




## Article

# Allelopathic Potential of Aqueous Extracts from Fleagrass (*Adenosma buchneroides* Bonati) against Two Crop and Three Weed Species

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**Abstract:** This study aimed to assess the phytotoxic potential of fleagrass (*Adenosma buchneroides*) on weeds and crops. We assessed the effects of applying aqueous extracts of fleagrass on the seed germination and seedling growth of three weeds (*Bidens pilosa*, *Paspalum thunbergia*, and *Bromus japonicus*) and two crops (*Oryza sativa* and *Zea mays*). The influence of six doses of fleagrass aqueous extract on seed germination and seedling growth was assessed through a Petri dish experiment. The aqueous extract of fleagrass was qualitatively characterized using widely targeted metabolomics analysis and found to mainly comprise flavonoids, phenolic acids, alkaloids, polysaccharides, phenylpropanoids, terpenoids, phenolamides, and quinones. The mean IC<sub>50</sub> for crop seed germination was 168,796, and the mean IC<sub>50</sub> for weed seed germination was 11,454. The inhibition effect on the tested species, from highest to lowest, followed the order of *B. japonicus* > *B. pilosa* > *P. thunbergii* > *O. sativa* > *Z. mays*. These results indicate the remarkable species-specific sensitivity of seed germination and seedling growth to fleagrass extract treatment, and that crops are more tolerant than weeds. Elucidation of the details of the fleagrass–weed/crop interaction can serve as a basis for intercropping fleagrass with crops in weed management strategies aimed at controlling weeds.

**Keywords:** *Adenosma buchneroides*; seed germination; allelopathy; aqueous extract



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## 1. Introduction

Weeds are one of the largest global biological constraints of crop production [1]. Weeds compete with crops for space, soil nutrients, water, light, and other growth requirements, and cause huge yield losses of –34% among major crops [1–3].

Weed management is a basic component of crop production, and many methods for weed control are employed in arable land worldwide [4]. Conventional weed control methods include hand weeding, mechanical tillage, and the application of herbicides [5]. These methods serve to reduce weed density and improve crop productivity [6]. However, despite the benefits of these practices in improving crop productivity, there are also certain challenges associated with them. The major challenges in hand weeding are decreasing availability and the increasing cost of labor [7]. In mechanical weed control, the soil structure is disturbed and the soil fertility is depleted due to additional soil turnover [1]. Similarly, challenges in the application of herbicides for weed control include the emergence of herbicide-resistant weeds and the side effects on environmental, human, and animal health [8]. Pan et al. revealed the resistance of the global weed *Echinochloa crus-galli* to the acetolactate-synthase-inhibiting herbicide penoxsulam and cross resistance to acetyl-coenzyme-carboxylase-inhibiting herbicides [9]. Another study systematically explained

changes in the use of 381 pesticides over 25 years, finding that “applied pesticide toxicity shifts toward plants and invertebrates, even in genetically modified crops” [10]. These challenges associated with conventional weed control methods make it essential to develop sustainable weed management practices [1].

There is a long history of using biological methods to control weeds in traditional agricultural systems [11,12]. Among them, crop rotation and intercropping are common and effective weed control methods. In shifting agriculture systems, approximately 20% of the weed biomass tends to be left undisturbed by farmers for weed management, and this was a practice common in Mayan agriculture in Mexico [13]. In China, intercropping has a 1000-year-old history and remains widespread in modern Chinese agriculture, and it is a practice that can suppress weeds because of the resulting higher biodiversity in comparison with monoculture [14]. Various studies have confirmed that these practices have inhibitory effects on weeds growing in different cropping systems [15,16]. Phytotoxic substances (allelochemicals), which are produced and released by plants, have an impact on coexistent species and can therefore play an important role in agroecosystems [11,17,18]. These allelochemicals can be released into the environment by rain leaching from aerial parts, which can affect the development of other plants [19].

*Adenosma buchneroides*, known as fleagrass, is an aromatic annual herb in the Plantaginaceae family. It was traditionally intercropped with upland rice and maize by the Hani people in Yunnan province, southwest China, and is an important herb of the Hani people, being especially used as an insect repellent [20–22]. In recent years, with its commercial development, large-scale organic cultivation of fleagrass has begun in the Xishuangbanna area, where it is mainly intercropped with upland rice or maize.

There is a very interesting phenomenon between fleagrass and weeds in the intercropping system. At the seedling stage of fleagrass, weed growth is luxuriant. However, at the vegetative stage of fleagrass, weeds appear to be suppressed. More importantly, weed intensity is reduced when crops are intercropped with fleagrass. This raises the question of whether fleagrass has allelopathic potential on weeds, and, if so, whether it can also impact intercropping crops.

