American Journal of Plant Sciences, 2013, 4, 2332-2339
Published Online December 2013 (http://www.scirp.org/journal/ajps)
http://dx.doi.org/10.4236/ajps.2013.412289

Allelopathic Effect of Seed and Leaf Aqueous Extracts of *Datura stramonium* on Leaf Chlorophyll Content, Shoot and Root Elongation of *Cenchrus ciliaris* and *Neonotonia wightii*

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Received September 29th, 2013; revised November 1st, 2013; accepted November 12th, 2013

ABSTRACT

Pot experiment was carried out to determine the allelopathic effects of *Datura stramonium* on leaf chlorophyll content, root and shoot elongation, fresh and dry weight of two wild plant species: *Cenchrus ciliaris and Neonotonia wightii*. Different concentrations (0%, 25%, 50%, 75% and 100%) from seed and leaf extracts of *D. stramonium* were used to investigate the allelopathic effects of *D. stramonium* on growth of tested species. The total chlorophyll content of *N. wightii* was significantly reduced in all plants treated with both aqueous seed and leaf extracts of *D. stramonium*. In *C. ciliaris*, the total chlorophyll content was also significantly reduced for those plants treated with aqueous seed extract and leaf extract from *D. stramonium*. Relative to the control treatments, there was greater reduction in root and shoot length which was observed in higher concentrations of aqueous seed and leaf extracts. Fresh and dry weight of tested species significantly decreased after being treated with both seed and leaf aqueous extracts of *D. stramonium*. It was found that the allelopathic effect of aqueous seed and leaf extracts from *D. stramonium* on tested species was concentration-dependent. The inhibitory effects on all tested species increased as the concentration of both extracts increased from 0% to 100%. This study concluded that aqueous seed and leaf extract of *D. stramonium* have allelopathic effects on leaf chlorophyll content, root and shoot length, fresh and dry weight of grass (*C. ciliaris*) and legume (*N. wightii*) species.

Keywords: Chlorophyll Content; *Datura stramonium*; Photosynthesis; Allelochemicals; Allelopathy; Inhibitory Effect

1. Introduction

Chemical exudates from some plants to the environment have been reported as causative agents of allelopathy on growth of neighboring plants and thus affecting normal growth in their natural environment [1-5]. The allelopathic potential of some plants through the release of allelochemicals has either deleterious or beneficial effects on other plants associated in same locality [6]. Allelopathy has been defined in several literatures as the act of one plant to inhibit or stimulate the growth of other neighbouring plants through the release of allelochemicals to the surrounding environment [1,4,7-9]. The action of allelochemicals on plants is diverse and it involves a large number of biochemical reactions resulting into their modifications and finally affects the overall growth of both target plants and others in the vicinity [6,10]. Amongst other parameters, total chlorophyll content, shoot and root length are highly affected by activities of the allelochemicals released to the environment.

Chlorophylls are complex molecules positioned to suit for light absorption, energy and electron transfer, the functions carried out in photosynthesis process [2]. These molecules are key biochemical component of pigment-protein complexes embedded in the photosynthetic membranes that are responsible for photosynthesis process [2,11]. Leaf chlorophyll content has been used as one of the elemental parameters in understanding the response of the plant to the environmental stress in which it inhabits [12,13]. Therefore, it is likely that healthy plants which are able to grow well are also expected to have

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larger quantity of chlorophyll than unhealthy ones [13]. The decrease in leaf chlorophyll content due to allelopathic effects has been reported [2,14,15]. Apart from blocking the biosynthetic pathway of chlorophyll, allelochemicals can stimulate the degrading pathway of chlorophyll and reduce its accumulation which in turn affects photosynthesis process and diminishes the total plant growth [2]. However, the photosynthesis process in plant is affected because plant's photosynthetic potential is directly proportional to the amount of chlorophyll present in the leaf tissues which play an important role in photochemical reactions [12,16]. It has been reported that the allelochemicals released to the environment by poisonous plants have significant effects on photosynthesis and respiration which have been recognized as the best-characterized results of allelopathic interactions [10]. Apart from total chlorophyll content, the effects of allelochemicals released by invasive alien species on root and shoot growth of neighbouring plants have been reported [4,17-20]. Furthermore, allelochemicals pose great effects on whole root system of plant such as reduction in number of roots, swelling or necrosis of root tips, lack of root hairs, reduced dry weight accumulation and lowered reproductive capacity of plant [17]. *D. stramonium* as an invasive alien species contains a series of allelochemicals including atropine, hyoscyamine, and scopolamine [21-24], which affects the growth and development of shoot and root of other plant species. Generally, when plants are exposed to stress condition posed by allelochemicals, their growth and survival are highly affected [17]. Considering the importance of legume and grass species in the survival of biodiversity, a study was conducted to explore the allelopathic effects of *D. stramonium* on growth of two wild plant species: *Cenchrus ciliaris* and *Neonotonia wightii*.

