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Invasiveness of *Alternanthera bettzickiana* – Is Allelopathy a Factor?

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Authors' contributions

This work was carried out in collaboration between both authors. Author TA designed the study, carried out the experiments, collected literature, performed statistical analysis and wrote the first draft of the paper. Author MVM managed literatures and modified the article to final form. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of the experiment was to investigate the allelopathic potential of *Alternanthera bettzickiana* (Regel) Voss*.*

Study Design: Experiment on effect of aqueous extract, incorporation and mulching of *A. bettzickiana* on crop seedling growth was carried out in completely randomized design with five treatments replicated five times. Varying concentrations and time of application of weed extract were also tried in an experiment laid out in factorial completely randomized design with a total of twenty treatment interactions replicated thrice.

Place and Duration of Study: Experiments were carried out at Kerala Agricultural University, Thrissur, during 2021- 2022.

Methodology: Rice and cowpea were used as test crops in all the experiments. A laboratory study was carried out with test crops sown in petri plates to assess the effect of aqueous extract of the weed at 0%, 2%, 4%, 6% and 8% concentration on germination of the crops. Effect of mulching and incorporation of *A. bettzickiana* at the rate of 0, 1, 2, 4 and 6 tonnes per hectare on crop growth was studied as pot culture experiments. To assess the influence of time of application of weed extract on seedling growth, extracts at various concentrations of 0%, 2%, 4%, 6% and 8% were applied at 0, 6, 15 and 21 days after sowing of test crops in trays.

Results: Only the treatment of 8% aqueous extract of weed caused a significant reduction in seedling vigour of cowpea, while rice was found to be unaffected. Incorporation and mulching of *A.*

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bettzickiana showed no significant effect on germination and growth of both the test crops. Time of application of weed extract at various concentrations also had no significant effect on seedling growth of both rice and cowpea.

Conclusion: *Alternanthera bettzickiana* possessed no strong allelopathic potential as exhibited by other species of the genera like *A. philoxeroides*, *A. sessilis etc*.

Keywords: Alternanthera bettzickiana; allelopathy; germination; seedling vigour.

1. INTRODUCTION

Allelopathy is defined as any direct or indirect, harmful or positive effect that one plant has on another through the synthesis of chemical substances that are released into the surrounding environment [1]. Allelochemicals are secondary metabolites produced in the plant system including alkaloids, terpenoids, flavonoids, and catechins that may promote or impede the growth of neighbouring plants. These compounds are found in various amounts in leaves, roots, stems, pollen, and other plant parts, and they are discharged into the environment via volatilization, root exudation, leaching or during decay [2]. They obstruct cell division and elongation, DNA, RNA, protein, and plant hormone synthesis, photosynthesis and respiration, and hence the plant's general growth and development [3].

Allelopathy is thought to be a key factor in the successful invasion of weeds in a given area, as it inhibits the growth and spread of native species by interfering with their physiological activities. Strong allelopathic potential have been exhibited by major invasive weeds like *Parthenium hysterophorus, Ageratum conyzoides, Lantana camara etc* through the release of various compounds like phenolics, caffeic acid, sesquiterpene lactones, methyl coumarins and umbelliferones which help in their dominance in several plant communities [4-6].

Alternanthera is a genus in Amaranthaceae family consisting of a large number of invasive species like *A. philoxeroides, A. sessilis, A. bettzickiana, A. pungens* etc. *A. philoxeroides* and *A. sessilis* can grow in diverse habitats and can spread quickly through vegetative propagation by creeping stolon [7]. *Alternanthera bettzickiana* (Regel) Voss. is a spreading perennial herb which is commonly known as calico plant. It originated in tropical America and has lately been designated as an invasive weed in many parts of the world. In India, it is found throughout the plains, degraded deciduous forests and wastelands in the southern and north-eastern states, especially in Kerala, Karnataka, Tamil Nadu and Assam [8]. Allelopathic potential of other species in the genus like *A. philoxeroides* and *A. sessilis* has been reported in several studies. HPLC analysis of whole plant aqueous extract of these weeds revealed the presence of phenolic compounds like ferrulic acid, chlorogenic acid, coumaric acid, vanillic acid and 4-hydroxy-3- methoxybenzoic acid [9]. GCMS analysis of leaf leachates of *A. ficoidea* confirmed the presence of phenolic compounds like tetramethyl-2-hexadecanol, tetraenoic acid, octadecatrienoic acid, methyl ester and phytol [10]. *A. bettzickiana* has been spreading at a very rapid rate in the uplands of Kerala, and the dominance of this species in uncropped areas raises the probability of it having allelopathic activity. With this background, the current study was undertaken to investigate the allelopathic potential of *A. bettzickiana* which is now a major weed of several upland crops of Kerala.

