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Article

Allelopathic Effect of Lotus and Arrowhead Weed on Wheat, Wild Oat and Milkthistle Germination

Efeito Alelopático do Lótus e da Erva-Flecha na Germinação de Trigo, Aveia Selvagem e Cardo-Mariano

GUL, B.[1]*
IJAZ, S.[1]
KHAN, H.[1]

ABSTRACT - An experiment was conducted to investigate the allelopathic effects of lotus (*Nelumbo nucifera* Gaertn.) and arrowhead (*Sagittaria sagittifolia* (Brummitt & Powell): L.) on seed germination of wheat and two associated weeds, namely, wild oat (*Avena fatua* L.) and milk thistle (*Silybum marianum* (L.) Gaertn.). The experiment was laid out in a Completely Randomized Design during May, 2014. Dried biomasses of lotus and arrowhead leaves were soaked in distilled water for 48 hrs for extract preparation. The extracts were applied at a rate of 5 and 10% concentrations to the seeds of wheat, wild oat and milk thistle at 25 °C in 10 cm wide Petri plates. A control treatment where only distilled water was applied to the seeds, was included for comparison. Data were recorded on germination (%), shoot length (cm), shoot and root fresh weights (g). The results revealed that 10% lotus extract reduced germination of wild oat (0.00%) and milk thistle (13.3%) but comparatively enhanced that of wheat (20%). By contrast, arrowhead totally inhibited germination of wheat and wild oat as compared to milk thistle (16.66%). Similarly, 10% lotus extract favored shoot length and root weight of wheat and milk thistle, whereas 100% inhibited wild oat. By comparison, arrowhead completely inhibited shoot length and root weight of wheat and wild oat at 10% conc. and enhanced milkthistle (3.00 cm and 3 g). A higher lotus extract conc. affected wild oat more than wheat and milkthistle, while arrowhead weed affected almost all test species. In conclusion, these aquatic weeds can be used as mulch in wheat in nearby fields of the infested aquatic water bodies for wild oat control, weed biomass disposal and nutrient addition to the soil.

Keywords: aquatic weeds, allelopathy, *Nelumbo nucifera* Gaertn., *Sagittaria sagittifolia* (Brummitt & Powell): L., *Avena fatua* L., *Silybum marianum* (L.) Gaertn.

*RESUMO - Foi conduzido um experimento para investigar os efeitos alelopáticos do lótus (**Nelumbo nucifera** Gaertn.) e da erva-flecha (**Sagittaria sagittifolia** (Brummitt & Powell): L.) na germinação de sementes de trigo e duas plantas daninhas associadas: aveia silvestre (**Avena fatua** L.) e cardo-mariano (**Silybum marianum** (L.) Gaertn.). O experimento foi instalado em um delineamento completamente casualizado em maio de 2014. As biomassas secas das folhas de lótus e erva-flecha foram embebidas em água destilada por 48 horas para preparo. Os extratos foram aplicados com concentração de 5 e 10%, nas sementes de trigo, aveia selvagem e cardo-mariano a 25 °C em placas de Petri de 10 cm. Para fins de comparação, foi incluído um tratamento controle no qual as sementes receberam apenas a aplicação de água destilada. Foram obtidos os dados de germinação (%), comprimento da parte aérea (cm), peso fresco da parte aérea e da raiz (g). Os resultados revelaram que o extrato de lótus a 10% reduziu a germinação da aveia*

*** Corresponding author:**
<bakhtiargul@aup.edu.pk>

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[1] Department of Weed Science, The University of Agriculture, Peshawar, Pakistan.

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selvagem (0,00%) e do cardo-mariano (13,3%) mas, em comparação, aumentou a do trigo (20%). Por outro lado, a erva-flecha inibiu totalmente a germinação do trigo e da aveia selvagem em comparação com o cardo-mariano (16,66%). Da mesma forma, o extrato de lótus a 10% favoreceu o comprimento da parte aérea e o peso da raiz do trigo e do cardo-mariano, enquanto a concentração de 100% inibiu a aveia selvagem. Em comparação, a erva-flecha inibiu completamente o comprimento da parte aérea e o peso da raiz do trigo e da aveia selvagem na concentração de 10%, mas aumentou os mesmos parâmetros no cardo-mariano (3,00 cm e 3 g). Uma concentração mais alta do extrato de lótus exerceu um maior efeito na aveia silvestre do que no trigo e no cardo-mariano, enquanto a erva-flecha exerceu efeito sobre quase todas as espécies testadas. Em conclusão, estas plantas daninhas aquáticas podem ser usadas como cobertura vegetal no trigo em campos próximos aos recursos hídricos infestados para controle da aveia selvagem, eliminação da biomassa de plantas daninhas e adição de nutrientes ao solo.

