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Research article

Use of by-products from the industrial distillation of lavandin (*Lavandula x intermedia*) essential oil as effective bioherbicides

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ABSTRACT

Weeds are one of the main problems causing losses in agricultural crops, which are nowadays mainly combated by the massive use of chemical herbicides. The development of new effective, sustainable, environmentally and health-friendly bioherbicides is a fundamental need worldwide. In this work, hydrolates and lavandin distilled straws produced during the distillation of the essential oil of lavandin (*Lavandula x intermedia*) were tested as potential bioherbicides. The weeds used were brome, annual ryegrass (monocotyledons), goosefoot and mat amaranth (dicotyledons) and the crops wheat, barley (monocotyledons), lentil and vetch (dicotyledons). The herbicidal capacity of both by-products was studied by applying the hydrolates *in vitro* on seeds and mixing the distillation straws with the growing substrate. Lavandin hydrolates significantly inhibited germination and growth of the four weeds used, being also phytotoxic for monocotyledonous crops, but hardly showed a phytotoxic effect on the dicotyledonous crops (lentil and vetch). With respect to lavandin distilled straws, they had an allelopathic effect of growth inhibition on all weeds and crops used in the work. In both by-products, lysophosphatidylcholine was identified as one of the major metabolites, while coumaroyl hexoside and feruloyl hexoside were identified as major metabolites only present in the straws. So far, only the phytotoxic capacity of the metabolites lysophosphatidylcholine and coumaroyl hexoside had been described. Therefore, by-products from the industrial distillation of lavandin could be used in the development of effective and sustainable bioherbicides, due to the allelopathic capacity of the metabolites present.

1. Introduction

Currently, the world population reaches 8.1 billion people (Worldometer, 2023). By 2050, it is estimated that the world population will have reached 10 billion people, which will require a 50% increase in agricultural productivity to feed them (Tripathi et al., 2019). The main causes of agricultural productivity loss today are pathogens, pests and weeds as biotic stresses, and drought as abiotic stress, causing losses in quantity and quality of 20–40% and up to 50%, respectively (Mesterházy et al., 2020).

Weeds cause crop problems due to competition for water, nutrients and solar radiation, which can lead to severe yield losses (Monteiro and Santos, 2022). These biotic stresses are controlled either mechanically, by tillage, which leads to increased erosion, or by herbicides (Monteiro and Santos, 2022). The massive use of herbicides in agriculture is associated with serious environmental and health problems, through contamination of water, soil, food and atmosphere (Kudsk and Streibig, 2003). Some of the diseases associated with accidental herbicide exposure in humans include birth defects, cancers, diabetes, respiratory disorders, neurological damage, developmental disability, reproductive disability, weaker immune system, and endocrine system disparity (Rani et al., 2021; Cavalier et al., 2023); although more factors than exposure to synthetic herbicides may be involved in these negative health consequences. In addition, another problem associated with uncontrolled herbicide use is the development of resistance by weeds, which induces the use of more herbicide, adding to environmental and health problems

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(Hasanuzzaman et al., 2020). In recent years, new and more sustainable weed control strategies are being developed (Korres et al., 2019). For example, mulching, covering the soil with wastes or synthetic materials to prevent the emergence of weeds, cover crops or living mulches, occupying the space that weeds could occupy between crop periods of interest, solarization, although it is harmful to beneficial soil organisms, or livestock grazing (Monteiro and Santos, 2022). Along with these strategies, the European Union is also committed to the development of others, such as the use of machine learning for the targeted application of herbicides, the use of bioherbicides from microbial and plant origin (allelopathy) and crop rotation (Tataridas et al., 2022). These strategies are included in the European Green Deal and the Farm to Fork strategy, which aims to reduce the use of chemical pesticides by 50% by 2030 (Montanarella and Panagos, 2021).

Furthermore, the evolution of herbicide resistance in crop weeds presents one of the greatest challenges to agriculture and new modes of actions (MOAs) are badly needed (Peterson et al., 2018; Baucom, 2019). However, relatively few new MOAs have been recently discovered (Qu et al., 2021). Natural compounds have been identified as potential source of new MOAs because they offer an unparalleled source of structural diversity, with little overlap with synthetic compounds generated by traditional organic synthesis in the laboratory (Duke and Dayan, 2022; Berestetskiy, 2023).

Bioherbicides are defined as organisms (plant pathogens, insects or plants) or compounds produced by them (phytotoxins or plant extracts) with the ability to act as a natural means of weed control (Hasan et al., 2021). The current need for the use of more environmentally and health friendly pesticides has led to an increase in the development of bioherbicides, with mainly those derived from microorganisms already available on the market (Hasan et al., 2021). However, their actual implementation in the market is still very limited, due to the fact that they are not able to compete with respect to the spectrum and price of chemical herbicides (Marrone, 2023). When the bioherbicide is produced by the use of other plants, by the release of root exudates or by the use of plant extracts and/or plant residues, it is called allelopathy. The production of allelopathic compounds by plants is a completely natural process to prevent the growth of potential competitors for resources and space around them, mainly by volatilization, root exudation or decomposition of dead plant biomass. About 1200 allelopathic compounds are currently known, mainly alkaloids (produced by the families Asteraceae, Apocynaceae, Boraginaceae or Fabaceae), phenolic compounds (produced by the families Apiaceae, Rutaceae, Asteraceae or Fabaceae), and terpenes (produced by the families Brassicaceae, Asteraceae or Myrtaceae) (Kostina-Bednarz et al., 2023).

The genus *Lavandula* includes several species cultivated industrially for ornamental and aromatic purposes. In particular, lavandin (*Lavandula x intermedia*) is the hybrid between true lavender (*Lavandula angustifolia* = *Lavandula officinalis*) and spike lavender (*Lavandula latifolia*) (Pokajewicz et al., 2023a). Lavandin is an herbaceous perennial dwarf shrub (40–80 cm) with violet flower spires that do not produce seeds (sterile hybrid) (Gallotte et al., 2020; Pokajewicz et al., 2023a). Within the genus, lavandin is the crop that produces the highest yields of flowers and the highest concentration of essential oils: 100 kg of essential oil/ha vs. 15–40 kg for lavender (Gallotte et al., 2020). Essential oils are produced by the conical cells lining the petals and sepals, being the main components in lavandin the oxygenated monoterpenes camphor and eucalyptol (or 1,8-cineole) (Héral et al., 2021). Having a less sophisticate sensory profile than lavender, lavandin is used for industrial perfumery, providing aroma in detergents and air fresheners (Pokajewicz et al., 2023b).

The essential oils are obtained from lavandin by distillation of flowers and stems, in order to release the lipids from the glandular structures. In this industrial process a supernatant oil (designated essential oil) in amounts ranging of 6–10 % v/w dry matter is obtained as a product, and as by-products the aqueous phase (designated as floral water or hydrolate) and the plant biomass (designated as lavandin distilled straws), both rich in bioactive compounds (Lesage-Meessen et al., 2015). Some of the uses described for lavandin hydrolates include as antimicrobial and nematicide, but there are several papers describing its phytotoxic ability against canola, wheat, subterranean clover, ryegrass, and radish (reviewed by Pokajewicz et al., 2023b). Regarding distilled straws, current uses include mulching to protect trees and crops against rain and soil moisture loss, and as an antifungal when mixed with soils (Lesage-Meessen et al., 2015).

As noted above, it is necessary to search for new biological alternatives that allow the control of weeds (especially those herbicide-resistant biotypes) without harming the environment and health, being wastes from the lavandin industry a possible alternative. The objectives of this work were based on studying the allelopathic capacity of hydrolates and wastes of plant biomass (distilled straws) from lavandin distillation on different weeds and crops of interest. In addition, metabolomic analysis of both raw materials (hydrolates and straws) was performed in order to identify the common major metabolites possibly involved in the results achieved.

