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Allelopathic Effects of *Pistia stratiotes* (Araceae) and *Lyngbya wollei* Farlow ex Gomont (Oscillariaceae) on Seed Germination and Root Growth

Jehangir H. Bhadha[1], Timothy A. Lang[2], Odiney M. Alvarez[1], Mihai C. Giurcanu[3], Jodie V. Johnson[4], Dennis C. Odero[5] & Samira H. Daroub[1]

[1] Soil and Water Science Deptment, University of Florida, Belle Glade, Florida, USA

[2] Agronomy, University of Florida, Belle Glade, Florida, USA

[3] Statistics Department, University of Florida, Belle Glade, USA

[4] Chemistry Department, University of Florida, Gainesville, USA

[5] Weed Science, University of Florida, Belle Glade, Florida, USA

Correspondence: Jehangir H. Bhadha, Everglades Research and Education Center, University of Florida, Belle Glade, FL-33430, USA. Tel: 1-561-993-1711. E-mail: jango@ufl.edu

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Abstract

Pistia stratiotes and *Lyngbya wollei* are the two most common aquatic weeds that flourish in farm canals within the Everglades Agricultural Area of Florida. Identifying a useful application of these weeds would not only address important environmental concerns, but would also be an incentive for farmers to harvest it. The objective of this study was to determine use of *P. stratiotes* and *L. wollei* as soil amendments for stimulation of seed germination and root growth in different plant species. The effects of different rates of dried and grounded *P. stratiotes* and *L. wollei* on germination and root length of snap bean, corn, sorghum, common lambsquarters, and rice were evaluated using a controlled petri-dish incubation bioassay study. Overall, both amendments had a negative allelopathic effect on germination of all species. The highest reduction in germination of 80 and 43% by *P. stratiotes* and *L. wollei* respectively was observed on corn. Rice was the most tolerant to allelopathic effects that emanated from both amendments. There was a significant positive increase in rice root length in response to *P. stratiotes* rate over the two-week period. This study shows that *P. stratiotes* can be used as a potential bio-fertilizer to stimulate early growth of rice.

Keywords: allelopathy, allelopathic index, bio-fertilizer, bio-herbicide, filamentous algae, germination, root length, sustainable agriculture, water lettuce

1. Introduction

The growth of floating and certain submerged aquatic vegetation is a primary management concern throughout Florida’s aquatic systems. In fiscal year 2005-2006, the US Department of Environmental Protection’s Bureau of Invasive Plant Management used approximately $173,000 to control these plants in springs, primarily using chemical herbicides (Evans, 2008). These aquatic vegetation are also commonly found in agricultural drainage canals where they cause various environmental problems, such as restricted drainage/irrigation flow and low dissolved oxygen levels (Bhadha et al., 2014) which can result in anaerobic conditions causing fish-kills, changes in aquatic communities, noxious odors, and health hazards (Mcpherson et al., 1976). There are many different species of aquatic vegetation such as water hyacinth (*Eichhornia crassipes* (Mart.) Solms), duckweed (*Lemna spp.*), torpedograss (*Panicum repens* L.), and alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) that flourish in farm canals within the Everglades Agricultural Area (EAA). The most abundant and problematic aquatic vegetation of concern in farm canals in the EAA are *Pistia stratiotes* L. (water lettuce) and *Lyngbya wollei* (Farlow ex Gomont) (filamentous algae) (Daroub et al., 2012; Evans, 2008; Bottcher & Izuno, 1994). *P. stratiotes* is a floating aquatic leaved macrophyte and *L. wollei* is a cyanobacterium characterized by entangled, long mat-forming filaments. Management practices used to reduce the negative impact of these two species include chemical, biological, and mechanical control (Mossler & Langeland, 2006). While nearly 90% of aquatic weeds within the EAA are chemically controlled, some research has recommended mechanical harvesting of these plants for concomitant removal of sequestered nutrients and for their use as soil amendments (Evans, 2008).

