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Do Aqueous Extracts from Buckwheat Compromise Seed Germination and Initial Development of *Bidens pilosa* and *Euphorbia heterophylla*?

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Abstract

Fagopyrum esculentum Moench (buckwheat) is a dicot species from the Polygonaceae family used as a cover crop in agricultural systems featured with a remarkable allelopathic potential for weed control, helping herbicide-resistance management and promoting substantial reductions in herbicide applications. The aim of this research was to examine the allelopathic potential of aqueous extracts from seeds and aerial part of buckwheat on seed germination and initial development of *Bidens pilosa* and *Euphorbia heterophylla.* Bioassay experiments were conducted under a completely randomized experimental design with four replications, containing 50 seeds each. Both weed seed species were harvested in a soybean field, and seed viability was previously assessed. Seeds were exposed to four concentrations (0, 25, 50, and 100%) from extracts of seeds (ES) and aerial part (EAP) of buckwheat. Germination speed index (GSI) in *B. pilosa* and *E. heterophylla* was daily evaluated throughout 14 and 16 days, respectively, whereas percentage of germination, abnormal seedlings, as well as non-germinated seeds, root (RL) and aerial part length (APL), and total dry matter (TDM) were rated at final germination test. EAP reduced the GSI, especially under the 100% concentration. Germination percentage was lower and abnormal seedlings increased for both weed species when seeds were exposed to EAP concentrations greater than 25%. However, ES did not impinge upon *E. heterophylla* germination. EAP and ES reduced the APL, RL, and TDM for concentrations greater than 50%, except for ES which did not affect *E. heterophylla* development. Both extracts from buckwheat have a high capacity to inhibit germination and compromise seedling development, culminating in such a potential alternative for *B. pilosa* and *E. heterophylla* management in agricultural systems.

Keywords

Fagopyrum esculentum Moench, Plant Allelopathy, Allelochemicals, Weed Management, Bioherbicides

1. Introduction

Fagopyrum esculentum Moench (buckwheat) is an annual dicotyledon species belonging to the Polygonaceae family that have high phenotypic plasticity, good development in poorly fertility soils and short life cycle, making possible its utilization either alone or in consortium with other cover crops in agricultural systems at Southern Brazil [1]. Additionally, rapid crop establishment and good closing of vegetative canopy are the main plant characteristics that might promote weed management in such a way as to reduce crop development and plant population density [2].

Although weed control has been evolved significantly over the last few decades from herbicides discovery and biotechnology events use, interference levels of weeds have been increasing strongly in cropping systems due to herbicide resistance and control failures [3]. A successful and intensive herbicide use in conjunction with absence of other management practices has been imposing strong selection pressure of herbicide-resistant weeds, reducing the herbicide options for certain weed infestations, especially when there are no herbicides with new modes of action available in the market to warrant good control levels [4]. In Brazil, 53 cases of herbicide resistance are reported for 28 weed species, including *Bidens pilosa* L. (hairy beggarticks) and *Euphorbia heterophylla* L. (wild poinsettia) in light of being two troublesome annual species commonly found in soybean, beans and corn production fields. Chemical control failures within hairy beggarticks populations include acetolactate synthase enzyme (ALS) and photosystem II (PSII) inhibiting herbicides, whereas wild poinsettia can exhibit populations with cross- and multiple-resistance to ALS and protoporphyrinogen IX oxidase (PPO) inhibiting herbicides, currently with a number of cases of resistant-biotypes to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) inhibiting herbicides, highlighting a critical scenario that demands changes in management strategies [5].

Integrated management practices to control the weed herbicide-resistant must be conducive to reductions in population density, aiming to minimize the competition effects and inherent problems from intensive usage of chemical control in agricultural systems [6] [7]. Therefore, allelopathy is an important tool with low environmental impacts that can suppress seed germination and development of weed species by means of chemical substances release from a plant to neighbors, promoting the manipulation and regulation of weed populations and lesser herbicide usage over time [8] [9] [10]. Thus, allelopathy is defined as a common biological phenomenon established between organisms in detrimental of their interactions to be detected between receptor and donor, from which one particular organism renders biochemical compounds that impinge upon growth, survival, development, and also reproduction of other organisms in turn [11] [12].