2. Materials and methods

2.1. Plant material used

In this work, four different species of weeds and four crops were used, each group including two monocotyledonous and two dicotyledonous species. The weeds used were brome (*Bromus rigidus*; Poaceae family), annual ryegrass (*Lolium rigidum*; Poaceae family) (both monocotyledons), goosefoot (*Chenopodium album*; Amaranthaceae family) and mat amaranth (*Amaranthus blitoides*; Asteraceae family) (both dicotyledons). The weeds seeds were obtained from weed seed bank of the Higher Technical School of Agricultural Engineering of Palencia (Spain). The crops used were wheat (variety Rimbaud) (*Triticum aestivum*; Poaceae family), barley (variety Mendiola) (*Hordeum vulgare*; Poaceae family) (both monocotyledons), lentil (variety Guareña) (*Lens culinaris*; Fabaceae family) and vetch (variety Filón) (*Vicia sativa*; Fabaceae family) (both dicotyledons). The crops seeds were kindly provided by the Agrarian and Agri-Food Technology Center ITAGRA (Palencia, Spain).

2.2. Study of lavandin hydrolates and distilled straws as bioherbicides

The lavandin hydrolates and distilled straws were provided by the farmers' society "Palentina de Aromáticas" (Ampudia, Palencia, Spain). In order to study the possible allelopathic capacity of lavandin hydrolates and distilled straws, different methodological approaches were used. With the hydrolates, a study of *in vitro* weed/crop germination was carried out.

For the *in vitro* germination study of the four weeds and the four crops in contact with the hydrolates, seeds were deposited in 12 cm diameter Petri dishes with Whatman® qualitative filter paper (Sigma-Aldrich, USA). Five seeds were deposited per Petri dish, covered with filter paper on top and underneath. The five different treatments were applied on the filter papers: 0% (control with distilled water), 25%, 50%, 75% and 100% (different concentrations of hydrolates and distilled water; v/v). Four replicates were made per treatment (20 seeds in total per treatment). Seeds were incubated in a growth chamber at 25/20 °C with a 16/8 h light/dark photoperiod. Daily germination data were taken and radicle and plumule lengths of seedlings were measured.

On the other hand, in order to analyze the possible allelopathic effect of lavandin straw against weeds and crops, this by-product was mixed with various proportions of peat, where the seeds were sown. Pots of 75 mL were used, filling them with the different treatments with respect to the v/v ratio of peat and straws: 1:0 (as a control without lavandin straws), 4:1, 2:1, 1:1 and 1:2. One seed was sown per pot, with 12 seeds per treatment. Data were taken on day of seedling emergence and stem length of emerged seedlings 45 days after sowing.

2.3. *Metabolomic analysis of lavandin hydrolates and distilled straws*

The analysis of metabolites present in lavandin hydrolates and straw was performed according to the methodology described by Rodríguez et al. (2023), with modifications. The material to be analyzed was 50 mg of hydrolates lyophilized or dry powder from the straws, which were dissolved in 500 mL of 80% aqueous methanol and then sonicated for 15 min. After centrifugation for 10 min (16,000×*g*, at room temperature), the extract was filtered through a 0.20 μm micropore PTFE membrane and placed in vials for further analysis. For the analysis of metabolomic composition, we employed ultra-performance liquid chromatography combined with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC–Q–TOF–MS/MS) using a Thermo Dionex Ultimate 3000 LC (Thermo Fisher Scientific, Waltham, MA, USA) and a Bruker Compact™ mass spectrometer featuring a heated electrospray ionization (ESI) source. The chromatographic separation was carried out on an Intensity Solo 2 C18 column (2.1 × 100 mm, 1.7 μm pore size; Bruker Daltonics, Billerica, MA, USA) utilizing a binary gradient solvent system composed of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient profile was as follows: 3% B from 0 to 4 min, increasing from 3% to 25% B from 4 to 16 min, then from 25% to 80% B from 16 to 25 min, followed by a transition from 80% to 100% B from 25 to 30 min, maintaining 100% B until 32 min, then returning from 100% to 3% B from 32 to 33 min, and finally holding at 3% B until 36 min. The injection volume was set at 5 μL, with a flow rate of 0.4 mL/min and the column temperature maintained at 35 °C. Mass spectrometry analysis was conducted over a spectral acquisition range of 50–1200 m/z. Both positive and negative polarities of the ESI mode were utilized under specific conditions: gas flow at 9 L/min, nebulizer pressure at 38 psi, dry gas flow at 9 L/min, and a dry temperature of 220 °C. The capillary and end plate offsets were configured to 4500 V and 500 V, respectively. External calibration of the instrument was conducted using a calibration solution containing 1 mM sodium formate/acetate in a 50/50 mixture of iPrOH and H_2O, with 0.2% formic acid, directly infused into the source. Prior to sample injections, the stability of the LC-qTOF system was assessed through three consecutive injections of chloramphenicol (ESI–mode; $\Delta RT = 0.02$ min; $\Delta m/z = 0.002$) and triphenyl phosphate (ESI + mode; $\Delta RT = 0.02$ min; $\Delta m/z = 0.001$). A calibration solution was injected at the start of each run, and all spectra were calibrated before conducting statistical analyses. MS/MS analysis was carried out using the previously established accurate mass and retention time, employing varying collision energy ramps ranging from 15 to 50 eV for fragmentation. The T–Rex 3D algorithm from MetaboScape 4.0 software (Bruker Daltonics, Billerica, MA, USA) was utilized for peak alignment and detection, as well as for creating both positive and negative bucket tables.

2.4. *Statistical analysis*

Probability of not germinating based on the nonparametric estimator Kaplan–Meier was analyzed with the "Survival" package (Therneau, 2023). Probability of not germinating curves were created with the "Survfit" function and the differences between the curves were tested with the "Survdiff" function. Analyses of variance (ANOVAs) and multiple comparison procedures were performed to test the effects of hydrolates and straws of lavandin on growth seedlings. As the data violated two of the ANOVA assumptions (normality and homogeneity of variances) nonparametric statistics were performed. Several nonparametric methods have been suggested for environmental studies (Ferraro et al., 2024), however robust statistical methods were applied in this study (García-Pérez, 2010). In particular, heteroscedastic one-way ANOVAs were performed using the generalized Welch procedure and a 0.1 trimmed mean transformation. The ANOVAs were carried out using the "Wilcox' Robust Statistics (WRS2)" package (Mair and Wilcox, 2020). All analyses were performed using R software environment (R Foundation for Statistical Computing, Vienna, Austria).

Statistical analysis of metabolomic data was performed using the web-based software Metaboanalyst (Chong et al., 2019). In order to remove non-informative variables, data were filtered using the interquantile range filter (IQR). Moreover, Pareto variance scaling was used to remove the offsets and adjust the importance of high- and low-abundance ions to an equal level. The resulting three-dimensional matrix (peak indices, samples and variables) was further subjected to statistical analysis. Univariate analysis (one-way ANOVA) with a *p* value ≤ 0.05 was carried out to find differentially expressed metabolites. Using the Volcano Plot (VP) approach, which measure differentially accumulated metabolites based on *t*-statistics and fold changes simultaneously, we also highlighted the metabolites with a $|\log2(FC)||\log2(FC)| \geq 1$ and statistically significant difference (FDR $\leq$0.05) between lavandin hydrolates and straws.

3. Results

3.1. *Study of lavandin hydrolates as bioherbicides*

In order to determine the possible allelopathic capacity of lavandin hydrolates on different weeds and crops, an *in vitro* study was carried out. *In vitro*, with respect to weeds, a significant reduction in germination was observed in all the species used with the application of hydrolates at 100% concentration (Fig. 1). In *B. rigidus*, the hydrolate concentration of 25% caused a significant increase in the probability of non-germination compared to the water control. Increasing the hydrolate concentrations to 50% and 75% caused an even greater and significant increase in the probability of non-germination compared to 25%, being even greater and significantly different at 100% hydrolate concentration (Fig. 1). In L. *rigidum*, the 50%, 75% and 100% hydrolate concentrations caused significantly lower germination compared to the water control. In addition, increasing hydrolate concentrations of 50%, 75% and 100% caused significantly different and lower germinations (Fig. 1). In dicots, a smaller effect of hydrolates on weed was observed, requiring higher concentrations to inhibit germination. In *C. album*, hydrolate concentrations of 75% and 100% significantly inhibited germination, with no significant differences between the two concentrations (Fig. 1). Finally, in the case of *A. blitoides*, 50%, 75% and 100% hydrolate concentrations significantly reduced germination compared to the water control, with no significant differences between the different concentrations (Fig. 1).

