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RESEARCH ARTICLE

The allelopathic potential of buckwheat (*Fagopyrum esculentum* Moench) on common crops and weeds: insights from an in vitro and a pot experiment

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Abstract

Backgrounds and aims Allelopathic species can be key components of successful integrated weed management. We evaluated the suppressive potential of buckwheat (*Fagopyrum esculentum*), simulating the effect allelochemicals exert through crop residues or root exudates on weeds, with minimal harm to crops.

Methods We investigated the effect of two buckwheat (BW) water extracts (weight ratio 1:5 and 1:10) on seed germination and root growth of weeds (*Echinochloa crus-galli*, *Cynodon dactylon*, *Amaranthus retroflexus*, *Setaria italica*) and crops (barley, chickpea, cress, lentil, quinoa, soybean and tobacco) in Petri dishes. Then, a pot experiment was conducted to test BW's root exudates on the growth of weeds (*Abutilon theophrasti* and *Amaranthus retroflexus*) and crops (chickpea and lentil), either intercropped with BW, or supplied with leached water from BW pots. The content of quercetin and rutin was assessed in all BW samples.

Results Both experiments demonstrated a suppressive effect of BW, with intensity varying by species susceptibility. Quercetin and rutin were found in ground buckwheat and in water extracts, but only quercetin in leached water. In the germination experiment, osmotic potential of the water extracts may also be a factor reducing germination rate, but the effect was not unequivocal.

Conclusion This study confirms the potential of BW for weed suppression in low-input systems: 1) the inhibition of weed germination from BW water extracts can support its use as mulch from crop residues; 2) since some crops were also affected in both experiments, BW use in intercropping or cover cropping should be adapted to the crops. Further research is needed to understand the mechanisms of buckwheat allelopathy and to test these results in field conditions.

Keywords Agroecology · Weed control · Intercropping · Allelopathy · Integrated weed management

Introduction

Chemical herbicides represent the most common solution for weed management, however, their long-term use as the primary weed control method has led to issues including the contamination of groundwaters (Guzzella et al. 2006), the

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development of herbicide-resistant weeds (Owen 2016; Peterson et al. 2018), the presence of residues in food products (EFSA et al. 2024) and biodiversity losses (Storkey et al. 2012). Given the adverse effects related to herbicides, in the last decades the number of approved herbicides has been restricted, while the development of new herbicides has been limited (Westwood et al. 2018). Integrated Weed Management (IWM) has emerged as an approach to diversify weed control methods by implementing preventive and alternative direct methods, e.g. crop rotation, cover crops, dead mulching, precision tillage, robotics (Lechenet et al. 2017), or by exploiting the allelopathic properties of plants and microorganisms (Jacquet et al. 2022) which inhibit weed seed germination, root elongation or overall plant growth (Scavo and Mauromicale 2021).

In agriculture, allelopathy has been adopted in various ways, including directly exploiting the capability of crops to release allelochemicals into the soil (e.g. intercropping and cover cropping), incorporating crop residues into the soil (e.g. mulching and green manuring), and producing allelochemical-based bioherbicides (Scavo and Mauromicale 2021; Kostina-Bednarz et al. 2023) such as those based on coumarins or phenoxypyridine.

Intercropping has been demonstrated to be an effective strategy for mitigating weeds competition as intercropped plants are more effective at competing for resources (Gu et al. 2021). Furthermore, in intercropping systems, weed management is frequently achieved by introducing companion species with allelopathic properties. Allelopathic weed-suppressive capacity has been observed in many crops, including rice, wheat, barley, sunflower, canola, sorghum, alfalfa and buckwheat (Olofsdotter et al. 2002; Aslam et al. 2017; Rawat et al. 2017; Rehman et al. 2019; Farooq et al. 2020; Hussain et al. 2021; Virili et al. 2024). For example, grain legumes, which are susceptible to weeds, benefit from intercropping with allelopathic crops, as demonstrated by cases like chickpea and wheat (Banik et al. 2006), as well as in the case of lentil with wheat and oat (Fernandez et al. 2015). Although allelopathy has been investigated for the suppressive effects on weeds, there is still a limited understanding of how allelopathic crops may affect main crops (Belz and Hurle 2004; Golisz et al. 2007a; Ferreira and Reinhardt 2010).

Buckwheat (*Fagopyrum esculentum* Moench, *Polygonaceae*), hereafter indicated with BW, is a spring–summer pseudo-cereal which also has a recognised weed suppressive ability through allelopathy (Iqbal et al. 2003). BW is adaptable to different climates and low-input systems, and it is gaining attention as a suitable crop for alternative cropping systems (Virili et al. 2024). Quantification of BW allelochemicals has been carried out on plant residues, seed flour, and soils containing incorporated plant residues. These phytochemicals are diverse and belong to a broad ensemble of organic compounds, including alkaloids, benzenoids, dihydroxybenzoic acids, fatty acids, hydroxycinnamic acids, phenolic acids, phenylpropanoids, and flavonoids (Iqbal et al. 2003; Golisz et al. 2007a, 2007b, 2008; Suzuki et al. 2015; Szwed et al. 2020a, 2020b). Among the flavonoids, the most relevant compounds are (-)-epicatechin, (+)-catechin, quercetin, and rutin (Falquet et al. 2015). The abundance of each specific allelochemical varies depending on the tissue type (roots, stems, leaves, inflorescences, and seeds) and the cultivar (Golisz et al. 2008; Vieites-Álvarez et al. 2024).

Among all allelochemicals, the more investigated in BW are quercetin and rutin. As all flavonoids, these low molecular weight phenolic compounds are biologically active. They are transported within and between plant tissues and are specifically released by roots into the rhizosphere where they are involved in plant-plant interactions (Weston and Mathesius 2013). Quercetin and rutin have been demonstrated to inhibit seed emergence and plant growth (Golisz et al. 2007a, 2008, Kalinova et al. 2006, 2009, Szwed et al. 2020a, 2020b, Fernández-Aparicio et al. 2021). Because of its allelopathic properties, BW has been traditionally used by farmers for weed management as a cover crop (Falquet et al. 2015). It has been demonstrated that the incorporation of BW residues in the soil has a significant effect on weed suppression (Kumar et al. 2009; Masilionyte et al. 2017; Szwed et al. 2019). Kumar et al. (2009) investigated through a pot-bioassay, the potential allelopathic effects of incorporating fresh BW residues on the suppression of *Amaranthus powellii* S.Watson. Moreover, Masilionytė et al. (2017) showed that weed biomass was most effectively suppressed in systems where white mustard and buckwheat were used as cover crops. Szwed et al. (2019) investigated the links between some metabolic indicators of oxidative stress in four weed species, including barnyard grass to BW root residues, all showing strong growth inhibition, compared to plants grown in bare soil. In a further investigation Szwed et al. (2020a) carried out a similar experiment on a wider ensemble of weeds, and compared the effect of BW root residues and of the entire BW plant residues, recording a significant inhibition effect on the emergence and growth of weed seedlings.

