Allelopathic Potential of Selected Grasses (Family *Poaceae*) on the Germination of Lettuce Seeds (*Lactuca sativa*)

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Abstract

Allelopathy is the production of chemicals (allelochemicals) of a plant which can influence the growth and development of another plant that can be either negative by reducing germination or positive by increasing growth. This study was conducted to (1) determine the allelopathic potential of the following grasses (Family Poaceae), Chloris barbata, Eleusine indica and Saccharum spontaneum in Tigbauan, Iloilo on the germination of lettuce (Lactuca sativa) seeds; (2) determine the grass extracts that can inhibit or induce the germination; and (3) determine if there is a significant difference on the allelopathic potential of the grass extracts on the germination of lettuce seeds in each assay used. Filter paper and soil germination assays were the growth assays used. Results of the study revealed that the grasses used had significantly reduced and differ in their germination rate of lettuce seeds at 0.05 level of significance using One-Way Analysis of Variance and post hoc Tukey analyses. Both germination assays used, showed that S. spontaneum has the lowest germination rate. Eleusine indica has the highest germination rate in filter paper assay while Chloris barbata in soil germination assay. The grass extracts used have an allelopathic potential by decreasing the germination rate of lettuce seeds. This study suggested that allelopathic activity is one feature of attributes that enable invasive species to dominate in habitat space of communities they are present. Identifying these attributes and clarifying the relative importance could enhance our ecological understanding of the problematic species and facilitate the management of plant invasions.

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Keywords: *Allelopathy, germination, Lactuca sativa, Poaceae*

1. Introduction

One of the fascinating but controversial processes in plant ecology is the mediation of competitive interactions among plants by plant-released chemicals which can suppress the growth of other plants, a process known as allelopathy [16, 17]. Allelopathic effects have been attributed to a number of exotic invasive plants [13] and recent research has also suggested the possibility that some invaders may possess novel chemicals that are more phytotoxic to naïve and non-adapted native plants, soil microbes or herbivores in the invaded range than adapted species in the invader's native range [1]. Such biographical differences in the effects of phytotoxic, antimicrobial or defense biochemistry have been proposed as a mechanism for invasion the "Novel Weapon Hypothesis" [2].

Invasive species move aggressively into natural areas, reducing the richness and diversity of plant communities, and altering ecosystem function [11]. Although much is known about how invasive species impact native plant communities [7], less is known about the mechanisms by which invasive species establish and maintain site dominance [20]. Some promising recent work has suggested that belowground processes are important during plant invasion and may be a key to understanding how invaders proliferate in native ecosystems [13]. Compelling evidence of allelopathic effects of non-native invasive plants has come from a variety of species and regions [2].

The defense chemicals or secondary metabolites of plants can serve several types of functions. These “allelochemicals” are specific biomolecules which are released mainly through root exudation and biomass decomposition [21]. These can be insecticidal, antimicrobial, herbicidal and some possess other types of biological activities [30]. These beneficial, bioactive chemical substances are found in abundance in plant species. Exploring the residence time of allelochemicals in the field has the potential to improve our understanding of interactions among plants. Furthermore, it is imperative to utilize indigenous occurring grasses in our locality as an effective herbicide to the cultivated crops. Through allelopathic screening, it would be a practical tool for weed management in organic farming systems.

Plant communities dominated by *Poaceae* are called grasslands [8]. It is estimated that they comprise twenty percent of the vegetation that covers the earth [8]. Their divergence is estimated to have taken place 200 million years ago [24]. Most of the grasses are considered to be invasive. Their unrestricted proliferation has had many dire consequences such including crop loss in the case of agricultural weeds, decrease in biodiversity and determination of overall environmental quality [10]. Three common grasses described by Sajise et al (1976) can be found in dry grasslands and open waste places and fallow lands. They are further characterized by Carballo et al (1985).