2. Materials and Methods

2.1. Experimental Site

Experiments to determine the allelopathic effects of leaf and seed aqueous extracts of *Datura stramonium* on leaf chlorophyll content, shoot and root elongation of grass and legume species; *Cenchrus ciliaris* and *Neonotonia wightii* respectively were conducted at Nelson Mandela African Institution of Science and Technology (NM-AIST) in Arusha, Tanzania.

2.2. Collection of Seeds, Leaves and Sample Preparation

Seeds and leaves of matured *D. stramonium* plants were collected in April, 2013 in Ngorongoro Conservation Area (NCA); a protected area located in the northern part of Tanzania. With regards to test species, seeds of *N. wightii* were collected at Tropical Pesticides Research Institute (TPRI) while seeds of *C. ciliaris* were collected from the field (NCA). Both seeds and leaves were stored in a room with constant temperature of 25˚ centigrade at NM-AIST laboratory in Arusha, Tanzania ready for experiments. Leaves of *D. stramonium* were dried in air for 20 days and then crushed in to powder form. Dried seeds of matured plants of *D. stramonium* were also crushed in to powder form.

2.3. Preparation of Concentrations

The powdered sample from leaves was weighed to get 50 g. The mixture was then made by mixing 50 g of powdered leaves and 500 ml of distilled water in a conical flask. With its mouth closed, the conical flask was kept for 72 hours in a room at a constant temperature of 25 degrees centigrade to allow the auto extraction of plant metabolites. The mixture was purposely kept in dark room to avoid undesired reaction that might be caused by direct light. The same procedures were used to obtain the mixture of 50 g of powdered seeds and 500 ml distilled water. Both mixtures were filtered using muslin cloth to get stock solution with concentration of 0.1 g/ml (100%). From stock solution, four concentrations; 25%, 50%, 75% and 100% were then made on the basis of percentage.

2.4. Seeds Germination in Pots

Seeds of *N. wightii* and *C. ciliaris* were sterilized by washing them in Sodium hypochlorite 5% for 2 minutes to avoid the effects of fungal contamination. Sterilized seeds were then washed thoroughly three times in distilled water before planting. Ten seeds from each tested native species were then planted in pots (30 cm diameter and 3 kg by weight) and then treated with different concentrations of leaf and seed extracts prepared from *D. stramonium*. The experiment consisted of 80 pots where 16 pots were treated with distilled water as control (T1) and 64 pots were treated with *D. stramonium* seed and leaf aqueous extracts separately based on the concentration level (25%, 50%, 75% and 100%). However, 32 pots were treated with aqueous leaf extract while the other 32 pots were treated with aqueous seed extract of *D. stramonium*. Each treatment had four replicates. All pots were randomly repositioned time to time to avoid the effect of sunlight. After 9 weeks of growth, the experiments were terminated and plants of *N. wightii* and *C. ciliaris* from each pot were harvested and final required measurements were taken.

2.5. Determination of Leaf Chlorophyll Content

A plant leaf of each species; *C. ciliaris and N. wightii*

from the tip was collected from each pot. One hundred (100) mg of the middle portion of fresh leaf slices were placed in a 15 ml vial containing 7 ml Dimethyl sulphoxide (DMSO) and then incubated at 4˚C for 72 hours. The extracts were diluted to 10 ml with DMSO after incubation. The use of DMSO is a simple extraction technique as it extracts chlorophyll from plant tissue without grinding or maceration [25]. A 2 ml sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (2800 UV/ Visible Spectrophotometer) was used to determine the absorbance values at 645 nm and 663 nm relative to a DMSO blank. The values obtained from samples were then used in the equation proposed by Arnon (1949) [26] to determine the total leaf chlorophyll content against DMSO blank, expressed as mg L^{-1} as follows:

Chlorophyll total ($Chl_t = 20.2D_{645} + 8.02D_{663}$).