It has been reported that incorporation in the soil of BW roots only, has a higher allelopathic potential than incorporating residues of both roots and aerial biomass (Szwed et al. 2019, 2020a). However, it has also been reported that BW's roots do not affect the content of unbound free phenolic allelochemicals in the soil, while content in the soil of bounded phenolic compounds increased after 37 days of BW residue decomposition (Szwed et al 2020b). Despite these results, research investigating the allelopathic effects promoted by the root exudates of BW is limited (e.g. Gfeller et al. 2018; Fernández-Aparicio et al. 2021) or denies this hypothesis (e.g. Wirth and Gfeller 2016; Szwed et al. 2020b). In a study published by Gfeller et al. (2018), it was reported that BW suppressed the growth of redroot pigweed, goosefoot and barnyard grass. The same authors reported situations where weeds suppression was observed but they were unable to prove any relation to allelopathy since the concentration of the compounds in the soil was low (Wirth and Gfeller 2016). The same results were supported by further evidence, suggesting that the allelopathic effect of BW might be due to the whole ensemble of compounds produced, as well as to the microorganisms present in the soil (Szwed et al. 2020b). However, despite its weed suppressing activity has been widely investigated, the mechanisms of action have not been clearly identified yet.

Regardless of these controversies, various studies have reported the efficacy of using water extracts from BW tissues as bioherbicides (e.g. Iqbal et al. 2003; Kalinova et al. 2007; Szwed et al. 2019; Fernández-Aparicio et al. 2021; Cechin et al. 2023). In vitro experiments using aqueous extracts obtained by centrifugation of lyophilized plant materials (stems, leaves, and roots) of dry BW have been proven to inhibit the germination and root elongation of various weeds, including *Amaranthus retroflexus* L., *Chenopodium album* L., *Echinochloa crus-galli* L., *Portulaca oleracea* L. (Falquet et al. 2015). Exposure to BW water extracts have been shown to negatively affect the secondary metabolism of many weed species. This suggests that water extracts could be used reliably to manage herbicide-resistant weeds (Cechin et al. 2023). Despite these findings, the full extent of BW suppressive capability remains uncertain.

In the present study, we aimed to confirm the allelopathic properties of BW retrieved from the literature by: i) examining the effects of BW water extracts on seed germination and seedling growth under in vitro conditions, and characterize the chemical composition of allelopathic compounds, ii) unravel the BW effects on weed and crop growth by disentangling BW allelopathy due to root exudates from interspecific competition. Our findings may help to define implications and perspectives related to integrating allelopathic species, like buckwheat, in diversified cropping systems (cover crop and/or intercropping) to support IWM.

The hypothesis behind this study is that allelopathic substances produced by BW are released into the environment through both root exudates and decomposition of BW's crop residues. We expect these allelochemicals to have a significant inhibitory effect: i) at different stages of growth; ii) on different parts of the plant; iii) on weeds, while causing minimal damage to the crop.

Materials and methods

Effect of buckwheat water extracts on crop and weed seed germination: in vitro assay

Buckwheat extract preparation

BW extracts were obtained by adapting the protocol of Carrubba et al. (2020). Plants of BW *cv* Panda were collected on the 20th October 2022, in the fields of a farm situated in Buttrio (Udine, Italy, 45.98 N, 13.39 E), when plants were at the full flowering stage. The entire plant was collected, including the roots. Plant material was oven-dried at 60 °C for 72 h and then ground in multiple steps, from blade mill to ball mill. The obtained powder was stored in paper bags in a dark and dry environment at room temperature until its use.

Water extracts were prepared by soaking 200 g of BW powder in 1 L of distilled water (weight ratio of 1:5) and stirring at 150 rounds per minute for 5 h. The water extracts were strained with filter paper and refrigerated at 4 °C until their use. The 1:10 water extract was obtained by diluting the previously prepared extract (1:5) with an equivalent volume of distilled water. For each water extract, pH and Electrical Conductivity (EC) were measured using the HI5521 (HI1131B Glass-body combination pH electrode, 0–13 pH range) and HI5522 (HI76312 Four-ring conductivity Probe, 0–200 mS/cm range) instruments (Hanna Instruments) and osmotic pressure was measured using the WP4C Instrument (Meter Group).

Experimental design

A germination test in petri dishes was carried out from April to June 2023 in the laboratories of the University of Udine, to test the allelopathic effect of buckwheat water extracts on crops and weeds. Seeds from seven crops were tested: chickpea (*Cicer arietinum,* L.), lentil (*Lens culinaris,* Medik.), soybean (*Glycine max,* L.), quinoa (*Chenopodium quinoa* Willd.), barley (*Hordeum vulgare,* L.), garden cress (*Lepidium sativum,* L.) and tobacco (*Nicotiana tabacum,* L.); and seeds from four weeds: red pigweed (*Amaranthus retroflexus,* L.*),* barnyard grass (*Echinochloa crus-galli,* (L.) P. Beauv.*),* bermudagrass *(Cynodon dactylon,* (L.) Pers.*)*, foxtail millet (*Setaria italica,* (L.) P. Beauv.). These species were chosen because of their abundance and their detrimental role in local cropping systems.

The weed seeds were collected in the fields of the experimental farm of the University of Udine (46.035N, 13.224E), Italy, on the 07th of October 2022 and kept in a dark and dry environment at room temperature after a two-week vernalization period at 4 °C. To break physical dormancy, a gentle mechanical scarification was used on seeds from all species before germination.

To prevent mold, seeds were sterilized by washing them with 20% sodium hypochlorite for 3 min, followed by five rinses with distilled water to remove any residue. The washing and rinsing operations were performed in sterile 25 ml test tubes, which were gently inverted during the process. The germination test was conducted beforehand.

Each species was tested under three dilution treatments: control (CNT) of distilled water; BW water extracts at concentration 1:10 (1 g of buckwheat residues and 10 mL of water – d01); buckwheat water extracts at concentration 1:5 (1 g of buckwheat residues and 5 mL of water – d02). Germination tests were conducted in sterile petri dishes with a diameter of 90 mm. Larger petri dishes of 150 mm diameter were used for the biggest seeds (e.g., chickpea, soybean). Each Petri dish contained 15 seeds soaked in 10 mL of water/extract poured onto filter paper in three rows of five seeds each, spaced as evenly as possible from one another. Each species *x* dilution treatment was replicated five times from April to June 2023 using 165 Petri dishes (11 species × 3 water extracts dilutions × 5 replications). The Petri dishes were kept in a growth chamber for seven days at 12 h of light/dark, 25 °C during the daylight and 20 °C at night to simulate spring/summer germination conditions.