Allelopathic compounds are synthetized by secondary metabolism of the plants from pathways of shikimate, acetate-mevalonate, malonic acid, and methylerythritol phosphate, such as alkaloids, terpenes, flavonoids, coumarins, quinones, phenols, and other carbon molecules can help with integrated management of weeds or natural products as a source of new herbicides in the near future [11] [12]. Direct releasing of allelopathic substances in agricultural environment might come from root exudation, volatilization, and leaching processes, whereas microbial decomposition of plant residues present on soil surface is considered indirect release, triggering biochemical substances with high potential to weed control in sustainable agricultural systems [13]. These compounds can interact alone or associated with soil microbiote, resulting in distinct allelopathic effects on an organism [14]. For instance, several results have been reporting phytotoxic effects on weed seedlings exposed to allelochemical compounds, triggering disturbs on root and shoot growth, cell cycle disturbance and oxidative damage that substantially reduce crop photosynthetic activity [9] [10]. On the other hand, plant extracts have major potential as an innovative tool to manage weeds in comparison to herbicides, with numerous amounts of unknown compounds in nature with capacity of agricultural uses, exhibiting good efficiency and lower negative impacts on agroecosystems [15].

The discovery of phenolic compounds from buckwheat extracts produced in variable amounts depending on tissue/part and phenological development stages of the plant might trigger adoption of new strategies for weed management [16]. Recently, allelopathic potential of buckwheat against seed germination and initial development has been reported in *Amaranthus retroflexus* L. (redroot pigweed) and *Chenopodium album* L. (common lambsquarters) [17], *Echinochloa crusgalli* (L.) *P. beauv.* (barnyardgrass) and *Galium aparine* L. (catchweed bedstraw) [18], and suppressed annual weeds in winter cereals [19], with variable amounts of compounds present between tissue/parts of buckwheat plant, and consequently, inhibitory potential of extracts leads to different biological responsiveness. In sensitive plants, growth inhibition is associated to disturbs related to secondary metabolism, increase of peroxidase enzyme activity and increasing of phenolic compounds found in plant tissues of the weed [18], demonstrating higher potential to control weed resistant-populations to herbicides.

However, information on allelopathic potential of buckwheat and their effects on germination and growth of weeds is still lacking in the literature considering its commercial use as a cover crop and compounds to bioherbicides. The aim of the current study was to assess the allelopathic potential of aqueous extracts from seeds and aerial part of buckwheat on both germination inhibition and initial development of *Bidens pilosa* and *Euphorbia heterophylla.*

2. Materials and Methods

2.1. Seeds Material and Aqueous Extracts

Buckwheat seeds (cultivar IPR92 Altar) were obtained by a grower from Palmeira, State of Paraná, Brazil. This cultivar is largely sown as a cover crop at his production fields. For obtention of extracts from aerial part (EAP), seeds were sown in plastic pots with 5 L of volumetric capacity, filled with soil samples classified as a typical Oxisol (USDA Soil Taxonomy) in mixture with substrate (sphagnum peat and vermiculite composition) at a 2:1 proportion. After emergence, plant thinning was performed to maintain 15 plants per pot. Buckwheat plants were kept in a greenhouse for 70-days after emergence up until harvest of aerial part of the plant. Aerial part was harvested containing stems and leaves, and buckwheat seeds were dried off in a forced air oven for 72-h under a constant temperature corresponding to 40˚C. Dry samples were ground separately in a knife mill grinder for 2 min at 1400 rpm to get a fine powder (1 mm) from each plant part. Distilled water turned out to be the solvent employed for extraction from samples of aerial part and seeds. The parts of the plants taken as extracts were ground, weighed and then 10 mL of distilled water was added to each gram of solute in order to form mother solutions. The solution was placed in bottles previously sterilized with 70% alcohol and protected with aluminum paper to preclude photodegradation of sensitive compounds owing to the incidence of solar radiation.

EAP and ES mother solutions were identified and kept under dark conditions for 24-h at 25˚C, and afterwards kept for 24-h under cold conditions of 10˚C, totaling 48-h for a full extraction. Subsequently, extracts were filtered with a gauze cloth and filter paper, and mother solution at 100% concentration was preserved in sterilized bottles protected from light, and kept under cold conditions to promote water dilution, aiming to obtain needed concentrations for germination tests.

Previously to germination test performance, hairy beggarticks and wild poinsettia seeds were harvested in a soybean production field and correctly identified at taxonomy level. Mechanical scarification of wild poinsettia seeds was performed with sandpaper dishes (grit size equal to 220) to break seed dormancy. A rough surface was maintained under seed contact using a weight of 400 g placed over the sandpaper to produce the same work power, rotating it either counter-clockwise or clockwise five times each (α = 1800˚) to promote seed tegument scarification and standardize weight on top of the seeds to prevent uneven scarification. Hairy beggarticks seed dormancy release was performed under cold stratification, in which seeds were kept at 10˚C for seven days. Seed viability of hairy beggarticks and wild poinsettia was of 82% and 70%, respectively.