Regarding the crops, the *in vitro* germination study also reported that in all of them, the 100% concentration of hydrolates significantly inhibited seed germination, compared to the control with water. In *T. aestivum*, all hydrolate concentrations significantly inhibited seed germination compared to the control. However, this germination inhibition is greater and significantly different among the different concentrations as they increase from 25% to 100% (Fig. 2). In *H. vulgare*, hydrolates concentrations between 50% and 100% significantly inhibited seed germination compared to the control, and the 100% concentration significantly inhibited germination more than the rest of the treatments (Fig. 2). With respect to dicotyledons (*L. culinaris* and *V. sativa*), the effect of lavandin hydrolates was similar in both crop species: only the 100% concentration of hydrolates significantly inhibited germination, compared to the rest of the treatments (Fig. 2).

In the *in vitro* study, radicle and plumule length of germinated seedlings were also analyzed. In *B. rigidus*, the absence of germination with the 100% lavandin hydrolates treatment prevented the measurement of seedling development. A significant decrease in radicle and plumule length was reported with the 25–75% hydrolates concentration treatments compared to the water control. In addition, radicle length was significantly reduced with the 75% hydrolates treatment compared to the 25 and 50% concentration treatments (Fig. 3). With respect to *L. rigidum*, the application of all concentrations of lavandin hydrolates significantly reduced radicle and plumule length compared to the water control. Moreover, radicle and plumule growth was significantly lower

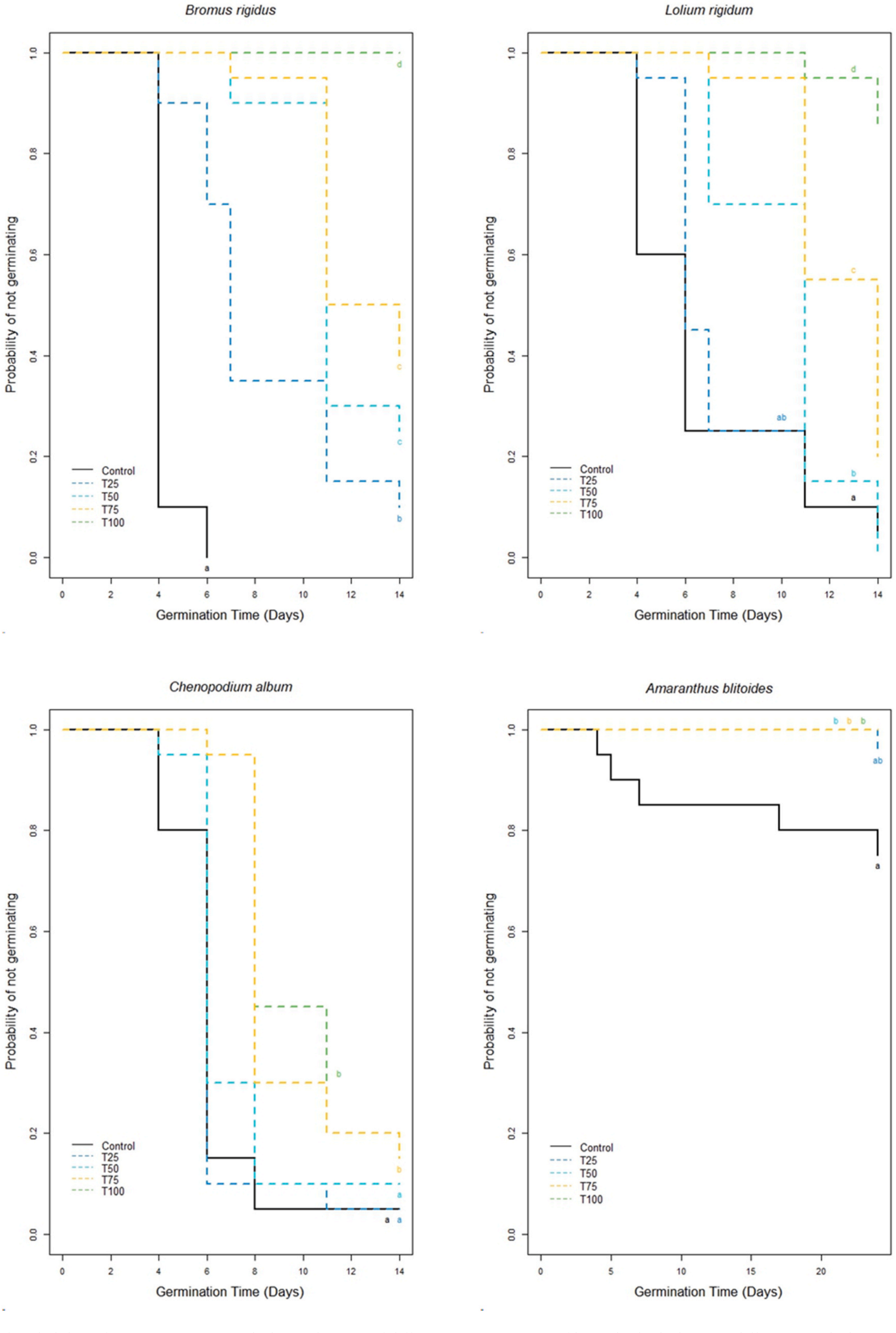


Fig. 1. Curves of probability of non-germination with the application of different concentrations of lavandin hydrolates on different weeds: brome (*Bromus rigidus*), annual ryegrass (*Lolium rigidum*), cocksfoot (*Chenopodium album*) and mat amaranth (*Amaranthus blitoides*). C: control with distilled water; T25: 25% hydrolates in distilled water (v/v); T50: 50% hydrolates in distilled water (v/v); and T100: 100% hydrolates in distilled water (v/v). Means with the same letter were not significantly different according to the Kaplan-Meier estimator ($\alpha \leq 0.05$).