Chloris barbata (L.) Sw. is a slender, wiry erect hardy grass reaching a height of about 1 m with long thin leaves and flowering stalk located at the end of the stem. It is a competitor to cultivated plants because it produces many tillers. *Eleusine indica* is an invasive species in the grass family *Poaceae*. It is a stout, erect smooth grass that grows from 10 cm to 1m high, with leaves that are long and curved when dry; leaf sheaths are flattened; numerous spikelets are filled with grains. *Saccharum spontaneum*, a gregarious grass, height 1 to 2.5 cm; underground rootstocks are stout; leaves are sharp, length about 0.5 to 1m long, 6 to 15 mm wide; tiny spikelets are along branches of inflorescence, with long white silky hair at the base, fruits are carried by the wind when ripe.

One of the most studied aspects of allelopathy is its role in agriculture [18]. Current research is focused on the effects of weed on crops, crops on weeds and crops on crops [18]. This research further shows the possibility of using allelochemicals as growth regulators and natural herbicides to promote sustainable agriculture [4]. It has been found that allelopathy offers great potential to (1) increase agricultural production, (2) decrease harmful effects of modern agricultural practices such as the indiscriminate use of pesticides on soil health and productivity on the environment, and (3) to maintain soil productivity and a pollution-free environment for our future generations. It is likely that in the near future, allelopathy will be used in crop production and protection, agroforestry and agrohorticutural practices in developed and developing countries [22]. It may become one of the strategic sciences to reduce the environmental pollution and to increase agricultural production in sustainable agriculture of the twenty-first century. For weed management, it is interesting to note the inhibition of one plant (the weed or weeds) by another (usually the crop) through the production of allelochemicals [9]. These allelochemicals may be actively produce by a growing plant or arise from the residues after death. The effects of allelochemicals may be reduced or enhanced by microorganisms. According to Davies (2004), such an effect can be negative (reduced by germination) or positive (increased growth). It is a must to explore the residence time of allelochemicals released by plants in different media, episodic exposure of plants to allelochemicals and the effect of allelochemicals in the two assays that will lead to improve our understanding of interaction among plants (Inderjit et al., 2008). Work on this would recognize that allelopathic activity is most likely one feature in a suite of attributes that enable invasive species to dominate habitat space in communities they invade. Identifying these attributes, and clarifying the relative importance of each, will enhance our ecological understanding of the problematic species and facilitate the management of plant invasions (19). This study was conducted to determine the allelopathic potential of three abundant grass species in Tigbauan, Iloilo on the germination of lettuce seeds (*Lactuca sativa*). It also seeks to determine the grass extracts that can inhibit or induce the germination of lettuce seeds in the two types of bioassay used. Finally, determine if there is a significant difference on the allelopathic potential of the grass extracts on the germination of lettuce seeds in the two bioassays used.

2. Methodology

2.1 Identification and Collection of Grass Species. Proper identification and characterization of the three grasses, *Chloris barbata, Eleusine indica and, Saccharum spontaneum* were based on Guidebook to Grassland Plants: A Resource Material for Biology Teachers by Carballo et al (1995). Confirmation of the said grasses was done at West Visayas State University by a botany and plant systematics instructor. The grasses, *Chloris barbata* and *Elusine indica* were collected along the roadside of barangay Sta. Lucia, Tigbauan, Iloilo and *Saccharum spontaneum* was collected near the river bank of Sibalom river in the same place on January 5, 2011.

2.2. Extracting Bioactive Components from Plant. Leaves and culms of the three grasses were used in the study. They were cut into smaller pieces and soaked in methanol as solvent in a glass container (300 g of sun-dried parts of each grass in 150 ml of methanol). The soaking process was allowed to stay at room temperature for 48 hours on January 6-8, 2011. Afterwards, the methanol extract was filtered. The filtrate was evaporated using a rotary evaporator until the methanol evaporated. The concentrated extract was partitioned into two parts and used for two separate bioassays, soil germination and filter paper germination assays.