Prior to evaluating the role of using nuisance aquatic vegetation such as *P. stratiotes* and *L. wollei* as soil amendments and source of nutrients for crops, it is vital to first evaluate their potential allelopathic effects on seed germination and root growth of these crops. The concept of "allelopathy" was first addressed by Hans Molisch to describe both the beneficial and detrimental chemical interactions of plants and microorganisms (Molish, 1937). The subject of allelopathy has presently received much attention from scientists, with an increasing interest in recent years driven by the recognition that agro-ecological applications of allelopathy may provide beneficial alternatives to synthetic herbicides for weed management (Romeo & Weidenhamer, 1999). Numerous studies indicate the presence and emission of allelopathic compounds from aquatic plant species (Gross, 2003; Pflugmacher, 2002). Several allelopathic compounds such as α-asarone, hydroxyl fatty acids, and steroid derivatives have been isolated from *P. stratiotes* (Alliotta et al., 1991). These compounds inhibited growth of seventeen algal cultures (Alliotta et al., 1991). *L. wollei* has been shown to produce various saxitoxins and derivatives, collectively called paralytic shellfish toxins, which are highly potent neurotoxic alkaloids that are known to inhibit nerve conduction and muscle contraction by blocking sodium channels (Jaiswal et al., 2008; Mihali et al., 2011). In addition, Chauhan et al. (1992) and Bagchi et al. (1993) found that filamentous cyanobacterium *Oscillatoria sp*. produced and released allelopathic compounds that inhibited other cyanobacteria and chlorophytes. Limited information is available on the potential allelopathic effects of aquatic vegetation on crop growth and development because aquatic vegetation is not typically considered as possible nutrient source to grow crops due to its very high water content. Albeit, some farmers do mechanically harvest the floating aquatic vegetation and replenish it back on their fields without knowledge of any potential allelopathic effects.

The maintenance of soil organic matter pools generally requires a sustainable input of biomass. A variety of organic amendments have been used successfully to augment native organic matter inputs. These include manure (Kolenbrader, 1974; Mishra et al., 1974; Meek et al., 1982), composted municipal waste (Mays et al., 1973), and sewage sludge (King & Dunlop, 1982; Mcintosh et al., 1984). With each of these soil amendments, an attempt was made to solve the problems of waste disposal and meet a growing agronomic need. Aquatic weeds can serve as a reservoir of organic material for soil amendment and as a source of organic fertilizer particularly for sandy soils bordering the EAA. This study was conducted to investigate the effect of dried and grounded *P. stratiotes* and *L. wollei* applied directly on seeds of plant species commonly associated with the EAA. The specific objectives of the study were to (i) evaluate the effects of six rates of dried amendments of the two species on germination and root growth of snap bean (*Phaseolus vulgaris* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* (L.) Moench), and common lambsquarters (*Chenopodium album* L.), and (ii) measure the degree of allelopathy between the five plant species based on an allelopathic index. Snap bean, corn, rice, and sorghum are crops while common lambsquarters is a weed species. Results from this experimental study will help determine the extent of damage or stimulus that dried aquatic weed causes when applied on plant seeds.

2. Method

A petri dish experiment was conducted at the Everglades Research and Education Center in Belle Glade, Florida, to evaluate the effects of *P. stratiotes* and *L. wollei* amendments on seeds of five plant species. Fresh *P. stratiotes* and *L. wollei* were collected from local farm canals within the EAA and dried in a hot room at 50 °C for 72 hours. The dried biomass was finely grounded to 1 mm particle size to be used as amendments. Germination assays were conducted using 10 seeds of snap bean and corn, and 20 seeds of sorghum, rice, and common lambsquarters. Six treatment rates of 0.03 g, 0.06 g, 0.12 g, 0.25 g, 0.50 g, and 1.00 g of the two amendments were added to the seeds. A control treatment consisting of no amendment (0.00 g) was also included. Seeds of each species and the amendments were placed on 10 cm diameter Petri dishes lined with filter paper and moistened with 20 ml of distilled water. Petri dishes were sealed with Parafilm paper to prevent desiccation and placed in growth chamber with day/night temperature of 25 °C, and 78% relative humidity. The experiment was arranged as a completely randomized design with three replication of treatments. Seeds of snap bean, corn, sorghum, and common lambsquarters were considered germinated when the emerging radicles were visible. Rice seeds were considered germinated when the coleoptile tip first became visible (Fageria, 2014). Percentage germination and root length were measured one week after initiation of the experiment. However, rice root length was measured one more time after two weeks of the treatment application due to delayed germination.

Extraction of allelochemicals from P. *stratiotes* and L. *wollei* was done using 1-g dry sample that had been sifted through a size-50 mesh (297 µm) and treated using 20 ml methanol (1:20 extraction ratio) under ultrasound-assisted extraction at room temperature. The extraction solution was first filtered through regular filter paper to remove the residual sediment and then filtered with a 0.22 µm organic filter membrane to prevent interference from particles. The solvent was refrigerated at 4 °C to be analyzed under a High Performance Liquid Chromatograph Mass Spectroscope (HPLC-MS, Agilent-ThermoFinnigan). Both samples were analyzed via reverse phase C8 HPLC/UV/ESI-MS with both (+) and (-) ionization. Numerous HPLC/(+) and (-)ESI-MSn (tandem mass spectrometry) were conducted on the components of the *P. stratiotes*, while only the HPLC/(+)ESI-MSn was done on *L. wollei*.