2.2. Germination Testing with Aqueous Extracts from Buckwheat

The experiment was carried out at a seed pathology laboratory belonging to Crop Science and Crop Protection Department of the State University of Ponta Grossa between April and July of 2021, using a randomized block experimental design with four replications. Hairy beggarticks and wild poinsettia seeds were subjected to four extracts concentrations of buckwheat (control, 25%, 50% and 100%) from aerial part and seeds, totaling 16 treatments. Fifty seeds collected from each weed species were placed individually into a transparent polyethylene box, measuring 11 × 11 × 3.5 cm, where seeds were distributed over two sheets of blotter paper, previously moistened with distilled water at an amount equivalent to 2.5-fold the paper weight, and re-moistened at four and ten days after the beginning of the test. Germination test was carried out in a chamber of biochemical oxygen demand (BOD) for 14 and 16 days for hairy beggarticks and wild poinsettia, respectively, and kept under alternating temperatures and photoperiod (20˚C for 16-h in dark and 30˚C for 8-h in light), according to the Brazilian Rules for Seed Testing [20].

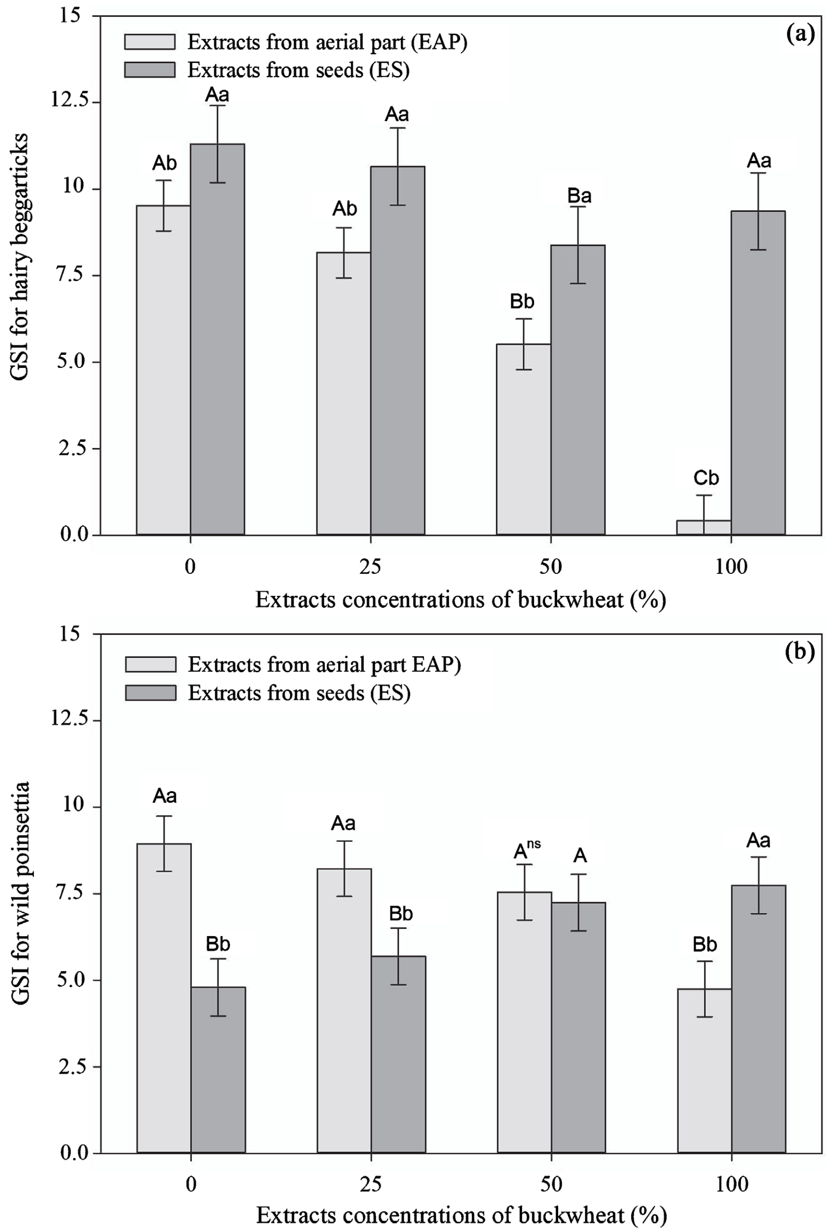
Germination speed index (GSI), germination (G) and abnormal seedlings (AS) percentage, non-germinated/death seeds (NGDS) percentage, root (RL) and aerial part (APL) length and total dry mass (TDM) of seedlings were evaluated. Germination of seedlings was daily counted to calculate the GSI by means of the equation proposed by Edmond and Drapala [21] and described as $GSI = (N_1/D_1) + \dots + (N_n/D_n)/T$, where N_1 = number of germinated seedlings on the first day; N_n = number of germinated seedlings on the n day; D_1 and D_n = observation performed on the first and n days after the beginning of the test, respectively; T = total number of evaluated days.