2. MATERIALS AND METHODS

Laboratory study and pot experiments were conducted at Kerala Agricultural University, Thrissur, during 2021-2022 to assess the allelopathic potential of *Alternanthera bettzickiana* (Regel) Voss. Rice and cowpea were used as test crops in the experiments.

2.1 Effect of Aqueous Extract of *A. bettzickiana* on Seed Germination

For preparing aqueous extract, whole plants including root, stem and leaves were collected and thoroughly washed to remove adhering soil. Cleaned materials were then macerated and steeped in water at 1:1 ratio. It was then shaken continuously for 1 h in an electric shaker and then filtered through a muslin cloth to get extract with 100% concentration (w/v). This was used as stock solution and diluted to desired concentrations of 2, 4, 6, 8 and 10 %. Eight ml of aqueous extracts of various concentrations were poured into Petri plates lined with filter paper. Petri plates with tap water served as control. Ten seeds each of rice and cowpea were then placed in these Petri plates and germination was observed. The experiment was laid out in completely randomized design with five replications.

2.2 Effect of Time of Application of Aqueous Extract of *A. bettzickiana* on Seedling Growth

Test crops were sown in plastic trays of equal size filled with equal quantity of soil. Experiment followed factorial completely randomized design with concentration of extract as first factor and time of application as second factor. To find out the amount of extract to be applied to each tray, quantity of water required for attaining field capacity was worked out before treatment application and was found to be 350 ml. Aqueous extract was prepared as mentioned earlier and the stock solution was diluted to desired concentrations of 2, 4, 6 and 8%. Extracts were applied at 0, 6, 15 and 21 days after sowing (DAS).

2.3 Effect of *A. bettzickiana* Incorporation and Mulching on Growth of Test Crops

Pot culture experiment was conducted to assess the effect of *A. bettzickiana* incorporation and mulching on growth of seedlings. Completely randomized design was adopted with four replications. Pots of uniform size were filled with equal amount of soil and 10 seeds each of rice and cowpea were sown. Mulching or incorporation was done at the rate of 0, 1, 2, 4 and 6 t/ha. Quantity of weed to be used as mulch or to be incorporated into the soil was calculated based on the diameter of the pots. To assess the effect of incorporation, *A. bettzickiana* was air dried, chopped into pieces of 5 to 10 cm length and required quantity of material was incorporated into the soil in the pots, before sowing of test crops. Mulching was done with freshly collected weed, which was chopped and applied after sowing of the crops.

2.4 Germination and Seedling Growth of Test Crops

Number of seeds germinated out of total number of seeds sown was recorded and germination percentage was calculated. Number of days required for the germination of 50 % of the seeds sown was also recorded. Seedling length and biomass of seeds sown in Petri dishes were recorded at 14 DAS. Root length, shoot length and dry weight of seedlings raised in pots were recorded at 15 and 30 DAS. For seeds sown in trays, shoot length, root length and seedling dry weight was observed at 30 DAS. Seedling vigour index was calculated by multiplying germination percentage with total seedling length (cm) and seedlings with higher vigour index were considered to be more vigorous [11].

2.5 Statistical Analysis

Significant difference between the treatments for various parameters was analysed using one-way ANOVA. Probability level used for the study was P<05 for all the statements reported in this study. All the tests were performed using GRAPES 1.1.0 statistical package [12].