The degree of allelopathy between the five plant species was estimated as an allelopathic index (AI) based on the average slope of application rate on the standardized percent germination and standardized root length for each plant species. Thus, the effect of treatment rate on both the percent germination and root length were taken into account while computing the AI:

$$AI = \frac{m_{pg}+m_{rl}}{2} \tag{1}$$

where m_{pg} is the slope (linear effect) of rate on standardized percent germination and m_{rl} is the slope (linear effect) of rate on the standardized root length. Note, that if the positive and negative effects cancel out, then the index is 0; if the positive effect is bigger than the absolute value of the negative effect, then the index is positive; and the index is negative, if the negative effect is bigger than the absolute value of the positive effect. To calculate AI, percent germination and root length were first standardized by subtracting each sample value from the sample mean and then dividing by the standard deviation for each response. This was followed by fitting the analysis of covariance (ANCOVA) models to assess the linear effects of rate on the standardized germination and root length, respectively. The AI index was calculated as the average of the two slopes (linear effects) as shown in equation (1). The AI index enabled assessment of the overall allelopathic effect of the amendment rate for each plant species by weed type combination.

2.1 Statistical Analysis

Statistical analysis was conducted using SAS (version 9.3 of the SAS System). Summary statistics, such as sample means, sample standard deviations, and standard errors across all five plant species and six treatment rates were calculated using PROC MEANS of SAS and the error bar graphs were plotted using PROC GPLOT of SAS. A three factor full factorial linear model for germination and root length response variables versus three categorical predictors (plant species, weed type, and amendment rate) were fitted. Since there are 5 plant species, 2 weed types, 7 rates (including the control rate), and 3 replications (petri-dishes) per treatment, the experimental data set has 5 × 2 × 7 × 3 = 210 observations in all. Because of evidence of heterogeneous variance across the plant species, we fitted the following three factor full factorial normal linear model with heteroscedastic errors:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl} \tag{2}$$

where μ is the overall mean; α_i, β_j, and γ_k are the main effects of plant species, weed type, and amendment rate, respectively; $(\alpha\beta)_{ij}$, $(\alpha\gamma)_{ik}$, and $(\beta\gamma)_{jk}$ are the two-way interaction effects; $(\alpha\beta\gamma)_{ijk}$ are the three-way interaction effects; and $\varepsilon_{ijkl} \sim N(0,\sigma_i^2)$ are the independent and heteroscedastic normal errors.

To quantify the effect of rate on the response, we also fitted an ANCOVA model for germination and root length against two categorical predictors, plant species and weed type, and the quantitative predictor, amendment rate. As there was evidence of heterogeneous variance across the species groups, we fitted the following ANCOVA model with heteroscedastic errors:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma X_{ijk} + (\alpha\gamma)_i X_{ijk} + (\beta\gamma)_j X_{ijk} + (\alpha\beta\gamma)_{ij} X_{ijk} + \varepsilon_{ijkl} \tag{3}$$

where μ is the overall mean; α_i and β_j are the main effects of plant species and weed type, respectively; γ is the overall linear effect of rate; $(\alpha\gamma)_i$ and $(\beta\gamma)_j$ are the linear effects of rate adjusted for plant species and weed type groups, respectively; $(\alpha\beta\gamma)_{ij}$ are the linear effects of rate adjusted for the plant species by weed type groups; and $\varepsilon_{ijkl} \sim N(0,\sigma_i^2)$ are the independent and heteroscedastic normal errors.

The linear and the ANCOVA models were fitted in using the PROC GLIMMIX of SAS. The results of the F tests for fixed effects were checked and then a Duncan's test of multiple comparisons with the control rate treatment carried out. Then, a Tukey's multiple comparison procedure (including the lines display) to separate the rate means for each combination of species by amendment type was carried out. This approach of statistical analysis is more powerful than the alternative of carrying out a multiple comparison test for the rate means by fitting a separate model for each species by amendment type. Goodness of fit tests for the fitted models was conducted using PROC UNIVARIATE of SAS by carrying out the Kolmogorov-Smirnov and Cramer-von Mises tests of normality of the standardized residuals. There was no strong evidence against the normality of the standardized errors for all models.