To answer these questions, we applied aqueous extracts of fleagrass to two types of crops and assessed the seed germination and seedling growth of three weeds. Elucidation of the fleagrass–weed/crop interaction can serve as a basis for intercropping fleagrass with crops in weed management strategies aimed at controlling weeds.

## 2. Materials and Methods

### 2.1. Plant Materials

Fleagrass (500 g fresh shoots) were collected in full bloom from Xishuangbanna, Yunnan, China (21°49' N and 101°05' E). For the purpose of the allelopathic experiment with crops and weeds, two crops (rice (*Oryza sativa* L.) and maize (*Zea mays* L.)) and three weeds (*Bidens pilosa* L., *Paspalum thunbergii* Kunth ex Steud., and *Bromus japonicus* Hoult.), which are very common in these fields [23,24], were selected as test plants. The seeds of these plants were collected from the same experimental field of fleagrass in November 2020.

### 2.2. Preparation of Aqueous Extract

Fresh fleagrass shoots were cut into 5 mm pieces; 10 g of the fresh sample and 100 mL of distilled water were added to an Erlenmeyer flask and shaken with an incubator shaker (ZWYC-2932, Zhicheng, China) at 20 °C for 24 h. They were then filtered through absorbent cotton and filter paper, and the solution was stored in a refrigerator at 4 °C.

### 2.3. Widely Targeted Metabolomics Analysis

The extract preparation and metabolite profiling characterization of the fleagrass aqueous extracts were carried out by Wuhan Metware Biotechnology Co., Ltd. (Wuhan, China). The sample was analyzed using an Ultra Performance Liquid Chromatography–electrospray ionization–tandem mass spectrometry (UPLC–ESI–MS/MS) system, following

the standard processes [25]. Three replicates were used. The quantification and annotation of metabolites were performed based on the methods by Yang et al. [26].

#### 2.4. Seed Germination Bioassay

The aqueous extracts were further diluted to concentrations of 5, 10, 25, 50, and 100 g/L with distilled water for the final assay. The different concentrations of extracts were added to Petri dishes (9 cm) lined with two sheets of filter paper, and distilled water was used as a control treatment. On one Petri dish, 20 seeds from only one species were added, and five Petri dishes were processed for each of the crop and weed species. Petri dishes sealed with Parafilm were incubated with a day length of 12 h, and day and night temperatures of 30 and 25 °C, respectively. The number of germinated seeds was checked every 24 h. Seeds were considered to have germinated if their root or coleoptile reached a length of at least 2 mm. The whole experiment was repeated three times.

#### 2.5. Seedling Growth Development

The seedlings were allowed to grow under the conditions indicated in Section 2.4. The shoot heights and root lengths, as well as the fresh and dry weights of the two crop species (at 7 days) and the three weeds (at 10 days), were measured using the WinRHIZOTM system (2013 e, Regent Instruments, Quebec, QC, Canada).

#### 2.6. Data Measurement

To determine the effects of the aqueous extracts on seed germination and seedling growth on targeted plants, the indices of germination percentage (*GP*), germination index (*GI*), shoot height (*SH*), root length (*RL*), fresh weight (*FW*), dry weight (*DW*), response index (*RI*), half maximal inhibitory concentration (*IC*<sub>50</sub>), and allelopathy synthesis effect (*SE*) were calculated using the following formulae:

$$GP (\%) = \text{seeds germinated} / \text{total seeds} \times 100.$$

$$GI = \sum G_i / T_i,$$

where  $G_i$  is the number of emerged radicles at time  $i$ , and  $T_i$  is the number of days from planting.

$$RI = 1 - (C/T) \ (T \geq C); RI = (T/C) - 1 \ (T < C),$$

where  $C$  is the mean value of the control, and  $T$  is the mean value of each extract treatment.  $RI > 0$  indicates promotion of growth,  $RI < 0$  indicates inhibition, and the magnitude of  $RI$  values reflects the intensity of the allelopathic effect [27].

The  $IC_{50}$  value is the fleagrass concentration that inhibits germination of 50% of five target plants, and was calculated by SPSS 17 [28].

$$SE = (RI_{GP} + RI_{GI} + RI_{SH} + RI_{RL} + RI_{FW} + RI_{DW}) / 6,$$

where  $RI_{GP}$  is the  $RI$  value of the seed germination percentage,  $RI_{GI}$  is the  $RI$  value of the germination index,  $RI_{SH}$  is the  $RI$  value of shoot height,  $RI_{RL}$  is the  $RI$  value of root length,  $RI_{FW}$  is the  $RI$  value of the fresh weight, and  $RI_{DW}$  is the  $RI$  value of the dry weight.  $SE > 0$  indicates the promotion of growth,  $SE < 0$  indicates inhibition, and the magnitude of  $SE$  values reflects the intensity of the allelopathy synthesis effect [29].