Palavras-chave: plantas daninhas aquáticas, alelopatia, *Nelumbo nucifera* Gaertn., *Sagittaria sagittifolia* (Brummitt & Powell): L., *Avena fatua* L., *Silybum marianum* (L.) Gaertn.

INTRODUCTION

Aquatic weeds are a big problem in water utilization in fresh water bodies of irrigated plains of Khyber Pakhtunkhwa, Pakistan. They create a great deal of problems to water bodies and hazards to local sites by creating unhealthy conditions, lowering biodiversity and providing favorable conditions for invasive plants. Blockage of waterways causes seepage, damage to the water body, siltation and floods hence blockage control is necessary. Invasive plants quickly learn to adapt in response to changed environmental conditions (Sakai et al., 2001), and spread rapidly, causing losses up to almost \$120 billion year^{-1} in the United States (Pimentel et al., 2005). However, chemical control of such plants causes environmental pollution and harms non-target species; moreover, agrochemicals used in aquatic ecosystems cause several other problems, e.g., eutrophication and water bloom. Mechanical control is safer than herbicide use in terms of pollution management but leftover biomass of weeds (specially perennial weeds) may cause new infestations and eutrophication; on the other hand, their disposal increases weed control costs. Modern mechanical weed control tools that reduce dependence on herbicides and cause low environmental impact are not available to developing countries. Conversely, knowledgeable use of fertilizers can minimize the release of nutrients into natural waters and eutrophication (Boyd and Massaut, 1999) and will indirectly assist with management of aquatic weeds. However, on the contrary, unwise fertilizer use will cause the production of huge biomass of aquatic weeds. After having been produced, such enormous biomass of aquatic weeds should be utilized for the benefit of humanity in terms of food, feed, fiber, medicine and mineral supplement, as a soil nutrient and as mulch for weed control, depending on the type of aquatic weed and water body. Therefore, in a sustainable economy, the use of biological raw materials is a completely new approach, required in any field from research and development to production and economy (Naik et al., 2010). One such approach is to use weed biomass as mulch. Although mulch has enormous benefits, it may have some allelochemicals that may affect crop/weed germination and growth in the form of delayed germination, weak roots/shoot, necrosis, root rolling, no root hairs, lower biomass and stunted growth. These allelochemicals may affect recipient plants at cellular or molecular level. However, allelopathy can also be used in weed management if the biomass is safe for the crop and detrimental to the weeds. Possible options are stubble mulch, whole plant mulch, crop rotation and intercropping. The prevalent allelochemicals are alkaloids, phenols, terpenoids and glycosides, which might be present in part or in the entire structure of plants (Corbu, 2007).

The use of allelopathy can also be implemented for weed management in wheat (*Triticum aestivum* L.), which is the major staple crop of Pakistan. The biomass of aquatic weeds (lotus and arrowhead weed) can be safely used as mulch in wheat without the risk of regeneration and further infestation, as wheat is the staple food of most people in Pakistan and needs more attention in terms of weed losses (Jabran et al., 2011). About Rs. 146 billion per annum is lost because of weeds alone (Ahmed and Shaikh, 2003). As a result of direct and poor weed control practices, 15-25% losses occur in wheat (Tunio, 2001) and other pests may also occur. Pakistan is far behind developed countries in terms of wheat production (8th largest producer). It accounts for 9.9% of the value added in agriculture and 2.0% of the GDP. The area under wheat cultivation is 9.26 million ha with a production of 25.482 million tons (Pakistan, 2015-2016). However, wild oat (*Avena fatua*) is a troublesome weed and the strongest competitor against wheat; crop mimicry makes it even more competitive (Mennan, 2003); in addition, there are other noxious weeds such as milk thistle etc.

Nelumbo nucifera has a strong allelopathic effect on certain plants because it has low fats, higher proteins, minerals, carbohydrates, vitamins (Sridhar and Bhat, 2007) and a great deal of bioactive compounds hence it can be utilized for weed management (Zhang et al., 2015). Similarly, *Sagittaria sagittifolia* is another aquatic weed which grows in shallow waters (Hickman, 1993); it also has bioactive compounds that can be explored for weed control in the form of aqueous extracts, e.g., mulberry extracts, which resulted in complete inhibition of wild oat germination (Jabran et al., 2010).