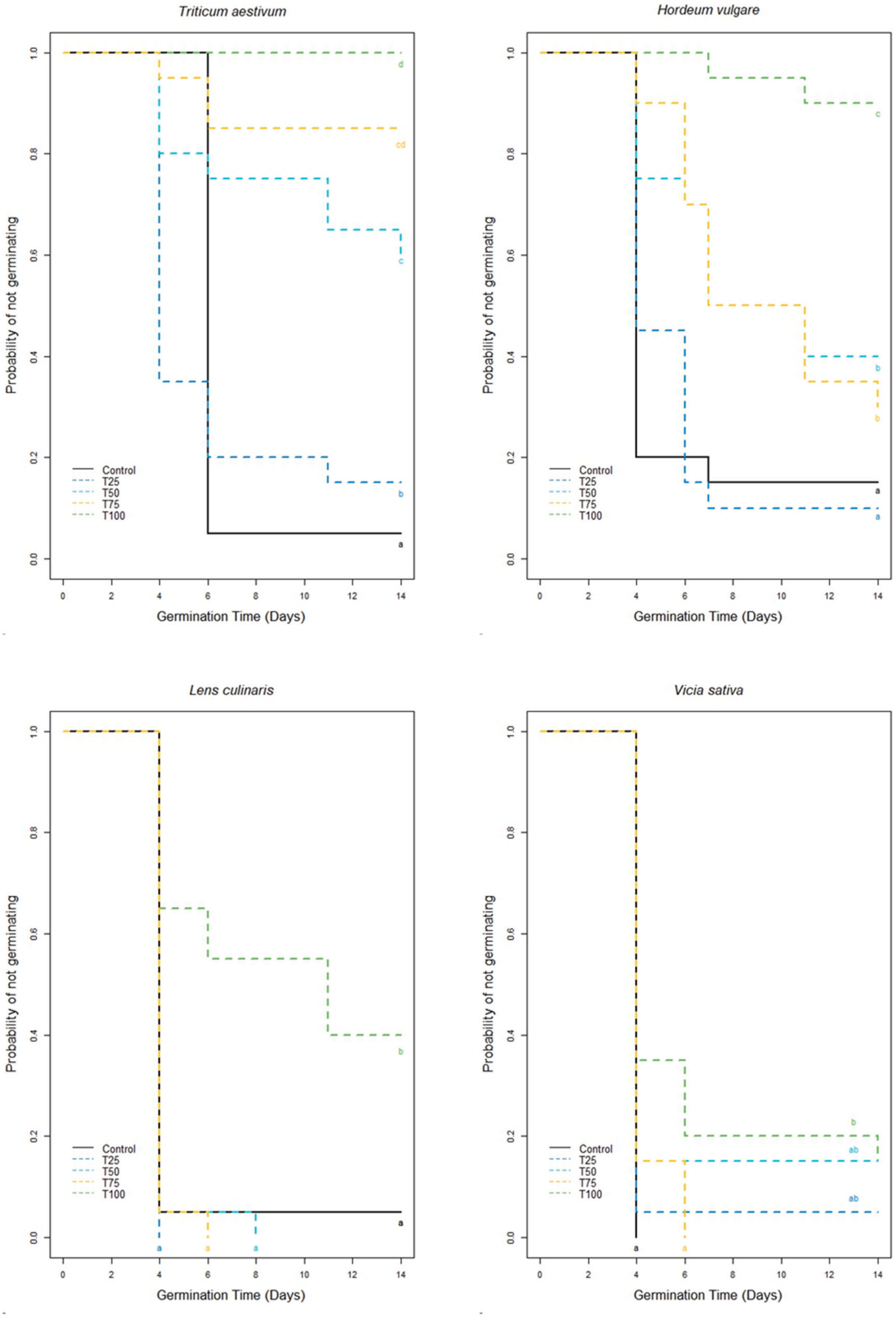


Fig. 2. Curves of probability of non-germination with the application of different concentrations of lavandin hydrolates on different crops: wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lentil (*Lens culinaris*) and vetch (*Vicia sativa*). C: control with distilled water; T25: 25% hydrolates in distilled water (v/v); T50: 50% hydrolates in distilled water (v/v); and T100: 100% hydrolates in distilled water (v/v). Means with the same letter were not significantly different according to the Kaplan-Meier estimator (α ≤ 0.05).

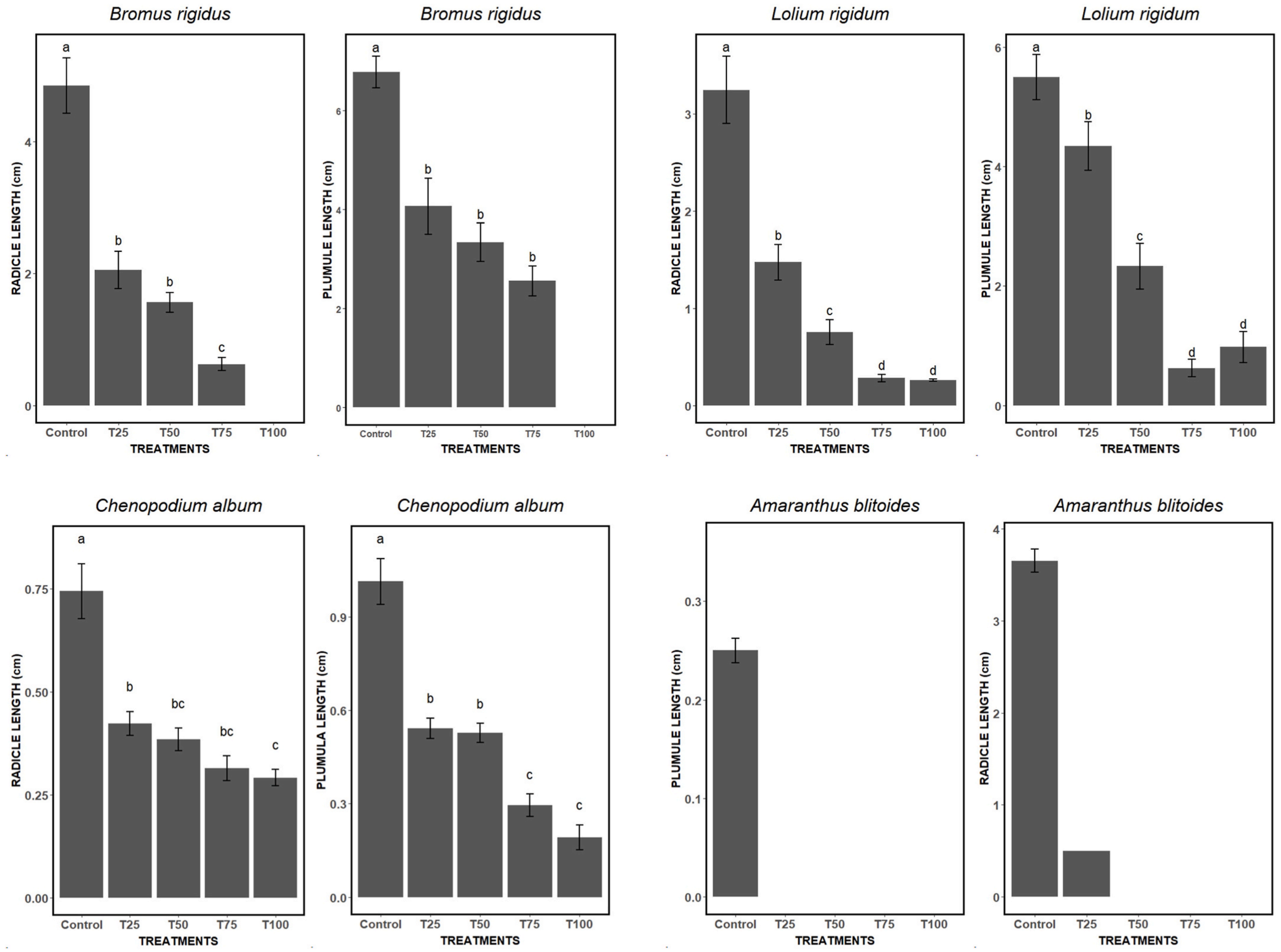


Fig. 3. Radicle and plumule length of the weeds brome (*Bromus rigidus*), annual ryegrass (*Lolium rigidum*), cocksfoot (*Chenopodium album*) and mat amaranth (*Amaranthus blitoides*) with the application of different concentrations of lavandin hydrolates on different weeds: C: control with distilled water; T25: 25% hydrolates in distilled water (v/v); T50: 50% hydrolates in distilled water (v/v); and T100: 100% hydrolates in distilled water (v/v). Means with the same letter were not significantly different according to the Kaplan-Meier estimator ($\alpha \leq 0.05$).

as the concentration of lavandin hydrolates increased, up to the concentrations of 75 and 100% in hydrolates, with no significant differences between them (Fig. 3). In *C. album*, significantly reduced radicle and plumule length was also reported in all seedlings treated with lavandin hydrolates. Radicle length was significantly reduced with the 100% concentration of hydrolates compared to the 25% concentration, however, the 50% and 75% concentrations did not produce significant differences compared to the other two hydrolate concentrations. In the plumule, the 75 and 100% concentrations of lavandin hydrolates significantly reduced growth compared to the 25 and 50% concentrations (Fig. 3). Finally, within the weeds, the absence of *A. blitoides* seed germination under different hydrolates concentrations prevented measurements of radicle and plumule length and the existence of significant differences compared to the water control (Fig. 3).

As far as crops are concerned, a different response was observed with lavandin hydrolates depending on whether they were monocotyledonous or dicotyledonous. In *T. aestivum*, the absence of seed germination with the 100% lavandin hydrolate treatment prevented growth measurements. The application of 25–75% hydrolate concentrations significantly reduced radicle and plumule length, and was significantly lower with the 50 and 75% concentrations (Fig. 4). With the other monocot, *H. vulgare*, the application of 50–100% concentrations of lavandin hydrolates significantly reduced radicle growth compared to the water control, being even significantly lower with the 100% concentration. However, in the plumule only the 75 and 100% concentrations of hydrolates significantly reduced growth compared to the water control (Fig. 4). Within dicots, in *L. culinaris*, 50–100% lavandin hydrolates concentrations significantly reduced radicle growth compared to the water control. However, in the plumule, only 50 and 100% hydrolate concentrations significantly reduced its growth (Fig. 4). Finally, in *V. sativa*, the application of different concentrations of lavandin did not cause a significant reduction in radicle and plumule growth compared to the water control (Fig. 4).