#### 2.7. Statistical Analysis

Data were shown as the mean  $\pm$  SE (standard error) ( $n = 3$ ). Statistical analysis was performed using a one-way ANOVA followed by the least significant difference test (LSD. test) (for multiple groups) or the two-tailed Student  $t$ -test (for two groups), and the “car” and “agricolae” packages in the statistical software R (version 4.0.3) [30,31]. If a  $p$ -value  $< 0.05$ , the result was considered to have a statistically significant difference.

### 3. Results and Discussion

#### 3.1. Qualitative Composition of the Aqueous Extract

The aqueous fleagrass extract was qualitatively examined using a UPLC–ESI–MS/MS system for the presence of composition as detailed in the Materials and Methods section. In total, 685 metabolite compounds were identified, which were various flavonoids, phenolic acids, alkaloids, polysaccharides, phenylpropanoids, terpenoids, phenolamides, and quinones. Among them, flavonoids were the richest, followed by phenolic acids and alkaloids. Luteolin-7,3'-di-O-glucoside was the most abundant flavonoid, 6-O-caffeoyl-D-glucose the most abundant phenolic acid, stachydrine the most abundant alkaloid, and numerous interesting terpenoids were also identified, such as ursolic acid and asiatic acid.

The qualitative chemical analysis of the aqueous extract of fleagrass showed that besides the large number of flavonoids, there was also a reasonable variety of phenolic acids, alkaloids, and terpenoids. These phytochemical constituents of fleagrass are partially similar to those identified in Ma [32], in which the chemical compositions of methanol and aqueous methanol extracts of fleagrass were investigated. However, further analyses using chemical standards are needed in order to verify the identity of the metabolite compounds in fleagrass.

#### 3.2. Effects on Seed Germination

Data from the germination trials in both crop and weed seeds treated with aqueous extracts are shown in Tables 1 and 2.

**Table 1.** Effects of fleagrass aqueous extracts on the seed germination percentage of five target plants.

Target Plant	Index	Concentration of Aqueous Extracts (g/L)						IC <sub>50</sub>
		Control	5	10	25	50	100	
<i>O. sativa</i>	GP	100 ± 0 <sup>a</sup>	99 ± 1 <sup>a</sup>	100 ± 0 <sup>a</sup>	99 ± 1 <sup>a</sup>	98 ± 1.225 <sup>a</sup>	25 ± 6.325 <sup>b</sup>	82.298
	RI		−0.01	0	−0.01	−0.0101	−0.75	
<i>Z. mays</i>	GP	93.33 ± 4.41 <sup>a</sup>	96.67 ± 1.67 <sup>a</sup>	93.33 ± 3.33 <sup>a</sup>	81.67 ± 3.33 <sup>b</sup>	80 ± 5.77 <sup>c</sup>	68.33 ± 1.67 <sup>d</sup>	255.240
	RI		0.03445	−0.03575	−0.125	−0.1724	−0.1964	
<i>B. pilosa</i>	GP	80 ± 6.45 <sup>ab</sup>	91.25 ± 4.27 <sup>a</sup>	77.5 ± 4.33 <sup>b</sup>	68.75 ± 6.57 <sup>b</sup>	2.5 ± 1.44 <sup>c</sup>	0 <sup>c</sup>	21.638
	RI		0.1233	−0.0313	−0.1406	−0.9726	−1	
<i>P. thunbergii</i>	GP	57.5 ± 4.33 <sup>a</sup>	53.33 ± 6.67 <sup>a</sup>	50 ± 9.35 <sup>a</sup>	31.25 ± 7.47 <sup>b</sup>	7.5 ± 4.33 <sup>c</sup>	2.5 ± 2.5 <sup>c</sup>	7.794
	RI		−0.0725	−0.1304	−0.4565	−0.8594	−0.95	
<i>B. japonicus</i>	GP	45 ± 6.45 <sup>a</sup>	47.5 ± 11.09 <sup>a</sup>	27.5 ± 10.31 <sup>ab</sup>	15 ± 9.57 <sup>bc</sup>	2.5 ± 2.5 <sup>c</sup>	0 <sup>c</sup>	4.930
	RI		0.0526	−0.3889	−0.6667	−0.9474	−1	

Notes: GP, germination percentage; GP (%) = seeds germinated / total seeds × 100. RI, response index;  $RI = 1 - (C/T)$  if  $(T \geq C)$ ;  $RI = (T/C) - 1$  if  $(T < C)$ , where C is the mean value of the control and T is the mean value of each extract treatment. Data are presented as mean values ± standard error. Statistical analysis: Different superscript letters in the same column indicate significant differences (one-way ANOVA, LSD-test).

