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Allelopathic Effect of Aqueous Extract of *Argemone mexicana* L on Germination and Growth of *Brachiaria dictyoneura* L and *Clitoria ternatea* L

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ABSTRACT

The present study was conducted in the laboratory to investigate the allelopathic effect of Mexican poppy (*Argemone mexicana* L.) to the germination and growth parameters of two native species *Brachiaria dictyoneura* L and *Clitoria ternatea* L. Different concentrations of leaf and seed aqueous extracts from *Argemone mexicana* (0%, 25%, 50%, 75% and 100%) were evaluated. Results showed that seed germination, root length, shoot length, seedling length, fresh weight and dry weight of *B. dictyoneura* and *C. ternatea* seedlings were significantly reduced by leaf and seed extracts compared with control treatments. Roots were more affected than shoots; and leaf extract was more suppressive than seed extracts. From the results, it is concluded that leaf and seed extracts have some allelochemicals with inhibitory effect on germination and growth of the tested plant species.

Keywords: Allelochemicals; Root Length; Shoot Length

1. Introduction

Invasive alien plants are among the important factors that influence plants growth parameters in and among farming systems and wildlife ecosystems. Integrity of farming system and natural ecosystems are threatened by alien invasive species which displace some of the native species and establishing mono-species in new habitat [1-3]. Production and release of allelopathic compounds (allelochemicals) by invasive species are factors that enhance its competitive ability over native species [4]. Plants can affect neighboring plants by releasing chemicals into the environment [5]. The Austrian plant physiologist Hans Molisch named this phenomenon "allelopathy" in 1937 [6]. Allelopathy refers to the effects of one plant on another plant or organisms through the release of chemicals into the environment [2,7]. Allelochemicals released from plants, imposing allelopathic influences are classified as secondary metabolites and are produced as offshoots of the primary metabolic pathways in plants [8]. Allelopathic effect of some invasive species over other species is stronger in introduced habitats than in native lands because in new habitat native species may not be as adapted to specific allelochemicals of invaders as species do in the native range [4]. Mechanistically, allelochemicals have a role in the determination of nutrient dynamics, mycorrhizae, soil chemical characteristics and microbial ecology [9,10]. Allelochemicals released by invasive species also affect native species through different pathways that includes interruption of plants nutrients uptake, change in membrane permeability [11], interference in cell division and elongation process in roots and shoots [12-14], interference in chlorophyll formation [15] protein synthesis inhibition [11,16] and change or inactivate the activity and functions of certain hormones and enzymes [17]. Hence, allelopathy has been considered as among of key factor to the success of invasive plant species over native species [2-4,18]. *Argemone mexicana* (Mexican poppy) is amongst the ecologically and economically destructive exotic invasive plant species in Tanzania affecting both natural and agricultural ecosystems [19]. *A. mexicana* is a herb plant with branches, which has naturalized widely in many tropical and subtropical regions although it is a native of tropical American [20]. It is a widespread annual weed primarily associated with agricultural crops and wastelands. It is a major weed of a number of crops in the tropics and warm temperate regions and is persistent as it produces a seed bank. *A. mexicana* is amongst invasive weeds reported to release allelochemicals which affect some other species within its vicinity especially crops in agricultural fields. Burhan and Shaukat [21] reported the inhibition effect of *A. Mexicana* extracts in seed germination, root and shoot growth of pearl-millet, mustard, wheat, carrot, corn and turnip caused by allelopathic compounds dissolved in extracts. Another study by Paul and Begum [22] indicated that seed germination percentage, root length, shoot length and seedling dry weight of blackgram (*Vigna mungo*) and rapeseed (*Brassica napus*) were significantly decreased with the increase of both root and leaf extracts of *A. mexicana*. Alagesaboopathi [23] also reported that there was decrease in seed germination and inhibition of plumule and radicle length of *Sorghum bicolor* seedlings with increase in *A. mexicana* leaf extracts concentration due to allelopathic potentialities. Paul and Begum [24] reported allelopathic effect of *A. mexicana* on germination and seedling growth of Lentil (*Lens culinaris*). Allelopathic effect of *A. mexicana* retarded the growth of tomato plants at higher concentration [25].

All these researches have reported inhibitory effect in seed germination, root length and shoot length and other primary growth parameters caused by allelochemicals present in aqueous extracts. Although allelopathic effect of *A. mexicana* on crops (such as beans and maize) has been reported, little information is available on their influence on wild plant species. Therefore this study was conducted to investigate the allelopathic effect of leaf and seed aqueous extracts of *A. mexicana* on germination and seedling growth of *B. dictyoneura* and *C. ternatea* which are important pasture for wildlife and livestock. The data from this study will help in current efforts to eradicate alien invasive species (*A. mexicana*) in wildlife protected areas in Tanzania.

