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Allelopathic Influences of Lower Plants on Barley (*Hordeum vulgare* L.) Seed Germination and Some Growth Characteristics

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Abstract

The present research aimed to study the effects of lower plant residues of (*Cladophora glomerata*, *Chara* sp., and *Ceratophyllum demersum*) with three treatments of 0.5, 1%, and 1.5% as an allelopathic agent on the barley (*Hordeum vulgare* L.) germination, growth properties, chlorophyll content, and relative growth rate (RGR). Statistically, a completely randomized design (CRD) was used with four replications, and Duncan's test was applied to compare means. Results showed that using *Chara* sp. at a dose of 1.5% significantly ($P \leq 0.05$) increased shoot weight, number of spikes, and the total weight of seed, while spike length significantly increased at a 1% dose. *Cladophora* sp. at 1.5% dose significantly($P \leq 0.05$) increased plant length, shoot weight, spike length, number of spikes, and the total weight of seed compared to the control plant. However, the flag leaf length was increased significantly ($P \leq 0.05$) at 1% treatment. *Ceratophyllum demersum* at 1% and 1.5 % doses ($P \leq 0.05$) causes increases in the total chlorophyll content, and chlorophyll a. The highest inhibition percentage was observed at a 1% dosage of *Cladophora glomerata* residues.

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1. Introduction

Allelopathy is the process by which one plant can stimulate or inhibit other plant growth through the production of allelochemicals [1] or can be defined as non-nutritional chemicals produced by one plant that affect the growth and health of other species [2].

Barley (*Hordeum vulgare* L.) is an annual herbaceous monocotyledonous grass from the Poaceae family, it was one of the first food crops to be domesticated since the dawn of civilization [3-6]. It ranks in the top three cereal crops in the world, after maize, wheat, and rice. It has 14 chromosomes and is a self-pollinating diploid species [7].

Lower plant residues are considered biostimulants that can be applied in different ways, such as foliar application or addition to soil or use in seed germination by imbibition method it can be used as fertilizer to induce plant growth, and also contain active compounds, which affect the physiology of plants through increasing plant growth, yield, and quality of crops [8].

Schaffer and Andersen (2018) mention that aquatic weeds or filamentous algae, such as *Cladophora* sp. or *Pithophora Oedogonium*, may consume nearly all available nitrogen or produce toxins that prevent the growth of barley. Another study in which a lower vascular plant was used on *Solanum lycopersium* and *Capsicum annum* plants by spray methods at different stages led to an increase in the plant size of pepper and tomato by 30% and 20%, respectively [9].

Prazukin, *et al.* [10] studied the application of algae powder to increase significantly plant height, leaf number and yield of Moongbean (*Phaseolus radiata*). Nofal Nofal, *et al.* [11] determined that using two wheat types and two irrigation techniques in combination with foliar sprays containing 1.5 g/L of the algal extract significantly improved wheat growth in sandy soil conditions.

The *Ceratophyllum demersum* and *Cladophora glomerata* work as nitrogen sinks in aquatic ecosystems, having allelochemical impacts on other organisms. Participating in suppressing the photosynthesis of competing primary producers seems to be a common method of action [12]. Lewandowska, *et al.* [13] revealed that the foliar application of the *Ceratophyllum* sp. extracts considerably increased the yield parameters, including the plant height, number of first branches, the weight of 1000 seeds, and yield, compared to the control.

However, Michalak, *et al.* [14] reported that the algal extracts were tested largely on wheat (*Triticum aestivum* L.; Poaceae) and garden cress (*Lepidium sativum* L.; Brassicaceae) to increase root thickness and above-ground biomass as well as chlorophyll and carotenoid content in plant shoots.

This study aims to know the allelopathic effect of some lower plant residues (*Cladophora* sp., *Chara* sp., and *Ceratophyllum demersum*) on seed germination, vegetative, reproductive, chlorophyll content and RGR of barely.