3. Results

3.1 Allelochemical Characterization

Three distinct groups of chemicals (bioflavonoids, nitrogen-containing compounds, and chlorophyll and related pigments) were identified in *P. stratiotes* (Figure 1). Based on the specific molecular weight (MW), some of the bioflavonoid chemicals identified were vicenin (apigenin-6,8-di-C-glycopyranoside (MW-594)), lucenin-2 (luteolin-5,8-di-C-glucoside (MW-610)), lucenin-1 and 3 (MW-580), isovitexin (apigenin-6-C-glucoside (MW-432)), vitexin (apigenin-8-C-glucoside (MW-432)), orientin (luteolin-8-C-glucoside (MW-448)), luteolin-7-glycoside (MW-448), and cyanidin-3-glucoside (MW-448). In addition there were other diglycosides present. The “derivatives” of lucenin and vicenin were detected and appear to be esters of coumaric acid and caffeic acid. In *L. wollei*, only one distinct group of chemicals, chlorophyll and related pigments were observed which emerged between retention time 45 and 60 minutes (Figure 2). Based on the MW, the chemicals identified are likely lipids, triacylglycerides and chlorophyll related compounds.

3.2 Allelopathic Effect on Seed Germination

In general, the application of both *P. stratiotes* and *L. wollei* as amendments had an overall negative effect on germination of all plant species (Table 1). A significant decrease ($p \leq 0.05$) in germination was observed on all plant species with increasing rates of *P. stratiotes* (Table 1). A significant decrease in germination was also observed in sorghum, beans, and corn with the application of *L. wollei*. The largest decline in germination was observed in corn whereby the *P. stratiotes* resulted in 80% reduction in germination at the 1.00 g rate. Both, snap beans and corn had the largest decrease in germination of 43% with *L. wollei* treatment application. Rice showed the least reduction in germination of 18% at the maximum amendment rate with *P. stratiotes* and a decrease of 5% with *L. wollei*. Common lambsquarters showed a significant 50% decrease in germination with *P. stratiotes* and a non-significant reduction with *L. wollei*. Sorghum seeds showed significant decrease in germination of 20% and 27% with *P. stratiotes* and *L. wollei*, respectively. It should also be noted that the germination percentages for the seeds without the weed amendment (control) were highly variable and ranged between 52% (common lambsquarters) and 100% (corn).