2.3. Growth Bioassays. There were two types of bioassay used in the study which were done on January 11, 2011 in the biological laboratory room of Iloilo Doctors' College. (1) Soil germination assay. This assay was done to identify allelopathic interaction in more natural settings with plants growing in soil. Soil sample was collected in Barangay Parara Sur, Tigbauan, Iloilo on January 9, 2011 in home garden. The soil was identified to have no history of exposure to any grass species used in the experiment. For soil bioassay, 50 g soil sample (sandy loam, pH acidic) was each placed in a petri dish and irrigated with 15 ml of the grass extract. Soil treated with 15 ml distilled water served as the control. Preliminary experiments made by Inderjit and Rajeswari (2010) suggested that 15 ml solution was required to moisten 15 g soil, which did not create stagnated conditions. Ten lettuce seeds with 90% germination, Lollo Rossa, a purple-leafed variety were placed on control or treated soils. (2) Filter paper germination assay. For this bioassay, Whatmann number one filter paper in four layers was used instead of soil sample. Each treatment was replicated three times. Ten ml of each extract was irrigated in each petri dish, then added with ten lettuce seeds in each.

Lettuce was chosen because it is a sensitive species widely used as an assay species in alellopathic research [14]. The treated seeds were incubated at 18-25^{0}C, 12h photoperiod as a specific growth requirement of the assay species. A high concentration was used because it is close to the mean concentration of the pulse measured by Perry et al (2007) and because preliminary experiments indicated that a pulse of this magnitude would be necessary to achieve even trace amounts after several days [15]. Pure concentration was also used in soil because it may affect due to transformation of the pure form through chelation, sorption, oxidation, microbial processes or other unknown soil effects [15]. The effect of each grass species was observed by measuring the percent seed germination after a week. Treatments were compared for each species using one-way ANOVA and Post ANOVA Tukey tests by the aid of SPSS v11.5.

3. Results and Discussion

3.1. Results

Assay number 1. Allelopathic potential of the three grass species extracts on the germination of lettuce seed using filter paper assay. The control group has completely induced the germination of lettuce seeds after a week. Among the experimental groups, most of the lettuce seeds were induced by *E. indica* extract while most inhibition of germination occurred in *S. spontaneum* extract. This is shown in Figure 1.