**Table 2.** Effects of fleagrass aqueous extracts on the seed germination indices of five target plants.

Target Plant	Index	Concentration of Aqueous Extracts (g/L)					
		Control	5	10	25	50	100
<i>O. sativa</i>	GI	17.78 ± 0.31 <sup>a</sup>	17.34 ± 0.36 <sup>a</sup>	17.54 ± 0.26 <sup>a</sup>	16.21 ± 0.28 <sup>b</sup>	9.66 ± 0.47 <sup>c</sup>	0.95 ± 0.3 <sup>d</sup>
	RI		−0.0247	−0.0135	−0.0883	−0.4429	−0.9458
<i>Z. mays</i>	GI	19.22 ± 1.74 <sup>ab</sup>	20.84 ± 1.34 <sup>ab</sup>	15.38 ± 1.29 <sup>bc</sup>	14.32 ± 1.07 <sup>c</sup>	13.26 ± 1.72 <sup>c</sup>	8.33 ± 1.81 <sup>d</sup>
	RI		0.0777	−0.1998	−0.2549	−0.3637	−0.4584
<i>B. pilosa</i>	GI	13.2 ± 1.47 <sup>a</sup>	13.51 ± 0.26 <sup>a</sup>	9.76 ± 0.89 <sup>b</sup>	3.83 ± 0.43 <sup>c</sup>	0.05 ± 0.03 <sup>d</sup>	0 <sup>d</sup>
	RI		0.0229	−0.2606	−0.7099	−0.9963	−1
<i>P. thunbergii</i>	GI	2.67 ± 0.08 <sup>a</sup>	2.3 ± 0.36 <sup>a</sup>	2.38 ± 0.67 <sup>a</sup>	0.89 ± 0.21 <sup>b</sup>	0.24 ± 0.16 <sup>b</sup>	0.05 ± 0.06 <sup>b</sup>
	RI		−0.1386	−0.1086	−0.6667	−0.8957	−0.979
<i>B. japonicus</i>	GI	8.32 ± 1.47 <sup>a</sup>	8.26 ± 0.26 <sup>a</sup>	3.2 ± 0.89 <sup>b</sup>	1.58 ± 0.43 <sup>b</sup>	0.05 ± 0.03 <sup>b</sup>	0 <sup>b</sup>
	RI		−0.0072	−0.6154	−0.8101	−0.9939	−1

Notes: GI, germination index;  $GI = \sum Gi/Ti$ , where Gi is the number of emerged radicles at time i, and Ti is the number of days from planting. RI, response index;  $RI = 1 - (C/T)$  if  $(T \geq C)$ ;  $RI = (T/C) - 1$  if  $(T < C)$ , where C is the mean value of the control and T is the mean value of each extract treatment. Data are presented as mean values ± standard error. Statistical analysis: Different superscript letters in the same column indicate significant differences (one-way ANOVA, LSD-test).

Crop seeds are more tolerant than weeds to fleagrass aqueous extracts. Among two crops, rice and maize, the aqueous extracts have less effect on the germination rate of rice than maize. As shown in Table 1, the effects of the aqueous extracts on rice germination were not different to controls except for the highest concentration (100 g/L). At the highest concentration (100 g/L), the fleagrass aqueous extracts did not completely block germination but caused a significant decrease in germination percentage and a 75-point reduction in RI. Meanwhile, among the weeds, at the  $\geq 10$  g/L concentration, treatment with the aqueous extracts showed a significant concentration-dependent inhibitory effect. Conversely, it slightly promoted germination in maize and two of the weeds (*B. pilosa* and *B. japonicus*) at the lowest concentration (5 g/L).

As shown in Table 2, under the lowest-concentration treatment (5 g/L), the fleagrass aqueous extracts caused no significant changes in germination indices in crop and weed seeds compared with controls. Conversely, in maize and *B. pilosa*, it induced a slight increase in germination indices, with RI values of 7.77 and 2.29, respectively. When the extract concentration reached 25 g/L, the germination indices of all tested species decreased significantly, and as the concentration increased, the germination index showed a downward trend.

These results have shown that fleagrass aqueous extracts can have different effects at different concentrations. At higher concentrations, they have inhibitory effects, while at low concentrations, they have stimulatory effects. Similar to the previous studies, BAO et al. found that the seed germination rate of *Elymus nutans* was significantly promoted by water extracts from *Medicago sativa* at a concentration of 5.5%, while it was inhibited at a concentration of 14.5% [29].