2. Material and Methods

2.1. Preparation of the Aqueous Extract Solution

Seeds and leaves of *A. mexicana* plants were collected at Ngorongoro Conservation Area (NCA). NCA is one of wildlife protected areas in Serengeti Ecosystem which has been invaded by *A. mexicana*. The Seeds and leaves were separately washed thoroughly with distilled water and air dried at room temperature for eight days. Seeds and leaves were separately crushed in a blander into powdery form and 100 g of these crushed materials were soaked separately in a corked, conical flask containing 1000 mL (1 Liter) of distilled water for 72 hours and filtered through Whatman filter paper No. 1. The extract was diluted to obtain the concentrations of 25 $g{\cdot}L^{-1}$ (25%), 50 $g{\cdot}L^{-1}$ (50%), 75 $g{\cdot}L^{-1}$ (75%), and 100 $g{\cdot}L^{-1}$ (100%) while the distilled water was used in the control treatment.

2.2. Determination of Germination Percentage, Root Length, and Shoot Length

The seeds of *Brachiaria dictyoneura* were collected from Ngorongoro Conservation Area and that of *Clitoria ternatea* were obtained from the National Plant Genetic Resources Centre of Tanzania at Tropical Pesticides Research Institute (TPRI)—Arusha Tanzania. The seeds were washed three times in running tap water to remove any impurities and afterwards surface sterilized with 5% of sodium hypochlorite for two minutes then rinsed four times with distilled water. Toxicity of aqueous extract of *A. mexicana* was tested against *Brachiaria dictyoneura* and *Clitoria ternatea* whereby 10 seeds of each target species were placed in 9 cm autoclave petri dishes lined up with double layer of whatman No. 1 filter paper and 10 mL of test extract for each concentration (25%, 50%, 75%, and 100%) of leaves and seeds. The seeds treated with distilled water were taken as a control (T_0) while seeds in petri dishes with four different concentrations (T_1 = 25%, T_2 = 50%, T_3 = 75%, T_4 = 100%) of *Argemone mexicana* extract constituted a four sets of treated seeds for each species (*Brachiaria dictyoneura* and *Clitoria ternate)*. The petri dishes were covered by caps and kept inside the cupboards at room temperature. The experimental design was a randomized entire block with four replications for each treatment. Extract/distilled water were added to moisten the seeds when required.

Seeds were observed every day and number of germinated seeds was recorded (germination count) whereas the emergence of the radicle from the seed was considered as criterion for germination to be recorded. Germination was recorded every day (at 24 hours intervals) over a 10-day period. On 15th day after germination the root length, shoot length and seedling length were measured by using digital caliper while fresh weight and dry weights of each seedling were measured by digital weighing balance. The dry weight of each seedling was measured after placing them in an oven maintained at 55˚C for 5 days.

2.3. Statistical Analysis

Percentage of inhibition/stimulation effect on germination and root and shoot elongation over control (T_0) was calculated using the equation used by Signh and Chaudhary [26]:

Inhibition (−) or stimulation (+) = [(Germinated seeds in extracts − Germinated seed in control)/Geminate seeds in control] × 100.

The data from the experiment was analyzed using the software of STATISTICA program 2013. When significant differences were detected by the analysis of variance (ANOVA), mean values of root length, shoot length, fresh weight and dry weight of seedlings were used to compare treatment means at p = 0.05 according to Fisher's Least Significant Difference (LSD).

3. Results

3.1. Effects of Aqueous Extracts of *A. mexicana* on Seed Germination

Table 1 presents mean seed germination percentages of *B. dictyoneura* and *C. ternatea* treated with different concentrations of seed and leaf aqueous extracts of *A. mexicana*. Seed and leaf aqueous extracts of *A. mexicana* significantly ($p \leq 0.01$) affected the germination of *B. dictyoneura* and *C. ternatea* seeds. Mean seed germination percentage of control treatments was high compared with seeds treated with aqueous extracts. The mean seed germination percentage was decreasing with increase in aqueous extracts concentration (**Table 1**). The mean germination percentage of *B. dictyoneura* seeds was 65%, 50%, 12.5% and 7.5% when treated with concentrations of 25%, 50%, 75% and 100% leaf extracts respectively. Therefore when compared with control, the leaf extracts at the concentrations of 25%, 50%, 75% and 100% significantly inhibited ($p \leq 0.001$) germination of *B. dictyoneura* by −31.6%, −47.4%, −86.8% and −92.1% respectively. In seed extract, the percentage germination of *B. dictyoneura* were also significantly reduced ($p \leq 0.001$) with increase in concentration whereby at the concentration of 25%, 50%, 75% and 100% aqueous seed extracts, the germination was 70%, 50%, 32.5% and 17.5% respectively. Relative to control treatment, the germination percentages were significantly reduced with increase in seed extracts concentration from 25%, 50%, 75% and 100% and this inhibited germination of *B. dictyoneura* by −24%, −45.9%, −64.8% and −81.1% respectively. The similar trend was observed in seed germination of *C. ternatea* whereby at concentration of 25%, 50%, 75% and 100% of leaf extract, the percentage germination were 77.5%, 77.5%, 37% and 25% respectively.