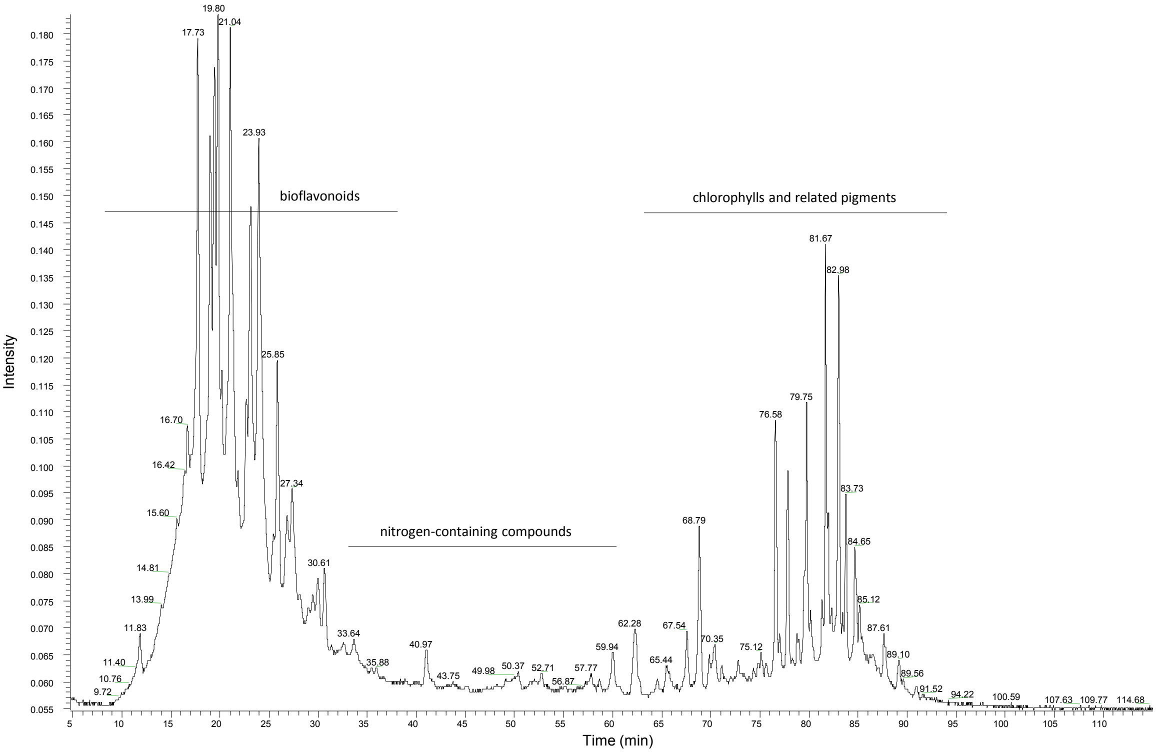

Figure 1. *P. stratiotes*: HPLC/280 nm UV chromatogram. There were three distinct regions (bioflavonoids, N-containing compounds, and chlorophyll and related pigments) as indicated by the horizontal lines and labeling

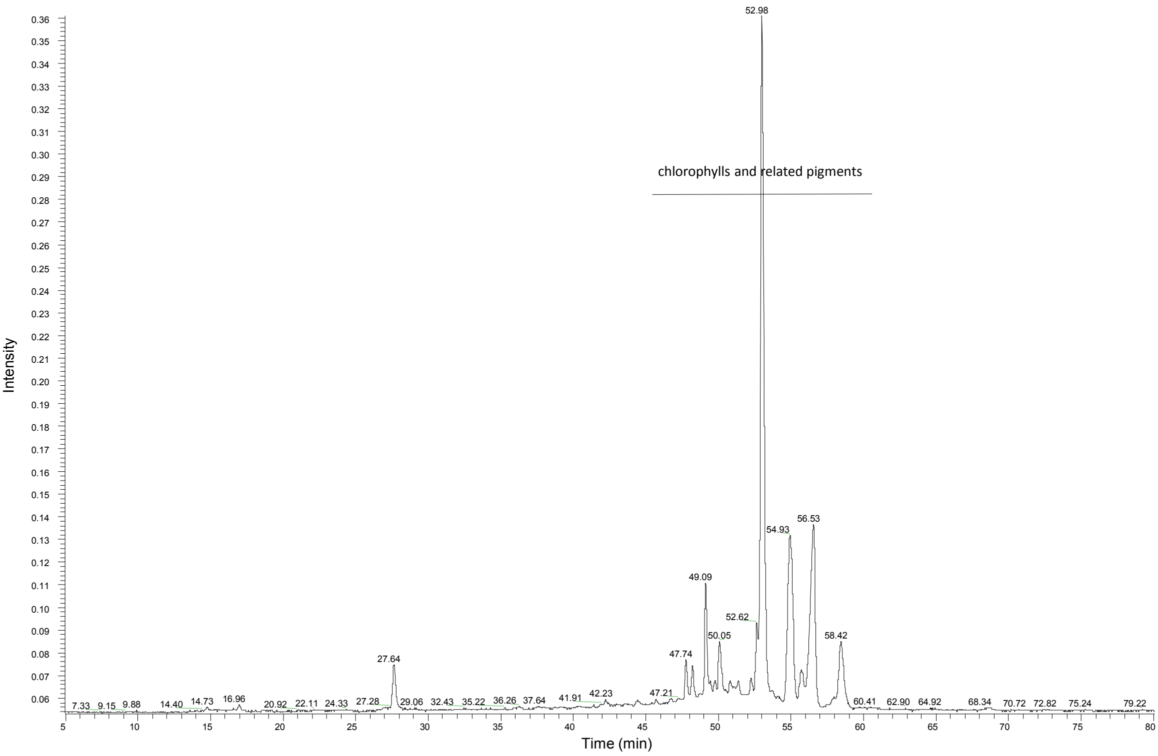

Figure 2. *L. wollei*: HPLC/280 nm UV chromatogram. There was one distinct region (chlorophyll and related pigments) as indicated by the horizontal line and labeling

Table 1. Tukey's multiple comparison procedure for percent germination and root length for common lambsquarters, sorghum, snap bean, corn, and rice