2. Materials and Methods

The samples of filamentous algae blanket weed or pond scum (*Cladophora glomerata*), stonewort (*Chara* sp.) and aquatic plants hornwort (*Ceratophyllum desmersum*) were collected from ponds (Guwer District) and streams (Shaqlawa Subdistrict) were dominant and blooming, and brought to the laboratory and cleaned from soil residues and suspended matter by washing them with water well. It was identified and classified using the classification key according to [15], and the collected algae were air-dried in a laboratory, hand-crushed, and pulverized in a mixer grinder. The algae powder was mixed with soil and placed into pots at a rate of 7 kg for each pot (Table 1). The experiment was conducted in the greenhouse of the Biology Department, College of Science, University of Salahaddin-Erbil, Iraq, following CRD design, with four replicates for each concentration. The treatments were prepared as the control treatment without any additives and treatments 0.5, 1, and 1.5% which is equivalent to 5, 10, and 15gm of algae and aquatic plant powders per kg of soil. *Hordeum vulgare* L. seeds were sown in pots with a 30 cm diameter and sandy loamy soil. In each pot, 7 seeds of barley were planted, and after growth, it was reduced to five plants for each pot. Selected crop growth at the time of harvest and overall yields were recorded. Bioassays were conducted on filter paper (12–15 cm) in a 140 mm diameter petri dish saturated with 4 ml of sterile distilled water to determine the allelopathic activity of lower plant residue on barley seedlings.

Several vegetative and reproductive traits, such as plant length (cm), flag leaf width and length (cm), shoot weight (gm), spike weight (gm), number of spikes, spike length (cm), number of seeds, total seed weight (gm), the weight of 100 seeds (gm), leaf area (cm^2), relative growth rate-(RGR) (gm/Kg/day), and chlorophyll content, were measured after the plant reached physiological maturity. The total chlorophyll content of leaves was extracted and measured according to [16].

Germination %, Inhibition %, Germination index%, and Plumule and radical elongation velocity are calculated as follows [17]:

$$Germination\ \% = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds\ sown}$$

$$Germination\ inhibition\ \% = \frac{Germination\ rate\ of\ control - germination\ rate\ of\ treatment}{Germination\ rate\ of\ control} * 100$$

$$\text{Germination index\%} = \frac{\%\ seed\ germination * root\ elongation}{100}$$

$$\text{Plumule or radicale elongation velocity } (\frac{\text{cm}}{\text{day}}) = \frac{Radical\ or\ plumule\ length\ (cm)}{Total\ number\ of\ days}$$

Table 1. Some chemical and physical properties of the soil used in the experiment.

Parameters	Measured units	Value
Clay	PSD g/Kg	241.6
Silt	PSD g/Kg	30.4
Sand	PSD g/Kg	728
Sandy Clay loam		1000
pH	----	7.92
EC	$dS.m^{-1}$	0.525
Organic Matter	%	0.34
Cation Exchange Capacity	$(Meq.100gm^{-1})$	18.99
	Available Nutrients	
Available Nitrogen	$(mg.Kg^{-1})$	234
Available Phosphorus	$(mg.Kg^{-1})$	17.18
Available Potassium	$(mg.Kg^{-1})$	12.41
Available Calcium	$(mg.Kg^{-1})$	33.0
Available Magnesium	$(mg.Kg^{-1})$	63.5

Four replications were used in the experiments with the Completely Randomized Design (C. R. D.) design, and the means were compared using the Duncan test at a significant 5% level [18].

3. Results and Discussion

As it is obvious from Table (2) using *Chara* sp. concentration at a dose of 1.5% significantly ($P \leq 0.05$) increased shoot weight, number of spikes, spike length and the total weight of seed. Also, spike length significantly increased at a 1% dose compared to the control plant. At the same time, it did not show any significant difference from other studied growth characteristics. Similar results were obtained [19] on *Zea mays* and [20] on rice. The increased vegetative characteristics of plants may be due to a considerable amount of micronutrients and amino acids in algae residue such as N, Mg, Ca, and K which are responsible for growth enhancement [21]. However, leaf area and RGR decreased non-significantly compared to the control treatment.

Table 2. Effect of different doses of *Chara* sp. on some vegetative and reproductive growth of barley (*Hordeum vulgare* L.) crop.