At concentrations of 25, 50, 75 and 100%, the germination of *C. ternatea* was significantly ($p \leq 0.001$) reduced by −20.5%, −20.5%, −62.1% and −74.4% respectively. Variation in mean germination percentage of *C. ternatea* was caused by inhibition effect that was increasing with increase in concentrations of leaf extracts when compared with the control treatment.

Mean germination percentages of *C. ternate* were also significantly reduced ($p \leq 0.01$) by −24.2%, −26.3%, −36.8% and −44.7% when treated with concentrations of 25%, 50%, 75% and 100% seed extracts respectively. Generally, germination inhibition was greater with 100% extract than with the other concentrations.

3.2. Effects of Aqueous Extracts of *Argemone mexicana* on Roots and Shoots Elongation (mm)

Root and shoot lengths of 15 days old seedlings of *C. ternatea* and *B. dictyoneura* treated with leaf and seed aqueous extracts of *Argemone mexicana* were much shorter in comparison with those of the control. The length of roots and shoots of *C. ternatea* and *B. dictyo neura* was decreasing with increase in concentrations of seed and leaf extracts.

Table 1. Effect of seed and leaf extracts of *A. mexicana* on germination percentage of *B. dictyoneura* and *C. ternatea*.

	B. dictyoneura		*C. ternatea*	
Extract Concentrations	Seed Extracts	Leaf Extracts	Seed Extracts	Leaf Extracts
T_0 (Control)	92.5 ± 4.78d	95 ± 5c	95 ± 2.88c	97.5 ± 2.5c
T_1 (25%)	70 ± 4.08c	65 ± 5b	72.5 ± 4.78b	77.5 ± 8.54b
	(−24)	(−31.6)	(−24.2)	(−20.5)
T_2 (50%)	50 ± 5.77b	50 ± 5.77b	70 ± 7.07ab	77.5 ± 4.79b
	(−45.9)	(−47.4)	(−26.3)	(−20.5)
T_3 (75%)	32.5 ± 4.78ab	12.5 ± 6.29a	60 ± 9.12ab	37 ± 2.5a
	(−64.8)	(−86.8)	(−36.8)	(−62.1)
T_4 (100%)	17.5 ± 8.54a	7.5 ± 4.79a	52.5 ± 6.29a	25 ± 9.57a
	(−81.1)	(−92.1)	(−44.7)	(−74.4)
One way ANOVA (F Statistics)	26.2037^{***}	46.1357^{***}	6.35^{**}	23.156^{***}

Values presented are means ± SE. *, **, *** = significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at p = 0.05 according to LSD. Values in the parenthesis indicates percentage of the inhibitory (−) effects in comparison with control (T_0).

Roots length of *B. dictyoneura* treated with concentration of 25%, 50%, 75% and 100% seed extract were 20.4, 16.4, 10.7 and 8.3 mm long respectively (**Table 2**). Root elongation inhibition percentages of seedlings treated with 25%, 50%, 75% and 100% of seed extracts was −29.4%, −43.3%, −63% and −71.3% respectively. Leaf extracts also significantly ($p \leq 0.001$) reduced roots elongation in comparison with control by −36.1%, −50.3%, −66%, and −64.6% when treated at concentrations of 25%, 50%, 75% and 100% respectively.

In leaf extracts, the shortest roots were recorded at higher concentrations whereas longest was recorded in the control treatment. The shoot lengths of *B. dictyoneura* were significantly ($p \leq 0.001$) reduced by −20.5%, −54.2%, −67.3% and −68.6% at concentrations (seed extracts) of 25%, 50%, 75% and 100% respectively. The tallest shoot of 60.5 mm long was observed in control treatment while at concentrations of 25%, 50%, 75% and 100%; shoot length was 48.1, 27.7, 19.8 and 19 mm respectively. In leaf extracts, the tallest shoot of 60.1 mm was measured in control treatment compared with 46.8, 25.5, 19.9 and 18.3 mm long, which was recorded in concentrations of 25%, 50%, 75% and 100% respectively. In comparison with control, shoot lengths were significantly ($p \leq 0.001$) reduced by −22.1%, −57.6%, −66.9% and −69.6% in leaf extracts with concentration of 25%, 50%, 75% and 100% respectively.