	Germination (%)			**Root Length (cm)**		
	Rate (g)	***P. stratiotes***	***L. wollei***	**Rate (g)**	***P. stratiotes***	***L. wollei***
Common lambsquarter	0.00	51.7 a	51.7 ba	0.00	3.1 a	3.1 a
	0.03	36.7 ba	58.3 a	0.03	2.9 a	2.1 b
	0.06	50.0 ba	46.7 ba	0.06	2.5 ba	1.5 cb
	0.12	33.3 bac	48.3 ba	0.12	2.6 bc	1.3 cb
	0.25	26.7 bc	41.7 ba	0.25	1.3 dc	1.1 c
	0.50	11.7 dc	35.0 ba	0.50	0.7 de	1.4 cb
	1.00	1.7 d	31.7 b	1.00	0.0 e	0.8 c
Sorghum	0.00	80.0 ba	80.0 ba	0.00	5.2 ba	5.2 c
	0.03	86.7 a	90.0 a	0.03	7.4 a	7.8 ba
	0.06	86.7 a	88.3 a	0.06	7.3 a	7.6 ba
	0.12	75.0 bac	88.3 a	0.12	6.7 a	8.2 ba
	0.25	73.3 bac	83.3 ba	0.25	6.9 a	7.8 ba
	0.50	65.0 bc	66.7 bc	0.50	6.8 a	9.3 a
	1.00	60.0 c	53.3 c	1.00	3.3 b	5.9 bc
Snap bean	0.00	73.3 a	73.3 a	0.00	1.8 ba	1.8 b
	0.03	73.3 a	53.3 ba	0.03	0.8 ba	5.6 a
	0.06	63.3 a	50.0 ba	0.06	4.1 a	5.4 ba
	0.12	46.7 ba	40.0 ba	0.12	0.5 ba	4.3 ba
	0.25	36.7 bac	50.0 ba	0.25	0.9 ba	5.4 ba
	0.50	23.3 bc	33.3 b	0.50	1.1 ba	3.8 ba
	1.00	0.0 c	30.0 b	1.00	0.0 b	2.4 ba
Corn	0.00	100.0 a	100.0 a	0.00	7.9 ba	7.9 a
	0.03	93.3 a	93.3 a	0.03	11.9 a	12.3 a
	0.06	83.3 a	80.0 ba	0.06	10.6 a	10.9 a
	0.12	83.3 a	73.3 ba	0.12	12.7 a	11.3 a
	0.25	73.3 a	56.7 bc	0.25	11.7 a	8.0 a
	0.50	36.7 b	33.3 c	0.50	8.1 ba	8.3 a
	1.00	20.0 b	56.7 bc	1.00	3.6 b	9.7 a
Rice	0.00	95.0 a	95.0 a	0.00	3.8 a	3.8 a
	0.03	98.3 a	98.3 a	0.03	3.8 a	3.4 a
	0.06	98.3 a	93.3 a	0.06	4.2 a	3.7 a
	0.12	95.0 a	96.7 a	0.12	4.4 a	3.8 a
	0.25	95.0 a	98.3 a	0.25	3.9 a	3.6 a
	0.50	88.3 ba	100.0 a	0.50	3.7 a	3.3 ba
	1.00	76.7 b	90.0 a	1.00	2.3 b	2.4 b

Note: The separation of the rate means was performed for each type of amendment (P. stratiotes and L. wollei) applied directly. Different alphabets correspond to significant differences ($p \leq 0.05$).

3.3 Allelopathic Effect on Root Growth

The application of *P. stratiotes* had a significant effect on the root length for common lambsquarters, sorghum and rice, all showing significantly shorter roots at the end of one week incubation period with increasing amendment rate (Table 1). Although statistically significant, the change in the root length for rice was the lowest in magnitude among all plant species (1.5 cm). Sorghum developed longer roots which were 2.2, 2.1, 1.5, 1.7, and 1.6 cm in length with application of *P. stratiotes* at 0.03, 0.06, 0.12, 0.25, and 0.50 g, respectively compared to the control rate. There was an increase of 2.3 cm in snap bean root length with application of *P. stratiotes* at 0.06 g. Root length measurements were highly variable in corn when *P. stratiotes* was used as an amendment. Corn developed longer roots which were 4.0, 2.7, 4.8, 3.8, and 0.2 cm in length with application of *P. stratiotes* at 0.03, 0.06, 0.12, 0.25, and 0.50 g, respectively. These results show that application of *P. stratiotes* resulted in significant stimulation of root length in sorghum, snap bean, and corn at rates < 1.00 g.