3. RESULTS AND DISCUSSION

3.1 Effect of Aqueous Extract of Weed on Seed Germination

Application of aqueous extract of *A. bettzickiana* at various concentrations did not show any prominent effect on seed germination of rice and cowpea. Germination percentage of rice ranged between 86 and 94 per cent and that of cowpea ranged between 96 and 100% (Fig. 1). This may be due to low concentration of secondary metabolites produced by *A. bettzickiana* to cause inhibition of germination of the crops. Kleinowski et al. [13] also reported no variation in germination of lettuce seeds when treated with aqueous extract obtained from leaves and stem of *A. philoxeroides*. But 60% reduction in germination of pearl millet when treated with 20% aqueous extract of *A. sessilis* was reported by Elavazhagan [14].

Time required for half of the seeds to germinate also showed no significant variation among the treatments in rice. In case of cowpea, seeds treated with 8% extract took significantly longer time of 2.6 days for half of the seeds to germinate, whereas those in control Petri dishes germinated in 2 days (Fig. 1). Delay in germination of seeds of parthenium treated with stem, leaf and root extracts of *A. philoxeroides* at higher concentrations has been reported by Safdar et al. [15]. Vidhu et al. [16] also reported reduction in germination percentage and speed of germination in green gram when treated with leaf extracts of *Alternanthera brasiliana* and reduction in germination was more prominent at higher concentrations of the extract.

Allelochemicals cause delay in seed germination due to their interference with protein, starch and oil metabolism, which provide energy during germination [17].

Seedling length, biomass and vigour index of rice seeds exhibited no significant difference between the treatments, while all these parameters varied significantly in case of cowpea (Table 1). In cowpea, significantly higher seedling length of 28.9 cm and biomass of 48 g was observed in control. Higher seedling vigour index of 2894.6 was recorded in control which was on par with 2% and 4% extract application. Lowest seedling length, biomass and vigour index of 23.1 cm, 38.5 mg and 2265.1 respectively was observed in seed treatment with 8% extract of the weed (Table 1). This is in line with the findings of Popoola et al. [28] who reported significant reduction in plumule and radicle length of cowpea, under laboratory conditions, when treated with aqueous extract of weeds like *Tridax procumbens* and *Chromolaena odorata.* Application of 20% extract of *A. sessilis* in the growing medium caused 34.4% reduction in root length and 66% reduction in shoot length in pearl millet [14]. Studies indicate that allelochemicals are water soluble and get accumulated in seeds when imbibed along with water. Therefore reduction in seedling length and biomass can be attributed to reduced rate of cell division due to accumulated allelochemicals which impede the functioning of cytokinin and auxin [19].