The allelopathic effect of seed and leaf extract of *A. mexicana* on root and shoot lengths of *C. ternatea* is shown in **Table 3**. Root lengths of *C. ternatea* were reduced with increase in concentration of seed extracts. The mean root length of *C. ternatea* treated with distilled water (control) was 35 mm long while, the root length of seedlings treated with 25%, 50%, 75% and 100% extract concentrations was 31.3, 24.5, 24.3 and 16.9 mm long respectively. Root length inhibition caused by seed extract at concentrations of 25%, 50%, 75% and 100% was −10.6%, −30%, −30.6% and −51.7% respectively. Root elongation of *C. ternatea* was also significantly ($p \leq 0.001$) hindered by different concentration of leaf extract whereby elongation inhibition was −26.5%, −49.9%, −63.6% and −83.5% at the concentrations of 25%, 50%, 75% and 100% respectively. Mean root length in control treatment was 39.3 mm while in concentrations of 25%, 50%, 75% and 100%, the mean root length was 28.9, 19.7, 14.3 and 6.5 mm respectively.

In comparison with control treatment, the allelopathic effect of leaf extract of *A. mexicana* reduced significantly ($p \leq 0.01$) the shoot length of *C. ternatea.* At concentrations of 25%, 50%, 75% and 100%, the shoot length was 81.4, 74.8, 94.5 and 13.6 mm respectively. Shoot length inhibition caused by leaf extracts was −22.5%, −28.8%, −10% and −87% in response to extract concentrations of 25%, 50%, 75% and 100% respectively. Seed extracts of *A. mexicana* also reduced shoot length of *C. ternatea* but it was not significant.

3.3. Effects of Aqueous Extracts of *A. mexicana* on Seedling Length (mm)

Mean seedlings length of *C. ternatea* and *B. dictyoneura* treated with different concentrations of leaf and seed extracts of *A. mexicana* were much shorter in comparison with those of the control treatment (**Table 4**). Different concentrations of seed extracts significantly ($p \leq 0.05$) reduced seedling length of *C. ternatea* by −8.5, −10.5, −23.6 and −31.8% in response to seed extract concentrations of 25%, 50%, 75% and 100% respectively. Mean

Table 2. Effect of seed and leaf extracts of *A. mexicana* on root and shoot elongation and seedling growth of *B. dictyoneura*.

	Seed Extracts		Leaf Extracts	
Extract Concentrations	Root Length (mm)	Shoot Length (mm)	Root Length (mm)	Shoot Length (mm)
T_0 (Control)	28.9 ± 1.31d	60.5 ± 0.43d	28.8 ± 0.75d	60.1 ± 1.15d
T_1 (25%)	20.4 ± 0.01c	48.1 ± 1.68c	18.4 ± 0.085c	46.8 ± 1.14c
	(−29.4)	(−20.5)	(−36.1)	(−22.1)
T_2 (50%)	16.4 ± 0.9b	27.7 ± 1.41b	14.3 ± 0.59b	25.5 ± 1.13b
	(−43.3)	(−54.2)	(−50.3)	(−57.6)
T_3 (75%)	10.7 ± 0.23a	19.8 ± 0.96a	9.80 ± 1.33a	19.9 ± 0.41a
	(−63)	(−67.3)	(−66)	(−66.9)
T_4 (100%)	8.3 ± 0.31a	19.0 ± 0.25a	10.2 ± 0.51a	18.3 ± 1.29a
	(−71.3)	(−68.6)	(−64.6)	(−69.6)
One way ANOVA (F Statistics)	109.7704^{***}	254.767^{***}	82.252^{***}	269.857^{***}

Values presented are means ± SE. *, **, *** = significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at P = 0.05 according to LSD. Values in the parenthesis indicates percentage of the inhibitory (−) effects in comparison with control (T_0).

Table 3. Effect of seed and leaf extracts of *A. mexicana* on root and shoot elongation (mm) of *C. ternatea*.