Germinated seeds were assumed as all seedlings with radicle/hypocotyl development equal or greater than the seed size and not affected by extracts compared with control. Seedlings with unable development/structure to growth normally were considered abnormal, whereas NGDS included deteriorated seeds and those dead shortly after germination. RL and APL were measured with a ruler from 10 seedlings randomly chosen for each replication, which samples were packaged in paper bags to dry off in a force air oven at 60˚C for 72-h to quantify the TDM, with values in milligrams of 10 seedlings.

Experimental data were subjected to Shapiro-Wilk test to verify data normality and therefore assessed to analysis of variance ($p < 0.05$). The allelopathic potential of buckwheat under the influence of different extract concentrations was compared by Duncan test ($p < 0.05$), whereas effects between extracts of each part of the plant was detected by the F test ($p < 0.05$).

3. Results and Discussion

Analysis of variance along with application of F test evidenced that concentrations of ES or EAP negatively impacted either seed germination or initial development of *B. pilosa* and *E. heterophylla* (Figures 1-4). GSI in hairy beggarticks was lower only at concentrations of 50% and 100% for ES and drastically reduced



*Uppercase letters compare effects of concentrations from each extract source by means of the Duncan test ($p < 0.05$). Lowercase letters refer to effects between different extracts from each concentration detected by the T test ($p < 0.05$). [ns]Non-significant. Error bars represent the least significant difference (LSD).

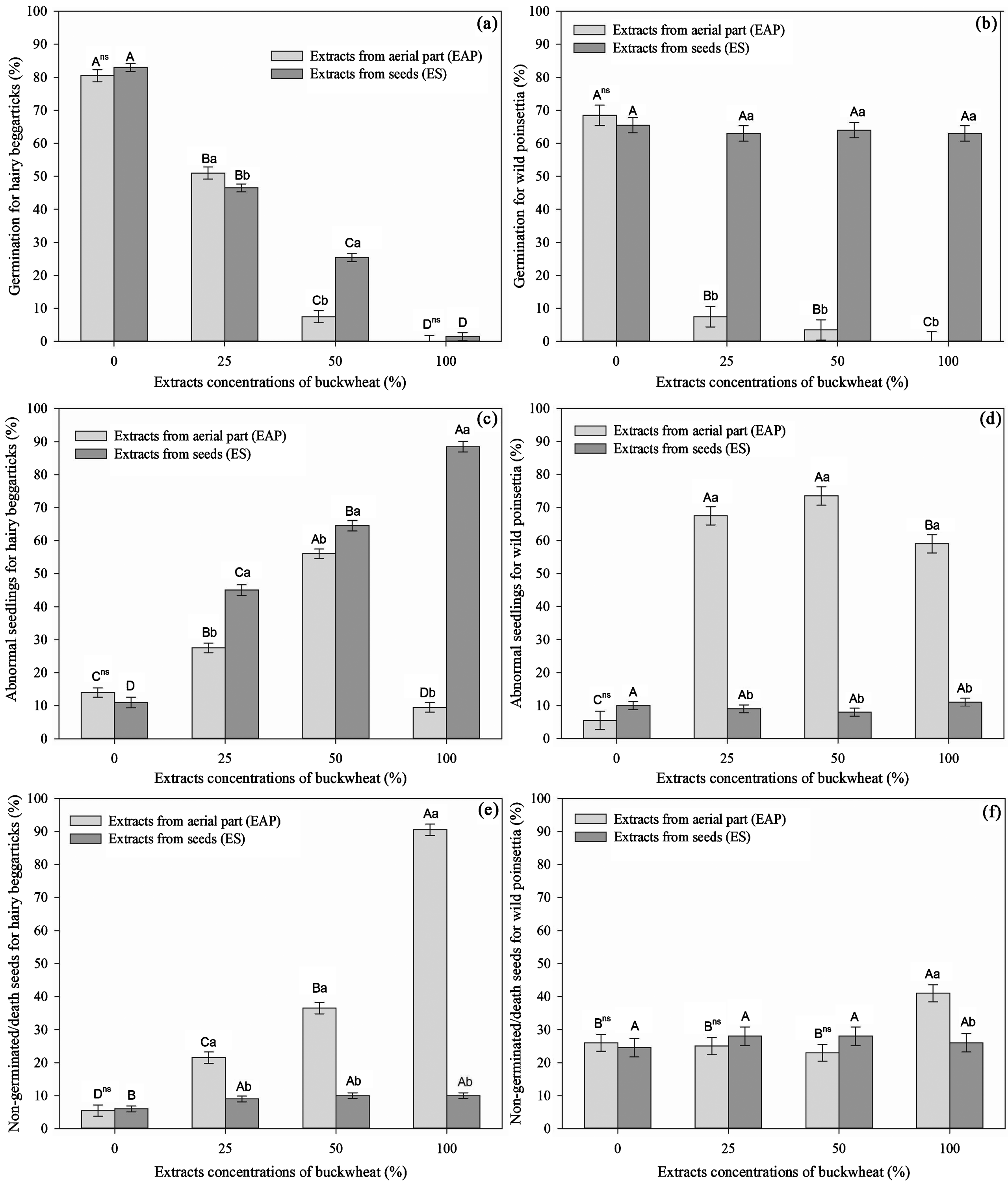
Figure 1. Germination speed index in hairy beggarticks (a) and wild poinsettia (b) after exposure to different concentrations of extracts from seeds (ES) and aerial part (EAP) of buckwheat.