3.2. Study of lavandin distilled straws as bioherbicides

The possible allelopathic effect of straws from lavandin distillation was analyzed by mixing them with peat and establishing weeds and crops. First, seedling emergence was quantified, with very different results among the plant species used (Figs. 5 and 6). In *B. rigidus*, a monocotyledonous weed, none of the mixture proportions of lavandin straws significantly modified the probability of emergence, compared to the control (Fig. 5). However, in *L rigidum* the peat/straws ratios of 1:1 and 1:2 caused a significant reduction in seedling emergence compared to the other treatments (Fig. 5). With respect to dicotyledonous weeds, only the 1:2 peat/straws ratio caused a significant reduction in the

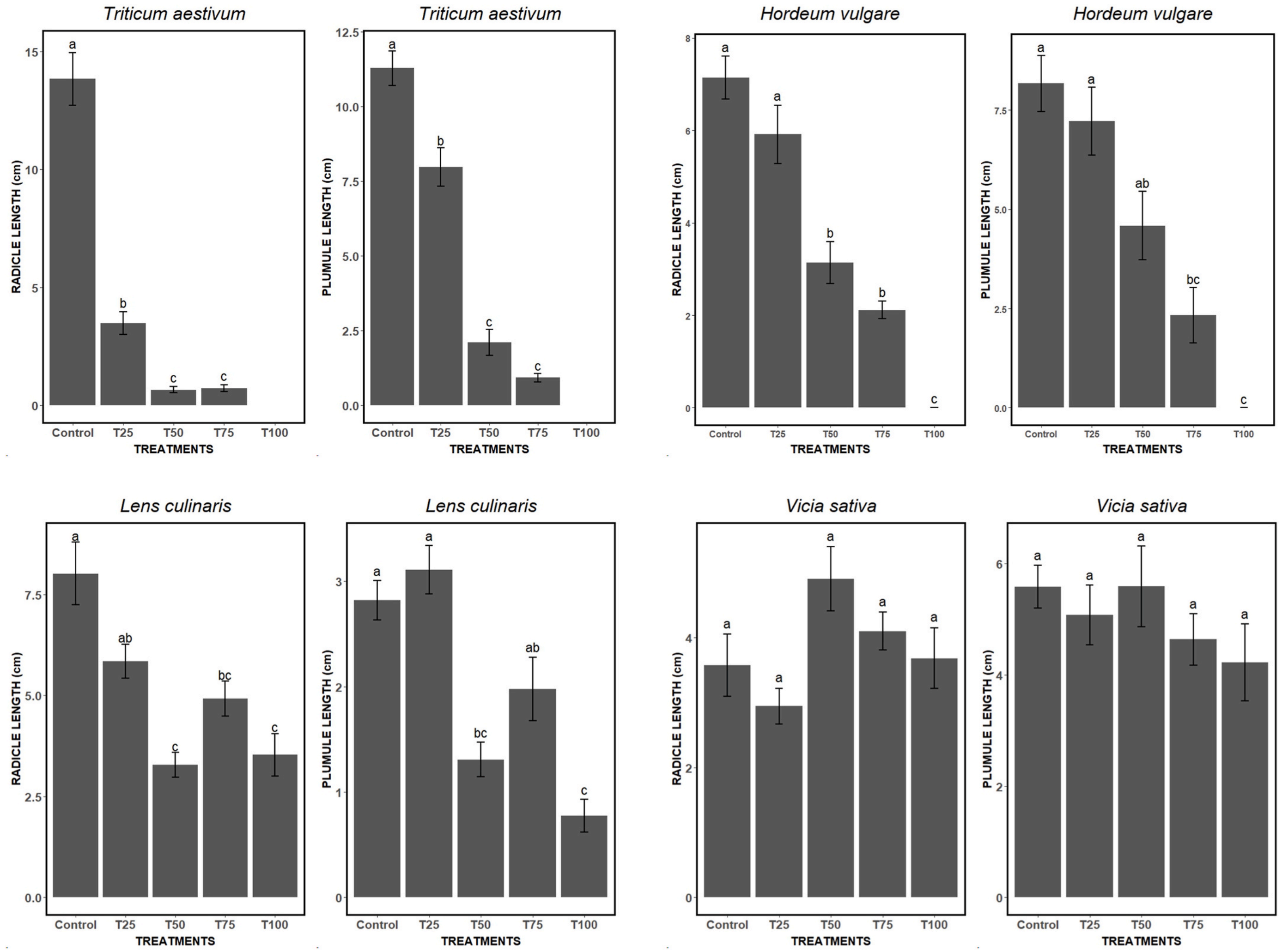


Fig. 4. Radicle and plumule length of the crops wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lentil (*Lens culinaris*) and vetch (*Vicia sativa*) with the application of different concentrations of lavandin hydrolates on different weeds: C: control with distilled water; T25: 25% hydrolates in distilled water (v/v); T50: 50% hydrolates in distilled water (v/v); and T100: 100% hydrolates in distilled water (v/v). Means with the same letter were not significantly different according to the Kaplan-Meier estimator (α ≤ 0.05).

emergence of *C. album*, compared to the peat-only control (Fig. 5). In *A. blitoides*, treatments with peat/lavandin straws ratios of 2:1, 1:1 and 1:2 significantly reduced the germination of the weed compared to the peat treatment. In addition, the 1:2 ratio caused significantly less reduction compared to the other treatments (Fig. 5).

As far as crops are concerned, although to different degrees, the inclusion of lavandin straws in the growing medium negatively affected seed germination. In *T. aestivum*, only the 1:1 and 1:2 peat/straws ratios significantly reduced seed emergence compared to the control. In this regard, only the 1:2 ratio significantly reduced seed emergence compared to the other treatments (Fig. 6). In the other monocot, *H. vulgare*, treatments with peat/straws ratios from 2:1 to 1:2 significantly reduced germination compared to the peat-only control. However, only treatments with the 1:1 and 1:2 ratios significantly reduced germination compared to the 4:1 ratio (Fig. 6). In the case of dicots, the response to lavandin straws was similar. In both L. *culinaris* and *V. sativa*, peat/straws ratios between 2:1 and 1:2 significantly reduced their germination compared to the other treatments. In addition, the treatment with the 1:2 ratio significantly reduced the germination of both crops compared to the other treatments (Fig. 6).

Along with germination, stem length data were also collected from emerged seedlings (Fig. 7). In both weeds (Fig. 7a) and crops (Fig. 7b), all proportions of lavandin straws applied in the growing substrate significantly reduced stem length. Furthermore, in the weed *B. rigidus* (Fig. 7a) and the crop *L. culinaris* (Fig. 7b), the 1:2 and 1:1 peat/straws ratios, respectively, led to reduced stem growth compared to the other treatments. On the other hand, the absence of germinated seedlings under any of the peat/straws ratios prevented the collection of stem length data: 1:2 (except in *B. rigidus*) and 1:1 (in the weed *A. bitiloides* and the crop *V. sativa*) (Fig. 7).

3.3. *Metabolomic study of lavandin hydrolates and distilled straws*

In order to characterize the main metabolites present in lavandin hydrolates and straws we performed a non-targeted metabolomics analysis. From 1429 features submitted to statistical univariate analyses, 633 were significative different between both materials. 589 metabolites were present in higher concentration in the vegetal material, while only 44 were in higher concentration in hydrolates (Fig. 8). Of the major metabolites in both materials, one of these metabolites is present only in hydrolates, three only in straws, and six in both (Table 1). When possible, a molecular formula was assigned to each metabolite based on the exact mass and the isotopic pattern. Tentative identification was performed based on the molecular formula and MS/MS fragmentation pattern. We were able to tentatively assign compound names to three out of ten metabolites: lysophosphatidylcholine (16:0), coumaroyl

