

RESEARCH ARTICLE

EFFECTS OF AQUEOUS LEAF EXTRACTS OF *AGERATUM CONYZOIDES* AND *BIDENS PILOSA* ON GERMINATION AND EARLY GROWTH OF TWO LOCAL RICE GENOTYPES FROM KOHIMA, NAGALAND

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Abstract: Weeds may affect crop establishment not only through competition for resources but also through chemical interactions. This study evaluated the effects of aqueous leaf extracts of *Ageratum conyzoides* L. and *Bidens pilosa* L. on seed germination and early seedling growth of two locally cultivated rice genotypes, *Thevür* and *Rosolha*. A factorial experiment was conducted in a completely randomized design with three replications. Root length (RL), shoot length (SL), root-to-shoot ratio (R/S), seedling vigor index (SVI), total seedling length (TSL), mean germination time (MGT) and germination percentage (GP) were evaluated. Extract concentration significantly affected SL, RL, R/S, SVI, TSL and MGT ($P < 0.001$), whereas GP was not significantly affected. *Rosolha* showed greater RL, R/S and SVI than *Thevür* ($P < 0.001$). Across weed species, seedlings exposed to *B. pilosa* had greater RL, R/S and SVI than those exposed to *A. conyzoides*, indicating stronger growth inhibition by *A. conyzoides* under the conditions tested. Significant genotype $\times$ concentration interactions were observed for SL ($P < 0.001$), TSL ($P = 0.002$) and MGT ($P = 0.020$), with reductions in growth and greater delays in germination at higher concentrations being more pronounced in *Thevür*. A significant weed species $\times$ concentration interaction was also observed for MGT ($P = 0.042$). Overall, the responses of the two rice genotypes to aqueous leaf extracts depended strongly on extract concentration, with *Thevür* showing greater reductions in early seedling growth at higher concentrations.

Keywords: Angami, Allelopathy, Wet terrace rice, Weeds, Aqueous leaf extract

INTRODUCTION

Weeds compete with crops for light, water, nutrients and space, thereby constraining crop production (Kaur *et al.*, 2018). Weed competition can reduce rice yield and may result in yield losses of about 50-60% in puddled transplanted rice and 70-80% in direct-seeded rice (Dass *et al.*, 2017). Crop losses associated with weeds are not attributable solely to competition for resources but may also involve weed-crop signalling and plant-plant interactions (Horvath *et al.*, 2023). One such form of interaction is allelopathy where secondary compounds produced by plants influence neighbouring plants. These allelochemicals are usually released from plant tissues through the processes such as leaching, volatilization, root exudation and decomposition of plant residues (Weston and Duke, 2003; Singh *et al.*, 2021). The effects of allelochemicals are not uniform and depend on the sensitivity of the plant species and concentration of the allelochemicals (Liu *et al.*, 2025). Depending on these factors, allelochemicals can either inhibit or sometimes at low concentrations stimulate seed germination and seedling growth (An *et al.*, 2005).

Several studies using bioassays and controlled growth experiments have reported the allelopathic potential of *A. conyzoides* L. and its inhibitory effects on the growth and development of several crop plants (Kong *et al.*, 2004; Singh *et al.*, 2003). Likewise, *B. pilosa* L. has been reported to possess allelopathic properties, with bioassays and root-exudate experiments which demonstrated inhibitory effects on other plants (Khanh *et al.*, 2009). Its allelopathic activity has also been investigated in relation to weed management and rice production, where the extracts suppressed the germination, growth and density of paddy weeds (Krumsri *et al.*, 2015). Collectively, these studies indicate that both *A. conyzoides* and *B. pilosa* have the potential to influence the germination and early growth of neighbouring plants through allelopathic interactions. Aqueous weed extracts are commonly used in laboratory bioassays to evaluate the potential phytotoxic effects of donor plants on seed germination and early seedling growth, with previous studies demonstrating that their effects on rice vary depending on weed species, plant parts used, and extract concentrations (Chopra *et al.*, 2017; Lalbiakdika *et al.*, 2022).

Rice is widely cultivated under traditional wet-terraced systems in parts of Kohima district,

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Nagaland. A field survey on these fields recorded 22 weed species representing 19 genera and 12 families, demonstrating the diversity of weeds associated with the local rice-growing system (Konyak *et al.*, 2022). Information on the possible allelopathic effects of locally occurring weeds on locally cultivated rice genotypes in this system remains limited. Therefore, this study evaluated the effects of aqueous leaf extracts of *A. conyzoides* and *B. pilosa* at different concentrations on seed germination and early seedling growth of two locally cultivated rice genotypes, *Thevür* and *Rosolha*, under controlled laboratory conditions.