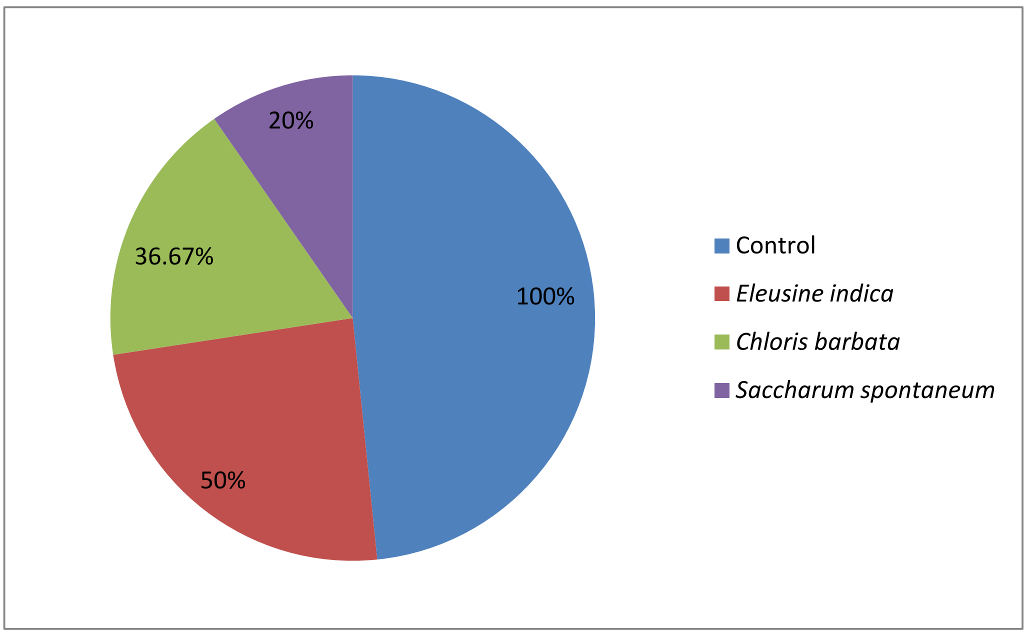

Figure 1. Percentage of Germination in Filter Paper Assay

From the One-Way ANOVA result, grass extracts used show a significant allelopathic potential on the germination of lettuce seeds in filter paper assay ($F_{3,8}$=428.00, P<0.05).

Furthermore, Tukey test for pairwise comparison in Table 2 shows that there is a significant difference between the control and the treatments used; *E. indica* and the control, *C. barbata* and *S. spontaneum; C. barbata* and control, *E. indica* and *S. spontaneum*; and *S spontaneum* and control, *E. indica* and *C. barbata*. This implies that treatments and the control group significantly differ in their allelopathic potential in either inhibiting or inducing germination of lettuce seeds.

Assay number 2. Allelopathic potential of the three grass species extract on the germination of lettuce seed using soil germination assay. The control group yields similar result with the filter paper assay. Among the experimental groups, most lettuce seeds were induced to germinate in *C. barbata* while most lettuce seeds are inhibited to germinate in *S. spontaneum*. This is shown in Figure 2.

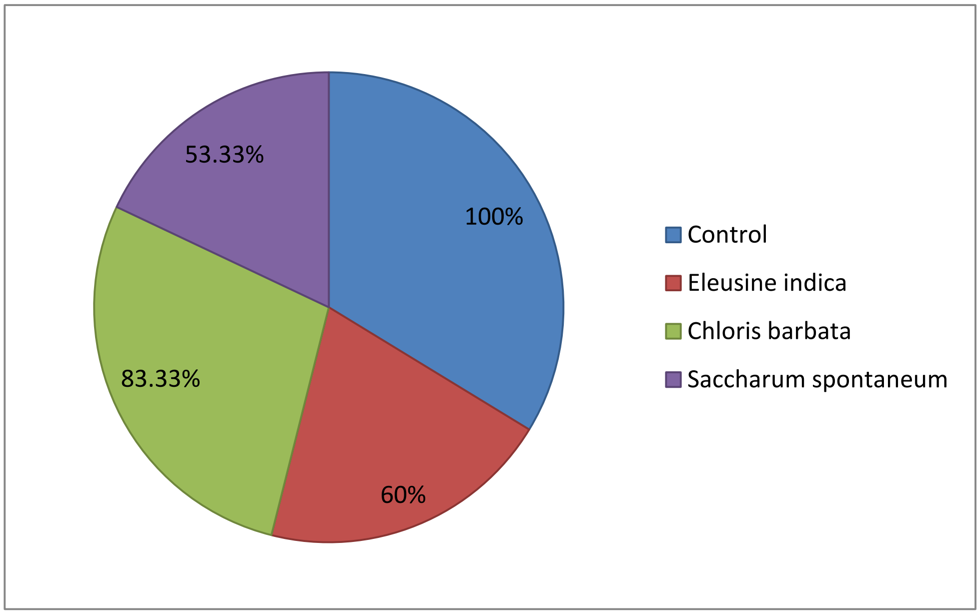

Figure 2. Percentage Germination in Soil Germination Assay

From the ANOVA result, grass extracts used show a significant allelopathic potential on the germination of lettuce seeds in soil germination assay ($F_{3,20}$=15.578, P<0.05) .

The result of the study demonstrated an allelopathic effect or growth inhibition on lettuce seeds (*Lactuca sativa*) under the two types of assay used. It also indicates the involvement of chemical factors in the growth inhibitory and stimulatory effects of the three grasses against lettuce seeds germination. Although there is no individual isolation of chemicals responsible for the growth inhibition and stimulation found in the extracts used, it is evident that multiple chemicals are involved. A plant released chemical may not inhibit plant growth at a given concentration but may have important ecological role in terms of influencing soil microbial and chemical factors [17, 31].