as a function of an increase in concentration when EAP was used, with GSI corresponding to 0.43% at 100% concentration (**Figure 1(a)**). In wild poinsettia, GSI was lower for EAP and directly proportional to concentration, with a value equivalent to 4.74 whenever seeds were exposed to 100% concentration treatment (**Figure 1(b)**). Conversely, increasing in ES concentration provided a higher GSI in wild poinsettia, with an average value of 7.49% at 50% and 100% extract concentration (**Figure 1(b)**).

Adverse effects of allelopathy in weeds might delay seed germination by reducing GSI due to inhibitory effects and vigor losses [22]. For cropping systems, delay of weed seed germination triggers reduced competition with a given crop, causing lower impacts on crop yield owing to reduced interference level. Thus, release of allelochemical compounds by the plants might stimulate toxicity for their neighbors, affecting both development and initial competition due to pre-empts the resources away by the crop [23].

Additionally, extracts from seeds and aerial part of buckwheat reduced the percentage of germination in hairy beggarticks, regardless of the concentration adopted (**Figure 2(a)**). Reduction in germination was of 30% when seeds were exposed to 25% of ES and EAP in comparison to control, whereas 100% concentration for both extracts dropped seed germination to 99%. Besides, EAP was more efficient to reduce seed germination at 50% concentration for the ES. In wild poinsettia, extracts from seeds did not affect germination percentage, irrespective of concentration. In contrast, 25% of EAP concentration plunged seed germination to 60%, with values nearly nil at 100% concentration (**Figure 2(b)**). According to Falquet *et al.* [24], the high inhibitory potential of buckwheat extracts reduced seed germination and compromised seedling development. Similarly, several allelochemical compounds from decomposing residues of buckwheat plants in Poland production fields exhibited strong negative effects on barnyardgrass by reducing either germination or growth plant [25].

Direct impacts of buckwheat extracts on hairy beggarticks seedlings were reported for percentage of abnormal seedlings, with substantial increase in values at concentrations up to 50% of EAP and at all ES concentrations, exhibiting maximum values of 60% and 87%, respectively (**Figure 2(c)**). Similar biological responses took place in wild poinsettia for EAP, with an average maximum value of 70% whenever seeds were exposed to 25% and 50% concentrations (**Figure 2(c)**). ES did not significantly impinge upon abnormal seedlings percentage, with values next to 10% at all concentrations (**Figure 2(d)**). Sytar *et al.* [16] found distinct amount of allelochemical compounds produced in buckwheat, emphasizing allelopathic potential on both germination and initial seedling development. Hence, a number of substances synthetized from secondary metabolism, such as phenolic, flavonoid, tannins and other carbon compounds with distinct functional groups might inhibit seed germination by conditioning seedling development and playing a pivotal role in supplying compounds to new herbicides [10].