MATERIALS AND METHODS

Fresh leaves of *A. conyzoides* and *B. pilosa*, along with seeds of the two rice genotypes, *Thevür* and *Rosolha*, were collected from Jotsoma Village, Kohima, Nagaland. The weed leaves were thoroughly rinsed with tap water, followed by several washes with sterile distilled water to remove adhering debris. The samples were spread on blotting paper and oven-dried at 48°C until completely dry and then ground into a fine powder. Subsequently, 15 g of powdered leaf material was suspended in 150 mL of distilled water and extracted for 24 h with continuous shaking (Chopra *et al.*, 2017). The extract was then filtered through Whatman No. 1 filter paper. The resulting undiluted extract, corresponding to 100 mg mL⁻¹ of dry leaf material was designated as the stock extract (100% extract). Working concentrations of 25%, 50% and 75% were prepared by diluting the stock extract with distilled water, following Khasabulli *et al.* (2018). The prepared aqueous extracts were stored at 4°C until use (Yusoff and Ismail, 2015) and, in the present experiment, were used within 24 h of preparation.

The rice seeds were soaked overnight and surface-sterilized by immersing them in 5% NaOCl for 20 minutes, followed by several rinses with sterilized distilled water (Wu *et al.*, 2010). The experiment was arranged as a 2 × 2 × 5 factorial in a completely randomized design, comprising two rice genotypes (*Thevür* and *Rosolha*), two weed species (*A. conyzoides* and *B. pilosa*) and five concentration levels (control, 25%, 50%, 75% and 100%). Each Petri dish was considered an independent experimental unit and three Petri dishes were used per genotype × weed × concentration combination. Ten sterilized seeds were placed in each 9 cm Petri dishes lined with sterile filter paper and treated with 5 mL of the respective aqueous extracts. For the control treatment, 5 mL of distilled water was added. The seeds were moistened daily with about 5 mL of the corresponding extract to maintain the moisture. The Petri dishes were then incubated at 25°C for germination (Masum *et al.*, 2016). Seeds were considered germinated when the radicle length

exceeded 2 mm (Haugland & Brandsaeter, 1996). The experiment was terminated after 15 days.

The length of the roots and shoots of all the germinated seeds was measured. Total seedling length (TSL) was calculated as the sum of root length and shoot length. Germination percentage (GP) and mean germination time (MGT) were then calculated using the method described by Ellis and Roberts (1981).

The germination percentage was calculated as:

$$GP = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Mean germination time (MGT) was calculated using the following formula:

$$MGT = \frac{\sum(Dn)}{\sum n}$$

where *n* represents the number of seeds that germinated on a particular day, and *D* represents the number of days elapsed from the beginning of the germination experiment. Germination was recorded at 24-h intervals throughout the experimental period.

Seedling vigor index (SVI) was calculated according to the method described by Abdul-Baki and Anderson (1973), using the following formula:

$$SVI = \text{Germination percentage} \times \text{total seedling length}$$

Data analysis

The experimental data were analysed using the mixed procedure with *P* ≤ 0.05 in SAS (SAS® Studio, SAS Institute Inc., 2024). Genotype (G), weeds (W), concentration (C) and their interactions were considered fixed effects, whereas replication was treated as random effects. Least-squares means (LS-means) were estimated for the effects and interactions of interest, and pairwise comparisons were performed using the Tukey-Kramer adjustment at *P* ≤ 0.05.

RESULTS AND DISCUSSIONS

Effects of genotype, weed species and extract concentration on seedling growth and germination

The effects of rice genotype, weed species, extract concentration and their interactions on shoot length (SL), root length (RL), root-to-shoot ratio (R/S), seedling vigor index (SVI), total seedling length (TSL), mean germination time (MGT) and germination percentage (GP) are presented in Table 1. Rice genotype significantly affected RL, R/S and SVI (*P* < 0.001), while weed species significantly affected RL (*P* = 0.025), R/S (*P* = 0.018), SVI (*P* = 0.024) and MGT (*P* = 0.005). Extract concentration significantly affected SL, RL, R/S, SVI, TSL and MGT (*P* < 0.001), but not GP (*P* = 0.419). Significant genotype × concentration (G × C) interactions were observed for SL (*P* < 0.001), TSL (*P* = 0.002) and MGT (*P* = 0.020), whereas the weed species ×

concentration ($W \times C$) interaction was significant only for MGT ($P = 0.042$). The other two and three-way interactions were not significant. Germination percentage was not significantly affected by genotype, weed species, extract concentration or their interactions (Table 1).