Data collection

Measurements were taken according to the protocols of Emino and Warman (2004). After 7 days of incubation, we counted the number of seeds germinated (N_G) in each petri dish and calculated the germination percentage (G, %). Seeds were then photographed on graph paper and the primary root length (L_R) was measured using the ImageJ v1.54d software (Schindelin et al. 2012). The Relative Seed Germination (RSG, %) and the Relative Root Growth (RRG, %) were calculated as:

$$RSG = \frac{N_G}{mean\, N_G\, in\, control\, (pure\, water)} \times 100$$

$$RRG = \frac{L_R}{mean\, L_R\, in\, control\, (pure\, water)} \times 100$$

The Germination Index (GI, %) was then calculated as:

$$GI = \frac{RSG \times RRG}{100}$$

Effects of buckwheat on growth of companion species: in-pot experiment

Experimental design

The experiment was conducted in a greenhouse at the facilities of the University of Udine (46.08 N, 13.20 E) during the spring of 2023. A total of four species were tested, two crops: chickpea cv Sultano and lentil cv Itaca; and two weeds: velvetleaf (*Abutilon theophrasti* Medik.) and red pigweed (*Amaranthus retroflexus* L.). Each species was tested under three experimental treatments: i) monoculture (MC); ii) intercropped with buckwheat cv Panda (IC); iii) grown in a staircase device (SD), an experimental tool specifically designed to separate the effect of allelopathy from competition (Mahé et al. 2022). The staircase device operates by allowing water used for irrigation to flow through a pot containing buckwheat into pots with the tested plants (chickpea, lentil, velvetleaf and red pigweed) as shown in Fig. 1. All treatments were replicated four times and placed in a randomized block design.

Sowing was conducted on the 21st of April 2023 in 3.5 L pots, with a surface area of 225 cm^2 (15 cm × 15 cm) and depth of 25 cm. Pots were filled with a field-collected sifted clay-loam (USDA textural soil classification) (clay 28%, silt 30% and sand 42%), with pH 5.01, organic carbon 13.8 mg g^{-1}, total nitrogen content 0.16%, organic matter 2.4%. BW, lentil, pigweed, and velvetleaf were sown at a density of 4 plants per pot in pure stands (MC and SD), and at 2 plants per plot when intercropped (IC). These plant densities were calculated from the common field sowing density of the densest species, i.e. buckwheat, with a seed density between 150–200 seeds per square meter. Chickpea was sown at 2 plants per pot in the pure stands and one plant per pot when intercropped.

Emergence occurred on the 24th of April for BW, chickpea and lentil, and on the 26th of April for *A. retroflexus* and *A. theophrasti*. Plants were irrigated three times per week with 0.5 L per pot. In the SD treatment, pure stands of BW were grown in pots fitted with perforated saucers connected to filtered funnels, allowing the collection of leachates free of soil residues. Each pot was fully irrigated (~ 1–1.5 L per pot). Leaching was completed within 30 min, after which, the leachate from all pots was collected and pooled into a tank and subsequently used to water the test species.

Data collection

The experiment length was of 66 days after sowing. Three destructive samplings were performed in the intercropping system, based on BW phenology: on the 16th of May 2023 at fifth full leaf development; on the 6th of June 2023 at full flowering; on the 26th of June 2023, at seeding onset. The SD treatment was sampled only at seeding onset due to lack of replicates for destructive measurements. At each sampling, plants were removed from the soil and the roots were carefully cleaned in shallow trays. The root system was separated from the shoot. Samples were oven-dried at 60 °C for 72 h to measure aboveground dry biomass (DW$_{ABV}$, g plant^{-1}) and root dry biomass (DW$_R$, g plant^{-1}). Competitive Balance Index (CBI, Wilson 1988) was calculated as:

$$CBI_x = \ln\left(\frac{\frac{DW_{ABV}\,of\,x\,in\,IC}{meanDW_{ABV}\,of\,x\,in\,MC}}{\frac{DW_{ABV}\,of\,BW\,in\,IC}{meanDW_{ABV}\,of\,BW\,in\,MC}}\right)$$

where ‘x’ is the tested companion species. The CBIx values < 0 indicate a competitive effect of BW on the

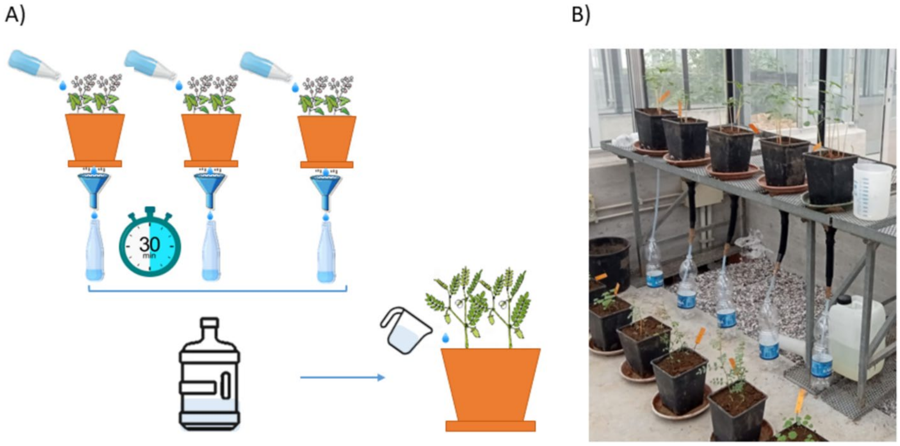

Fig. 1 Illustration of the staircase device used in the pot experiment to separate the effect of allelopathy from the effect of competition. **A**) collection of the leachate after irrigation from buckwheat pots and distribution of the leachates in the pots with crops and weeds; **B**) photo of the device during the experiment in 2023

companion species, and CBIx values > 0 indicate a competitive effect of the companion crop on biomass production of BW.

Allelochemical analysis

The contents of allelochemicals were measured in samples of ground BW used to prepare the water extracts, as well as in the three samples of the d02 solution used to test the seeds in the germination test. Additionally, three aqueous samples of the leached water from BW pots in the SD treatment were analyzed, collected during the last irrigation, along with soil samples collected at the last sampling from the rhizosphere of each pot. All analytical samples were stored frozen at –20 °C until they were analyzed.

Samples were analyzed using high-performance liquid chromatography (HPLC) to assess the content of quercetin and rutin, two of the most abundant allelochemicals found in BW (Iqbal et al. 2022). The allelochemicals in the samples of ground BW and soil (200 mg) were extracted with 15 mL of $CH_3OH:H_2O$ (80:20), agitated using a vortex, sonicated for 20 min, centrifuged for 20 min at 6000 rpm, and filtered through syringe filters (PTFE 0.2 µm, 25 mm). Samples of d02 solution and leached water were only filtered through syringe filters and directly injected in HPLC. The quantification was carried out using an external standard calibration curve. The chromatographic conditions were as follows: flow rate: 0.8 ml/min; mobile phase: $CH_3OH:H_3PO_4$ 0.4% (50:50); injection volume: 25 µl; column: Spherisorb ODS 2 5 µm (Waters) maintained at 30 °C; Detector: a diode-array detector (PDA) set at 360 nm. For each matrix, 3 replicates were analyzed.