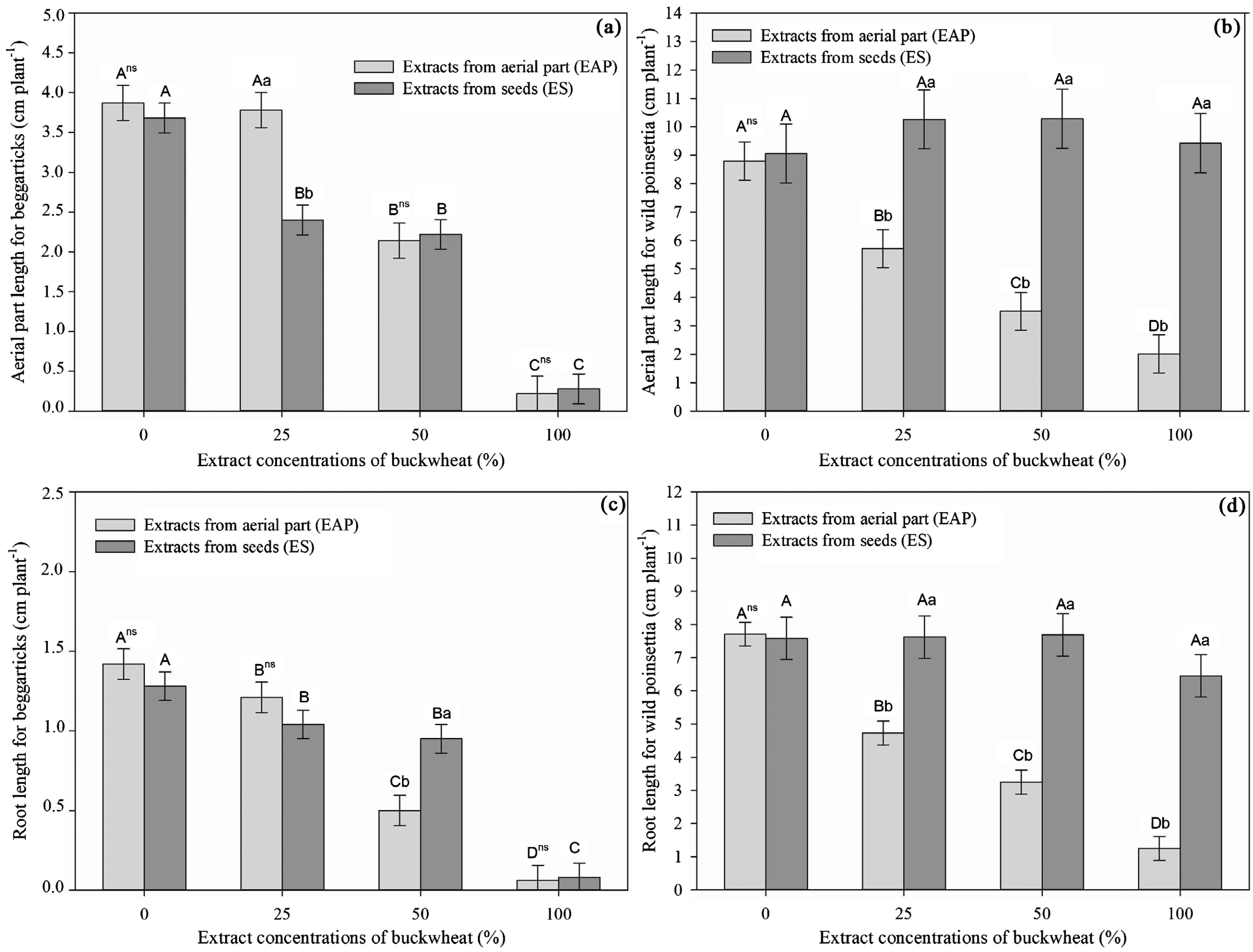
*Uppercase letters compare effects of concentrations from each extract source by means of the Duncan test ($p < 0.05$). Lowercase letters refer to effects between different extracts from each concentration detected by the T test ($p < 0.05$). ^{ns}Non-significant. Error bars represent the least significant difference (LSD).

Figure 2. Germination percentage (a) and (b), abnormal seedlings (c) and (d) and non-germinated/death seeds (e) and (f) in hairy beggarticks and wild poinsettia after exposure to different concentrations of extracts from seeds (ES) and aerial part (EAP) of buckwheat.