The application of *L. wollei* had a significant effect on the root length for common lambsquarters and rice, both showing significantly shorter roots at the end of one week incubation period (Table 1). While the average root length of common lambsquarters in the control treatment was 3.1 cm, its length was reduced significantly to 0.8 cm at the 1.00 g amendment rate with *L.wollei* (Table 1).

Sorghum developed significantly longer roots which were 2.6, 2.4, 3.0, 2.6, and 4.1 cm in length with application of *L. wollei* at 0.03, 0.06, 0.12, 0.25, and 0.50 g, respectively. There was an average increase of 0.7 cm in sorghum root length with *L. wollei* at the maximum rate of 1.00 g. The application of *L. wollei* had a significant positive effect on the average root length of snap bean at 0.03 g. Corn developed longer roots with application of *L. wollei* at all rates.

Overall, rice seeds took longer than a week to germinate. During the first week of the experiment, there were no significant differences in root length with the application of *P. stratiotes* and *L. wollei* on rice, except for the maximum application rate (Figure 3). However, by the second week, rice roots grew longer than the control and showed significant longer roots when *P. stratiotes* was applied at 0.12, 0.50, and 1.00 g, respectively. This would suggest that the allelopathic compounds found in *P. stratiotes* did not affect rice roots. In comparison, the application of *L. wollei* had a variable effect on the root length of rice. The mean root length observed in the control using *L. wollei* was longer than all other amendment rates.

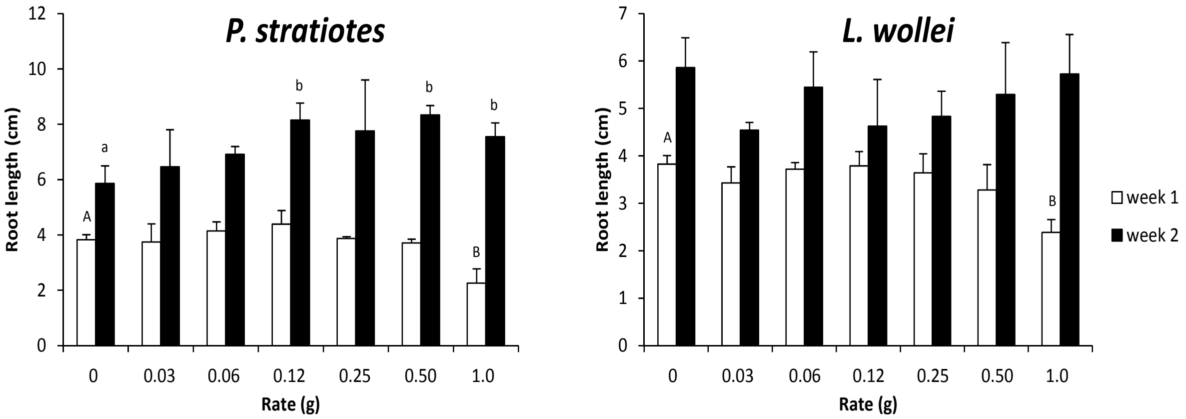

Figure 3. Average root length of rice seedling observed over two-week period for the *P. stratiotes* and *L. wollei* amendments in response to different application rates. Error bars represent standard deviation. Different alphabets correspond to significant differences ($p \leq 0.05$)

3.4 Degree of Allelopathy

The allelopathic index (AI) used to estimate the degree of tolerance of the individual plant species towards *P. stratiotes* and *L. wollei* was based on percent germination and overall root length. Higher AI values imply that the plant is more tolerant to the amendment whereas lower AI values imply that the plant is less tolerant towards the amendment. All the plant species were less tolerant to *P. stratiotes* compared *L. wollei* (Figure 4). Also, sorghum and rice did not show much difference between the two amendments with regard to tolerance. Rice was the most tolerant out of the five plant species (since the AI values for both amendments were the highest), while corn was the least tolerant (since the AI values for both amendments were the lowest). Rice may be naturally tolerant to allelochemicals from aquatic weeds, because it is the only crop that grows in flooded environments.