Statistical analysis

Statistical analyses were conducted using R software version 4.3.1 (R Core Team 2025). Data management and preprocessing were performed using the "dplyr" package (Wickham et al. 2014). Differences among treatments in the germination experiment were tested using a two-way analysis of variance (ANOVA) implemented through generalized linear models (GLMs) with a Gamma distribution and an inverse link function. Specifically, each response variable (i.e., G, RSG, RGG, or GI) was modeled as a function of species, treatment/solution, and their interaction. GLMs were generated with the "glm" function of the basic "stats" package (R Core Team 2025) setting the family parameter as "Gamma" with inverse link function. Models' performance was displayed with the "Anova" function of the "car" package (Fox and Weisberg 2018). Similarly, data collected from the pot experiment in greenhouse were analyzed using a three-way ANOVA implemented through generalized linear models (GLMs) with a Gamma distribution and a Log link function. The GLMs were generated accordingly with the "glm" function but setting the family parameter as log link function. Experimental factors were sampling dates, species and buckwheat water extracts treatment. In both experiments, model diagnostics included evaluation of dispersion, inspection of fitted and deviance residual patterns, and identification of influential observations using Cook's distance and leverage values.

Dispersion was calculated as the ratio between the deviance of the fitted model object calculated with the "deviance" function from the "stats" package, and the residual degrees-of-freedom extracted with the "dr.residual" function from the "stats" package. Inspection of fitted and deviance residuals patterns was conducted by plotting the extracted Pearson and deviance residuals values with the "residuals" function of the "stats" package specifying "deviance" and "pearson" type, versus the fitted values, which were extracted with the "fitted" function of the "stats" package. Normality of the residuals was assessed with a Q–Q plot of the deviance residuals implemented with the "qqPlot" function of the "car" package. Leverage and Cook's distance were calculated respectively with the "hatvalues" and "cooks.distance" functions of the "stats" package. As the models showed slight underdispersion, this was accounted for by specifying the estimated dispersion parameter when performing the marginal analysis of variance. This operation was conducted with the "Anova" function of the "car" package by specifying "type 2" analysis and the corresponding calculated dispersion value.

The differences for Competitive Balance Index between species were tested using a simple linear model two-way ANOVA, with sampling dates and species as response variables. The model was run with the "lm" function of the "stats" package and tested with the "Anova" function of "car". When interactions of fixed factors were significant ($p < 0.05$), differences between means were assessed using Tukey's Honestly Significant Difference test as post hoc. Tukey's test was assessed by getting the estimated marginal means with the "emmeans" function of the "emmeans" package (Lenth and Piaskowski 2025) and comparing them with the "pairs" function of the "stats" package by setting the adjustment factor as "tukey". Normality and homoscedasticity of the ANOVA model residuals were checked visually through quantile–quantile plot and residuals-fitted values plot. These plots were generated with the "qqPlot" function of the "car" package and the "plot" function of the basic "stats" package.

Finally, to test whether the seed size may affect the sensitivity to allelopathic substances, Pearson correlation analysis was performed between the measured thousand-seed weight of species and their average germination index value both with the d01 and d02 solutions. This analysis was conducted with the "cor.test" function of the "stats" package by setting the method "pearson".

Results

Germination tests

Regarding the water extracts used, measured rutin concentration was 30.11 ± 0.90 mg L^{-1} in ground BW and 6.15 ± 0.07 mg L^{-1} in the water extract d02, while quercetin concentration was 1.45 ± 0.02 mg L^{-1} in ground BW and 1.35 ± 0.02 mg L^{-1} in the d02 solution (supplementary material S1). Osmotic potential was respectively −033 MPa for d01 and −0.73 MPa for d02. The chromatograms showing the analyses that were achieved are reported in the supplementary materials.

An excessive development of mold was found on tobacco. Therefore, it was not possible to measure the root length and calculate Relative Root Growth (RRG) and the Germination Index (GI), however it was still possible to count germinated seeds and calculate germination percentage (G) and Relative Seed Germination (RSG).

Results of the analyses of variance for results of the germination tests are listed in Table 1, while differences between groups are reported together with mean values, corresponding standard error and dose–response slopes in Figs. 1, 2, 3 and 4. For all the tested parameters, the effects of species, treatment, and their interaction were statistically significant.

Reduction of seeds' G was observed in all species (Fig. 2). However, significant differences in treatments among the species were assessed only when the slope 'm' was lower than −18. The most tolerant species were bermudagrass ($m = -7.3$), barnyard grass (m = −15.3), barley (m = −6.7), lentil (m = −15.3) and soybean ($m = -2$). Accordingly, significant differences in Relative Seed Germination RSG (supplementary material S2) were assessed in redroot pigweed, cress, chickpea and tobacco (all $m = -50$), where in the d02 treatment no seeds germinated. The same result was observed for the Relative Root Growth RRG (Fig. 3) and Germination Index GI (supplementary material S3), where significant

Table 1 Results of the analyses of variance conducted on germination percentage (G, %), relative seed germination (RSG), relative root growth (RRG), and germination index (GI)

Parameter	Statistic	Experimental factors		
		Species	Treatment	Species*Treatment
G (%)	LR	418.01	290.15	240.84
	DF	10	2	20
	p-value	< 0.001***	< 0.001***	< 0.001***
RSG (%)	LR chi-square	60.92	124.19	1095.55
	DF	10	2	20
	p-value	< 0.001***	< 0.001***	< 0.001***
RRG (%)	LR chi-square	74.96	173.25	984.66
	DF	9	2	18
	p-value	< 0.001***	< 0.001***	< 0.001***
GI (%)	LR chi-square	23.18	294.49	649.66
	DF	9	2	18
	p-value	0.006**	< 0.001***	< 0.001***

For each experimental factor, the likelihood ratio (LR), degrees of freedom (DF), and corresponding p-value are reported. Significance: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