	Seed Extracts		Leaf Extracts	
Extract Concentrations	Root Length (mm)	Shoot Length (mm)	Root Length (mm)	Shoot Length (mm)
T_0 (Control)	35.0 ± 1.75d	106.7 ± 1.78a	39.3 ± 2.18d	105.0 ± 1.88b
T_1 (25%)	31.3 ± 3.0cd	98.4 ± 6.58ab	28.9 ± 1.51c	81.4 ± 8.44a
	(−10.6)	(−7.8)	(−26.50)	(−22.50)
T_2 (50%)	24.5 ± 1.51b	102.3 ± 5.86b	19.7 ± 3.11b	74.8 ± 10.12a
	(−30)	(−4.1)	(−49.9)	(−28.8)
T_3 (75%)	24.3 ± 4.24abc	83.9 ± 6.99ab	14.3 ± 0.88ab	94.5 ± 2.47ab
	(−30.6)	(−21.4)	(−63.6)	(−10)
T_4 (100%)	16.9 ± 2.03a	79.7 ± 12.02b	6.5 ± 0.0a	13.6 ± 0.0c
	(−51.70)	(−25.30)	(−83.5)	(−87)
One way ANOVA (F Statistics)	9.8^{***}	2.3 NS	23.688^{***}	10.6397^{**}

Values presented are means ± SE. *, **, *** = significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at p = 0.05 according to LSD. Values in the parenthesis indicates percentage of the inhibitory (−) effects in comparison with control (T_0).

Table 4. Effect of seed and leaf extracts of *A. mexicana* on seedling length (mm) of *B. dictyoneura* and *C. ternatea*.

	C. ternatea		*B. dictyoneura*	
Extract Concentrations	Seed Extracts	Leaf Extracts	Seed Extracts	Leaf Extracts
T_0 (Control)	141.7 ± 3.31b	144.2 ± 2.83c	89.4 ± 1.5d	88.8 ± 1.89d
T_1 (25%)	129.6 ± 6.56ab	110.4 ± 8.33a	68.5 ± 1.67c	65.2 ± 1.54c
	(−8.5)	(−23.4)	(−23.4)	(−26.6)
T_2 (50%)	126.8 ± 7.12ab	97.4 ± 13.79a	44.1 ± 2.25b	39.7 ± 1.65b
	(−10.5)	(−32.5)	(−50.7)	(−55.3)
T_3 (75%)	108.2 ± 11.23ac	108.8 ± 1.59a	30.5 ± 1.14a	29.6 ± 1.48a
	(−23.6)	(−24.5)	(−65.9)	(−66.6)
T_4 (100%)	96.6 ± 14.01c	20.2 ± 0.0b	27.3 ± 0.51a	28.5 ± 0.78a
	(−31.8)	(−86)	(−69.5)	(−67.9)
One way ANOVA (F Statistics)	4.07^{*}	14.362^{***}	267.277^{***}	233.903^{***}

Values presented are means ± SE. *, **, *** = significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at p = 0.05 according to LSD. Values in the parenthesis indicates percentage of the inhibitory (−) effects (%) in comparison with control (T_0).

seedling lengths of *C. ternatea* in control treatment was 141.7 mm long while at concentrations of 25%, 50%, 75% and 100% of seed extracts were 129.6, 126.8, 108.2 and 96.6 mm respectively. In leaf extracts, seedling length of *C. ternatea* was significantly ($p \leq 0.001$) reduced as extract concentrations was increasing. Inhibition percentages of seedling length caused by leaf extract concentrations of 25%, 50%, 75% and 100% were −23.4%, −32.5%, −24.5% and −86% respectively. Mean seedling length of *C. ternatea* at concentrations of 25%, 50%, 75% and 100% leaf extract of *A. mexicana* was 110.4, 97.4, 108.8 and 20.2 mm respectively. Hence seedling length was decreasing with increase in extract concentration relative to the control treatment in which the seedling length was 144.2 mm long. Mean seedlings length of *B. dictyoneura* treated with seed extracts *of A. Mexicana* were significantly ($p \leq 0.001$) reduced compared with control treatment by −23.4%, −50.7%, −65.9% and −69.5% in response to the concentrations of 25%, 50%, 75% and 100% respectively (**Table 4**). Increase in inhibition percentages caused the decrease in seedling lengths whereby in control treatment the mean seedling length of 89.4 mm long was recorded while at concentrations of 25%, 50%, 75% and 100% seedling length was 68.5, 44.1, 30.5 and 27.3 mm respectively. The similar trend was observed in seedlings treated with leaf extracts whereby significant ($p \leq 0.001$) reduction in seedling lengths was observed with increasing concentrations in the extracts. Mean seedling lengths of *B. dictyoneura* in the control treatment was 88.8 mm while those treated with concentrations of 25%, 50%, 75% and 100% leaf extracts had length of 65.2, 39.7, 29.6 and 28.5 mm respectively. Hence length inhibition impact caused by *A. Mexicana* leaf extract at concentrations of 25%, 50%, 75% and 100% was −26.6%, −55.3%, −66.6% and −67.7% respectively.