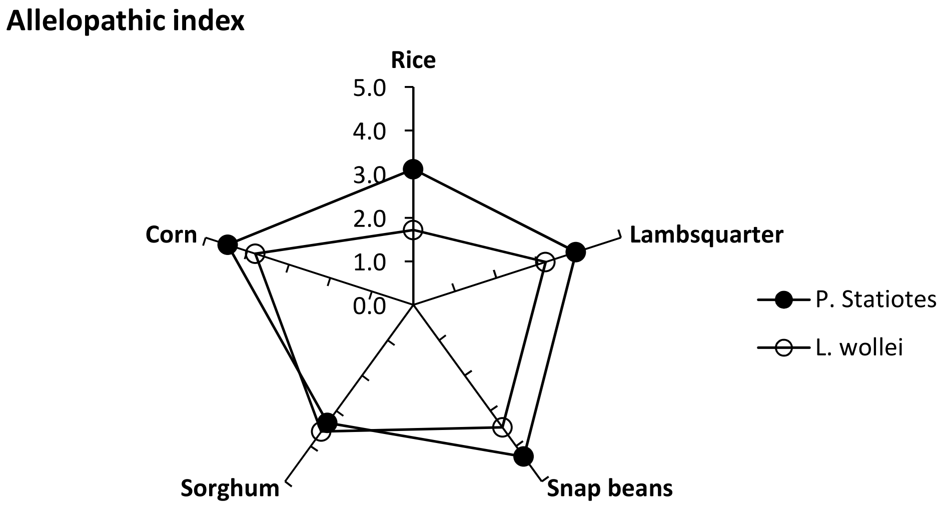

Figure 4. Allelopathic index showing the degree of plant species tolerance towards *P. stratiotes* and *L. wollei*

4. Discussion

Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals, such as phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone (James et al., 2013). Flavonoid glycosides are biologically active low molecular weight secondary metabolites typically realized into the rhizosphere by roots from where they can be involved in autotoxicity and allelopathy (Weston & Mathesius, 2013). Low molecular weight fatty acids which are components of triacylglycerols have been shown to be toxic to fungi because of their ability to alter pH (Seigler, 2006). The negative effect on germination could be attributed to allelochemicals including flavonoids and fatty acids present in the amendments that can affect many different cellular processes, such as disruption of membrane permeability (Galindo et al., 1999) and photosynthesis. Ayeni et al. (1997) showed that allelochemicals from residues of maize root and rice husk retarded seed germination rate, reduced radicle extension, and caused swelling of root tips of Bidens pilosa L. The possibility of suppressing the growth of common lambsquarters may have a useful practical application in controlling the plant species, which is often a common weed in many cropping systems (Hall et al., 2012). Previous studies have showed that certain rice cultivars contain allelopathic compounds that can suppress several aquatic weed species by inhibiting root growth by more than 90% (Seal & Pratley, 2010). These results show that rice is more tolerant to allelopathic compounds that maybe released by *P. stratiotes* or *L. wollei* compared to the other plant species used in this study.

The study was a good first step screening process to test the feasibility of using the amendments as bio-fertilizers and bio-herbicides. In general, it was observed that the finely grounded *P. stratiotes* and *L. wollei* had inhibitory effects on germination of all five plant species used in this study. Results showed that higher rates of the amendments caused greater inhibition of germination and root length germination, probably because these higher rates contained more allelochemicals. This suggests the suitability of the amendments as potential bio-herbicides if applied to control weed species such as common lambsquarters. In contrast, low application rates (0.03 g and 0.06 g) had a hormetic effect by enhancing root growth of corn, snap bean, and sorghum. The effect of allelochemicals extracted from *P. stratiotes* was tested on the growth and microcystin production and release by *M. aeruginosa*, a common aquatic blue green algae (Wu et al., 2013). The study showed that a low dose (20 – 60 mg L^{-1}) promoted algal growth while a high dose (100 – 200 mg L^{-1}) inhibited algal growth. Allelochemicals have been shown to result in hormetic responses in several plant species (Duke, 2011).

Rice showed the most tolerance to the amendments. Root length of rice had a significant positive increase in response to *P. stratiotes* at the end of the two-week period compared to the control. This illustrates that *P.*

stratiotes can be used as a potential bio-fertilizer to stimulate growth of rice. Within the EAA, there is tremendous potential to explore both the positive and negative effects of these aquatic plants on crops and weed species. The petri dish experiment created an appropriate controlled environment where the plants under study could only be affected by the presence of the amendment application rather than external factors such as soil, nutrients, and climate. The EAA consists of nearly 280,000 ha of farmland that spawns large quantities of floating and submerged aquatic vegetation within its farm canals year-round. Finding a useful application of the aquatic vegetation can be beneficial to sustainable farming practices within the region. Approximately 9,000 ha of land are used to grow rice (Coale et al., 1992), which is often rotated with sugarcane as a summer crop. With rice showing a positive response to the application of *P. stratiotes* and *L. Wollei*, these amendments may be beneficial in increasing the hectarage of rice production within the region.

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