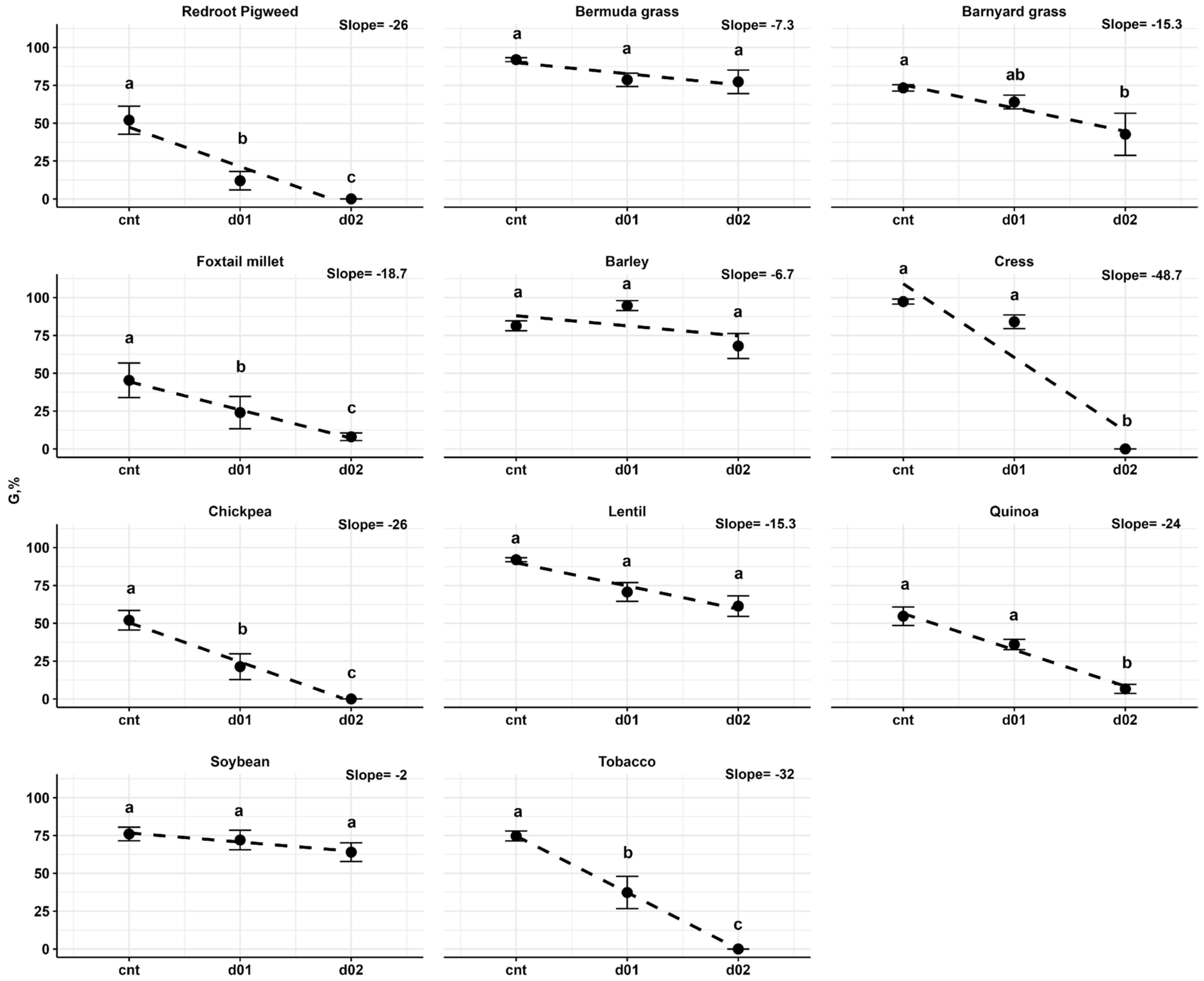

Fig. 2 Mean germination percentage (G %), and corresponding standard error limits, in response to water extracts concentrations (cnt control, d01 buckwheat water extracts at concentration 1:10 (100 g L^{-1}), d02 buckwheat water extracts at concentration 1:5 (200 g L.$^{-1}$)). Statistical differences are denoted by different letters. For each species the slope of the dose–response linear regression is specified (dotted line)

differences were observed in these species ($m > 49$), excluding tobacco.

Among the weeds, G in bermudagrass and barnyard grass was not strongly reduced in either treatment, although root length was approximately halved compared to the control (Fig. 2). In contrast, foxtail millet showed a significant reduction in RSG (S1), and in the d02 treatment, RRG was reduced by approximately 70% (Fig. 3). The least suppressed species was soybean, with a GI of 75.9% at d01 and 40.9% at d02. At d01, the three most tolerant species after soybean were weeds: foxtail millet (GI 57.0%), barnyard grass (GI 55.1%), and bermudagrass (GI 46.7%) as shown in supplementary material S3. Despite being the most resistant species, the germination reduction for all of these was close to 50% (Fig. 2).

A unique exception in seed response to BW extracts was observed in quinoa. Despite the significant reduction in seed germination, in the d01 treatment the measured values of RRG were higher than in the control. However, the differences were not statistically significant.

No significant correlation was assessed between thousand seed weight and GI for both d01 and d02 ($p > 0.05$).

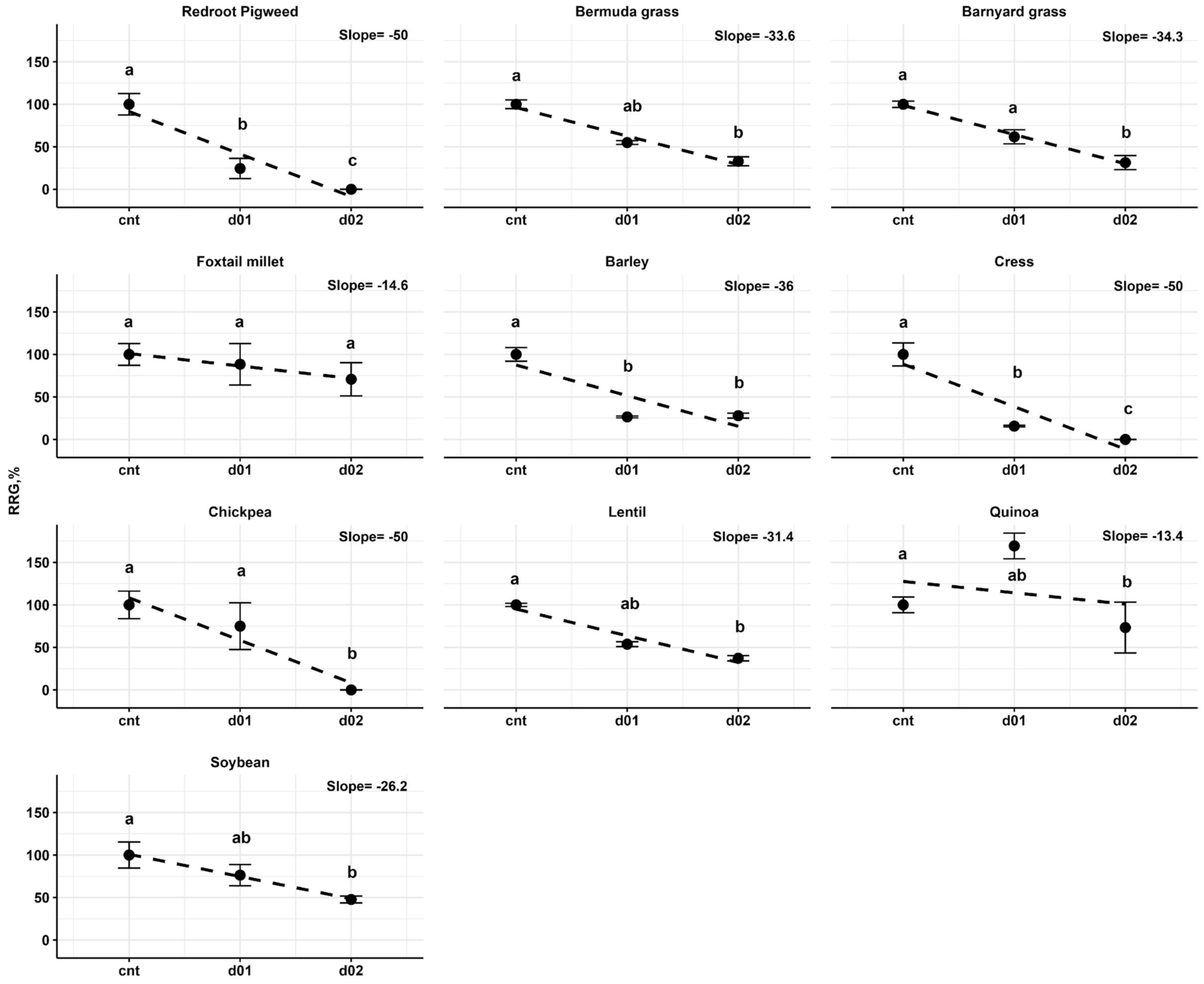

Fig. 3 Mean values with corresponding standard error of Relative Root Growth (RRG, %) measured during the experimentation (cnt control, d01 buckwheat water extracts at concentration 1:10 (100 g L^{-1}), d02 buckwheat water extracts at concentration 1:5 (200 g L.$^{-1}$)). Statistical differences are denoted by different letters. For each species the slope of the dose–response linear regression is specified (dotted line)

Greenhouse experimentation

Results of the analyses of variance on measured values of aboveground dry biomass (DW$_{ABV}$) and root dry biomass (DW$_R$) are listed in Table 2, Figs. 4–5 show the differences between the treatments for each species, along with the mean values and the corresponding standard error. For all the tested parameters, the effects of species, treatment and their interaction were statistically significant.