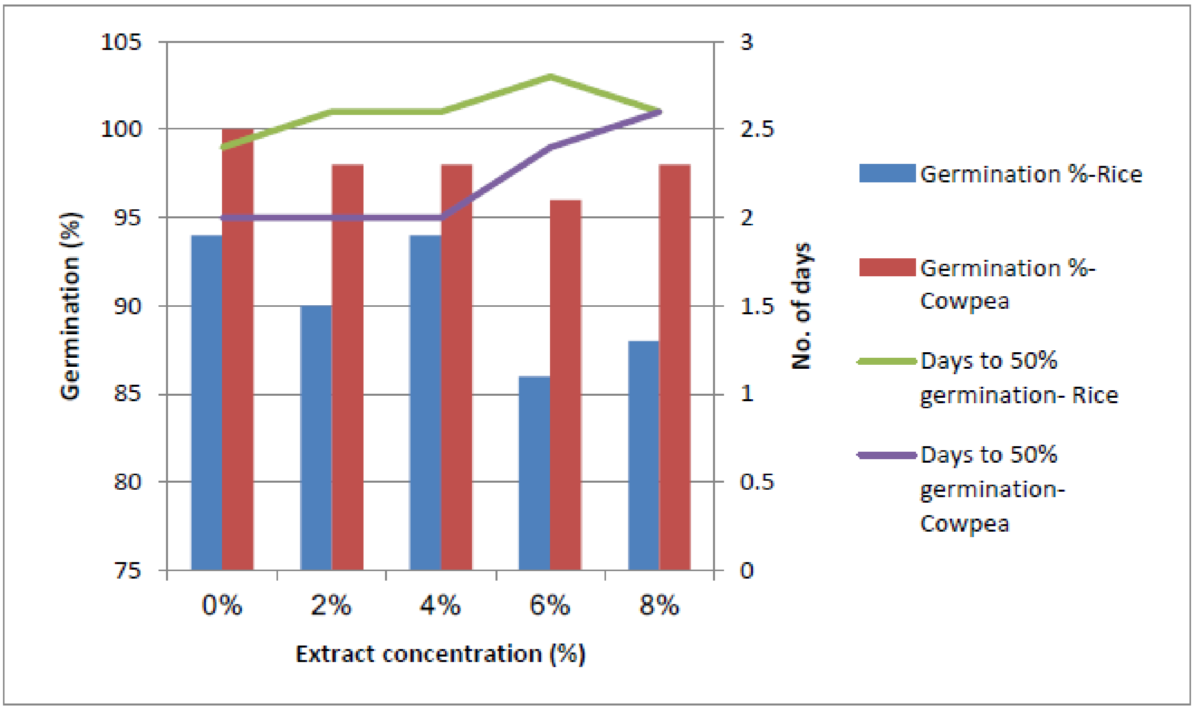

Fig. 1. Effect of aqueous extract of *A. bettzickiana* on seed germination of rice and cowpea

Table 1. Effect of aqueous extract of *A. bettzickiana* on seedling vigour of rice and cowpea

Extract concentration (%)	Seedling length (cm)		Seedling biomass (mg)		Vigour index	
	Rice	Cowpea	Rice	Cowpea	Rice	Cowpea
0	14.1 ±0.64	28.9 ± 0.9[a]	17.30 ± 1.4	48.0 ± 4.1[a]	1325.3	2894.6[a]
2	14.2 ± 0.81	27.8 ±1.3[ab]	17.53 ±1.5	45.0 ±5.2[ab]	1280.0	2727.7[a]
4	13.7 ± 0.62	26.2 ±1.1[bc]	16.70 ±1.0	41.3 ±3.6[bc]	1290.2	2497.8[a]
6	14.0 ±0.68	24.9 ±1.3[cd]	15.90 ± 1.1	39.6 ±3.5[bc]	1238.2	2389.6[ab]
8	13.9 ± 0.13	23.1± 2.3[d]	15.51 ±1.2	38.5 ±4.6[c]	1191.8	2265.1[b]
(*P*=.05%)	0.73	0.01	0.08	0.01	0.51	0.02

[*] *In a column, figures followed by the same letters do not differ significantly in DMRT; P>.05 indicates no significant difference between treatments*; N=5, mean± SD

3.2 Effect of Time of Application of Aqueous Extract on Seedling Growth

Shoot length, root length and dry weight of rice and cowpea sown in trays were observed at 30 DAS to assess the allelopathic effect of aqueous extract of *A. bettzickiana* during seedling growth. Application of aqueous extract of the weed at different concentrations did not show significant effect on the growth parameters of both rice and cowpea. Time of application of the extracts also showed no prominent effect on seedling growth of both the test crops. Interaction effect of extract concentration and time of application of extracts was also found to be non-significant (Figs. 2,3). This indicated that extract of *A. bettzickiana* at given concentrations has no inhibitory effect at any stage of seedling growth of rice and cowpea. This was in line with the results of pot experiments conducted by Prinsloo and Du Plooy [20], who reported no significant reduction in the growth of cabbage with application of aqueous extract of *Amaranthus cruentus*. But the results are in contrast with the findings of Abbas et al. [9] who reported prominent reduction in seedling growth parameters like root and shoot length, seedling vigour index and seedling biomass of rice when applied with different concentrations of aqueous extracts of *A. philoxeroides* and *A. sessilis.* Significant reduction in hypocotyl and radicle length and total seedling height of lettuce was reported by Kaliyadasa and Jayasinghe [21] when applied with dried plant material of *Ageratum conyzoides*, *Cassia occidentalis* and *Clidemia hirta* in the growing medium.