### 3.3. Effects on Seedling Growth

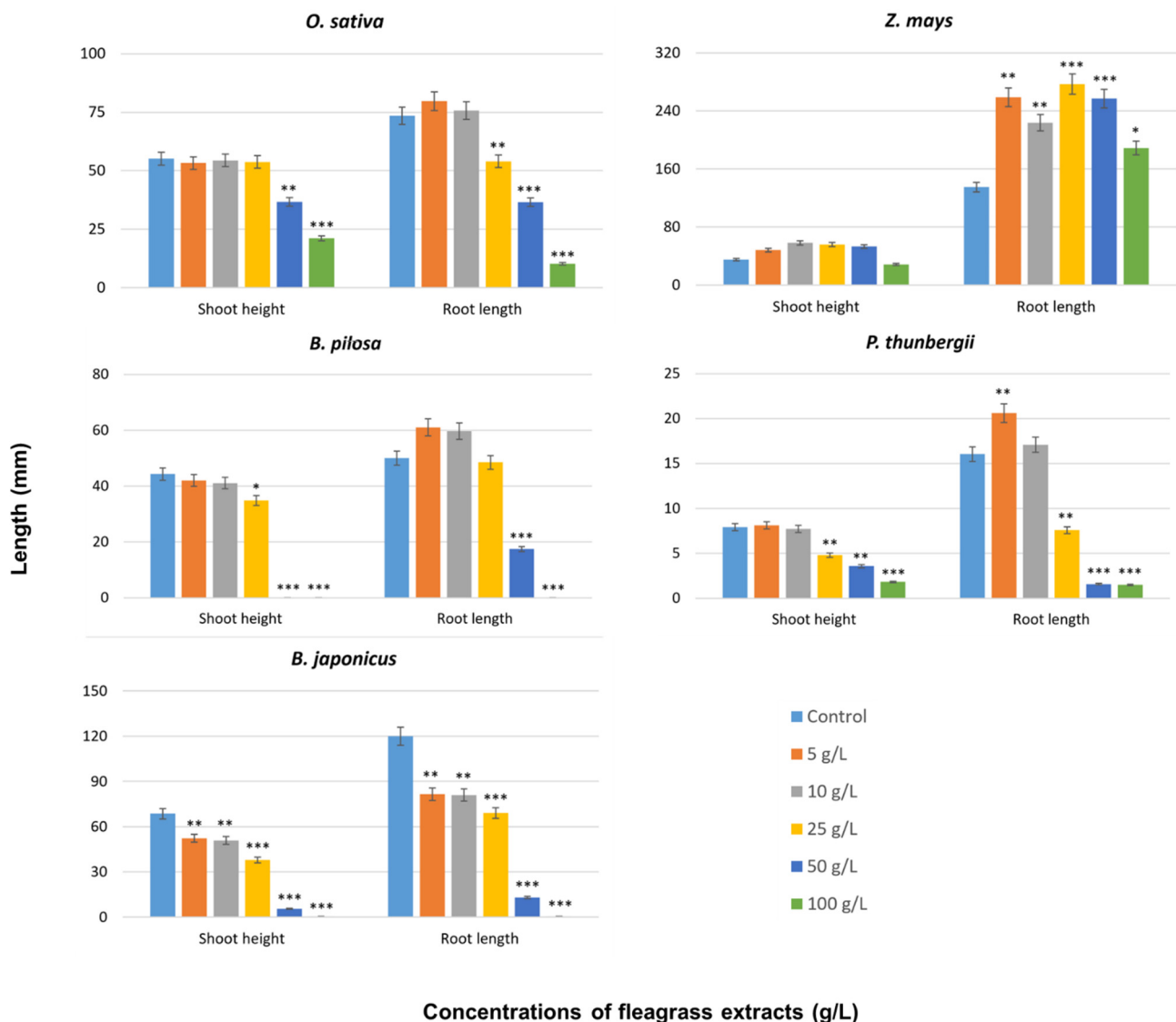
Shoot height, root length, fresh weight, and dry weight were measured to assess seedling growth in response to fleagrass aqueous extracts. As shown in Figure 1 and Table 3, the seedling growth of rice, *B. pilosa*, *P. thunbergia*, and *B. japonicus* were inhibited by fleagrass extracts at concentrations of 25 g/L and higher. Similarly, treatment at all concentrations of fleagrass extract caused a significant decrease in the shoot and root growth of *B. japonicus*. However, in maize, the fleagrass extract induced a significant decrease in root length, and the shoot height was not different compared to controls.

