Allelopathic effects of *Parthenium hysterophorus* on seed germination, seedling growth, fresh and dry mass production of *Alysicurpus glumaceae and Chloris gayana*

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Abstract

Laboratory study was conducted to investigate allelopathic effects of leaf and seed aqueous extract of *Parthenium hysterophorus* on seed germination, seedling growth, fresh and dry mass production of two native plant species; *Chloris gayana (a grass)* and *Alysicurpus glumaceae* (legumes species). Different levels of *P. hysterophorus* leaf and seed aqueous extracts concentrations (25%, 50%, 75% and 100%) were used to test its effect on the test species. Results revealed significant allelopathic effects of leaf and seed aqueous extract *of P. hysterophorus* on seed germination, roots and shoot length, fresh and dry weight of all tested species. However, it was found that *Alysicurpus glumaceae* was more susceptible to both seed and leaf aqueous extract of *Parthenium hysterophorus* compared with *Chloris gayana.*

Key words: Alien invasive, allelochemicals, allelopathic, grass and legume species

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Introduction

Parthenium hysterophorus is herbaceous annual weed plant found in many parts of Tanzania. According to Nath, (1988) *P. hysterophorus* originated from natural hybridization between *Parthenium confertum* and *Parthenium bipinnatifidum*. As a result of its allelopathic effect, *P. hysterophorus* can establish itself rapidly in an alien environment and suppresses the growth of other native species. It is is one of the best-known plant invaders in the world, a phenomenon linking allelopathy to exotic invasion (Pandey, 1994; Khanchan and Jayachandra, 1979). According to Evans (1997), the invasive ability and allelopathic properties of *P. hysterophorus* posses a great risk to disrupt ecosystem.

The concept of allelopathy was firstly widely studied in forestry ecosystems where it was initially discovered that many of the forestry species investigated had negative allelopathic effects on food and fodder crops (Olofsdotter, 1998)). In connection to these and many other findings Olofsdotter (1998) defined allelopathy as a direct influence of chemicals released from one plant on the development and growth of another plant. Jalali et al. (2013) considered both positive and negative influences of allelochemicals by defining allelopathy as the ability of plants to inhibit or stimulate growth of other plants in the environment by exuding chemicals. Allelochemicals are plant secondary metabolites normally released into the environment through volatilization, leaching, root exudation and decomposition of plant residues in the soil (Khalaj et al., 2013). Putnam (1988) listed 6 classes of allelochemicals isolated from more than 30 families of terrestrial and aquatic plants including alkaloids, flavonoids, cinnamic acid derivatives, cyanogenic compounds, benzoxazinones and ethylene. To link between allelochemicals and allelopathy Makoi and Ndakidemi (2012) noted in their study that most of the allelopathic effects in plants are known to result from allelochemicals released from plants.

Once these allelochemicals get dissolved in the soil they may come into contact with other element of different physical, chemical, biological and physiochemical

properties which may influence activity of allelochemicals and therefore either amplify or reduce their impact on recipient plants (Inderjit, 1996; Blum et al,. 1993; Nair et al,. 1990).. The extent of the allelopathic effects resulting from allelochemicals concentration in soil may be affected by other factors such as soil pH, organic matter content, nutrient and moisture content and microorganisms (Blum 1995).

Field experiments by Parker and Reichard (1998), have confirmed that the presence of alien species can threaten the persistence of native species probably because of the negative effects of competition from the invasive alien on native species populations. In essence, allelopathy have the potentials to disrupt aspects of plant ecology such as dominance and occurrence, plant succession, plant communities and diversity, growth and plant productivity. Bhadoria (2011) produced an extended list of readily visible effects of allelochemicals on the growth and development of other plants. These effects include inhibition or retardation of germination rate; darkening and swollen of seeds; reduction of root and shoot length; swelling or necrosis of root tips; curling of the root axis; discoloration, lack of root hairs; increased number of seminal roots; reduced dry weight accumulation; and lowered reproductive capacity.

Irrespective of whether a native species produces allelochemicals or not, it can be significantly affected by allelochemicals produced by alien plants. A study conducted by Kumar and Gautum (2008) revealed that although sunflower imparts high allelopathic effects on the environment, it is heavily affected by allelochemicals released by *P. hysterophorus.* This study aimed at establishing the allelopathic effects of *P. hysterophorus* on seed germination, seedling growth, fresh and dry mass production of *A. glumaceae and C. gayana*.