There are lesser seeds germinated in the filter paper assay. Relevant to this assay investigation would probably due to the absence of most mineral nutrients. For instance, it is known that when roots lack an external calcium supply, their membranes become much more permeable, possibly resulting in an unnaturally high release/ absorption of allelochemicals [12]. In soil germination assay, it reveals that the allelopathic potential of an allelochemical depends upon the biological, chemical and physical composition of the soil environment [16].

Abiotic and biotic conditions of soil used may play a significant role in the accumulation and phytotoxicity of an allelochemical [15]. It also suggests that growth media used is an important factor in determining phytotoxicity of an allelochemical [16].

Seed germination can be explained that under good growing conditions, allelopathy usually represent 5-10% of the of the total interference between species [5]. As stress becomes great, allelopathy increases in importance [5]. However variations in the inhibition or stimulation of germination can be explained that allelopathic chemicals can be damaging or stimulating depending upon dose – too much or too little can change growth [5]. This is best exemplified from the statement of Paracelsus (1493-1541) justly considered as the herald of modern toxicology, "All things are poison and not without poison; only the dose makes a thing not a poison."

One puzzling aspect of plant invasion is that the species in question are often infrequent and innocuous in native habitats, but proliferates aggressively and form near –monoculture stands in the invaded habitats [19]. Numerous explanations for plant invasiveness have been proposed, but one key component of the disparity in ecological activity may be that their invaded habitats, these plants contain secondary compounds that facilitate acquisition of habitat space [2, 13, 25). An allelopathic component in the invasion ecology of diffuse knapweed has been demonstrated [1] and preliminary works suggests that allelopathy may play a role in garlic mustard [25] and Japanese honeysuckle [27] invasions. Overall, a growing body of work supports the idea that allelopathy is a contributing factor in some plant invasions.

4. Conclusions and Recommendations

The findings of the study suggest that all grass extracts have reduced the germination of lettuce seeds in the two types of growth assays used. However, greater inhibition of germination is exhibited in filter paper assay and soil germination assay with *Saccharum spontaneum*. Likewise, all grass extracts have induced germination rate in the two types of assays used but significantly reduced the germination rate. The soil germination assay showed a higher percentage in the rate of germination as compared to the filter paper assay with *Chloris barbata* while *Eleusine indica* in the filter paper assay. Furthermore, there is a statistically significant difference on the allelopathic potential of the grass extracts used on the inhibition or induction of germination of lettuce seeds in each type of bioassay used. This explains that a plant released chemical may not inhibit that growth in a given concentration but may have important ecological role in terms of influencing soil and chemical factors [17, 29, 31].

The study describes that allelopathy increases the invasive potential of exotic plants which would contribute a great deal of general models of invasive susceptibility in natural system (13). Investigation of shifts in the tolerance f native species to the allelopathic effects of invader may yield new insights into the mechanisms that drive the dramatic transformation of the ecology of some species when they are introduced to new region of the globe [13].

Investigation is needed to isolate the individual chemical and to study their growth inhibitory and enhancing effects. There is a need for the examination of the ecological role(s) of the alleochemical in addition to its effect on plant growth. It is a must to design novel techniques to quantify allelochemicals in soil environment. Future studies on the adsorption and recovery of allelochemiclas from aqueous and soil solution might help to further understand its role in the soil environment [31]. Field application and testing allelopathy through improved methodology in the design is recommended. Other seeds and grass species are suggested to be used for further alleloapthic investigation. Characterization, elucidation and identification of different biochemicals of the grass species used are also recommended. There is a need also for the integration of soil microbial communities in allelopathic research so that its importance in plant-plant competitive interactions can be thoroughly evaluated [17].

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