Thus, the role of allelopathy for sustainable weed management in wheat is encouraging in terms of total inhibition of wild oat germination and biomass reduction. It is an eco-friendly weed management technique compared to herbicides. Allelochemicals act as bio-herbicides without damaging crop but further research is needed on them (Bajwa et al., 2013). Likewise, milkthistle is also a noxious weed of wheat. Awareness of milk thistle is necessary (Khan and Marwat, 2006) as it has become widespread in KPK, causing > 37% losses in wheat as well as harvesting problems (Khan et al., 2009). Seed dormancy in soil is > 3 years and seeds may germinate after wheat harvesting. Therefore, long-term management through allelopathy is recommended (Sindel, 1991). The current study was carried out to investigate the allelopathic effect of lotus and arrowhead on germination and growth of wheat and its associated troublesome weeds, and to utilize their enormous biomass for eco-friendly and economical weed control in wheat.

MATERIALS AND METHODS

An experiment was conducted in the Department of Weed Science, University of Agriculture Peshawar (UAP), throughout May, 2014, in order to investigate the allelopathic effect of two weeds, namely, lotus and arrowhead, on germination of wheat, wild oat and milk thistle. The experiment was laid out in a Completely Randomized Design (CRD) consisting of 15 treatments replicated three times. Plant samples (leaves) of lotus and arrowhead were collected at maturity in the district of Charsadda, Tarnab, Khyber Pakhtunkhwa, while seeds of wheat (variety Gaznavi 98), wild oat and milkthistle were obtained on the farm of the University of Agriculture (UAP). The plants species were identified by renowned weed science scientists at UAP. The collected samples were brought to the laboratory, washed with tap water, oven-dried at 60 ^{o}C for 24 hours and ground with a grinder. 100 g of the powder was soaked in one litre of distilled water at room temperature (25 ^{o}C) for 10% extract preparation. After 48 hrs, the solutions were filtered through Muslin cloth. The 10% extract solution was diluted to 50% to get 5% aqueous extracts.

The extracts were prepared in a sterilized and hygienic way. The extracts were shifted to sterilized flasks to avoid contamination and the flasks were sealed with aluminum foil. Ten viable and healthy seeds (after the seeds were scanned through an x-ray machine) of each test species were placed in separate, previously sterilized (autoclaved for 15 minutes at 121 ^{o}C) glass Petri dishes. Three layers of Whatman filter paper grade 181 with 9 cm diameter were used on the Petri dishes before placing the seeds on them. The seeds were soaked for 48 hrs into extracts of 5 & 10% of lotus and arrowhead as required by the treatment, respectively. In the control treatment, only tap water was used to facilitate seed germination. During the germination, water was added to each Petri dish as required by the germinating seeds.

The data were recorded after three weeks on the following parameters

Germination (%): Seed germination was calculated by counting the number of germinated seeds in each treatment and dividing it by the total number of seeds and converting it to% seed germination by multiplying the ratio by 100.

Root weight (g): Fresh root weight plant^{-1} was calculated of five representative plants with the help of an electronic balance and divided by 5 to get average fresh root weight plant^{-1}.

Shoot length (cm): Shoot length of five representative plants was calculated through a transparent ruler and divided by 5 to get average shoot length.

Shoot weight (g): Fresh shoot weight of five representative plants was calculated with the help of an electronic balance and then converted to average weight for statistical analysis.

Statistical analysis: The recorded data for each parameter was subjected to ANOVA techniques by using computer software MSTAT-C, which is appropriate for a completely randomized design, and means were compared by using the Least Significant Difference (LSD) test at 0.05 level of probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Seed germination (%)

Statistical assessment of the data revealed that the application of allelopathic extracts of lotus and arrowhead had a significant effect on seed germination (%) of the test species (wheat, wild oat and milkthistle), as shown in Table 1. Seed germination of the test species decreased when the extract concentration was increased. Among the test species, seed germination percentage of wild oat (1.99%) was more affected by various aqueous extracts of lotus and arrowhead than wheat (10.66%) and milk thistle (21.33%). Among the allelopathic plants, seed germination of all test species was suppressed by arrowhead (5.55%) at a higher concentration, as compared to lotus (11.11%) at the same concentration. Furthermore, the interaction data on seed germination of the test species as affected by two aquatic weed extract concentrations revealed that minimum seed germination (0.00%) was noted in wild oat at the 10% extract concentration of both lotus and arrowhead. By contrast, wheat was highly affected only by arrowhead, whereas high seed germination was found in milk thistle (40 and 20%) by the 5% concentration of both arrowhead and lotus, respectively. Thus, wild oat was significantly affected by milk thistle and wheat among the test species. These results are in line with the findings of Dhole et al. (2011) and Gu et al. (2008), who reported that different aquatic weeds extracts suppressed seed germination and also seedling growth of agronomic plants. Similar results have been found by other researchers who worked on aquatic weeds such as *A. philoxeroides* and *A. sessilis* and reported that both of them have allelopathic effects and inhibited seed germination during germination time; also, when used at an early stage in wheat crops, they suppressed plant growth of wheat and its associated major weeds (Zhang et al., 2009).