**Table 3.** Response index of extracts from fleagrass aerial parts on the growth of five target plant species.

Target Plants	RI	Concentration of Aqueous Extracts (g/L)				
		5	10	25	50	100
<i>O. sativa</i>	SH	−0.0331	−0.0125	−0.0246	−0.3334	−0.6169
	RL	0.079	0.0297	−0.2641	−0.5024	−0.8598
	FW	−0.1117	−0.0972	−0.085	−0.1676	−0.3161
	DW	0.0291	0.0224	0.0091	0.0937	0.119
<i>Z. mays</i>	SH	0.2748	0.3992	0.3738	0.3405	−0.1904
	RL	0.4791	0.3971	0.5134	0.4753	0.2864
	FW	0.211	0.1378	0.163	0.0714	−0.0615
	DW	0.0347	−0.0925	−0.1327	−0.1026	−0.1545
<i>B. pilosa</i>	SH	−0.053	−0.0728	−0.2139	−1	−1
	RL	0.1817	0.1625	−0.03	−0.65	−1
	FW	−0.2811	−0.4677	−0.5188	−0.8999	−1
	DW	0.2581	0.1154	0.0143	−0.8841	−1
<i>P. thunbergii</i>	SH	0.0245	−0.0241	−0.3935	−0.5474	−0.7694
	RL	0.2213	0.0614	−0.5275	−0.9013	−0.9065
	FW	0.2131	−0.1017	−0.1356	0.3867	−1
	DW	0.4167	0.2671	−0.2709	−0.1133	−0.7734
<i>B. japonicus</i>	SH	−0.2389	−0.2596	−0.4486	−0.9193	−1
	RL	−0.3203	−0.3244	−0.4245	−0.8916	−1
	FW	−0.1043	−0.2771	−0.5474	−0.9739	−1
	DW	0.0738	0.0529	−0.3002	−0.8768	−1

Notes: RI, response index;  $RI = 1 - (C/T)$  if  $(T \geq C)$ ;  $RI = (T/C) - 1$  if  $(T < C)$ , where C is the mean value of the control and T is the mean value of each extract treatment.  $RI > 0$  indicates promotion,  $RI < 0$  indicates inhibition, and the magnitude of RI values reflects the intensity of the allelopathic effect. SH, shoot height; RL, root length; FW, fresh weight; DW, dry weight.





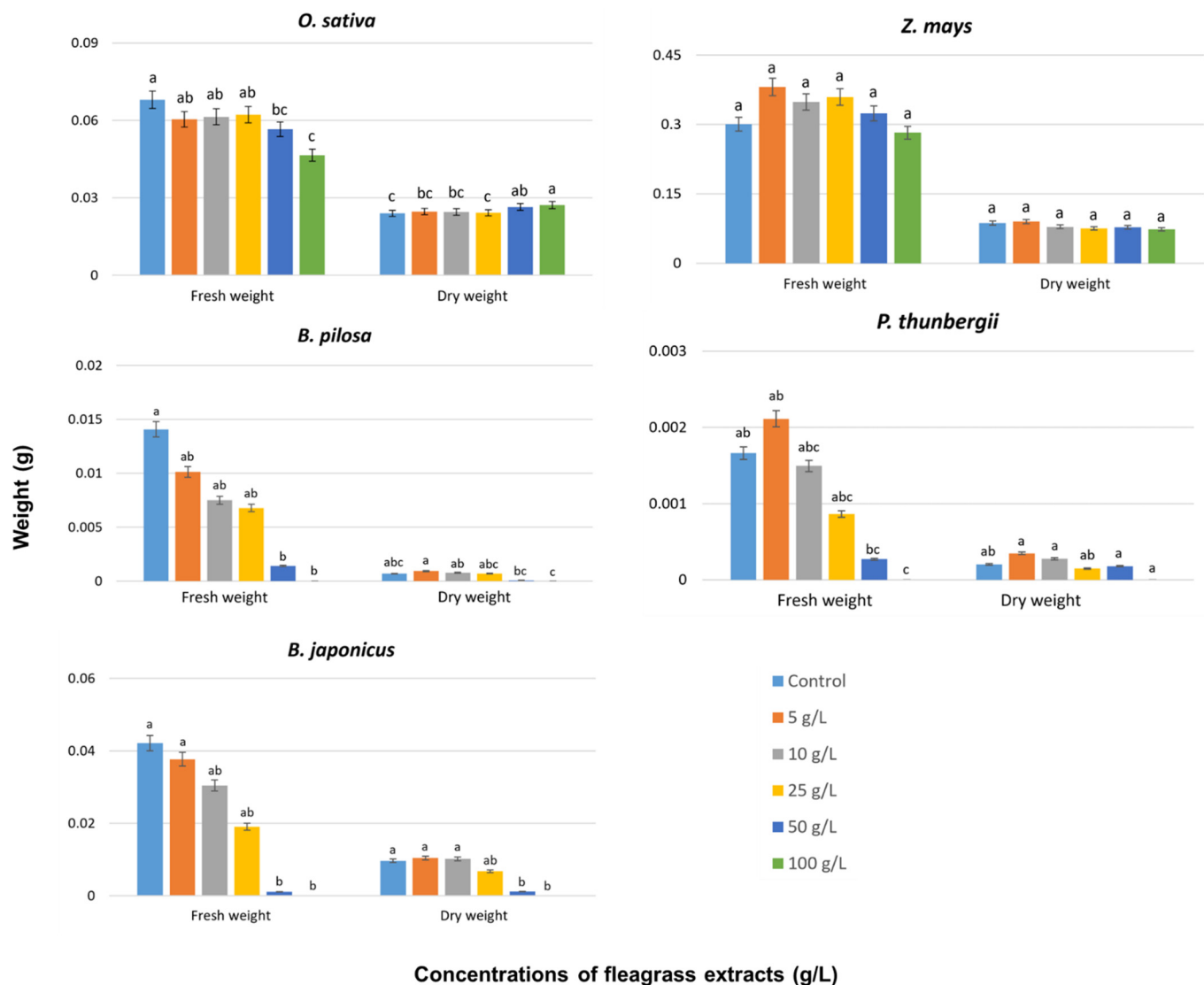
**Figure 1.** Effects of different concentrations of fleagrass extracts on shoot height and root length in five target plant species. The significant differences between treatments and control are indicated by asterisks: \*  $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