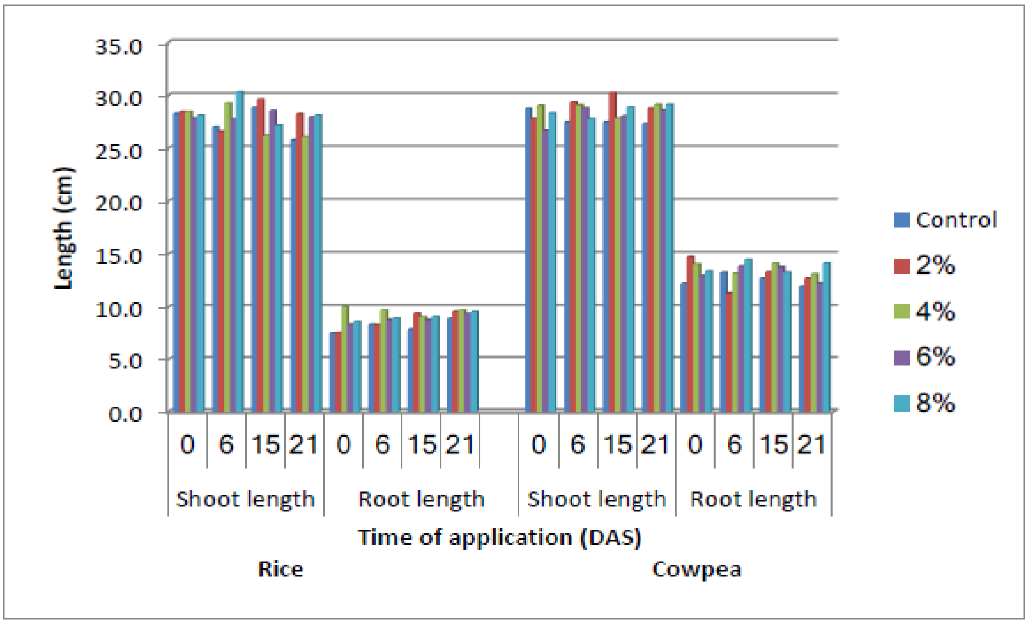

Fig. 2. Effect of time of application of aqueous extract of *A. bettzickiana* at various concentrations on seedling growth of rice and cowpea (DAS: Days after Sowing)

Table 2. Effect of incorporation and mulching with *A. bettzickiana* on seedling vigour index of rice and cowpea

Treatments (t/ha)	Seedling vigour index			
	Incorporation		Mulching	
	Rice	Cowpea	Rice	Cowpea
0	2217.7	3701.1	1936.3	3875.0
1	2237.6	3593.4	1950.6	3577.5
2	2015.4	3724.6	1934.4	3880.0
4	2251.0	3702.4	2044.9	3845.0
6	2033.5	3616.9	1934.5	3469.5
(*P*=.05%)	0.72	0.98	0.88	0.59