NGDS percentage was similar among all ES concentrations used in hairy beggarticks, with values lower than 10%. However, percentage of NGDS increased strongly when seeds were exposed to EAP, inhibiting 40% and 90% of seed germination at 50% and 100% extract concentrations compared to control (**Figure 2(e)**). Extracts from seeds and aerial part did not affect the NGDS in wild poinsettia, except for EAP at 100% concentration exhibiting values next to 57% and 2-fold greater than control treatment (**Figure 2(f)**). Inhibitory effects on watermelon germination were found for seeds exposed to vanillic acid, with significant amounts of this compound found in leaves and stems of buckwheat [16] [26]. Khanh *et al.* [27] reported that 100 and 200 ppm of palmitic acid from passion fruit *(Passiflora edulis*) aqueous extracts inhibited approximately 35% of germination in barnyardgrass, and completely the seed germination in radish (*Raphanus sativus* L.). In this way, our results are similar to others reported in the literature with regard to effects of buckwheat against weeds. Nevertheless, inhibitory potential on seed germination and seedlings growth depends on weed species, part of the plant used to prepare mother-solutions and extract concentration scrutinized in every single specific-bioassay.

By evaluating development of seedlings, use of extracts from seeds and aerial part reduced length of root and aerial part in hairy beggarticks, whereas only EAP negatively affected RL and APL in hairy beggarticks and wild poinsettia in comparison to control (**Figure 3**). Increasing concentration of both extracts reduced APL in hairy beggarticks, with values greater than 90% at 100% extract concentration. For control treatment, length of aerial part and root in hairy beggarticks was approximately 3.7 $cm \cdot plant^{-1}$, whereas use of ES and EAP at 100% concentration exhibited 0.3 and 0.4 $cm \cdot plant^{-1}$ for RL and APL, respectively (**Figure 3(a)** and **Figure 3(c)**). Conversely, APL and RL in wild poinsettia was lower only for seeds exposed to aerial part extracts of buckwheat compared to control, with values up to 75% lower at the maximum extract concentration (**Figure 3(b)** and **Figure 3(d)**).

Extracts from seeds did not affect significantly RL and APL compared to control, with mean value of APL equals to 9.8 $cm \cdot plant^{-1}$ and RL close to 7 $cm \cdot plant^{-1}$ (**Figure 3(b)** and **Figure 3(d)**). Recently, *Hesperozygis ringens* extracts containing oxygenated monoterpenes and known as pulegone exhibited a high allelopathic potential capable of negatively interfering both initial growth and plant development [28]. Similar responses were reported for *Ambrosia artemisiifolia* L. (common ragweed) extracts, which reduced initial development of soybean [29], whereas seeds of hairy beggarticks imbibed in aqueous extracts from *Sorghum bicolor* (shatter cane) and *Brachiaria brizantha* (palisade grass) reduced root and aerial part length, respectively [30]. Effects on root system and aerial part due to allelopathic compounds compromise establishment and seedling development of the plants, affecting plant competitiveness and impairing therefore life cycle of a specific-crop, especially when it comes to seed shedding into the soil system [31].



*Uppercase letters compare effects of concentrations from each extract source by means of the Duncan test ($p < 0.05$). Lowercase letters refer to effects between different extracts from each concentration detected by the T test ($p < 0.05$). ^{ns}non-significant. Error bars represent the least significant difference (LSD).

Figure 3. Aerial part (APL) and root (RL) length evaluated in hairy beggarticks (a) and (c) and wild poinsettia (b) and (d) after exposure to different concentrations of extracts from seeds (ES) and aerial part (EAP) of buckwheat.

Increasing buckwheat extract concentration from seeds and/or aerial part triggers reductions in accumulation of total dry matter in hairy beggarticks seedlings, reaching up to 99% at 100% extract concentrations in comparison to control (Figure 4(a)). By using 50% of ES, TDM was of 20% lower in hairy beggarticks, whereas 50% of extracts from aerial part reduced 50% TDM accumulation (Figure 4(a)). Wild poinsettia exposed to extracts from buckwheat seeds showed a reduction of 11% for TDM accumulation, regardless of extract concentration (Figure 4(b)). In contrast, EAP at 100% concentration plunged TDM roughly 50% in relation to control, and only 8% whenever seeds were exposed to 50% EAP concentration (Figure 4(b)). Similar results were reported for hairy beggarticks submitted to 200 $g \cdot L^{-1}$ of sunflower extracts extracted from leaves, with a strong reduction in seedling biomass accumulation [32]. Lower development of aerial part and roots was found in redroot pigweed and common