3.4. Effects of Aqueous Extracts of *A. mexicana* on Fresh Weight and Dry Weight (mg)

Mean seedling fresh weight and dry weights of *B. dictyoneura* are presented in **Table 5**. The fresh weight and dry weight of *B. dictyoneura* seedlings were significantly ($p \leq 0.001$) affected upon treatment with different concentrations of leaf and seed extracts of *A. mexicana*. In seed and leaf extract of *A. mexicana* the fresh weight and dry weight of *B. dictyoneura* decreased with increasing concentrations in the extracts.

The mean fresh weights of *B. dictyoneura* in the control treatment was 22.3 mg while at concentration of 25%, 50%, 75% and 100% seed extracts, fresh weight was 15.5, 12.1, 8.5 and 5.9 mg respectively. Hence, in comparison with mean fresh weight in the control treatment, the fresh weight of *B. dictyoneura* at 25%, 50%, 75% and 100% seed extracts concentrations were reduced by −30%, −45.7%, −61.9% and −73.5% respectively.

With leaf extracts of *A. mexicana*, the mean fresh weights of *B. dictyoneura* decreased with increase in extracts concentration. Mean fresh weight of seedling treated with leaf extract at concentrations of 25%, 50%, 75% and 100% was 22.7, 12.9, 10.7, 10.1 and 7.5 mg respectively. In comparison with mean fresh weight of control treatment, the mean fresh weights at concentrations of 25%, 50%, 75% and 100% leaf extracts was significantly inhibited by −43%, −52.9%, −55.6% and −67% respectively.

Phytotoxin of seed extract of *A. mexicana* also inhibited dry weight of *B. dictyoneura* at different concentra tion levels. The highest dry seedling weight of 1.97 mg was acquired in control treatment while at concentrations of 25%, 50%, 75% and 100% the mean dry weights were 1.4, 0.63, 0.29, and 0.17 mg respectively. The reduction percentage of seedling dry weight in the seed extract at concentrations of 25%, 50%, 75% and 100% were −28.9%, −68%, −85% and −91% respectively.

Results in **Table 5** illustrate that the leaf extracts also significantly ($p \leq 0.001$) decreased the seedling dry weight as compared with control treatment. The seedling dry weight of 1.8 mg was obtained in control treatment, while dry weights of 0.5, 0.33, 0.27 and 0.25 mg were recorded at concentrations of 25%, 50%, 75% and 100% respectively. The inhibition effect in dry weights of seedlings treated with leaf extracts at concentration of 25%, 50%, 75% and 100% were −72.2%, −81.7%, −85%, −86.1% respectively.

Mean fresh weight and dry weights in **Table 6**, demonstrated that seed and leaf extracts of *A. mexicana* sig nificantly decreased the seedling fresh and dry weight of *C. ternatea* as compared with control treatment.

Mean fresh weight of *C. ternatea* treated with seed extracts significantly decreased by −5%, −17%, −22% and −28% when treated with seed extract at concentrations of 25%, 50%, 75% and 100% respectively. Thereafter, the mean fresh weight of *C. ternatea* in the control treatment was 340 mg, while at concentrations of 25%, 50%, 75%

Table 5. Effect of seed and leaf extracts of *A. mexicana* on fresh and dry weight of *Brachiaria dictyoneura*.

	Seed Extracts		Leaf Extracts	
Extract Concentrations	Fresh Weight (mg)	Dry Weight (mg)	Fresh Weight (mg)	Dry Weight (mg)
T_0 (Control)	22.3 ± .29d	1.97 ± 0.25b	22.75 ± 1.89c	1.8 ± 0.11b
T_1 (25%)	15.6 ± 0.71c	1.4 ± 0.37b	12.97 ± 0.58b	0.5 ± 0.18a
	(−30)	(−28.9)	(−43)	(−72.2)
T_2 (50%)	12.1 ± 0.74b	0.63 ± 0.11a	10.71 ± 0.59ab	0.33 ± 0.19a
	(−45.7)	(−68)	(−52.9)	(−81.7)
T_3 (75%)	8.5 ± 1.28a	0.29 ± 0.01a	10.1 ± 0.59ab	0.27 ± 0.17a
	(−61.9)	(−85.3)	(−55.6)	(−85)
T_4 (100%)	5.9 ± 0.81a	0.17 ± 0.04a	7.5 ± 0.7a	0.25 ± 0.15a
	(−73.5)	(−91.4)	(−67)	(−86.1)
One way ANOVA (F Statistics)	37.8702^{***}	12.226^{***}	27.051^{***}	16.614^{***}

Values presented are means ± SE. *, **, *** = significance at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at p = 0.05 according to LSD. Values in the parenthesis indicates percentage of the inhibitory (−) effects in comparison with control (T_0).