The significant genotype effect on RL, R/S and SVI indicated differences in early seedling development between the two rice genotypes. *Rosolha* recorded significantly greater RL, R/S and SVI than Thevür, with mean RL of 5.30 cm compared with 4.00 cm, R/S of 0.65 compared with 0.49, and SVI of 537.95 compared with 406.84, respectively (Table 2). The higher R/S value in *Rosolha* indicates relatively greater root development in relation to shoot development. Similar differential responses of root and shoot growth under *B. pilosa* exposure have been reported by Hsueh *et al.* (2020), who observed alterations in shoot-to-root allocation in *Cyperus rotundus* seedlings exposed to *B. pilosa* var. *radiata* residues. However, the present experiment does not provide evidence for the physiological mechanisms underlying the genotype differences.

Weed species also significantly affected RL, R/S and SVI. Seedlings exposed to *B. pilosa* recorded greater RL, R/S and SVI than those exposed to *A. conyzoides*, with RL values of 5.00 and 4.30 cm, R/S values of 0.62 and 0.53, and SVI values of 507.82 and 436.97, respectively (Table 2). These differences correspond to approximately 16-18% higher values under *B. pilosa* across the three traits. Thus, under the conditions of the present experiment, *A. conyzoides* exerted the stronger inhibitory effect on early seedling development. This observation is consistent with Zumbado-Rodríguez *et al.* (2026), who reported that *A. conyzoides* leaf extracts reduced the initial growth of *Oryza sativa*, particularly plant length, although seedling emergence and biomass were not significantly affected. Their findings provide comparative evidence for the inhibitory potential of *A. conyzoides* on early rice growth, while the stronger inhibition relative to *B. pilosa* observed in the present study is specific to the experimental conditions used here.

Extract concentration affected wide range of the measured early seedling-growth traits. Root length decreased from 7.37 cm in the control to 1.41 cm at 100% extract, corresponding to an 80.9% reduction (Table 2). The control and 25% treatments did not differ significantly, whereas significant reductions

were observed at 50%, 75% and 100% concentrations. This concentration-dependent reduction in RL indicates that substantial inhibition of root growth occurred mainly at higher extract concentrations. The observed pattern is consistent with Bhatt *et al.* (2001), who reported increasing phytotoxicity of *A. conyzoides* and *B. pilosa* extracts with increasing concentration, and with Negi *et al.* (2020), who observed concentration-related reductions in rice root length following exposure to *A. conyzoides* extracts.

Root-to-shoot ratio declined from 0.94 in the control to 0.26 at 100% extract, representing a 72.3% reduction. The control differed significantly from the 25% treatment, whereas some adjacent concentration levels did not differ significantly from one another (Table 2). This indicates that the response was not characterized by a statistically significant decline at every successive concentration. Nevertheless, the overall reduction in R/S suggests that increasing extract concentration altered the relative development of roots and shoots. Hsueh *et al.* (2020) similarly reported that *B. pilosa* residues could alter relative root and shoot development, supporting the possibility of differential effects of weed-derived compounds on seedling organs.

Seedling vigor index showed a similar concentration-dependent response, decreasing from 744.81 in the control to 145.97 at 100% extract, representing an 80.4% reduction. The control and 25% treatments were statistically similar, whereas SVI was significantly reduced at 50%, 75% and 100% concentrations (Table 2). Negi *et al.* (2020) also reported concentration-related reductions in rice seedling vigor following exposure to *A. conyzoides* extracts. The consistent decline in RL and SVI at higher concentrations indicates that increasing extract concentration adversely affected early seedling development, although the magnitude of response varied among the rice genotypes and weed species.

Interestingly, germination percentage was not significantly affected by any of the experimental factors, whereas MGT was significantly affected by genotype, weed species and extract concentration, with significant $G \times C$ and $W \times C$ interactions (Table 1). This indicates that the treatments influenced the timing of germination more clearly than the final proportion of seeds germinating.