No significant difference was observed in DW$_{ABV}$ on the first sampling conducted 24 days after sowing. However, in both of the following sampling dates, DW$_{ABV}$ in MC was significantly higher than in IC and SD across all species. In *A. theophrasti*, DW$_{ABV}$ under the SD treatment was lower than in IC. No difference was recorded for chickpea between IC and SD, while DW$_{ABV}$ values for lentil and *A. retroflexus* in SD were significantly higher than in IC.

Statistical differences in DW$_R$ were assessed on the second sampling in redroot pigweed and chickpea, where DW$_R$ was lower in IC compared to the control MC. On the third sampling date differences between MC and IC were assessed in lentil only, while for velvetleaf no difference was assessed at all.

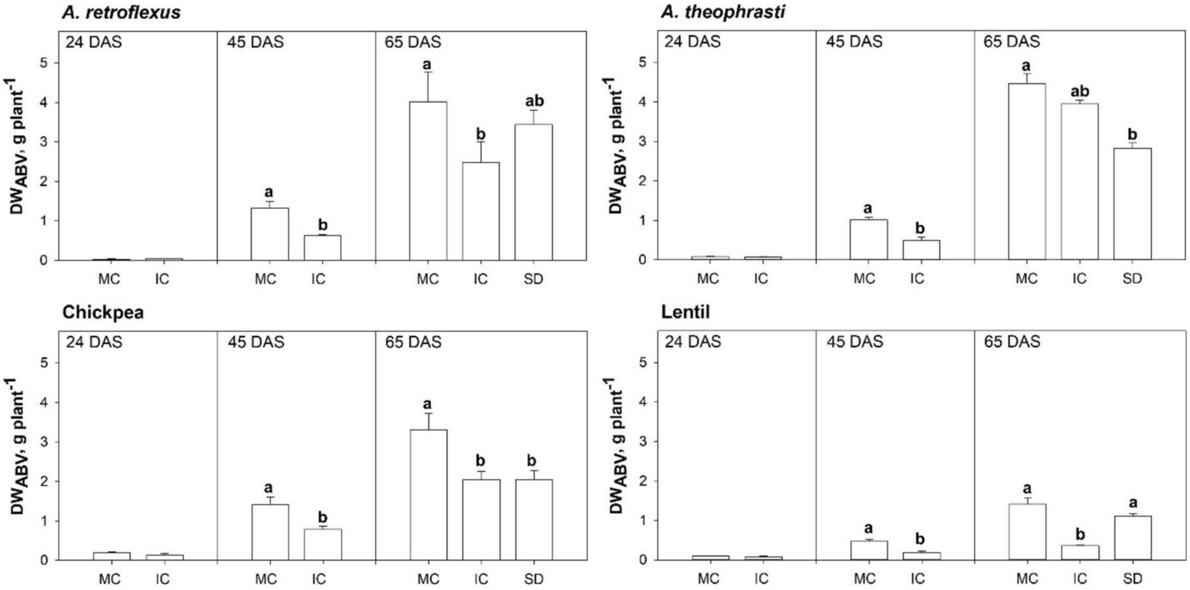

Fig. 4 Mean values with corresponding standard error of aboveground dry biomass (DW_{ABV}) measured in redroot pigweed, velvetleaf, chickpea, and lentil, under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Measurement conducted 24 (16th May 2023), 45 (6th June 2023) and 55 (26th June 2023) days after sowing (DAS). Statistical differences assessed by Tukey's post hoc test are specified by letters, otherwise not significant

Table 2 Results of the analyses of variance conducted on aboveground dry biomass (DW_{ABV}) and root dry biomass (DW_R)

	DW_{ABV}			DW_R		
Experimental factors	LR	DF	*p*-value	LR	DF	*p*-value
Dates of sampling	2368.55	2	< 0.001***	668.56	2	< 0.001***
Species	509.99	4	< 0.001***	306.62	4	< 0.001***
Treatments	24.07	2	< 0.001***	4.48	2	< 0.001***
Dates:Species	298.19	8	< 0.001***	539.85	8	< 0.001***
Dates:Treatments	28.45	2	< 0.001***	1.35	2	0.736
Species:Treatments	100.91	8	< 0.001***	48.98	8	< 0.001***
Dates:Species:Treatments	19.22	8	0.006**	32.33	8	< 0.001***

For each variable, the likelihood ratio (LR), degrees of freedom (DF), and corresponding p-value are reported. Significance: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Results of the analyses of variance for Competitive Balance Index (CBI) calculated from DW_{ABV} of crops and weeds in IC, are listed in Table 3, while differences between species are reported together with mean values and standard errors are represented in Fig. 6. All species behaved as poor competitors, as CBI values were systematically lower than 0 throughout the season. All factors and their interactions were statistically significant and differences were assessed on the last sampling, where velvetleaf had higher CBI than redroot pigweed and chickpea; lentil was significantly lower than the other species. Regarding the quantification of allelochemicals, rutin was not detected in the leached water, while quercetin concentration was found to be 1.05 ± 0.04 mg L^{-1} (supplementary material S1).

Discussion

In this study, we examined the allelopathic effect of buckwheat (BW) on various crops and weeds (Supplementary Material S4) in two experiments: i) in an in vitro assay to test the effect of BW water extracts on seed germination and seedling growth; ii) in a pot