Table 6. Effect of seed and leaf extracts of *A. mexicana* on fresh and dry weight of *Clitoria ternatea*.

	Seed Extracts		Leaf Extracts	
Extract Concentrations	Fresh Weight (mg)	Dry Weight (mg)	Fresh Weight (mg)	Dry Weight (mg)
T_0 (Control)	340 ± 15.31c	33.5 ± 2.08c	343 ± 7.9b	35.1 ± 1.64c
T_1 (25%)	321.7 ± 17.77bc	25.4 ± 3.07b	240.9 ± 30.9a	18.2 ± 1.83ab
	(−5)	(−24)	(−30)	(−48)
T_2 (50%)	282.5 ± 15.94ab	15.4.0 ± 2.26a	168.0 ± 29.9a	13.7 ± 1.71a
	(−17)	(−54)	(−51)	(−61)
T_3 (75%)	263.9 ± 47.52ab	13.9 ± 1.25a	144.8 ± 45.46a	13.7 ± 4.13a
	(−22)	(−59)	(−58)	(−61)
T_4 (100%)	243.4 ± 18.74a	11.9 ± 1.37a	134.5 ± 0.0a	10.2 ± 0.0a
	(−28)	(−64)	(−61)	(−71)
One way ANOVA (F Statistics)	4.305^{*}	16.25^{***}	8.99^{**}	65.87^{***}

Values presented are means ± SE. *, **, *** = significance at $p \le 0.05$, $p \le 0.01$, $p \le 0.001$ respectively. NS = not significant, SE = Standard error of mean. Means followed by similar letter in a column are not significantly different from each other at p = 0.05 according to LSD. Values in the parenthesis indicates the percentage of inhibitory (−)effects in comparison with control (T_0).

and 100% fresh weight was 321.7, 282.5, 263.9 and 243.4 mg respectively. Similar trend was evident with leaf extract whereby the mean fresh weight of *C. ternatea* decreased with increasing concentrations of A. *mexicana*. Mean fresh weight at concentrations of 25%, 50%, 75% and 100% leaf extract was 240.9, 168, 144.8 and 134.5 mg respectively. Relative to the control treatment, the mean fresh weight of *C. ternatea* treated with concentration of 25%, 50%, 75% and 100% leaf extract were significantly ($p \le 0.01$) reduced by −30%, −51%, −58% and −61% respectively Dry weight of *C. ternatea* was significantly ($p \le 0.01$) reduced by seed extracts compared with the control treatment (**Table 6**). The decrease in seedling dry weight with seed extracts was −24%, −54%, −59% and −64% in response to seed extracts concentrations of 25%, 50%, 75% and 100% respectively. Hence, the highest dry weight of 33.5 mg was acquired in the control treatment while at concentrations of 25%, 50%, 75% and 100% seed extracts, the dry weight were 25.4, 15.4, 13.9 and 11.9 mg respectively. In leaf extracts of *A. mexicana*, the trend of dry weights was similar to that of seed extracts whereby dry weight of *C. ternatea* decreased with increasing concentration. Dry weight of seedlings in the control treatment was higher (35.1 mg) than seedlings that treated with leaf extracts of different concentrations. Dry weights of *C. ternatea* seedlings were 18.2, 13.7, 13.7, and 10.2 mg in response to leaf extract concentrations of 25%, 50%, 75% and 100% respectively. Therefore, mean dry weights of *C. ternatea* were inhibited by −48%, −61%, −61% and −71% when treated with leaf extract at concentrations of 25%, 50%, 75% and 100% respectively.

4. Discussion

This study clearly shows that there is phototoxic effect of aqueous extracts of leaves, and seeds of *A. mexicana* on the germination and growth of *C. ternatea* and *B. dictyoneura.* Higher concentrations of leaf and seed extracts had a higher degree of germination inhibition. This might have been caused by some of allelochemicals present in leaf and seed aqueous extracts of *A. mexicana*. These results correlates with studies conducted by Burhan and Shaukat, [21] Paul and Begum [22] and Alagesaboopathi [23] who reported seed germination inhibition of *Sorghum bicolor*, carrot, wheat, mustard, turnip, pearl-millet, blackgram, rapeseed wheat and corn with increase in *A. mexicana* leaf and shoot extracts concentration. The germination reductions in these studies were related to the allelopathic potential of *A. Mexican*. In detailed studies, Rice, [27] revealed that some of allelochemicals interrupted the mitotic activity of young cells, resulting in the inhibition of seed germination. Similar to our study, several researchers have reported germination inhibition in some cultivated crop species. For instance, Chandra *et al.* [28] and Esmaeili *et al.* [29] reported that salicylic and vanillic inhibited seed germination of cowpea (*Vigna unguiculata*) and Barnyard grass (*Echinochloa crus-galli* L) respectively.