Table 1. Analysis of variance for the effects of rice genotype, weed species, leaf extract concentrations and their interactions on germination and early seedling growth.

Source of variation	SL (cm)	RL (cm)	R/S	SVI	TSL (cm)	MGT	GP
G	0.028*	<0.001***	<0.001***	<0.001***	<0.001***	0.206 ^{ns}	0.323 ^{ns}
W	0.311 ^{ns}	0.025*	0.018*	0.024*	0.393 ^{ns}	0.005**	0.323 ^{ns}
C	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.419 ^{ns}
G × W	0.221 ^{ns}	0.129 ^{ns}	0.200 ^{ns}	0.134 ^{ns}	0.105 ^{ns}	0.058 ^{ns}	0.323 ^{ns}
G × C	<0.001***	0.256 ^{ns}	0.592 ^{ns}	0.254 ^{ns}	0.002**	0.020*	0.419 ^{ns}
W × C	0.311 ^{ns}	0.520 ^{ns}	0.134 ^{ns}	0.537 ^{ns}	0.942 ^{ns}	0.042*	0.419 ^{ns}
G × W × C	0.078 ^{ns}	0.336 ^{ns}	0.716 ^{ns}	0.347 ^{ns}	0.165 ^{ns}	0.563 ^{ns}	0.419 ^{ns}

G = Genotype, W = Weed, C = Concentration, SL = Shoot Length, RL = Root Length, R/S = root-to-shoot ratio, SVI = Seedling Vigor Index, TSL = Total Seedling Length, MGT = Mean Germination Time, GP: germination percentage, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, ns = non-significant.

Genotype × concentration effects on shoot length and total seedling length

The significant G × C interactions for SL ($P < 0.001$) and TSL ($P = 0.002$) indicated that the two rice genotypes responded differently to increasing extract concentration. In *Rosolha*, SL was highest at 25% extract (9.89 cm) and declined to 7.13 cm at 100%. In *Thevür*, SL increased from 7.79 cm in the control to 10.89 cm at 25%, followed by a decline to 3.71 cm at 100% (Table 3). Thus, the reduction in SL at higher concentrations was more pronounced in *Thevür* than in *Rosolha*.

A similar pattern was observed for TSL. In *Rosolha*, TSL decreased from 16.88 cm at 25% extract to 9.29 cm at 100%, whereas in *Thevür* it decreased from 17.18 cm to 4.36 cm over the same concentration range (Table 3). The greater reduction in *Thevür* indicates that this genotype was more strongly affected by increasing extract concentration with respect to overall seedling growth. The significant G × C interaction confirms that the effect of concentration on SL and TSL depended on rice genotype.

The response across concentrations was non-monotonic. In both genotypes, relatively high SL and TSL values occurred at 25% extract, followed by reductions at higher concentrations. Negi et al. (2020) similarly reported variety-dependent responses of rice to *A. conyzoides* extracts, with the direction and magnitude of response varying with both rice variety and extract concentration. The higher-concentration reductions observed in the present study are also broadly consistent with the findings of Bandeira et al. (2024), who reported reductions in root and hypocotyl length with increasing concentrations of *B. pilosa* leaf extract in lettuce and chicory. Although the species used in those experiments differed from the present study, the results provide supporting evidence for concentration-dependent effects of *B. pilosa* extracts on early seedling development. The relatively high

SL and TSL observed at 25% in the present study therefore indicate a non-monotonic response, while the mechanism responsible for this pattern cannot be determined from the present measurements alone.

Taken together, the significant G × C interactions demonstrate that the response of rice seedlings to extract concentration differed between the two genotypes. The divergence was particularly evident at 75% and 100% extract, where *Thevür* showed substantially lower SL and TSL than *Rosolha*. Thus, genotype-specific responses became more apparent at higher extract concentrations.

Genotype × concentration and weed × concentration effects on mean germination time

Mean germination time was significantly affected by the G × C interaction ($P = 0.020$). In *Rosolha*, MGT increased from 2.17 days in the control to 4.00 days at 100% extract, whereas in *Thevür* it increased from 1.17 to 4.83 days. Significant genotype differences were observed at the control, 50%, 75% and 100% concentrations, with *Thevür* showing greater MGT than *Rosolha* at the higher concentrations (Fig. 1a). These results indicate that increasing extract concentration delayed germination and that the magnitude of this delay differed between the two rice genotypes.