Table 1 **-** Germination (%) of wheat, wild oat, and milk thistle as affected by various concentrations of the aqueous extracts of Lotus and arrowhead weed

Target plant specie	Allelopathic plants used (water extracts)					Specie mean
	Nelumbo nucifera L.		*Sagittaria sagittifolia* L.		Control 0% conc.	
	10% conc.	5% conc.	10% conc.	5% conc.		
Triticum aestivum L.	20.00 b	16.66 c	0.00 h	6.66 f	10.00 e	10.66 b
Avena fatua L.	0.00 h	3.33 g	0.00 h	3.33 g	3.33 g	1.99 c
Silybum marianum L.	13.33 d	20.00 b	16.66 c	40.00 a	16.66 c	21.33 a
Concentration means	11.11 b	13.33 ab	5.55 c	16.66 a	9.99 b	

LSD values (0.05) for Species means = 1.99, Concentrations: 3.63 and Interactions = 2.17.

Shoot length (cm)

The analysis of data as influenced by the allelopathic plant extracts showed a significant effect on shoot length of all the test species, as shown in Table 2. By increasing extract concentration, shoot length (cm) was reduced. Among the test species, there was maximum reduction in shoot length (0.73 cm) in wild oat, followed by wheat (1.81 cm) as compared to

Table 2 - Shoot length (cm) of wheat, wild oat and milk thistle as affected by various concentrations of the aqueous extracts of lotus and arrowhead weed

Target plant specie	Allelopathic plants used (water extracts)					Specie mean
	Nelumbo nucifera L.		*Sagittaria latifolia* L.		Control 0% conc.	
	10% conc.	5% conc.	10% conc.	5% conc.		
Triticum aestivum L.	4.00 ab	1.42 abc	0.00 c	2.22 abc	1.44 abc	1.81
Avana fatua L.	0.00 c	1.33 abc	0.00 c	1.00 bc	1.33 abc	0.73
Silybum marianum L.	2.22 abc	1.88 abc	3.00 abc	4.66 a	1.55 abc	2.66
Concentration mean	2.07	1.54	1.00	2.62	1.44	

LSD values (0.05) for Interaction: 3.33.

milkthistle (2.66 cm). Average shoot length (1.00 cm) was more influenced by 10% conc. of *Sagittaria* followed by lotus (2.07 cm) as compared to 5% (2.62 cm) of the *Sagittaria* extract. The data regarding interaction of the test species showed that shoot length of wild oat was significantly affected (0.00 cm, each) by both lotus and arrowhead with 10% extract concentration. However, in contrary the shoot length of milkthistle and wheat (4.00 and 2.22 cm, respectively) was positively affected by 10% conc. of lotus, as compared to its 5% conc. (1.42 and 1.88 cm, respectively). While in case of *Sagittaria* the increase in extract concentration negatively affected all the test species. However, wild oat was more affected by both extracts of wheat and milkthistle, but *Sagittaria* affected both wild oat and wheat as compared to milkthistle. Our findings are similar to those of Benyas et al. (2010), who investigated negative effects of alellochemicals from different aquatic weeds on plant root/shoot growth.

Shoot weight (g)

The statistical analysis of data (Table 3) showed that shoot weight of wheat, wild oat and milkthistle was significantly influenced by allelopathic extracts of lotus and *Sagittaria*. Shoot weight decreased by increasing extract concentration. Shoot weight of milk thistle (0.11 g) was much more reduced than that of wheat (0.97 g) and wild oat (2.41 g). A higher concentration of lotus and arrowhead reduced average shoot weight of the test species (0.04 and 0.05 g, respectively) as compared to a lower concentration of both species (2.05 and 2.03 g, respectively). Interaction of various extracts with the test species revealed that shoot weight of wild oat (0.00 g, each) was highly influenced by 10% conc. of lotus as well as *Sagittaria* as compared to shoot weight of wild oat in control as well as 5% conc. of lotus (6.00 g, each). However, wheat shoot weight was highly influenced by 10% conc. of *Sagittaria* compared to control (0.04 g) whereas at and 5% conc. of lotus and *Sagittaria*, higher shoot weight was noted (0.08 and 4.7 g, respectively) as compared to control (0.04 g). The effect of different extracts and their concentrations were minimum on shoot weight of milk thistle and all the values were at par with each other. Wild oat was more affected than milkthistle and milkthistle than wheat. These results are in line with those of Benyas et al. (2010), who reported that various aquatic weeds have bioactive compounds capable of reducing shoot biomass of agronomic crops and other terrestrial noxious weeds.