For all target crop and weed plants, treatment with the lowest concentration of fleagrass aqueous extracts resulted in germination values that were not different from controls. In fact, there was a slight increase in the germination percentage and index in maize and *B. pilosa*. This partly explains the phenomenon of no dominance in the competition between fleagrass and weeds at the seedling stage. Similar results have also been reported by other researchers [33,34].

The effect of fleagrass aqueous extracts is concentration-dependent, and crop seeds are more tolerant than weeds. All target seeds were affected as a function of increasing concentrations of the treatment solution. The mean half maximal inhibitory concentration ( $IC_{50}$ ) of crop seed germination was 168.796, and the mean  $IC_{50}$  of weed seed germination was 11.454. This result is similar to the allelopathic effect of *Sonchus oleraceus* on crops and weeds. It was found that under treatment with the same concentration of *Sonchus oleraceus* aqueous extract, seed germination of *Brassica nigra* and *Melilotus indicus* was inhibited, but the crop *Trifolium alexandrinum* was not affected [35]. Similarly, another study on the essential oils from temperate-climate plants on the germination of weeds and crops based on Petri dish experiments reported that three crops—*Avena sativa*, *Brassica napus*, and

maize—were more tolerant than weeds to the majority of essential oils, and the kernels of maize were the most tolerant [36].

As shown in Figure 2 and Table 3, consistent with the results from the shoot and root length, in rice, *B. Pilosa*, *P. thunbergia*, and *B. japonicus*, it caused a significant decrease in fresh weight, especially, when a concentration of fleagrass extract up to 50 g/L. In contrast to the trend of dry weight, which increased with increasing fleagrass extract treatment concentrations, the fresh weight decreased. Meanwhile, in maize, the effect of fleagrass extracts on fresh and dry weight values was not different from controls.



**Figure 2.** Effects of fleagrass extracts on the fresh and dry weight of five target plants; significant differences (one-way ANOVA, LSD-test) are marked using different letters.

Seedlings of the two investigated crop species (maize and rice) grew poorly at the highest concentrations of fleagrass extract, but they still germinated. At high extract concentrations, seedlings grow poorly, and most of the nutrients are still stored in the seeds, so the fresh weight of the seedlings is reduced as the extract concentration is increased. However, the crop dry weight showed an upward trend with increasing extract concentrations. Similar results are also reported in other studies, for instance, *Galium aparine* L. aqueous extracts were reported to promote the seedling growth of maize [37].