Materials and Methods

Experimental site

Experiments were conducted at the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratories in Arusha, Tanzania.

Species Involved in the study

Three species were involved in the study. *A. glumaceae* and *C. gayana* which are native legumes and grass species respectively and an alien invasive *P. hysterophorus*.

Seeds Collection

Matured seeds of *C. gayana* and *P. hysterophorus* were collected from the field in Mwiba Wildlife Ranch, which is part of the greater Serengeti-Maasai Mara ecosystem and located northwestern side of Tanzania (It lies between S 03 57656 and E 3486983 on the west and S 03 50432 E 34 83561 on the western boundary). Seeds of *A. glumaceae* were obtained from seed bank at TPRI head office in Arusha.

Preparation of aqueous extract

Leaves of the vegetative full-grown *P. hysterophorus* plants were collected from the field and air-dried at room temperature (25^{o}C) for 20 days while seeds were collected from well-dried and matured plants in the field. The dried leaves and seeds were grinded separately to powder using laboratory blender. Distilled water was used as an extraction solvent whereby a 50g of powdered seeds/leaves were prepared and soaked in 500ml of distilled water. The mixture was kept in a conical flask with its top closed and stored in dark room for 72hrs at room temperature and thereafter filtered using muslin cloth to obtain a stock solution of 0.1g/ml concentration. The stock solution was then adjusted accordingly to obtain four different levels concentrations i.e. 25%, 50%, 75% 100% and denoted as T2, T3, T4 and T5 respectively. Distilled water was used as a control (T1).

Seed germination test

Before starting germination test, seeds were sterilized using sodium hypochlorite (5%) to remove any possible contaminations and then the seeds were washed thoroughly 4 times with distilled water.

Germination tests were done for the selected seeds of *A. glumaceae* and *C. gayana* whereby 10 seeds for each species were planted separately in Petri dishes with double layer towel paper. This experiment involved a total of 80 petri dishes 40pcs for each test species in which each treatment (T1, T2, T3, T4 and T5) was replicated 4 times to obtain 20 petri dishes for each native species for both leaf and seed aqueous extract of *P. hysterophorus.* Seeds of tested species were treated once with different levels of seed and leaf aqueous extract concentration of *P. hysterophorus*. Subsequently, seeds were then irrigated with distilled water regularly to retain moisture content and to allow seed to germinate.

Germination inhibition/stimulation

Percentages of inhibition/stimulation effect on seed germination over control (T1) were calculated using the formula proposed by Singh & Chaudhary (2011).

- Inhibition (-) or stimulation (+) = [(Germinated seeds in extracts - Germinated seed in control)/Geminated seeds in control] x 100.

Statistical analysis

Significance of the difference in germination percentage, root and shoot length, fresh and dry weight of seedlings under different treatments were tested and compared using One way Analysis of Variance (ANOVA). The analyses were done using a statistical package SATISTICA version 10. Significant means were compared at p=0.05 according to Fischer's least significant different test.

Results

Our findings demonstrated that *P. hysterophorus* exhibited significant allelopathic activities in all parameters measured (germination, shoot and root length, fresh and dry weight). As shown in Table 1, both leaf and seed aqueous extract significantly inhibited germination and reduced shoot and root length of *A. glumaceae*. The result obtained were concentration dependent as increasing seeds and leaf extract concentration significantly inhibited germination of *A. glumaceae*. The highest inhibitory effect (-96.7%) of seed extract compared with the control was recorded at T5 (100% concentration) while the lowest inhibitory effect (-38.3%) was observed at T2 (25% concentration). The results in Table 1 further shows that while the lowest inhibitory percentage value for leaf aqueous extract was (-61.5%) at T2 (25% concentration), this is nearly twice the value of the lowest inhibitory percentage in seed aqueous extract which is approximating -38.3% at same level of concentration T2 (25% concentration). The reduction pattern in shoot length and root length was highly similar for both leaf and seed aqueous extract as observed and recorded in Table 1.

Results presented in Table 2 indicate that both leaf and seed aqueous extract of *P. hysterophorus* significantly ($p < 0.001$) reduce dry and fresh weight of the *A. glumaceae*. The highest values of fresh weight were 121.8 mg and 91.4 mg for seed and leaf aqueous extract respectively compared with the control while dry weight values were 11.9 mg and 10.5 mg for seed and leaf aqueous extract respectively. The highest fresh weight value of 296.7 mg was observed at 0% concentration (T1 - control) for seed aqueous extract while the lowest value of 14.9 mg was recorded at 100% concentration (T5). Similarly, for leaf aqueous extract the highest fresh weight value of 235.7 mg was observed at 0% concentration (T1 – control) while the lowest value (16.5 mg) was observed at 100% concentration (T5). These results suggest that the fresh weight of *A. glumaceae* decreases significantly ($p < 0.001$) as concentration levels were increased. The lowest dry weight values were 1.8 mg and 2.45 mg at 100% concentration (T5) for seed and leaf aqueous extracts respectively.