The W × C interaction was also significant for MGT ($P = 0.042$). Under *A. conyzoides*, MGT increased from 1.67 days in the control to 5.33 days at 100% extract. Under *B. pilosa*, MGT increased from 1.67 to 3.50 days. The two weed species differed significantly only at 100%, when MGT was higher under *A. conyzoides* than under *B. pilosa* (5.33 versus 3.50 days) (Fig. 1b). The concentration-related delay in germination is consistent with Akter et al. (2023), who reported an increase in MGT of wheat treated with *A. conyzoides* root exudates. The present results therefore indicate that increasing extract concentration, particularly at 100%, delayed germination, with the magnitude of the response depending on both rice genotype and weed species.

Table 2. Effects of rice genotype, weed species and leaf-extract concentration on root length, root-to-shoot ratio and seedling vigor index of rice.

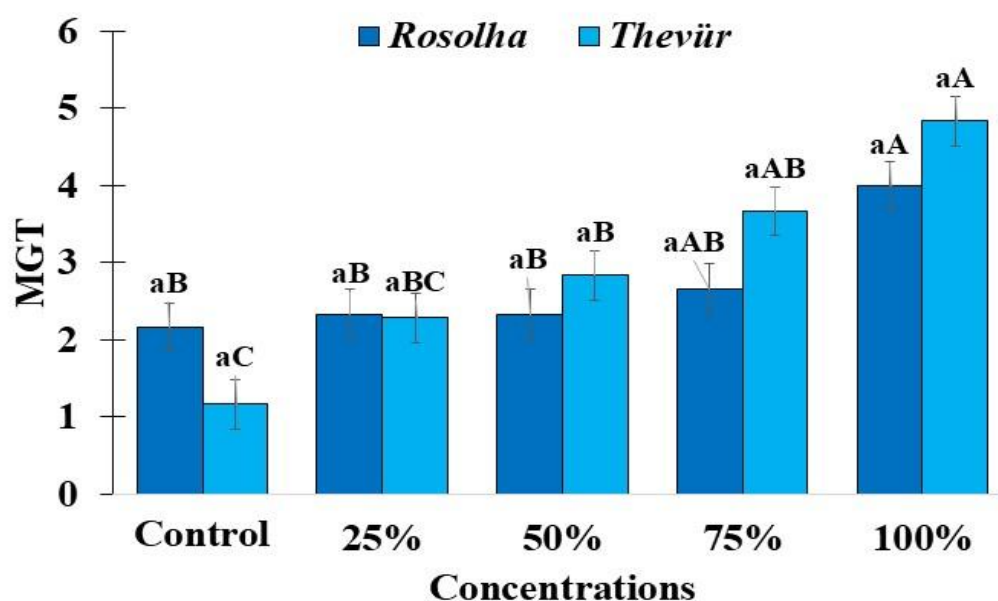
Factor	Level	RL (cm)	R/S	SVI
Genotype	<i>Rosolha</i>	5.30 ^a	0.65 ^a	537.95 ^a
	<i>Thevür</i>	4.00 ^b	0.49 ^b	406.84 ^b
Weed species	<i>Ageratum conyzoides</i>	4.30 ^b	0.53 ^b	436.97 ^b
	<i>Bidens pilosa</i>	5.00 ^a	0.62 ^a	507.82 ^a
Concentrations	Control	7.37 ^a	0.94 ^a	744.81 ^a
	25%	6.64 ^a	0.65 ^b	672.81 ^a
	50%	5.04 ^b	0.63 ^b	512.06 ^b
	75%	2.79 ^c	0.39 ^c	286.32 ^c
	100%	1.41 ^d	0.26 ^c	145.97 ^d

Values are LS-means. Within each factor and column, means followed by different lowercase letters are significantly different according to the Tukey-Kramer adjustment ($P \leq 0.05$). RL = root length; R/S = root-to-shoot ratio; SVI = seedling vigor index.

Table 3. Interactive effects of rice genotype and leaf-extract concentration on shoot length and total seedling length of rice

Genotype	Concentrations	SL (cm)	TSL (cm)
<i>Rosolha</i>	Control	7.93 ^{bcd}	15.98 ^{abc}
	25%	9.89 ^{ab}	16.88 ^{ab}
	50%	7.72 ^{cd}	12.98 ^{cd}
	75%	7.63 ^{cd}	11.66 ^{de}
	100%	7.13 ^{cd}	9.29 ^{ef}
<i>Thevür</i>	Control	7.79 ^{cd}	14.48 ^{abcd}
	25%	10.89 ^a	17.18 ^a
	50%	8.63 ^{bc}	13.45 ^{bcd}
	75%	6.12 ^d	7.68 ^{fg}
	100%	3.71 ^e	4.36 ^g

Values are LS-means. Within each column, means followed by different lowercase letters are significantly different according to the Tukey-Kramer adjustment ($P \leq 0.05$). SL = shoot length; TSL = total seedling length.