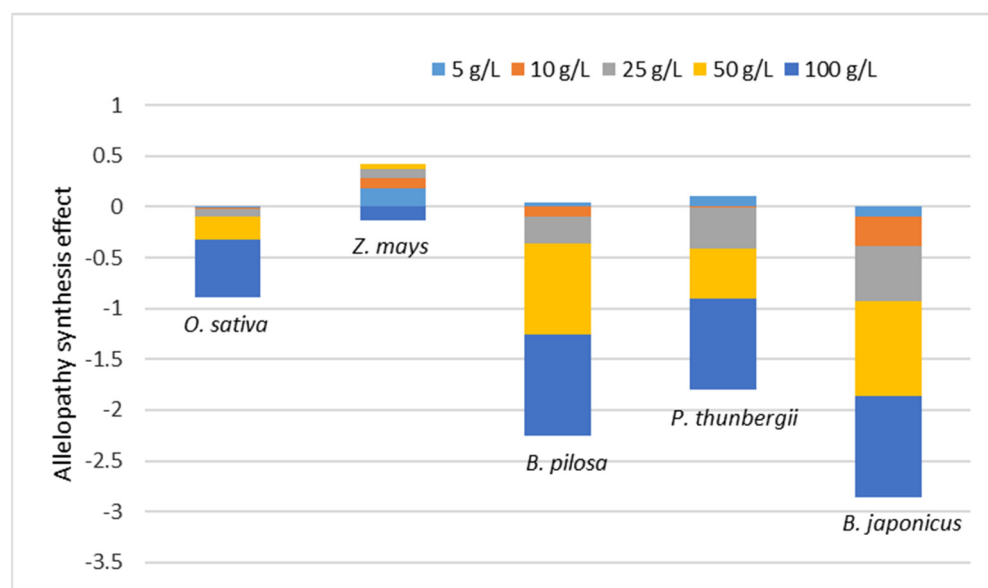
Taken together, these results indicated remarkable species-specific sensitivity of seed germination and seedling growth to fleagrass extract treatment. Crops are more tolerant

than weeds to fleagrass aqueous extract. Under the same concentration level of fleagrass extracts, there is a slight promotion of growth in crops, while there is significant inhibition of growth in weeds. As other studies have reported, allelopathic effects can vary from species to species [35,38].

In particular, the extract concentration of 25 g/L can be defined as the optimal concentration, since it is able to inhibit the seed germination and seedling growth of weeds with weak side effects on crops, or even stimulate germination and growth in the case of maize. These phenomena are similar to the allelopathic effects documented for *Artemisia vulgaris* extracts on potato and maize cultivation [39,40].

### 3.4. Allelopathy Synthesis Effect

The allelopathy synthesis effect was obtained for each target plant by combining the RI values of seed germination, germination index, shoot height, root length, fresh weight, and dry weight. As shown in Figure 3, the lowest concentration of fleagrass extract (5 g/L) had a promoting effect on *Z. mays*, *B. pilosa*, and *P. thunbergii*. In maize, all concentrations of fleagrass extract, except for the highest of 100 g/L, had a significant promoting effect on seed germination and seedling growth. The germination and growth of the other four target plants were inhibited by fleagrass extracts in a dose-dependent manner. The response order of the allelopathic sensitivity of target plants to aqueous extracts of fleagrass is *B. japonicus* > *B. pilosa* > *P. thunbergii* > *O. sativa* > *Z. mays*.



**Figure 3.** Allelopathy synthesis effect of aqueous extracts from fleagrass on five target plants.

Analysis of the allelopathy synthesis effect of fleagrass aqueous extracts showed that their phytotoxic activity on weeds was greater than that on crops and showed species specificity. Allelopathy is a process by which phytotoxic substances (allelochemicals) are produced and released by microorganisms or from the roots, leaves, flowers, stems, or seeds of plants. These chemicals have an impact on the coexistent species and can therefore play an important role in agroecosystems [11,17,18]. Currently, allelopathy is an important tool in the sustainable management of weeds, and these plants can be used to cover or smother weeds, or can be grown as companion crops, and allelochemicals can also be applied directly as natural herbs and pesticides [11,41,42]. Allelopathy can be a useful tool in weed control that neither harms the environment nor increases weed management costs [1]. Allelopathic weed control can be implemented by growing allelopathic crop cultivars, intercropping with allelopathic weed suppressing plants, growing allelopathic cover crops, applying allelopathic plant residues, and including allelopathic crops in rotation [1]. Fleagrass is rich in phenolic acids and terpenoids, which manifest as allelochemicals in crops,



such as sorghum [43–46], brassicas [47–49], and rice [50–53], as well as in sunflower [54], mugwort [40,55], and red clover [56]. From a practical application point of view, intercropping fleagrass with crops is an alternative tool for weed management, although field experiments are needed to verify whether fleagrass influences the yield and quality of primary crops when used in intercropping.

#### 4. Conclusions

This study reveals that fleagrass has allelopathic potential for the seed germination and seedling growth of weeds. Moreover, we found that crops were only slightly affected by fleagrass extracts, with a slight promotional effect observed on maize. Future research should be performed in the field to further unravel more details about the ecological role and significance of fleagrass applied in intercropping systems for weed control in addition to determining the effects on crops.

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