2.6. Determination of Root and Shoot Length

Final measurements were taken after nine weeks of growth of test species after termination of the experiment. Roots and shoots length of all plants in each replicate were measured in millimeters (mm) using ruler. The fresh weight of plants in each pot was determined and the averages were calculated. After recording the fresh weight, the plants were placed in labeled envelope and put them inside an oven maintained at 55˚C for 5 days. The dry weights of plants were recorded and the data obtained were analyzed.

2.7. Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to analyze data recorded from the study. Significant means were separated according to Fishers least significant difference (LSD) at $p = 0.05$. The software program STATISTICA version 10 was used to perform statistical analysis.

3. Results

The results presented in (**Tables 1**, **2** and **3**) showed clearly the effects of *D. stramonium* on growth parameters of grass and legume species (*C. ciliaris and N. wightii* respectively). Parameters which were investigated in this study include; leaf chlorophyll content, root and shoot length as well as fresh and dry weight.

In legume plants, the total chlorophyll content was ultimately affected and its accumulation was significantly reduced ($p < 0.001$) in all plants treated with both aqueous seed and leaf extracts of *D. stramonium*. The total chlorophyll content in grass species (*C. ciliaris*) was significantly reduced ($p < 0.001$) for those plants treated with aqueous seed extracts and ($p < 0.05$) in those treated with aqueous leaf extract from *D. stramonium* (**Table 1**). High inhibitory effects were exhibited at higher concentrations particularly in 100% concentration. In *N. wightii*, the total leaf chlorophyll content was significantly lowered to 9.8 mg/L in treatments involving seed extract while in leaf extract, the chlorophyll content was reduced to 8.4 mg/L. Compared with controls, the total leaf chlorophyll content in *Cenchrus ciliaris* was significantly lowered to 7.8 mg/L for aqueous seed extract and 8.8 mg/L for those treated with aqueous leaf extract from *D. stramonium*. The results showed that there was high chlorophyll accumulation in control pots whereby the highest value was 15.3 mg/L and 13.5 mg/L for *N. wightii* and *C. ciliaris* respectively. The capacity of

Table 1. The effects of aqueous seed and leaf extracts of *Datura stramonium* on total chlorophyll content of *Neonotonia wightii* and *Cenchrus ciliaris.*

Extract concentration (%)	Total chlorophyll content (mg/L)			
	Neonotonia wightii		*Cenchrus ciliaris*	
	Aqueous seed extract	Aqueous leaf extract	Aqueous seed extract	Aqueous leaf extract
T1 (0%) = control	15.2 ± 0.39 d	15.3 ± 0.43 d	13.5 ± 0.70 d	13.0 ± 0.72 c
T2 (25%)	13.4 ± 0.26 a	12.3 ± 0.14 c	11.6 ± 0.72 c	11.4 ± 0.74 bc
T3 (50%)	12.6 ± 0.28 a	10.8 ± 0.46 b	10.2 ± 0.12 bc	10.5 ± 0.67 ab
T4 (75%)	11.2 ± 0.23 c	9.4 ± 0.42 a	8.9 ± 0.60 ab	9.8 ± 0.64 ab
T5 (100%)	9.8 ± 0.17 b	8.4 ± 0.47 a	7.8 ± 0.58 a	8.8 ± 0.88 a
	One-way ANOVA			
F-statistic	57.735^{***}	44.216^{***}	14.651^{***}	4.670^{*}

Values presented are means ± SE. *, **, *** = significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. T1, T2, T3, T4 and T5 are levels of concentrations. Means followed by different letters in the same column are significantly different from each other at $P = 0.05$ according to Fishers Least Significant Difference test.