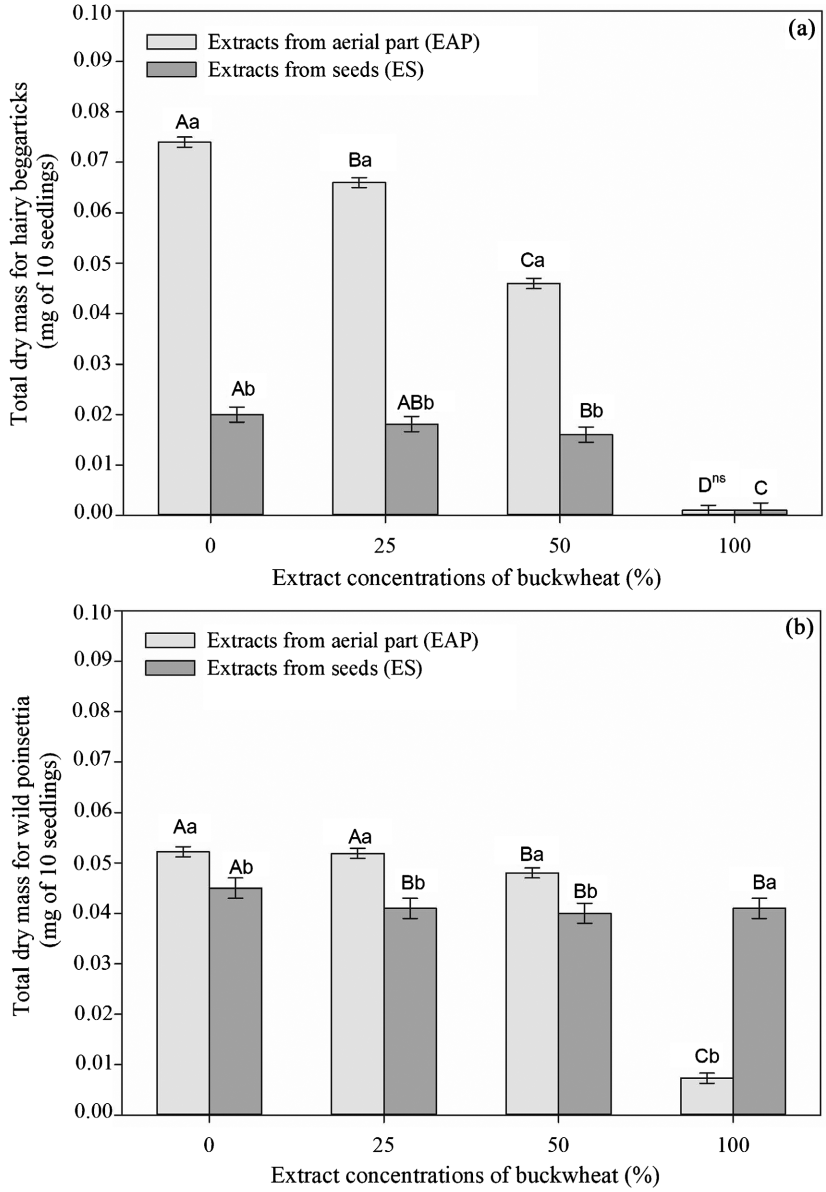


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Figure 4. Total dry matter (TDM) in seedlings of hairy beggarticks (a) and wild poinsettia (b) after exposure to different concentrations of extracts from seeds (ES) and aerial part (EAP) of buckwheat.

lambsquarters under buckwheat straw residues on soil surface [17], demonstrating that such a plant might be adopted as an alternative to weed management in agricultural systems.

In general, buckwheat extracts from seeds and aerial part negatively impacted seed germination and reduced seedlings development in both hairy beggarticks and wild poinsettia, depending upon extract concentration and part of the plant supplying aqueous extracts to be used previously as a mother-solution. This evidence on buckwheat extracts is ascribed to a promising alternative for development of new herbicides and as a tool to improve weed management in production fields grown around the world along with numerous plants resistant to herbicides. Thus, our outcomes epitomize a great opportunity for farmers to use buckwheat extracts in order to control weed infestation at their fields and mainly promote sustainable agriculture at commercial scales with environmental protection and rentability.

4. Conclusions

Extracts of buckwheat from seeds and aerial part negatively interfere with seed germination and seedling development in hairy beggarticks and wild poinsettia, with dependent effects on extract concentration from seeds or aerial part.

Aerial part extracts have a higher inhibitory capacity to affect seed germination parameters and seedlings initial development for both weed species, with better responses to concentrations greater than 50% aiming at assisting weed management by means of allelopathy.

Seeds extracts do not impair germination speed index, percentage of germination, abnormal seedlings, root and aerial length in wild poinsettia, irrespective of extract concentration.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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