Results presented in Table 3 demonstrate significant ($p<0.001$) allelopathic effect of both leaf and seed aqueous extract on seed germination, roots and shoot length of *Chloris gayana*. The inhibitory effect on seed germination and length reduction trends showed a significant concentration dependent effect whereby increased seed and leaf aqueous concentration led to increased inhibitory effect on germination and reduction in root and shoot length. The highest germination percentages were 97.5% and 100% in control treatment for seed and leaf aqueous extracts respectively. Inhibition percentage increased significantly ($p<0.001$) from -28.6 to -77.2% at 25% concentration and from -30.0 to -80.0% at 100% concentration for seed and leaf extract respectively. Similar trends were observed in shoot and root length reduction patterns whereby the highest values were recorded at T1 (control), which is equivalent to 48.7mm, and 49.9mm in shoots and 38.0mm and 41.5mm in roots for both seed and leaf aqueous extract respectively. Comparatively however, the results presented indicate that for both shoot and root length there is stronger inhibitory effect on leaf aqueous extract than seed aqueous extract. The lowest shoot and root length was 25.5mm and 19.0mm for seed aqueous extract while the lowest length for leaf aqueous extract was 21.3mm and 10.7mm for shoot and root length respectively.

Leaf and seed aqueous extract of *P. hysterophorus* showed significant allelopathic effect at $p<0.001$ on fresh and dry weight of *C. gayana* compared with the control treatment (Table 4). The lowest weight values in seed aqueous extracts were 7.8mg and 0.4mg for fresh and dry weight respectively compared with control while the lowest value in leaf aqueous extracts were 12mg and 0.3mg for fresh and dry weight respectively also compared with control. Generally, both fresh and dry weight decreased significantly with increased concentration of seed and leaf extracts of *P. hysterophorus*.

Discussion

In this study, we assessed the overall effect/response of the two native species across the whole range of allelochemical levels found in *P. hysterophorus.* As presented in our result, leaf and seed aqueous extract of *P. hysterophorus* had significant effect on seeds germination, reduction in shoot and root length as well as reduction of dry and fresh weight production. As the concentration levels increased, these effects were also escalated. These findings correspond with results by Mersie and Singh (1987) who found strong relation between increased aqueous extract concentrations of *P. hysterophorus* and increased toxicity to some agronomic crops and weed plants.

Both leaf and seed aqueous extracts imposed negative impacts on the test species. However, these effects were not the same for leaf and seed extract. This is in agreement with Sarkar et al. (2012) who argued that although allelochemicals are normally found throughout the plant body, its concentration may differs between plant parts. We found in our study that leaf aqueous extract of *P. hysterophorus* had slightly higher allelopathic effect on *A. glumaceae* plants than seed aqueous extract. Similar findings by Maharjan *et al*. (2007) showed that leaf aqueous extract of *P. hysterophorus* had the strongest allelopathic effect on seed germination than other vegetative parts. Tefera (2002) and Kanchan (1975) also demonstrated that the inhibitory allelopathic impact of leaf aqueous extract was more powerful than other vegetative parts of *Parthenium* plants. Similarly Srivastava *et al*. (1985) also reported that the aqueous extracts of leaves and inflorescences inhibited the germination and seedling growth of barley, wheat and peas. On the other hand Guzman (1988) argued that the differences in the intensity of inhibitory effect between plant parts could be attributed by the release of different kinds of phytotoxic compounds such as phenolics, sesquiterpenes and lactones, from root and vegetative part of living plants as well as from the achene by exudation.

Similar to our findings, Maharjan *et al*. (2007) also found that the germination of the tested species was significantly reduced with the increase in the

concentration of leaf aqueous extract of *P. hysterophorus* L. In this study, *P. hysterophorus* retarded the seedling growth and reduced the fresh and dry matter production. Related to these findings, Guzman (1988); Mersie and Singh (1988); Swaminathan et al. (1990); Evans (1997); Tamado et al. (2002) observed similar effect on maize and sorghum, multi purpose trees, pumpkin, and tomato.