P>.05 indicates no significant difference between treatments

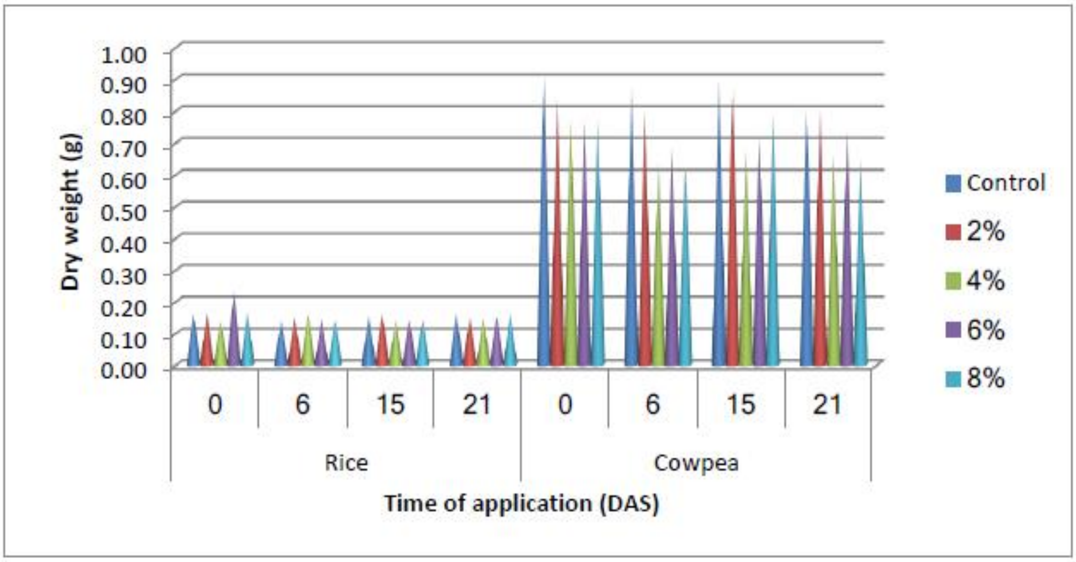

Fig. 3. Effect of time of application of aqueous extract of *A. bettzickiana* at various concentrations on seedling dry weight of rice and cowpea (DAS: Days after Sowing)

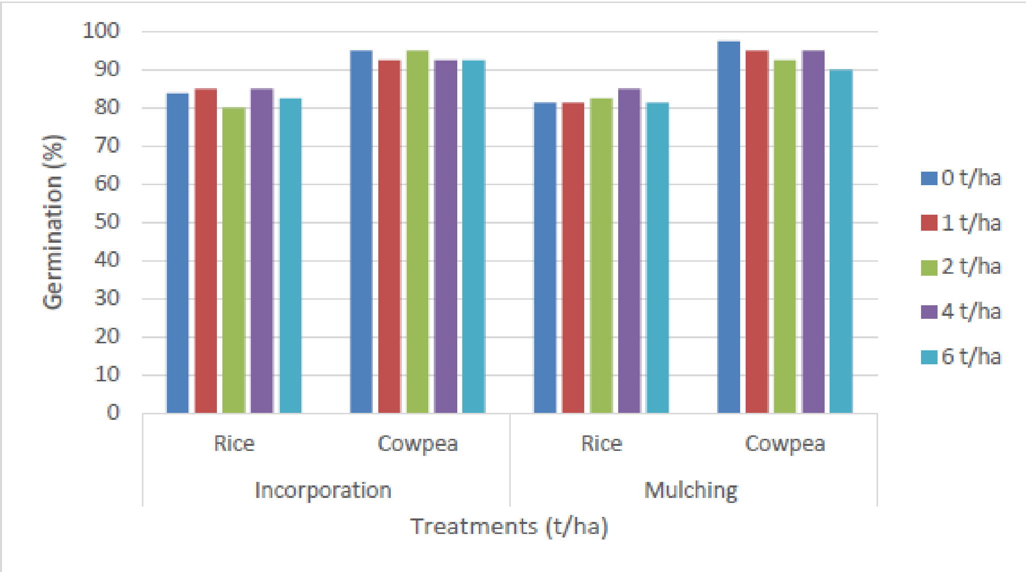

Fig. 4. Effect of incorporation and mulching with *A. bettzickiana* on seed germination of rice and cowpea

3.3 Seedling Growth

Incorporation of weed at various quantities in the soil showed no significant effect on root length, shoot length and seedling biomass for both rice and cowpea at 15 and 30 DAS. Rice recorded an average shoot length, root length and dry weight of 21.9 cm, 4.19 cm and 0.06 g respectively at 15 DAS, and 33.3 cm, 6.9 cm and 0.52 g respectively at 30 DAS (Fig. 5). Cowpea seedlings recorded an average shoot length of 29.6 cm, root length of 9.6 cm and dry weight of 0.53 g at 15 DAS. At 30 DAS, it exhibited an average shoot length of 33.8 cm, root length of 15.1 cm and dry weight of 1.84 g (Fig. 5).