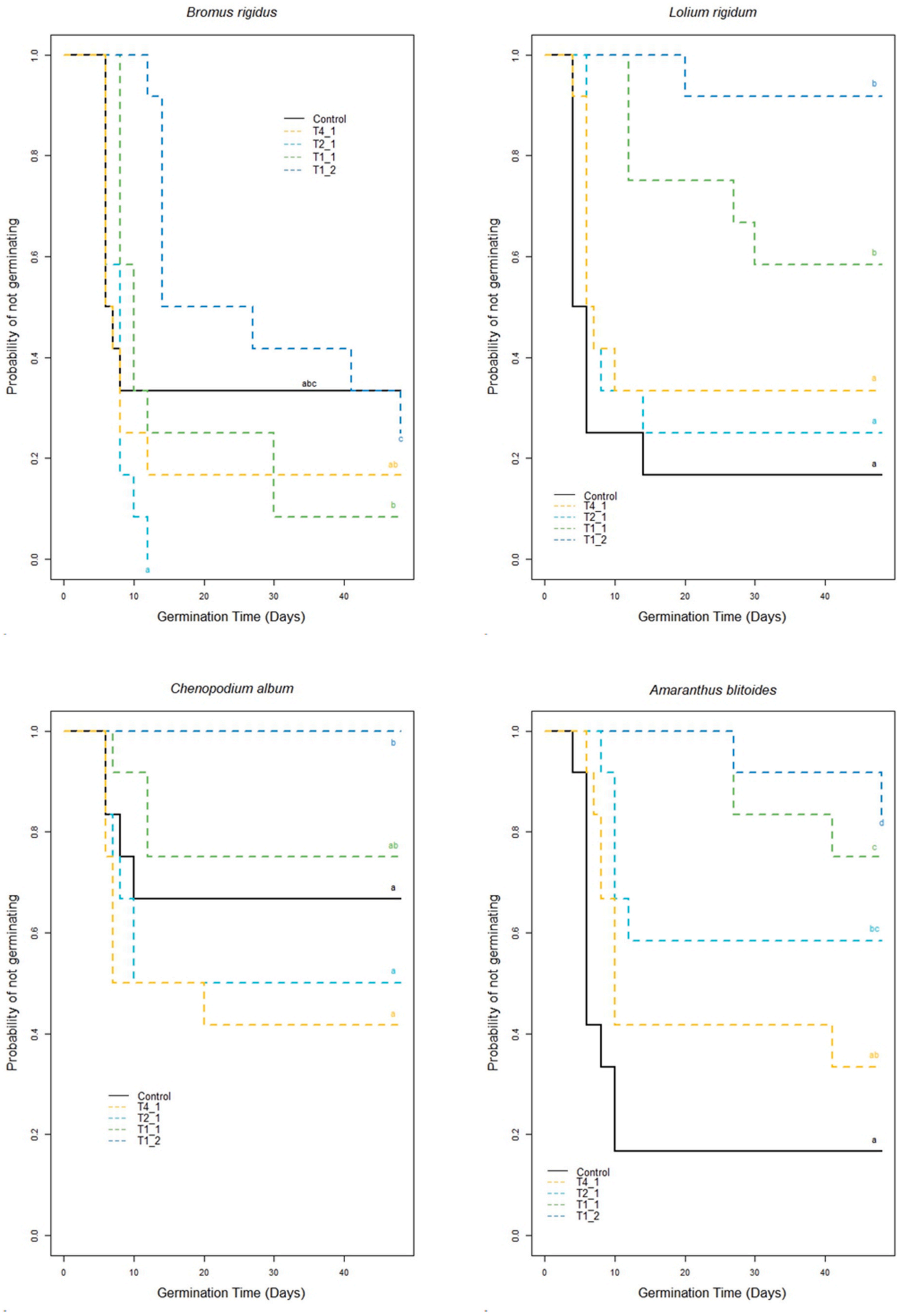
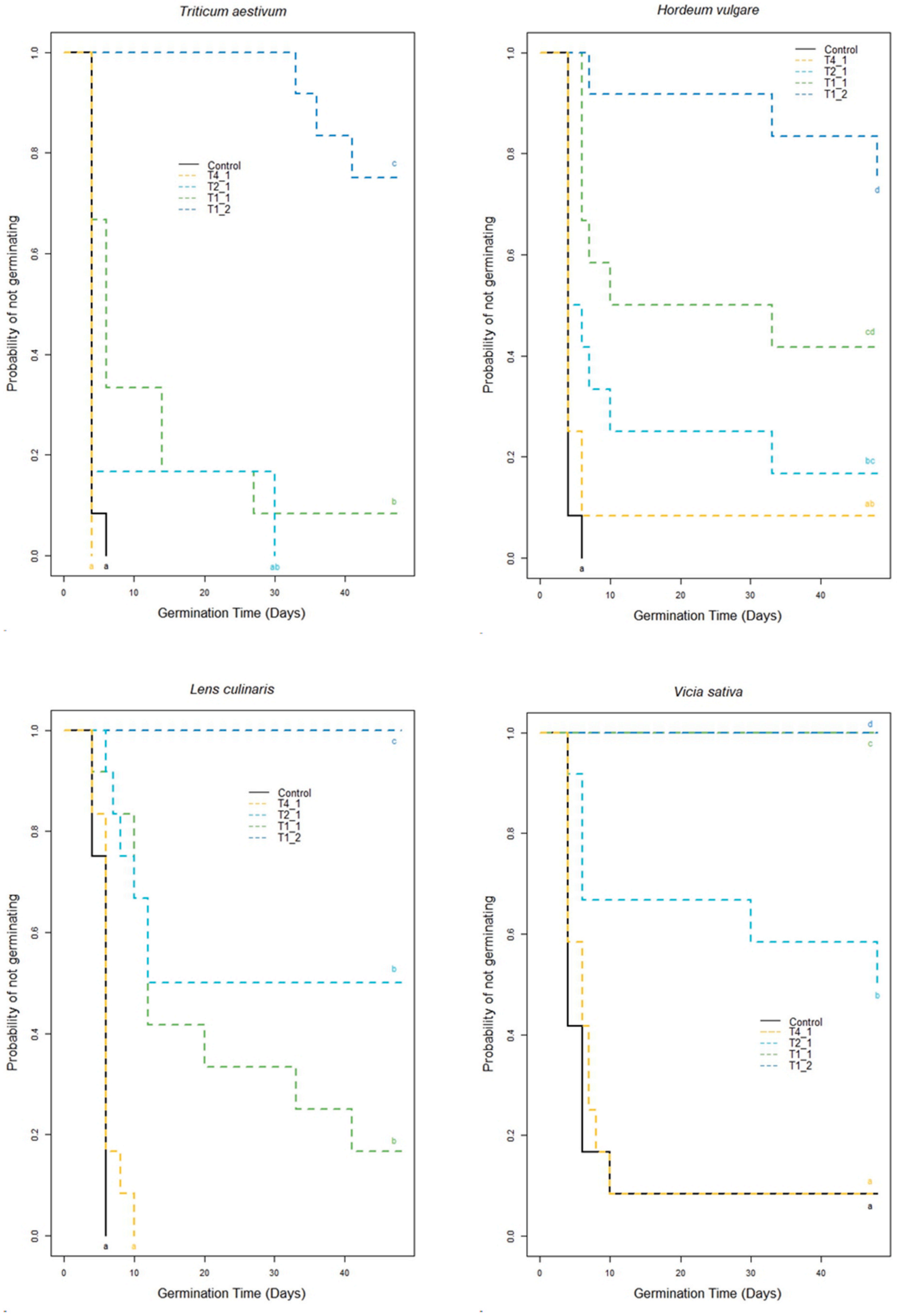


Fig. 5. Curves of probability of non-germination in weeds sown in different proportions of peat and lavandin straws (v/v): Control (1:0), T4_1 (4:1); T2_1 (2:1), T1_1 (1:1) and T1_2 (1:2). The weeds used were brome (*Bromus rigidus*), annual ryegrass (*Lolium rigidum*), cocksfoot (*Chenopodium album*) and mat amaranth (*Amaranthus blitoides*). Means with the same letter were not significantly different according to the Kaplan-Meier estimator ($\alpha \leq 0.05$).



Triticum aestivum

Hordeum vulgare

Lens culinaris

Vicia sativa

Fig. 6. Curves of probability of non-germination in crops sown in different proportions of peat and lavandin straws (v/v): Control (1:0), T4_1 (4:1); T2_1 (2:1), T1_1 (1:1) and T1_2 (1:2). The crops used were wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lentil (*Lens culinaris*) and vetch (*Vicia sativa*). Means with the same letter were not significantly different according to the Kaplan-Meier estimator ($\alpha \leq 0.05$).