(a)

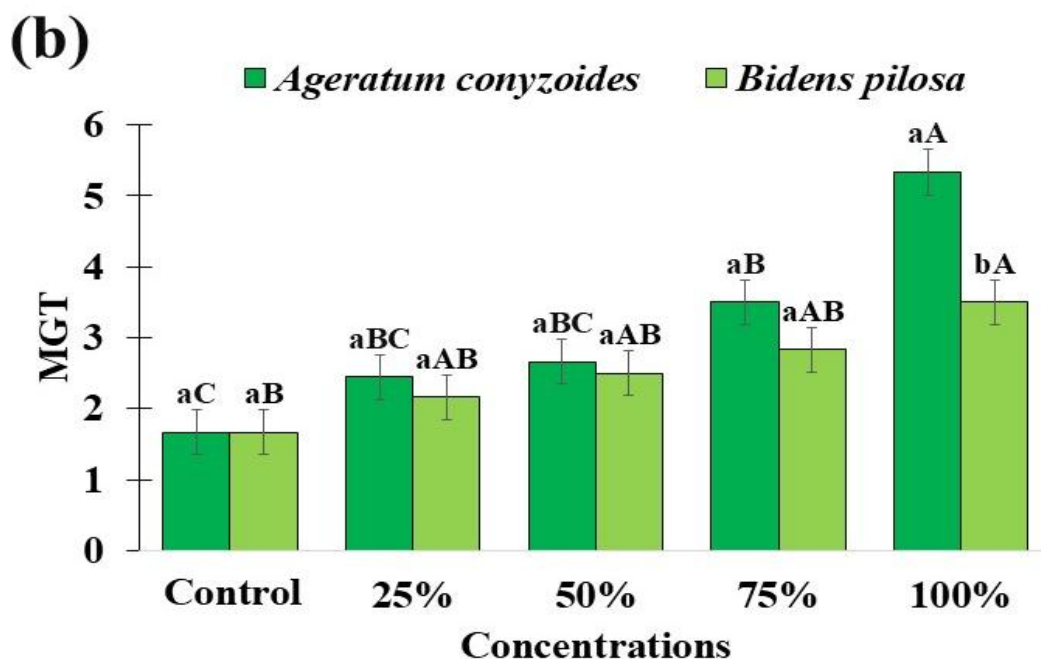


Fig. 1. Interactive effects of leaf-extract concentration with (a) rice genotype and (b) weed species on mean germination time (MGT) of rice. Bars represent LS-means $\pm$ SE. Different lowercase letters indicate significant differences among concentrations within the respective factor, while different uppercase letters indicate differences between genotypes or weed species at each concentration according to the Tukey-Kramer adjustment ($P \leq 0.05$).

CONCLUSIONS

The study demonstrated that aqueous leaf extracts of *A. conyzoides* and *B. pilosa* influenced early growth of the two rice genotypes in a concentration-dependent manner. Extract concentration significantly affected root length, root-to-shoot ratio, seedling vigor index, shoot length, total seedling length and mean germination time, whereas germination percentage was unaffected. *Rosolha* showed greater root development, root-to-shoot ratio and seedling vigor than Thevür. Significant genotype $\times$ concentration interactions for shoot length, total seedling length and mean germination time indicated genotype-dependent responses to increasing extract concentrations. *A. conyzoides* produced greater inhibition of RL, R/S and SVI than *B. pilosa* under the conditions of the experiment. Because the study was conducted using aqueous extracts under controlled laboratory conditions, further experiments using field-relevant exposure levels and plant residues are needed to determine the ecological relevance of these responses.

Credit Statement

Pudumi Rongpipi (PR): Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing-original draft

Zenwang Konyak (ZK): Investigation, Data curation, Data analysis, Methodology, Validation, Writing-review & editing

Keneizoulhou Kesiezie (KK): Conceptualization, Supervision, Methodology, Validation, Writing-review & editing.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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