Mulching with *A. bettzickiana* fragments at various quantities also produced no significant variations in shoot length, root length and dry weight of seedlings of rice and cowpea at 15 and 30 DAS. Average shoot length, root length and dry weight of rice seedlings was 19.7 cm, 4.08 cm and 0.05 g respectively at 15 DAS and 31.9 cm, 6.72 cm and 0.41 g respectively at 30 DAS (Fig. 6). Cowpea seedlings recorded an average shoot length of 28.8 cm and 32.7 cm respectively at 15 and 30 DAS. It exhibited an average root length of 10 cm and dry weight of 0.52 g at 15 DAS and 16.2 cm root length and 1.73 g dry weight at 30 DAS (Fig. 6).

Conversely, Abbas et al. [9] observed significant decrease in seedling growth parameters in rice when incorporated with *A. philoxeroides* residue at different quantities. Nadeem et al. [22] observed that soil incorporation of plant residues of *A. philoxeroides* at various quantities significantly reduced seedling growth parameters of maize, and this reduction was directly proportional to increasing quantities of weed residue. In the current study, incorporation or mulching with *A. bettzickiana* did not cause reduction in seedling growth which may be attributed to the inefficiency of the compounds released during the decomposition process of incorporated or mulched material to interfere with the growth of the crops.

Invasive species offer higher level of competition to the native species, when they are introduced into a novel community. Standish et al. [23] reported that increase in the density, biomass and spread of *Tradescantia fluminensis*, which is an invasive weed in New Zealand and Florida, caused dramatic reduction in the abundance and richness of native species in the invaded forest ecosystem by reducing light availability and competition for various resources. Infestation with several invasive and non-invasive exotic species exhibited great competitive effects on a native grass *Elymus canadensis* and caused significant reduction in its height and biomass when compared to those infested with native species and non- infested condition [24].

Thangamani et al. [25] observed that *A. ficoidea,* a close relative of *A. bettzickiana,* is a faster invader when compared to other upland invasive plants like *Lantana camara*, *Ageratum conyzoides* and *Chromolaena odorata* due to its capacity to produce enormous number of seeds as well as to propagate vegetatively through stem cuttings and rooted cuttings. Since the secondary metabolites produced by *A. bettzickiana* shows no significant allelopathic effects, dominance of this weed in an ecosystem may be explained by greater seed production capacity, ability for rapid regeneration and competitive potential [26].