Growth characteristics	Control	0.5%	1%	1.5%
Plant length (cm)	63.5 ± 7.25^{ab}	62.1 ± 1.63^{ab}	57.1 ± 1.74^{b}	74.3 ± 1.00^{a}
Length of flag leaf (cm)	6.94 ± 1.06^{a}	6.22 ± 1.09^{a}	8.61 ± 0.53^{a}	6.77 ± 0.93^{a}
Width of flag leaf (cm)	0.70 ± 0.07^{a}	0.544 ± 0.07^{a}	0.711 ± 0.04^{a}	0.655 ± 0.01^{a}
Shoot weight (gm)	2.23 ± 0.32^{a}	2.41 ± 0.09^{a}	2.60 ± 0.52^{a}	5.12 ± 0.66^{b}
Spike weight (gm)	0.89 ± 0.15^{a}	1.02 ± 0.03^{a}	1.04 ± 0.12^{a}	1.15 ± 0.06^{a}
Number of spikes	1.11 ± 0.11^{b}	1.33 ± 0.19^{b}	1.55 ± 0.11^{ab}	1.88 ± 0.11^{a}
Spike length (cm)	14.9 ± 0.44^{c}	15.1 ± 0.29^{bc}	16.4 ± 0.54^{a}	16.3 ± 0.16^{ab}
Number of seeds	13.4 ± 1.74^{a}	14.5 ± 0.40^{a}	14.2 ± 1.56^{a}	17.4 ± 1.12^{a}
Total weight of seeds (gm)	2.27 ± 0.25^{b}	2.95 ± 0.21^{b}	2.82 ± 0.44^{b}	5.36 ± 0.41^{a}
Weight of 100 seeds	5.49 ± 0.29^{a}	5.89 ± 0.08^{a}	5.95 ± 0.09^{a}	5.47 ± 0.02^{a}
Leaf area (cm^2)	3.75 ± 0.85^{a}	2.64 ± 0.739^{a}	4.63 ± 0.586^{a}	3.35 ± 0.507^{a}
RGR (gm/kg/day)	4.43 ± 1.07^{a}	1.47 ± 1.132^{a}	1.98 ± 1.776^{a}	1.82 ± 1.329^{a}

Results in Table (3) indicated that using *Cladophora* sp. at dose 1.5% significantly ($P \leq 0.05$) increased shoot weight, spike length, number of spikes, and the total weight of seed as compared to the control treatment. While, highest length of flag leaf was recorded at 1% treatment with significant differences ($P \leq 0.05$), as well as, shoot weight and number of spikes, spike length, and the total weight of seeds were increased. Clear increases in most plant growth characteristics were observed at 0.5%. An increase in vegetative and reproductive characteristics may be referred to as an algal extract containing all hormones and nutrients that are essential for plant growth [22].

Both algal residues (*Chara* sp. and *Cladophora* sp.) and in all concentrations led to increases in the shoot weight of barley plant. Similar results were obtained [23] on *Vigna radiate* and *Sesamum indicum* plants by [13]. The auxin found in algae, which is important for the division of the apical meristem, may cause an increase in plant length when employing algae [24]. Belkhadir, *et al.* [25] illustrated that the interaction of polysaccharides with leucine-rich repeat membrane receptors,

which can activate pathways that result in the control of numerous genes involved in cell growth, has increased plant growth in lower vascular plants.

Table 3. Effect of different doses of *Cladophora glomerata* on some vegetative and reproductive growth of barley (*Hordeum vulgare* L.) crop.

Growth characteristics	Control	0.5%	1%	1.5%
Plant length (cm)	63.5±7.25^{ab}	77.1±6.66^{ab}	76.6±4.28^{ab}	84.4±4.15^{a}
Length of flag leaf (cm)	6.94±1.06^{b}	6.72±0.24^{b}	10.7±0.96^{a}	8.66±0.60^{ab}
Width of flag leaf (cm)	0.70±0.07^{a}	0.65±0.011^{b}	0.85±0.055^{a}	0.71±0.048^{ab}
Shoot weight (gm)	2.23±0.32^{c}	5.95±1.54^{b}	8.82±1.47^{ab}	11.5±0.11^{a}
Spike weight (gm)	0.89±0.15^{a}	2.78±1.70^{a}	1.22±0.13^{a}	1.24±0.17^{a}
Number of spikes	1.11±0.11^{c}	2.05±0.33^{bc}	2.77±0.48^{b}	4.16±0.25^{a}
Spike length (cm)	14.9±0.44^{c}	15.5±0.38^{b}	16.1±0.78^{ab}	17.3±0.51^{a}
Number of seeds	13.4±1.74^{a}	16.9±1.61^{a}	17.6±0.76^{a}	17.7±2.23^{a}
Total weight of seeds (gm)	2.27±0.25^{c}	5.83±1.45^{b}	8.27±1.16^{ab}	10.1±1.03^{a}
Weight of 100 seeds	5.49±0.29^{a}	5.87±0.08^{a}	5.53±0.41^{a}	5.34±0.01^{a}
Leaf area (cm^2)	3.75±0.85^{b}	3.31±0.14^{b}	6.99±1.10^{a}	4.65±0.85^{ab}
RGR (gm/kg/day)	4.43±1.07^{ab}	1.48±0.49^{b}	2.78±0.25^{ab}	9.82±4.42^{a}

Different doses of *Ceratophyllum demersum* significantly ($P \leq 0.05$) decreased the vegetative and reproductive characteristics of barley plant except for the length of flag leaf increased significantly at a dose 0.5 % as it is clear from Table (4) while treatments showed a nonsignificant difference of a number of spikes and these results disagree with [26]. Both 1% and 1.5 % doses decreased ($P \leq 0.05$) leaf area and increased RGR significantly.