Table 2. The effects of aqueous seed and leaf extracts of *Datura stramonium* on growth of *Cenchrus ciliaris.*

Extract Concentration (%)	Aqueous seed extract					Aqueous leaf extract				
	Germination (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)	Germination (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
T1 (0%) = Control	100.0 ± 0.00 c	143.8 ± 5.51 c	69.3 ± 1.93 d	427.4 ± 21.36 c	73.3 ± 2.49 c	100.0 ± 0.00 c	170.0 ± 18.26 c	79.3 ± 4.71 c	458.0 ± 8.00 b	73.5 ± 1.72 c
T2 (25%)	60.0 ± 7.07 b	91.8 ± 11.85 b	60.8 ± 5.39 cd	344.6 ± 25.89 bc	53.3 ± 2.79 b	65.0 ± 6.45 b	98.0 ± 10.45 b	55.3 ± 5.33 b	368.2 ± 33.54 b	53.4 ± 0.79 b
T3 (50%)	50.0 ± 5.77 b	73.5 ± 8.66 ab	53.3 ± 1.25 bc	311.9 ± 26.94 b	45.3 ± 6.14 b	45.0 ± 6.45 ab	82.3 ± 9.78 b	47.3 ± 4.78 b	274.3 ± 32.16 a	37.1 ± 8.03 a
T4 (75%)	32.5 ± 6.29 a	71.3 ± 9.24 ab	45.8 ± 4.51 ab	130.3 ± 38.24 a	17.4 ± 2.78 a	47.5 ± 6.29 a	45.3 ± 3.97 a	33.3 ± 3.01 a	243.7 ± 34.48 a	32.0 ± 7.15 a
T5 (100%)	32.5 ± 6.29 a	50.3 ± 10.35 a	35.5 ± 2.03 a	109.6 ± 36.76 a	13.2 ± 3.65 a	30.0 ± 10.00 a	33.7 ± 2.60 a	28.0 ± 1.00 a	181.4 ± 38.54 a	32.6 ± 4.17 a
					One-way ANOVA					
F-statistic	23.750***	14.185***	12.296***	20.728***	41.322***	18.055***	22.810***	21.537***	12.174***	11.114***

Values presented are means ± SE. *, **, *** = significance at P < 0.05, P < 0.01 and P < 0.001 respectively. T1, T2, T3, T4 and T5 are levels of concentrations. Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers Least Significant Difference test.

Table 3. The effects of aqueous seed and leaf extracts of *Datura stramonium* on growth of *Neonotonia wightii.*

Extract Concentration (%)	Aqueous seed extract					Aqueous leaf extract				
	Germination (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)	Germination (%)	Shoot length (mm)	Root length (mm)	Fresh weight (mg)	Dry weight (mg)
T1 (0%) = Control	100.0 ± 0.00 d	297.5 ± 25.62 c	174.5 ± 17.68 a	1337.3 ± 32.09 c	196.0 ± 9.24 d	97.5 ± 2.50 d	319.5 ± 12.02 e	192.5 ± 9.85 e	1321.9 ± 22.75 e	175.3 ± 13.45 d
T2 (25%)	72.5 ± 4.79 a	231.0 ± 21.40 bc	159.0 ± 19.21 a	591.4 ± 61.16 b	67.4 ± 13.52 c	67.5 ± 6.29 c	243.3 ± 24.21 d	144.0 ± 5.55 d	840.0 ± 46.5 d	102.1 ± 11.92 c
T3 (50%)	62.5 ± 6.29 a	214.3 ± 30.06 ab	153.0 ± 19.93 a	495.3 ± 9.72 b	34.1 ± 8.88 b	50.0 ± 4.08 b	171.3 ± 16.72 c	119.0 ± 8.90 c	599.5 ± 64.38 c	46.8 ± 20.59 b
T4 (75%)	40.0 ± 4.08 c	141.0 ± 39.19 a	103.0 ± 9.41 c	269.8 ± 11.14 a	2.2 ± 0.08 a	40.0 ± 8.16 ab	101.8 ± 9.44 b	70.3 ± 2.29 b	364.6 ± 27.87 b	6.1 ± 2.18 a
T5 (100%)	25.0 ± 6.45 b	49.8 ± 4.09 d	50.3 ± 7.95 b	176.5 ± 19.04 a	1.2 ± 0.18 a	27.5 ± 4.79 a	39.1 ± 7.22 a	38.8 ± 1.89 a	145.8 ± 2.76 a	3.6 ± 0.88 a
					One-way ANOVA					
F-statistic	35.043***	12.547***	10.582***	196.117***	93.622***	24.308***	53.722***	84.874***	135.623***	35.04***

Values presented are means ± SE. *, **, *** = significance at P < 0.05, P < 0.01 and P < 0.001 respectively. T1, T2, T3, T4 and T5 are levels of concentrations. Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers Least Significant Difference test.

plants treated with aqueous seed and leaf extracts to accumulate chlorophyll decreased as the concentration of both aqueous seed and leaf extracts of *D. stramonium* increased (**Table 1**).

