (Heisey, 1996, 1999) reported that the
302 effect was rapidly lost (in 3 days) when incubated at 25°C in presence of non-sterile soil. The
303 breakdown of an herbicide in soil depends on several factors, including degradation acted by
304 microbes, which presence is usually supported by organic matter. This latter, in turns, can give
305 suitable surface for sorption of the herbicide, limiting its bioavailability. Both of the
306 abovementioned mechanisms are plausible explanations for the loss of phytotoxicity displayed in
307 this study in the cultivation substrate. Indeed, when the trial was performed on paper (*Experiment*
308 *2*), Ail was deeply active at low doses until 30 DAT on radish and 20 DAT on garden cress, while
309 on cultivation substrate lasted 30 DAT on radish, but using eight-times higher concentration (60 mg
310 L^{-1}). Ail remained highly toxic throughout 21 days if the soil was sterilised (Heisey, 1996). This
311 study also provided for the first time information on Ail degradation in soilless substrate, compared
312 to that in non-sterile soil. The half-life times for Ail degradation (DT_{50}) were similar in both
313 substrates, while the DT_{90} value was higher in the soil, meaning that longer time was needed to
314 degrade Ail. This is probably due to the lower amount of organic material in the soil tested. The
315 short persistence of the active principle could be an advantage for the environment and human
316 safety, but at the same time can be an hindrance, leading to a rapid loss of the herbicidal activity

(Bhowmik and Inderjit, 2003; Cutler and Cutler, 1999; Duke et al., 2000; Kohli et al., 1997; Narwal, 1994; Sladonja et al., 2015).

Conclusions

Ail showed a high phytotoxic activity, supporting evidence of *A. altissima* allelopathy, which contributes to the high invasiveness of the species. The phytotoxic activity was different based on the tested species, thus its efficacy can be variable and related to the species sensitivity. Further researches in this regard are thus needed to confirm Ail potential use in horticulture and urban green areas as natural herbicide.

Declaration of interest: none.

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Data in Brief: Ailanthone inhibition data on seed germination and seedling growth of *Lepidium sativum* L. and *Raphanus sativus* L. Demasi S., Caser M., Fogliatto S., Vidotto F., Trotta F., Scariot V.

Tables

Table 1. Summary of the bioassays performed to evaluate the phytotoxic activity of ailanthone (Ail).

Experiment No.	Ail concentration (mg L^{-1})	Container	Substrate[a]	Conditions[b]	Duration	Evaluation
1	1, 1.5, 2, 2.5, 5, 7.5, 10	Petri dish	P	D	96 h	IGe%[c]
2	1.5, 7.5	Plastic flask	P	L	10, 20, 30 DAT[e]	IGr%[d]
3	7.5, 30, 60, 90	Plastic flask	C and S	L	96 h	IGe%
4	30, 60, 90	Plastic flask	C and S	L	10, 20, 30 DAT	IGr%

[a]P=paper; C=cultivation substrate; S=soil. [b]D=dark, growth chamber, 25°C; L=12h photoperiod, growth chamber, 25°C. [c]IGe%= Index of Germination. [d]IGr%= Index of Growth. [e]DAT= days after treatment

Table 2. Physical and chemical characteristics of the cultivation substrate and non-sterile soil used in the *Experiments 3* and *4*.

Floradur® B Seed			Non-sterile soil		
pH ($CaCl_2$)		5.6	pH[a]		8.0
Salinity	g L^{-1}	0.8	Carbonates[b]	%	12.02
N ($CaCl_2$)	mg L^{-1}	140	C tot[c]	%	2.257
P (P_2O_5)	mg L^{-1}	80	N tot[c]	%	0.069
K (K_2O)	mg L^{-1}	190	CEC[d]	meq $100g^{-1}$	4.61
			Exchageable Ca	mg L^{-1}	1012
			Exchageable Mg	mg L^{-1}	27
			Exchageable K	mg L^{-1}	30
			Available P[e]	mg L^{-1}	9.5
			Clay	%	3.69
			Silt	%	7.60
			Sand	%	88.71

[a]ISO 10390; [b]ISO 10693; [c]ISO 10694; [d]cation exchange capacity, ISO 11260; [e]Olsen.

Table 3. Concentration required to reduce by 10%, 50% and 90% (ED_{10}, ED_{50} and ED_{90}) the IGe index and their ratio with lower and upper limits of the confidence interval for garden cress and radish (*Experiment 1*).

	Garden cress	Radish	ED ratio [a]	ED ratio lower limit	ED ratio upper limit
ED_{10}	0.16	0.41	0.40*	0.06	0.74
ED_{50}	1.06	1.19	0.89ns	0.63	1.16
ED_{90}	6.93	3.46	2.00ns	0.99	3.01

[a]ED ratio: calculated as EDx garden cress/EDx radish. The statistical relevance is provided (ns, non-significant; * $p \leq 0.05$).

Figure captions

Figure 1. Dose-response curve of index of germination (IGe%) of garden cress (*Lepidium sativum*) and radish (*Raphanus sativus*) in response to different concentrations of ailanthone on filter paper after 96 hours under controlled conditions (*Experiment 1*).

Figure 2. Index of growth (IGr%) of a) garden cress (*Lepidium sativum*) and b) radish (*Raphanus sativus*) in response to 1.5 (grey) and 7.5 mg L^{-1} (black) of ailanthone in filter paper 10, 20, and 30 days after treatment (DAT) under controlled conditions (*Experiment 2*). Similar upper case letters along the same treatment and similar lower case letters within the same group denote no significant differences according to Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

Figure 3. Index of germination (IGe%) of garden cress (*Lepidium sativum*) and radish (*Raphanus sativus*) in response to different concentrations of ailanthone on a) cultivation substrate and b) non-sterile soil in controlled conditions (*Experiment 3*). Similar lower case letters in garden cress and similar upper case letters in radish denote no significant differences among concentrations, according to Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

Figure 4. Index of growth (IGr%) of a) garden cress (*Lepidium sativum*) and b) radish (*Raphanus sativus*) in response to 30, 60 and 90 mg L^{-1} of ailanthone on cultivation substrate after 10, 20, and 30 days after treatment (DAT) (*Experiment 4*). Similar upper case letters along the same treatment and similar lower case letters within the same group denote no significant differences according to Tukey post-hoc test ($p < 0.05$). Bars indicate the standard error.

Figure 5. Degradation rate of ailanthone in non-sterile soil and cultivation substrate.