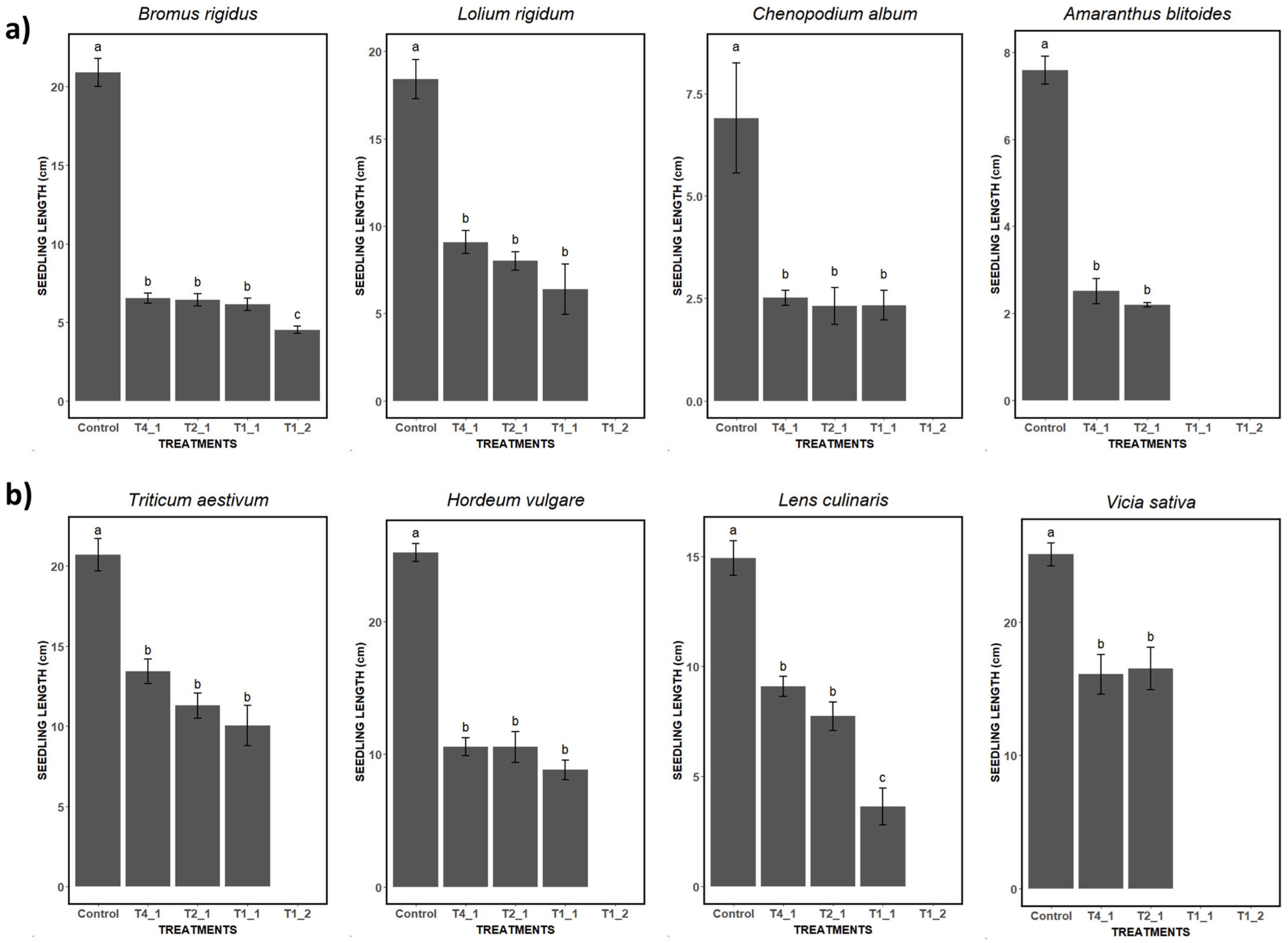


Fig. 7. Stem length of the weeds brome (*Bromus rigidus*), annual ryegrass (*Lolium rigidum*), cocksfoot (*Chenopodium album*) and mat amaranth (*Amaranthus blitoides*) (a), and crops wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), lentil (*Lens culinaris*) and vetch (*Vicia sativa*) with the application of different proportions of peat and lavandin straws (v/v): Control (1:0), T4_1 (4:1); T2_1 (2:1), T1_1 (1:1) and T1_2 (1:2). Means with the same letter were not significantly different according to the Kaplan-Meier estimator ($\alpha \leq 0.05$).

hexoside and feruloyl hexoside in straws (Table 1).

4. Discussion

The environmental and health problems derived from the massive and uncontrolled use of herbicides in agriculture lead to the need for the development of more sustainable and safer strategies. In this vein, the use of by-product hydrolates and straws derived from the distillation of lavandin essential oils could be a good alternative as bioherbicides. In the case of lavandin hydrolates, the *in vitro* study reported that they were efficient at different concentrations to reduce seed germination and seedling development of different weeds and crops, monocotyledons and dicotyledons. Specifically in crops, dicots (*L. culinaris* and *V. sativa*) were less affected by lavandin hydrolates than monocots (*T. aestivum* and *H. vulgare*). These results are in agreement with previous work, where lavandin hydrolates totally inhibited radish (*Raphanus sativus*) germination (Politi et al., 2020) and root development of canola (*Brassica napus*), subterranean clover (*Trifolium subterraneum*), wheat (*T. aestivum*) and annual ryegrass (*L. rigidum*) (Haig et al., 2009). Another aromatic plant, *Thymus vulgaris*, also showed an herbicidal effect (Konstantinović et al., 2022). However, in contrast to the results of the present study where two legume species (*L. culinaris* and *V. sativa*) were the least affected, both Haig et al. (2009) and Konstantinović et al. (2022) found that other legume species, such as alfalfa (*Medicago sativa*), subterranean clover (*T. subterraneum*) and white clover (*Trifolium repens*) were the most sensitive. This disparity in the results seems to point out that each hydrolate has unique properties that may be used to control specific weeds and, therefore, further studies should be carried out to identify the sensitive species, potential uses of each hydrolates and the mechanisms of action involved in the control.

In relation to our results with hydrolates and their possible real application, different bioherbicide hydrolates have been described so far, although very few with activity on weeds. The main plant species described as origin of allelopathic hydrolates are included in the genera *Angelica* (angelicas), *Croton* (marmeleiros) or *Salvia* (sages), reducing the germination and growth of cucumber, radish, broccoli, lettuce, tomato and flax crops, and the weed amaranth (*Amaranthus retroflexus*) (reviewed by Tavares et al., 2022). Therefore, our work provides new information on the use of hydrolates as bioherbicides, studying their effect on both weeds and crops.

The other by-product of the industrial distillation of lavandin was distilled straws. In this sense, our work reported an inhibitory effect of germination and development on all weeds and crops used, being the first work done so far with this by-product. The use of lavandin straws has not been much studied as potential bioherbicides due to their allelopathic capacity, highlighting the study carried out with rice straw in the inhibition of *Avena ludoviciana* (syn.: *A. sterilis* and *A. macrocarpa*) (common name: animated oat, sterile oat or wild oat) (Prachi and Rao,

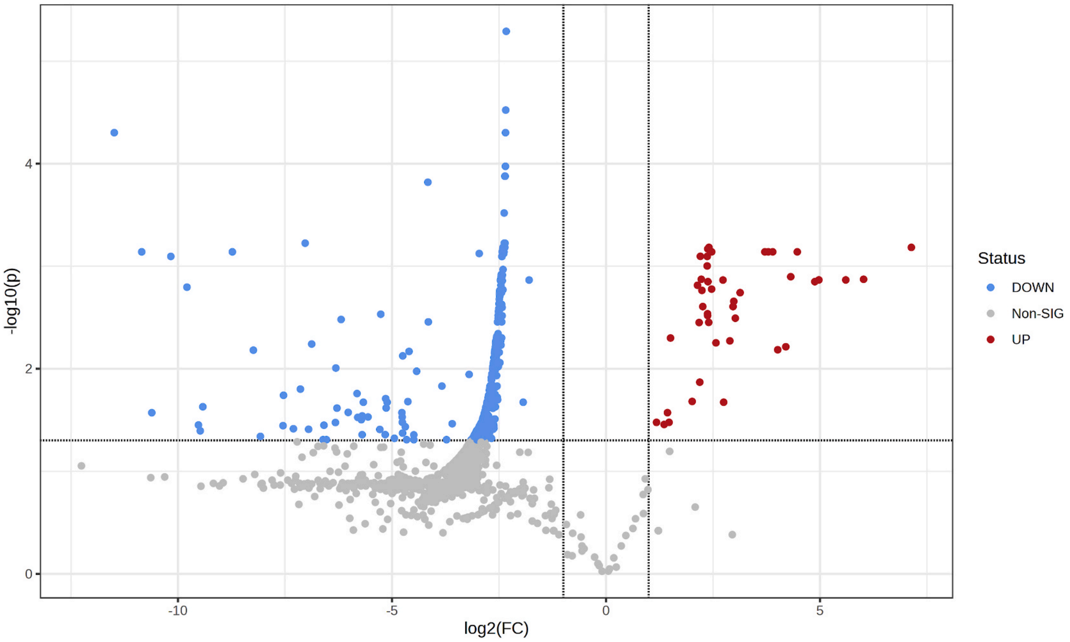


Fig. 8. Volcano plot representing the detected features in the non-targeted metabolomic analysis. The y-axis represents the negative decade logarithm of the significance value (*FDR*) and the x-axis represents the $\log_2$ of fold change (lavandin hydrolates vs. straws). Levels of features with a $-\log_{10}(p) \leq 1.3$ and a $|\log_2(FC)| \geq 1$ are considered to be differentially accumulated in both lavandin materials. Significantly up-regulated features are represented by red circles and down-regulated features are represented by blue circles. Grey circles represent insignificant features.