An et al. (2005) stated that there could be marked differences among species in their susceptibility towards the effects of allelochemicals.

Results from this study showed that both leaf and seed aqueous extract of *P. hysterophorus* showed stronger inhibition effect on *A. glumaceae* (legume) than on *C. gayana* (grass). Seed aqueous extract had the highest inhibition percentage value of -77.2% for *C. gayana* (equivalent to 30% germination) compared with the lowest inhibition percentage value of -96.7% (equivalent to 2.5% germination) for *A. glumaceae* in seed aqueous extract. Comparatively for leaf aqueous extract, the highest inhibition percentage was -80% (equivalent to 20.0% germination) for *C. gayana* compared with inhibition percentage of -92.6% (equivalent to 10.0% germination) for *A. glumaceae*. Likewise, Creamer et al. (2006) found in their study that barley residues with allelopathy added to the soil surface of field plots inhibited emergence of *Solanum ptycanthum* (eastern black nightshade) by 98% and *Setaria glauca* (a yellow foxtail grass) by 81% for 30 days after planting the weed species in a soil with allelochemicals.

Conclusion

In conclusion, this study revealed that both seed and leaf aqueous extract *of P. hysterophorus* has significant effect on seed germination, seedling growth, dry and fresh weight of the two native species of *C. gayana* and *A. glumaceae*. However, the degree of susceptibility towards increased levels of aqueous

extract concentration differs between the two species. *A. glumaceae* was more susceptible to both seed and leaf aqueous extract of *P. hysterophorus* compared with *C. gayana.* It is further concluded that establishment and spread of *P. hysterophorus* may impart severe damages to the two native species tested in this study and other close relatives.

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Table 1: The effects of seed and leaf aqueous extracts of *Parthenium hysterophorus* on seed germination, shoot and root length of *Alysicurpus glumaceae*

Treatment	Seed extract				Leaf extract			
	Germination (%)	Inhibition (%)	Shoot length (mm)	Root length (mm)	Germination (%)	Inhibition (%)	Shoot length (mm)	Root length (mm)
T1 (0%= control)	97.5 ± 2.50c	-	41.1 ± 1.43d	32.1 ± 0.74d	90.0 ± 4.08c	-	38.3 ± 1.24d	24.4 ± 0.38b
T2 (25%)	60.0 ± 4.08b	-38.3 ± 4.41c	32.6 ± 0.50c	28.5 ± 0.45a	35.0 ± 5.00b	-61.5 ± 4.61b	31.4 ± 0.61b	22.4 ± 0.47b
T3 (50%)	30.0 ± 7.07a	-69.4 ± 6.83a	30.9 ± 1.50bc	28.5 ± 0.56a	22.5 ± 7.50ab	-74.4 ± 9.29ab	26.6 ± 1.54ab	14.9 ± 0.50a
T4 (75%)	20.0 ± 4.08a	-77.2 ± 4.55a	27.7 ± 1.08ab	25.0 ± 0.49c	13.3 ± 3.33ab	-88.5 ± 5.14a	27.0 ± 2.38a	17.2 ± 1.54a
T5 (100%)	2.5 ± 2.50a	-96.7 ± 3.33b	24.0 ± 0.00a	17.5 ± 0.00b	10.0 ± 0.00a	-92.6 ± 3.7a	20.0 ± 0.70c	17.4 ± 1.95a
				One-Way ANOVA				
F-statistic	72.95***	20.95***	20.63***	41.33***	36.74***	4.80*	19.27***	24.60***

Values presented are means ± SE. *, *** denote significant at P ≤ 0.1 and P ≤ 0.001 respectively. The treatment T1, T2, T3, T4 and T5 are levels of concentrations whereby T1 = (0% control), T2= (25% conc.), T3 = (50% conc.), T4 = (75% conc.) and T5 = (100% conc.). Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers LSD test.