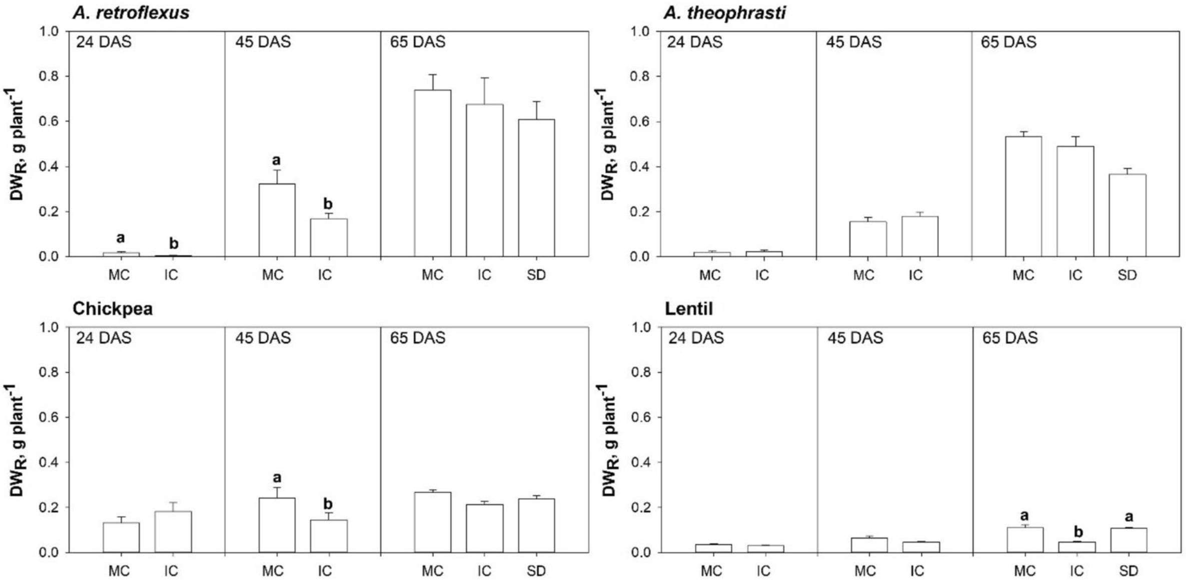

Fig. 5 Mean values with corresponding standard error of root dry biomass DW$_R$) measured in redroot pigweed, velvetleaf, chickpea, and lentil, under three different treatments: monoculture (MC), intercropping (IC) and staircase device (SD). Measurement conducted 24 (16th May 2023), 45 (6th June 2023) and 55 (26th June 2023) days after sowing (DAS). Statistical differences assessed by Tukey's post hoc test are specified by letters, otherwise not significant

Table 3 Results of the analysis of variance conducted competitive balance index (CBI)

Effect	Competitive Balance Index		
		LRDF	*p*-value
Sampling dates	1.6750	2	0.0037**
Species	2.3577	3	0.0017**
Sampling date:Species	3.6346	6	0.0012**

For each effect, the likelihood ratio (LR), degrees of freedom (DF), and corresponding p-value are reported. Significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

experiment to quantify, in soil conditions, the effects of BW allelopathic compounds through root exudates.

BW water extract inhibition effect on crop and weed germination and seedling growth

In the in vitro experiment, both concentrations of BW water extracts negatively affected all tested crops and weeds, reducing seed germination and root elongation and leading to consistent decreases in the estimated germination and root growth indexes. This finding is particularly interesting, considering that the measured levels of quercetin and rutin in the extracts were approximately 50 times lower than values typically reported in the literature (e.g. Golisz et al. 2007a, 2007b). Both rutin and quercetin are known to inhibit seed germination through several mechanisms like affecting the energy metabolisms in the first hours of imbibition (Takashi et al. 1998), the release of various antioxidant enzymes (Marusa Pergo & Ishii-Iwamoto 2011) or the regulation of several hormones (Kang et al. 2025). Our results are in accordance with the literature, suggesting a suppressive effect on seed germination, even though previously only evaluated for weeds (e.g. Kalinova et al. 2009, Falquet et al. 2015, Scavo and Mauromicale 2021, Vieites-Álvarez et al. 2023). Moreover, since no correlation was found between the thousand-seed weight and the germination index, any effect of seed size on susceptibility to allelopathic substances can be excluded.

However, several factors can impact the germination process, particularly the osmotic potential was reported to influence germination in allelopathy studies (Anderson and Loucks 1966; Haugland and Brandsaetler 1996). Other authors reported a positive role of quercetin on seed germination and seed vigor under osmotic stress (Yang et al. 2021). At

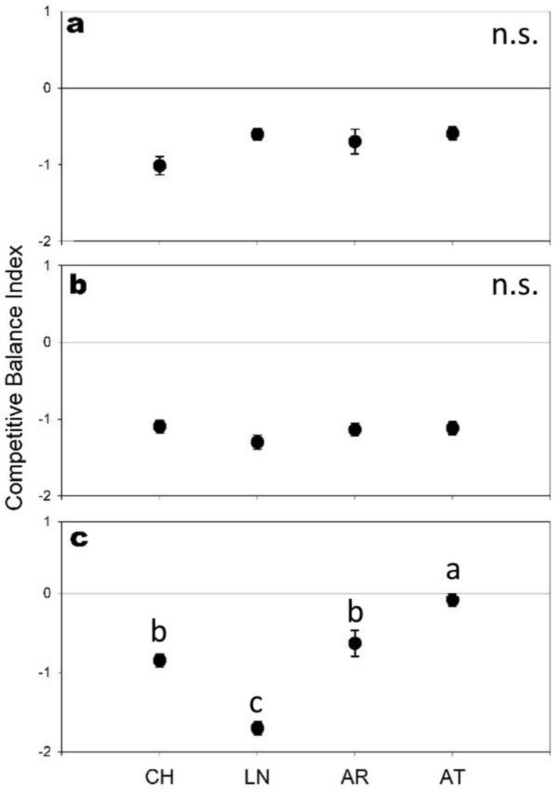

Fig. 6 Mean values with corresponding standard error of Competitive Balance Index (CBI) of chickpea (CH), lentil (LN), redroot pigweed (AR) and velvetleaf (AT) calculated from aboveground dry biomass measured on **a)** 24 days after sowing (16th May 2023); **b)** 45 days after sowing (6th June 2023); **c)** 65 days after sowing (26th June 2023). Statistical differences assessed by Tukey's post hoc test are specified by letters, otherwise not significant (n.s.)

the best of our knowledge, few studies in allopathy insert a positive control (e.g. with PEG solutions) to check the effect of osmotic potential disentangled from the effect of the allelochemicals, whereas this is a common practice in studies on the effect of salinity or drought on germination. For some of the species tested in this study, the literature reports an inhibitory effect of osmotic potential on germination when considering the osmotic potential retrieved for the two water extracts (d01: −0.33 MPa; d02: −0.73 MPa). This effect was particularly evident in the most concentrated solution (d02) for species such as redroot pigweed (Ghorbani et al. 1999), barnyard grass (Boyd and Van Acker 2004), chickpea (Abderemane et al. 2025) and soybean (Wijewardana et al. 2018). However, for other species like quinoa (de la Reguera et al. 2020), lentil (Turk et al. 2004) or bermudagrass (Tucker et al. 2017), osmotic potential did not show, according to literature, an inhibitory effect on germination at the values corresponding to the tested water extracts. Therefore, we can conclude that at least the reduced germination of quinoa can be ascribed to allelopathy rather than osmotic pressure. On the other hand, soybean was not negatively affected by the two water extract concentrations, but in literature (Wijewardana et al. 2018) it appears sensitive to the corresponding osmotic pressures. We hypothesize that there are both species-specific and cultivar-specific (Turk et al. 2004; Tucker et al. 2017; Abderemane et al. 2025) responses to osmotic pressure and that this aspect should be investigated in future studies on allelopathy.