Table 3 - Shoot weight (g) of wheat, wild oat and milk thistle as affected by various concentrations of the aqueous extracts of lotus and arrowhead weed

Target plant specie	Allelopathic plants used (water extracts)					Specie mean
	Nelumbo nucifera L.		*Sagittaria sagittifolia* L.		Control 0% conc.	
	10% conc.	5% conc.	10% conc.	5% conc.		
Triticum aestivum L.	0.06 b	0.08 b	0.00 b	4.70 a	0.04 b	0.97 ab
Avana fatua L.	0.00 b	6.00 a	0.00 b	0.08 b	6.00 a	2.41 a
Silybum marianum L.	0.06 b	0.08 b	0.15 b	0.24 b	0.06 b	0.11 b
Concentration mean	0.04 b	2.05 a	0.05 b	1.67 ab	2.03 a	

LSD values (0.05) for Species mean: 1.99, Concentration mean: 1.81 and Interaction: 1.67.

Root weight (g)

The statistical analysis of the data revealed that root weight of the test species (wheat, wild oat and milkthistle) were non-significantly influenced by the aqueous extracts of lotus and *Sagittaria*; however their interactions were significantly affected (Table 4). Root weight (g) was suppressed with increasing extract concentrations. Average root weight was not significantly affected; however, wheat (0.02 g) was significantly affected as compared to (0.09 g) milk thistle and wild oat (1.33 g). However, the mean extract concentrations were non-significantly different from each other, but the 10% conc. of arrowhead and lotus reduced average root weight (0.01 and 0.12 g) as compared to the 5% conc. of aqueous extract of arrowhead and lotus (0.03 and 1.14 g) with respect to control (1.12 g). This showed that arrowhead is more toxic to the test species at both concentrations than lotus regarding root weight reduction. Furthermore, the interaction between test species in terms of extract concentration showed that 10% conc. of *Sagittaria* highly affected both root weight of both wild oat and wheat (0.00%) as compared to milk thistle (0.03 g) while lotus highly affected wild oat (0.00 g) as compared to wheat (0.05 g) and milk thistle (0.32 g). The 5% conc. of lotus aqueous extract enhanced the root weight of all the test species as compared to control while the same conc. of *Sagittaria* negatively affected them when compared to control. Therefore, the root weight of all test species was affected up to a certain level. Lower root weight and root inhibition led to reduced water and nutrient uptake and consequently lower protein synthesis, respiration, cell division and thickness of seminal roots as well as delayed growth (Jafariehyazdi and Javidfar, 2011). These findings are in line with the results of (Mishra, 2015) who reported that aquatic weed extracts limited plant seed germination and root biomass and its elongation as well as some other metabolic activities of the test species.

Table 4 **-** Root weight (g) of wheat, wild oat and milk thistle as affected by various concentrations of the aqueous extracts of lotus and arrowhead weed

Target plant specie	Allelopathic plants used (water extracts)					Specie mean
	Nelumbo nucifera L.		*Sagittaria sagittifolia* L.		Control (0%) conc.	
	10% conc.	5% conc.	10% conc.	5% conc.		
Triticum aestivum L.	0.05 b	0.04 b	0.00 b	0.01 b	0.03 b	0.02
Avana fatua L.	0.00 b	3.33 a	0.00 b	0.01 b	3.33 a	1.33
Silybum marianum L.	0.32 b	0.05 b	0.03 b	0.08 b	0.01 b	0.09
Concentration mean	0.12	1.14	0.01	0.03	1.12	

LSD values (0.05) for Interaction: 1.67.

The aqueous extracts of lotus and arrowhead negatively affected germination percentage, shoot length, shoot and root weight of all the test species i.e. wheat and its associated troublesome weeds such as wild oat and milk thistle. By increasing the extract concentration of lotus, somewhat positive effects were found on wheat whereas wild oat was negatively affected by increasing the extract concentration of lotus as well as arrowhead weed. Thus, it is recommended that *Sagittaria sagittafolia* and *Nelumbo nucifera* biomass should be used as mulch in wheat fields infested with wild oat and milk thistle near infested water bodies for proper weed biomass disposal, weed control and nutrient addition to the soil.

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