Table 2: The effects of seed and leaf aqueous extracts of *Parthenium hysterophorus* on fresh and dry weight of *Alysicurpus glumaceae*

Treatment	Seed extract		Leaf extract	
	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
T1 (0%= control)	296.7 ± 11.88c	19.0 ± 0.67d	235.7 ± 29.42c	30.3 ± 6.23b
T2 (25%)	121.8 ± 7.16b	11.9 ± 0.75c	91.4 ± 21.05b	10.5 ± 2.19a
T3 (50%)	55.9 ± 16.88a	6.5 ± 0.94b	99.9 ± 27.37ab	4.6 ± 1.36a
T4 (75%)	26.4 ± 4.14a	4.3 ± 0.62ab	22.1 ± 3.61a	4.37 ± 0.75a
T5 (100%)	14.9 ± 0.00a	1.8 ± 0.00a	16.5 ± 0.25ab	2.45 ± 0.05a
			One-way ANOVA	
F-statistic	94.73***	64.49***	12.77***	10.48***

Values presented are means ± SE. *** Denote significant at P ≤ 0.001. The treatment T1, T2, T3, T4 and T5 are levels of concentrations whereby T1 = (0% control), T2= (25% conc.), T3 = (50% conc.), T4 = (75% conc.) and T5 = (100% conc.). Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers LSD test.

Table 3: The effects of seed and leaf aqueous extracts of *Parthenium hysterophorus* on seed germination, shoot and root length of *Chloris gayana*

Treatment	Seed extract				Leaf extract			
	Germination (%)	Inhibition (%)	Shoot length (mm)	Root length (mm)	Germination (%)	Inhibition (%)	Shoot length (mm)	Root length (mm)
T1 (0%=control)	97.5 ± 2.50d	-	48.7 ± 1.36d	38.0 ± 0.84d	100.0 ± 0.00d	-	49.9 ± 0.83e	41.5 ± 0.96d
T2 (25%)	70.0 ± 7.07c	-28.6 ± 5.78a	38.1 ± 1.37c	29.5 ± 0.61c	70.0 ± 7.07b	-30.0 ± 7.07c	41.8 ± 0.66d	26.5 ± 1.89c
T3 (50%)	57.5 ± 8.54bc	-41.4 ± 7.82a	33.5 ± 0.88b	25.8 ± 0.80a	52.5 ± 6.29ab	-47.5 ± 6.29bc	34.4 ± 0.42c	19.0 ± 0.81b
T4 (75%)	45.0 ± 6.45ab	-53.3 ± 7.82ab	28.7 ± 0.91a	23.5 ± 0.65a	40.0 ± 7.07a	-60.0 ± 7.07ab	28.7 ± 0.38b	13.9 ± 0.62a
T5 (100%)	30.0 ± 11.55a	-77.2 ± 10.98b	25.5 ± 2.7a	19.0 ± 2.37b	20.0 ± 7.07c	-80.0 ± 7.07a	21.3 ± 0.67a	10.7 ± 2.16a
				One-way ANOVA				
F-statistic	11.79***	6.19**	39.48***	44.13***	24.36***	9.35**	325.93***	74.00***

Values presented are means ± SE. **, *** Denote significant at P ≤ 0.01 and P ≤ 0.001 respectively. The treatment T1, T2, T3, T4 and T5 are levels of concentrations whereby T1 = (0% control), T2= (25% conc.), T3 = (50% conc.), T4 = (75% conc.) and T5 = (100% conc.). Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers LSD test.

Table 4: The effects of seed and leaf aqueous extracts of *Parthenium hysterophorus* on fresh and dry weight of *Chloris gayana*

Treatment	Seed extract		Leaf extract	
	Fresh weight (mg)	Dry weight (mg)	Fresh weight (mg)	Dry weight (mg)
T1 (0%= control)	36.7 ± 1.45e	3.9 ± 0.39b	44.9 ± 1.86d	5.4 ± 0.28d
T2 (25%)	26.7 ± 1.72d	3.3 ± 1.92ab	30.3 ± 1.81c	2.5 ± 0.43c
T3 (50%)	21.7 ± 0.80c	1.2 ± 0.10ab	20.7 ± 1.43b	1.2 ± 0.08b
T4 (75%)	13.8 ± 0.96b	0.8 ± 0.11a	14.9 ± 1.21ab	0.8 ± 0.08ab
T5 (100%)	7.8 ± 2.47a	0.4 ± 0.15a	12.0 ± 5.29a	0.3 ± 0.11a
		One-way ANOVA		
F-statistic	55.13***	2.74NS	23.29***	72.18***

Values presented are means ± SE. ***, NS Denote significant at P ≤ 0.1 and P ≤ 0.001 respectively. The treatment T1, T2, T3, T4 and T5 are levels of concentrations whereby T1 = (0% control), T2= (25% conc.), T3 = (50% conc.), T4 = (75% conc.) and T5 = (100% conc.). Means followed by different letters in the same column are significantly different from each other at P = 0.05 according to Fishers LSD test.