BW root exudates and inter-specific competition effects on weed and crop growth

In the pot experiment, an inhibitory effect of intercropped BW on plant growth was observed 45 days after sowing, corresponding to the full flowering stage of BW. The staircase device treatment was essential to distinguish between growth inhibition caused by interspecific competition versus allelopathy. Despite no allelochemical being detected in the soil samples, the analysis revealed that the leached water from the staircase device pot contained a concentration of quercetin comparable to the water extract used in the in vitro experimentation. The literature reports similar evidence supporting inhibition by the allelochemicals produced during the early growth stages of buckwheat (Iqbal et al. 2003; Kalinova et al. 2007, Falquet et al. 2015). Daryanavard et al. (2023) reported that flavonols, quercetin in particular, are able to affect both shoot and root growth through maintenance of ROS (Reactive Oxygen Species) homeostasis and inhibition of auxin transport. In our pot experiment, the reduction of the aboveground biomass of all the species tested was either greater (lentil, redroot pigweed) or equal (chickpea, velvetleaf) when they were intercropped with buckwheat (IC) compared to the staircase device (SD) pots. This was confirmed by the Competitive Balance Index (CBI) which was negative across all sampling dates for all the tested species (particularly lentil) in the IC treatment. Falquet et al. (2015) carried out a pot experiment investigating the effects of intercropping buckwheat with redroot pigweed, and reported a significant inhibitory effect, which they attributed to both competitive shading and root interactions, potentially involving allelopathy. Chickpea and velvetleaf were both negatively affected in the SD treatment, but only chickpea recorded significantly lower shoot biomass in the IC treatment compared to the sole crop control. Only the root biomass of lentil was reduced in the IC treatment, which suggests that buckwheat's effectiveness in suppressing the root growth of other tested species can be attributed more to competition for resources rather than the release of allelopathic compounds by root exudates. Other studies have reported changes in root size in pots (Gersani et al. 2001, Dou et al. 2025), while changes in root biomass may be better observed at a larger scale (Ajal et al. 2022; Shaheb et al. 2025). Furthermore, root biomass measurements may have been influenced by practical challenges related to root extraction and cleaning during destructive sampling, which could have increased the uncertainty and variability of the results. Further studies involving different soil substrates and pot depths may help quantify the relative contribution of interspecific competition and allelopathy and improve the reliability of these findings.

Implications for the use of BW in IWM through diversified cropping systems

The present study, carried out in controlled environments, confirms the weed-suppressive ability of buckwheat on weeds, and, for the first time, also on some crop species. The findings from both experiments support the use of cropping system diversification with allelopathic species like BW in the context of IWM (Riemens et al. 2022). Buckwheat used as dead mulch (i.e., crop residues) may inhibit weed germination and limit weed growth when used as cover crop or intercrop. These results are in agreement with the findings of Szwed et al. (2020a, b) and Kumar et al. (2009) in pot experiments. However, our results also indicate that the germination of some crop species may also be affected by buckwheat residues, highlighting the need for caution when buckwheat is used as a preceding crop in systems that conserve residues or rely on cover crops. This effect is species-specific, and Kumar et al. (2011a, b) did not find any negative effect of buckwheat residues on subsequent wheat. Similarly, Wirth and Geller (2016) conducted both field and pot experiments using soils previously cultivated with BW for varying durations. Their study found no influence of buckwheat on the germination or development of redroot pigweed and lettuce. The amount of time for which BW allelochemicals remain active and potentially detrimental to succeeding crops needs to be further investigated, as several authors (e.g. Macias et al. 2022) suggest that the degradation of allelochemicals can produce a diversity of compounds and interact with soil microbiome. This would allow to verify the trade-off between the positive weed control effect of BW dead mulching versus the potential negative effect on other crops when implementing temporal diversification with allelopathic species. Similarly, when considering spatial diversification practices, like intercropping, it remains important to assess if buckwheat competition for resources together with root exudates can limit weed emergence and growth while minimizing the trade-off with crop production.

Limitations and prospects

Despite these results, some limitations highlight the need for protocol improvement to confirm the present findings. For example, the authors encountered issues due to the development of fungi in the petri dishes because the water extracts could not be sterilized; furthermore, more shallow pots with an inert medium may have been more appropriate to reduce the degradation or soil adsorption of some polyphenols in the staircase device setting. Recent studies have also highlighted significant differences in allelochemical content between both tartary and common buckwheat, and between common buckwheat cultivars (Vieites-Alvarez et al. 2024). Future studies could focus on the application of water extracts from different buckwheat cultivars on seeds in different soil conditions. With this respect, the staircase device can be further used to better investigate the mechanisms related to allelopathy, like collecting intermediate sampling points on shoot and root data. Additionally, IC treatments should be compared both to the SD device, to systems separating roots with mesh and to systems separating shoots, to understand which system is best for disentangling belowground, and aboveground competition from allelopathy.

Up to now, field studies evaluating the suppressive effects of allelopathic crops in field are scarce, hence, more evidence is needed to compare the results obtained with field-based evidence (Mahé et al. 2022). For example, only a few studies have investigated the legacy effect of buckwheat mulch on the soil seed bank. Implications for intercropping systems involve testing species densities and spatial layouts, while prioritizing scalability in real farm conditions. Together, these findings would allow farmers to receive evidence-based recommendations in support of cropping system diversification including buckwheat as a preceding or companion crop.

Conclusions

The present study carried out a comprehensive investigation on the effect of buckwheat on the germination and growth of weeds and crops to investigate the potential use of this allelopathic crop for Integrated Weed Management (IWM). We performed two experiments to simulate the two mechanisms (i.e. crop residue decomposition and root exudates) underlying the allelopathic effect of buckwheat. To simulate the release mechanism of allelochemicals from buckwheat residues, we tested two concentrations of buckwheat water extracts (1:5 and 1:10 g BW/mL) in an in vitro assay on the seed germination of four weeds and, for the first time, seven crops. In the second experiment, we intercropped buckwheat with two weeds and two crops in pots to test the effect of exudates released by buckwheat roots into the rhizosphere. To disentangle the effect of competition and allelopathy in intercropping we also tested the effects of buckwheat leachate in a staircase device treatment. A negative effect of the water extracts was observed, but no clear difference was recorded between the crop and weed categories or with respect to the seed size. This inhibitory effect was hypothesized to be related to the presence of rutin and quercetin in the water extracts, although their osmotic potential has been identified in the literature as a potential confounding factor in the germination tests for redroot pigweed, barnyard grass and for specific cultivars of chickpea and soybean; therefore, the authors recommend including a positive control with PEG in future allelopathy studies. In the pot experiment, lentil appeared as the most negatively affected species both by competition and allelopathy, although all species suffered from the proximity of buckwheat.

While the inhibitory effect of buckwheat extracts on weed germination has been previously reported, it has often been limited to a small number of weed species. Our results broaden this evidence and can support the design of future field experiments evaluating the integration of buckwheat in arable cropping systems either through temporal (i.e., dead mulch, cover crop) or through spatial (intercropping) diversification for weed control while assessing its effects on crops.

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Data availability The datasets generated from and/or analysed in the current study are available on Zenodo with restricted access (https://doi.org/10.5281/zenodo.15533660).

Declarations

Competing interests All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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