In this study, shoot, root and seedling growth of *C. ternatea* and *B. dictyoneura* treated with different concentrations of seed and leaf extracts of *A. mexicana* were reduced with increasing concentrations. Reduction in seedling growth might have been caused by some of allelochemicals. According to studies by Cruz-Ortega *et al.* [12] Colpas *et al*. [13] and Cruz *et al.* [14] some allelochemicals from *A. mexicana* were reported to interrupt the process of cell division and elongation in roots and shoots which in turn reduced the seedling growth. Barkosky and Einhellig [30] also found that the growth of soybean seedlings was reduced with high concentrations of phydroxybenzoic acid. Chen *et al.* [31] postulated that exudates of vanillin and cinnamic acid posed allelopathic effect on egg plant seedling growth at high level concentration. Elsewhere, other researchers found that cinnamic acid was an allelochemical responsible for allelopathy for root growth in cucumber [32] and shoot and root length of cabbage seedlings [33]. Hence results on seedling growth found in this study conforms to findings reported by Alagesaboopathi [23]; Paul and Begum [24]; Jilani *et al.* [25]; Burhan and Shaukat [21] who reported allelopathic effect of *A. mexicana* on the growth of tomato, sorghum, lentil, carrot, wheat, mustard, turnip, pearlmillet, corn, blackgram and rapeseed.

In comparative analysis between control treatment and aqueous extract treatments, the root lengths were greatly inhibited than shoot lengths. The difference in the extents to which roots and shoots were affected by aqueous extracts in this study may be due to the contact of the roots with the filter paper, leading to constant absorption of the extract solution [34]. Also Nishida *et al.* [35] postulated that permeability of allelochemicals to root tissues is greater than in shoots. Chon *et al.* [36] reported that root length is good indicator of allelopathic effect of plant extracts because it is more sensitive to phytotoxic compounds than shoot growth. Keshavarzi *et al.* [37] also concluded that extracts has more impact on radicle length, because it has direct contact with radicle. Similar kind of observation was also reported by Sarkar *et al.* [34] who studied allelopathic effect of *Cassia tora* on seed germination and growth of mustard.

The present study also found that seed and leaf extract of *A. mexicana* significantly reduced fresh and dry weights of *B. dictyoneura* and *C. ternatea* with increasing extracts concentration. Decrease in fresh and dry weight might be attributed to allelochemicals inhibiting protein and carbohydrate synthesis and hence reducing seedling growth [11,16,38]. Similar to our study, Alagesaboopathi [23] also reported the decrease in fresh and dry weights of sorghum upon treatment with different concentrations of *A. mexicana* leaf aqueous extracts.

Overall, leaf extracts were found to have more allelopathic effect compared with seed extracts in most of parameters tested. Similar findings were also reported by Paul and Begum [22]; Cipollini and Flint [39] in blackgram, rapeseed, wheat and native woodland plants. Moreover, in this study the allelopathic effects of leaf and seed extracts of *A. mexicana* was stronger in grass species (*B. dictyoneura*) than in the leguminous species (*C. ternatea*). This observation might be due to the fact that some leguminous species has allelopathic potentials. Study done by Piyatida and Kato-Nunguchi [40] concluded that *C. ternatea* has inhibitory effect on the growth of lettuce (*Lactuca sativa* L), alfalfa (*Medicago sativa* L) and timothy (*Phleum pretence* L).

5. Conclusion

From the present study, it is evident that, *A. mexicana* has allelopathic effect on the germination, growth and development of *C. ternatea* and *B. dictyoneura*. The allelochemicals compounds in *A. mexicana* might have inhibited the seed germination, seedling growth in the tested plant species. However, the inhibition was dose dependent as there was great inhibition at higher extract concentrations. *A. mexicana* being allelopathic and invasive alien species in Tanzania it should be controlled in natural ecosystems and agricultural fields otherwise may suppress crops and native plant species. However, further research is needed to isolate and identify specific allelochemicals presented in *A. mexicana* and mechanisms which caused inhibition in the tested species.

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