Table 1
Tentative identification of major metabolites with a $|\log_2(FC)| \geq 1$ in lavandin hydrolates and straws. Metabolites are sorted by ionization mode.

m/z	Lavandin containing material	Neutral Mass	Ionization	RT (s)	Log2 (FC)	Fragments	Tentative identification	Molecular formula
5.648.293	Hydrolates	565.83770	$[M-H]^-$	47.35	2.46	130.9657, 146.9601, 174.9553, 192.9338, 304.9145, 434.8701	–	C13H3N4O12PS4
308.1514	Hydrolates and straws	307.14412	$[M+H]^+$	1437.60	4.47	70.049, 91.0554, 125.0154, 304.2989	–	C17H17N5O
22.893.223	Hydrolates and straws	227.92495	$[M+H]^+$	47.67	4.2	84.959, 98.975, 130.9306, 170.9227, 183.9961	–	n/a
32.710.848	Hydrolates and straws	328.11575	$[M-H]^-$	752.59	−11.49	106.0427, 121.0655, 138.0329, 147.0442, 165.0562	–	C11H16N6O6
47.134.242	Straws	470.33616	$[M+H]^+$	1543.87	−8.08	119.0839, 135.1155, 175.1487, 189.1655, 217.1587, 253.1907, 271.1976, 425.3406	–	C30H46O4
21.503.304	Hydrolates and straws	216.04031	$[M-H]^-$	56.50	−10.17	59.0145, 63.9608, 71.0149, 78.9697, 89.0245, 119.0334, 181.0765, 212.0725	–	C6H9N4O3P
496.33559	Hydrolates and straws	495.32831	$[M+H]^+$	1490.85	−9.03	86.0965, 104.1074, 124.997, 184.0765, 329.1683	Lysophosphatidylcholine (16:0)	C24H50NO7P
533.347	Hydrolates and straws	534.35428	$[M-H]^-$	1462.89	−8.73	146.067, 245.133, 326.1846, 487.3421	–	C31H50O7
325.09282	Straws	326.10010	$[M-H]^-$	641.09	−10.85	89.0243, 119.0494, 163.0401	Coumaroyl hexoside	C15H18O8
355.10335	Straws	356.11063	$[M-H]^-$	737.34	−2.36	89.0251, 119.0355, 134.0366, 49.0605, 193.0519	Feruloyl hexoside	C16H20O9

2013).

Therefore, this work has allowed to determine the effective allelopathic capacity of lavandin hydrolates and straws on different weeds and crops. This may allow the possible future development of commercial herbicides based on these by-products. However, it is important to consider not only the herbicidal effect against weeds, but also the reported allelopathic effect on crops. For this reason, these bioherbicides should be approached rationally in their agricultural use, for example, through background application, in the absence of field crops, or on woody crops, although their allelopathic effect on these should be studied. Other possible non-agricultural uses for these herbicides could be to prevent weeding in photovoltaic fields and in urban gardening.

With respect to metabolomic analysis, the by-products used in the present work show clear metabolic differences compared to other studies, with a lower diversity of major metabolites. In the hydrolates of this work, it was only possible to identify lysophosphatidylcholine as a major metabolite, however, other works identified coumarin and derivatives (Haig et al., 2009), linalool and 1,8-cineole (Politi et al., 2020) as major metabolites, without reporting the presence of lysophosphatidylcholine. Whereas in lavandin straws, the major metabolites identified in our work coincide with the major metabolites of other studies (with the exception of lysophosphatidylcholine, unique in our work). We succeeded in identifying the major metabolites coumaroyl hexoside and feruloyl hexoside, both derived from the previously described major metabolites in lavandin straws, coumarin and glycosylated ferulic acid (Lesage-Meessen et al., 2015; Marovska et al., 2024). This lower metabolic profile of our by-products is possibly due to the way they are obtained and stored. Previous studies analyzed hydrolates and straws obtained under laboratory conditions and controlled storage. However, our work was based on the use of real by-products from the lavandin oil distillation industry, and metabolites may be lost during the whole industrial process and prolonged-uncontrolled storage.

We identified the metabolite lysophosphatidylcholine present mostly in both hydrolates and lavandin straws. This fatty acid is a derivative of the major component of the cell plasma membrane, phosphatidylcholine (Liu et al., 2020). Its phytotoxic action has not been extensively studied so far, however, its damaging action on the normal functioning of the plasma membrane of corn roots (Bourdil et al., 1990) and soybean stems (Morré et al., 2013) has been described. Specifically, it was identified that lysophosphatidylcholine acts as a detergent that solubilizes the fatty components of the plant plasma membrane (determined by absorbance), In addition, lysophosphatidylcholine induces ATPase and reductase activity in plant membrane, as a plant response to osmotic and oxidative damage (determined by enzyme activity analysis) (Bourdil et al., 1990; Morré et al., 2013). Thus, the allelopathic effect described for our by-products could be related to the phytotoxic capacity of lysophosphatidylcholine.

In addition, we identified two major metabolites present only in lavandin straws, not in the hydrolates. Coumaroyl hexoside is a derivative of coumarin (benzopyrone), a compound described as possibly involved in the allelopathic capacity of lavandin hydrolates on different crops (Haig et al., 2009). This coumarin-derived metabolite has also been described by itself as potent phytotoxic in previous works. It was isolated from silverleaf nightshade (*Solanum elaeagnifolium*) seeds, reporting an 80% reduction in common purslane (*Portulaca oleracea*) biomass production (Balah, 2015, 2020). On the other hand, feruloyl hexoside is a derivative of ferulic acid, in its conjugation with sugars. This metabolite has been described as a potent antioxidant present in a large number of plant species (Shahidi and Chandrasekara, 2010), and even as a possible animal biocide (Azevedo et al., 2024). However, its allelopathic capacity has never been described before, being our work the first approach in this sense, although more research is needed to determine whether this metabolite has allelopathic potential. The phytotoxic activity described for these metabolites absent in the hydrolates, but present in the straws, could explain the different allelopathic capacity of both by-products.

5. Conclusions

As conclusions, the hydrolates obtained by industrial distillation of lavandin essential oil have allelopathic effect on the germination and growth of the four weeds used in this work, as well as on the monocot crops (wheat and barley) used. However, these hydrolates hardly have a phytotoxic effect on the dicotyledonous crops lentil and vetch. The lavandin distilled hydrolates had an allelopathic effect on the growth of all weeds and crops, affecting to a lesser extent the emergence of brome and goosefoot weeds. Possibly, this allelopathic effect of both by-products is a consequence of the major presence of the identified metabolite lysophosphatidylcholine.

Therefore, the by-products of the industrial distillation of lavandin could be used in the development of effective and sustainable bioherbicides, due to the allelopathic capacity of the metabolites present. However, their actual application requires studying their mode of application and purpose, in order not to affect non-target species, such as surrounding crops.

CRediT authorship contribution statement

Jorge Poveda: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Daniel Vítores:** Methodology, Investigation. **Tamara Sánchez-Gómez:** Methodology, Investigation. **Óscar Santamaría:** Writing – review & editing, Investigation. **Pablo Velasco:** Methodology, Investigation. **Irene Zunzunegui:** Methodology, Investigation. **Víctor M. Rodríguez:** Methodology, Investigation. **Baudilio Herrero:** Writing – review & editing, Investigation. **Jorge Martín-García:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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