The root and shoot lengths, fresh and dry weights of both test species were significantly reduced by aqueous extracts of *D. stramonium* (seed and leaf) (**Tables 2** & **3**).

Relative to controls, the greatest reductions in root and shoot length were observed at higher concentrations mostly in treatment 5 (100% conc.) of both aqueous seed and leaf extracts. Regarding *C. ciliaris*, the highest inhibitory effects were also observed at higher concentrations of aqueous seed and leaf extracts of *D. stramonium*. The lowest root length (35.5 mm and 28.0 mm) and shoot length (50.3 mm and 33.7 mm) of this grass species were seen in pots supplied with 100% of both aqueous seed and leaf extracts (**Table 2**). In comparison with plants in control pots (0%) which had higher root length (69.3 mm and 79.3 mm) and shoot length (143.8 mm and 170 mm), the length of treated plants were negatively influenced by the increase in level of concentration from 25% - 100% (**Table 2**). Concerning the legume species (*N. wightii*), the highest root length (174.5 mm and 192.5 mm) and shoot length (297.5 mm and 319.5 mm) were found in the control treatments contrast to pots which were treated with aqueous seed and leaf extracts of *D. stramonium*. The lowest root length (50.3 mm and 38.8 mm) and shoot length (49.8 mm and 39.1 mm) were seen in pots supplied with 100% of both aqueous seed and leaf extracts respectively (**Table 3**).

The results presented (**Tables 2** & **3**), clearly demonstrate the allelopathic effects of aqueous seed and leaf extract of *D. stramonium* whereby both fresh and dry weight of *C. ciliaris* and *N. wightii* were reduced when compared with control treatments. The weights of both native species under study were significantly reduced in both treatments which involved leaf and seed aqueous extracts of *D. stramonium*. The highest fresh weight values of *C. ciliaris* were observed in control treatments which were 427.4 mg and 458.0 mg while the lowest values (109.6 mg and 181.4 mg in aqueous seed and leaf extracts respectively) were found in higher concentration (100%). The same inhibitory effects were seen in dry weight where the highest weight values 73.3 mg and 73.5 mg were found in controls while the lowest values 13.2 mg and 32.6 mg were seen in pots supplied with 100% of both aqueous seed and leaf extracts of *D. stramonium* respectively (**Table 2**). In contrast with control treatments which had higher weight values, all *N. wightii* plants which were treated with different concentrations of seed aqueous extracts of *D. stramonium* exhibited high inhibitory effects. The highest fresh weight values of 1337.3 mg and 1321.9 mg and dry weight of 196.0 mg and 175.1 mg were observed in controls (**Table 3**). The lowest fresh weight values of 176.5 mg and 145.8 mg and dry weight values of 1.2 mg and 3.6 mg in plants treated with aqueous seed and leaf extracts respectively were found in pots supplied with 100% of both aqueous seed and leaf extracts.

4. Discussion

The aim of this study was to determine the allelopathic effect of leaf and seed aqueous extracts of *D. stramonium* on leaf chlorophyll content, root and shoot elongation of *C. ciliaris and N. wightii*. The results showed the reduction in total chlorophyll content and decrease in plant height due to reduced shoot and root elongation as a result of allelopathic effects of aqueous extracts from *D. stramonium.* Our results also conform to work by Oyerinde *et al*., (2009) [15] who revealed the decrease in chlorophyll a, chlorophyll b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which is a weed plant known to possess allelopathic characteristics. The data obtained strongly suggest that the decrease in leaf chlorophyll content of tested native species was influenced by the increase in concentrations of both aqueous extracts from *D. stramonium.* Similar to our study Siddiqui and Zaman (2005) [2] reported that the accumulation of chlorophyll and porphyrin contents of *Vigna radiate* (L.) wilczek seedlings was inhibited as the *Capsicum* leachates concentrations increased. Yang *et al*. (2002) [27] also found that the accumulation of chlorophyll and porphyrin contents of rice (*Oryza sativa*) seedlings were inhibited as the allelopathic phenolics concentration increased.