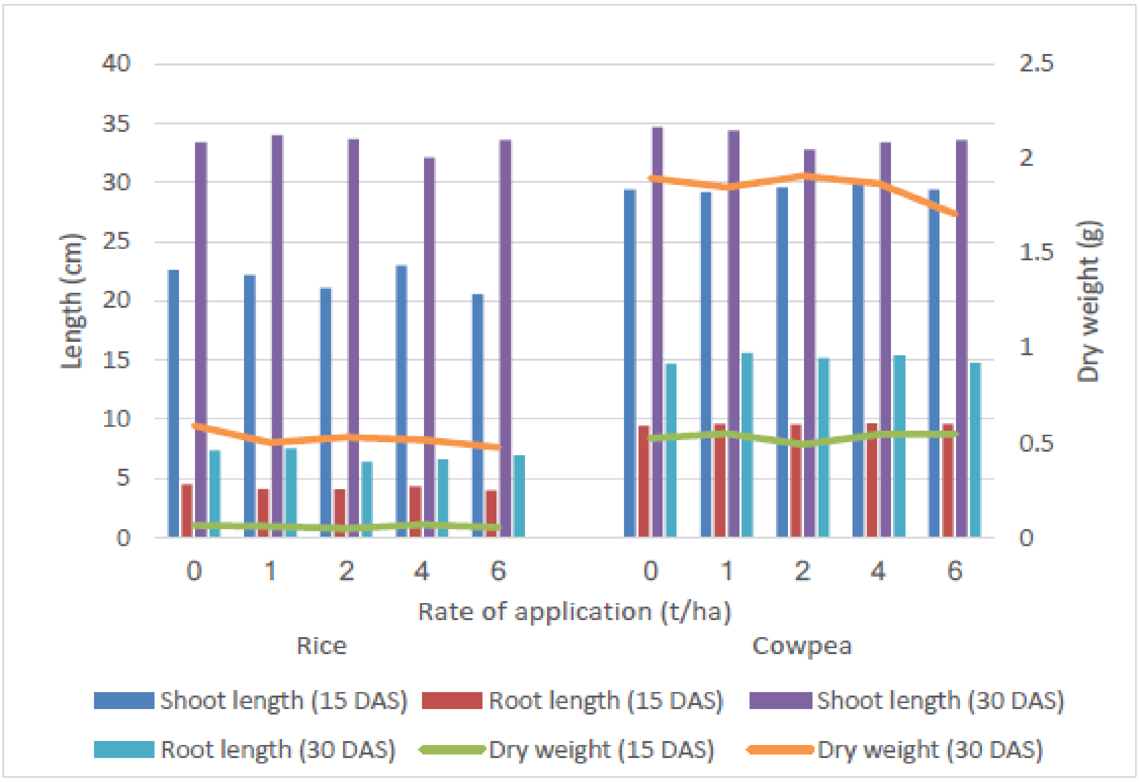

Fig. 5. Effect of incorporation of *A. bettzickiana* on seedling growth of rice and cowpea

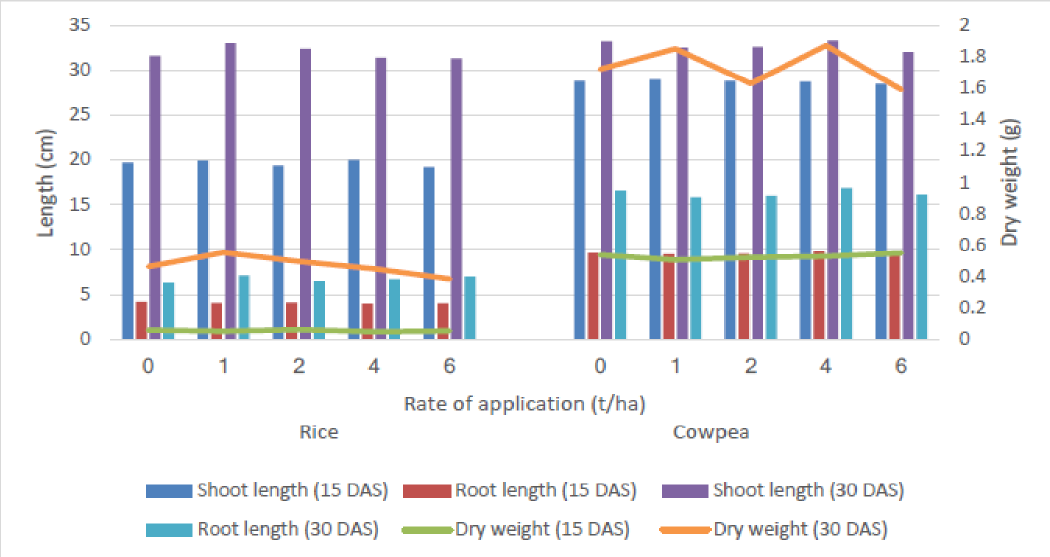

Fig. 6. Effect of mulching with *A. bettzickiana* on seedling growth of rice and cowpea

4. CONCLUSION

In this study, allelopathic effect of *A. bettzickiana* was observed only on seed germination and seedling vigour of cowpea under laboratory conditions. Pot experiments with aqueous extract application and incorporation and mulching of *A. bettzickiana* showed no significant effects on seed germination and seedling growth. This indicated that the secondary metabolites produced by *A. bettzickiana* may be in lower concentration or they did not possess strong allelopathic potential as exhibited by other species of the same genus like *A. philoxeroides*, *A. sessilis*, *A. brasiliana etc*. Since allelopathy provided no significant contribution to the dominance of *A. bettzickiana,* the faster growth rate and regeneration capacity of the weed might have facilitated smothering of native plant communities and therefore, its rapid spread.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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