The reduction in total chlorophyll at different concentrations of seed and leaf aqueous extracts of *D. stramonium* compared with those in controls might be attributed by presence of allelochemicals. The study carried out by Peng *et al.* (2004) [14] also pointed out that the allelochemicals produced by invasive species affects the photosynthesis and plant growth by destroying the chlorophyll. Our results are also in agreement with the findings of Stupnicka-Rodzynkiewicz *et al*. (2006) [28] and Hussain and Reigosa (2011) [20] which reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants. Based on the results obtained in this study, we can postulate that root and shoot length, fresh and dry weight of both tested species were also affected due to reduction in plants' capacity to accumulate chlorophyll which is an essential component of food manufacturing process; the photosynthesis.

The results showed that the root and shoot length of both test species was reduced by aqueous seed and leaf extracts of *D. stramonium*. The results obtained suggest that the reduction in root and shoot length might be attributed by allelochemicals present in both aqueous seed and leaf extracts of *D. stramonium.* Our results corroborate with Hussain and Reigosa (2011) [20] who found the inhibitory effect of allelochemicals (phenolic compounds) on root and shoot length of *Dactylis glomerata*, *Lolium perenne* and *Rumex acetosa*. Similarly, it has been reported that the effects of allelochemicals includes inhibition or retarded germination rate, shoot and root growth [4,6,17]. The allelopathic effects of both aqueous seed and leaf extracts of *D. stramonium* on shoot and root growth of both tested species increased as their concentration increased. Arowosegbe *et al*. (2012) [29] reported that the increase in concentrations of leaf and root extracts of *A. ferox* also increased inhibitory effect on root and shoot elongation of beetroot and carrot. Another study conducted by Ashrafi *et al*. (2008) [30] found that the allelopathicity on germination and growth of wild barley (*Hordeum spontaneum*) increased with the increasing concentrations of sunflower extracts. This inhibitory effects on root and shoot elongation of tested species in our investigation might have been also contributed by reduction in cell division [19], due to damage of cell membrane caused by allelochemicals [1]. The retarded germination, root and shoot length, fresh and dry weights which have been observed in our study might be influenced by damage of root and shoot cells due to interference of nutrient uptakes and other growth processes caused by allelochemicals found in seed and leaf extracts of *D. stramonium*. Moreover, our results showed more inhibitory effects on root than shoot length. This might due to direct contact of root with the extracts containing inhibitory chemicals. Similar findings were reported by Quasem, (1995) [31] who investigated the allelopathic effect of three *Amaranthus* spp. (Pigweeds) on wheat (*Triticum durum*).

The reduction in growth of root probably had effects on both physiological and biological functions of a plant such as anchoring, absorption of water and other essential nutrients required by plant for its survival. This might have contributed to the decrease in both fresh weight and dry weight [17] of the test species. Our results suggest that both tested species exhibited significant decrease in fresh and dry weight after being treated with aqueous seed and leaf extracts of *D. stramonium*. Similar observation was established by Sahoo *et al*. (2010) [32] who reported the reduction in dry weight of chilli, soybean, maize, rice and lady's finger at higher concentrations of aqueous leaf extract from *Mangifera indica* L. The reduction in both fresh and dry weight of both tested species may be contributed by allelochemicals present in both aqueous seed and leaf extracts of *D. stramonium*. Choyal and Sharma (2011) [33] also reported that allelochemicals may exert allelopathic effect on growth and yield of neighbouring plants. This study revealed that the reduction in weight of both grass and legume species might be attributed by various allelochemicals present in *D. stramonium*.

5. Conclusion

It can be concluded that aqueous seed and leaf extract of *D. stramonium* had allelopathic effects on chlorophyll content, root and shoot length, fresh and dry weight of grass (*C. ciliaris*) and legume (*N. wightii*) species. However, further investigations should be conducted in the natural environment where these species grow in close association. The isolation and identification of allelochemicals present in *D. stramonium* should be investigated to establish if they can be used as natural herbicide to control other invasive alien species.

6. Acknowledgements

Our special thanks to Tanzania Commission for Sciences and Technology (COSTECH) and The Nelson Mandela African Institution of Science and Technology (NM-AIST) for financing this research work.

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