As clarified in Table (5), treatments showed no significant difference in chlorophyll a, chlorophyll b. Meanwhile, for *Cladophora* sp., aside from a 1% dose, there are no discernible differences between the treatments in terms of total chlorophyll, chlorophyll a, and chlorophyll b. These findings contradict the results of [14]. For both algae residues (*Chara* sp. and *Cladophora* sp.), statistically non-significant differences ($P > 0.05$) were found between the control treatment and other treatments for chlorophyll content. Commonly doses of *Ceratophyllum demersum* showed significant induction ($P \leq 0.05$) for total chlorophyll at 1% and 1.5 % doses, chlorophyll a significantly increased at all studied doses (0.5% 1%, 1.5%) and chlorophyll b decreased at 0.5% and 1.5%. An increase in chlorophyll content is due to the presence of cytokinins hormone in algae which can inhibit senescence in leaves by decreasing the effect of ethylene or abscisic acid [27]. An increase in chlorophyll content agreed with the result obtained by [26].

Table 4. Effect of different doses of *Ceratophyllum demersum* on some vegetative and reproductive growth of barley (*Hordeum vulgare* L.) crop.

Growth characteristics	Control	0.5%	1%	1.5%
Plant length (cm)	63.5±7.25^{a}	40.5±1.44^{b}	40.0±0.57^{b}	39.0±0.00^{b}
Length of flag leaf (cm)	6.94±1.06^{a}	10.0±0.00^{a}	7.75±0.14^{b}	7.00±0.28^{b}
Width of flag leaf (cm)	0.70±0.07^{a}	0.50±0.00^{b}	0.40±0.00^{bc}	0.40±0.00^{bc}
Shoot weight (gm)	2.23±0.32^{a}	0.38±0.00^{b}	0.32±0.00^{b}	0.32±0.00^{b}
Spike weight (gm)	0.89±0.15^{a}	0.23±0.01^{bc}	0.43±0.00^{b}	0.08±0.01^{c}
Number of spikes	1.11±0.11^{b}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
Spike length (cm)	14.9±0.44^{a}	9.00±0.57^{bc}	9.25±0.14^{bc}	9.75±0.14^{b}
Number of seeds	13.4±1.74^{a}	3.50±0.28^{c}	7.50±0.28^{b}	1.00±0.57^{c}
Total weight of seeds (gm)	2.27±0.25^{a}	0.32±0.00^{b}	0.34±0.00^{b}	0.05±0.00^{b}
Weight of 100 seeds	5.49±0.29^{a}	4.86±0.00^{b}	4.60±0.00^{b}	3.60±0.00^{c}
Leaf area (cm^2)	3.75±0.85^{a}	3.75±0.00^{a}	2.33±0.04^{b}	2.10±0.09^{b}
RGR (gm/kg/day)	4.43±1.07^{b}	7.11±0.29^{ab}	9.36±0.098^{a}	8.74±0.26^{a}

Table 5. Effect of different doses of lower plants on barley (*Hordeum vulgare* L.) crop on total chlorophyll, chlorophyll *a*, and *b*.

Lower plants residues	Dosage	Germination%	Inhibition germination %	Germination index	Plumule elongation velocity (cm/day)	Radicle elongation velocity (cm/day)
	Control	100	0	0.657	1.398	1.397
Chara sp.	0.5%	26.6	73.4	0	0	0
	1%	16.6	83.4	0	0	0
	1.5%	20	80	0	0	0
Cladophora glomerata	0.5%	23.3	76.7	0	0	0
	1%	10	90	0	0	0
	1.5%	10	90	0	0	0
Ceratophyllum desmersum	0.5%	16.6	83.4	0	0	0
	1%	16.6	83.4	0	0	0
	1.5%	47.57	52.43	0	0	0

Table 6. Effects of *Lower plants* on seed germination of barely cultivar.

Lower plants residues	Dosage	Total Chlorophyll	Chlorophyll *a*	Chlorophyll *b*
Chara sp.	Control	0.224±0.01^{a}	0.164±0.004^{a}	0.059±0.012^{a}
	0.5%	0.199±0.04^{a}	0.159±0.036^{a}	0.039±0.007^{a}
	1%	0.228±0.01^{a}	0.182±0.017^{a}	0.045±0.003^{a}
	1.5%	0.201±0.02^{a}	0.137±0.034^{a}	0.064±0.011^{a}
Cladophora glomerata	Control	0.224±0.01^{a}	0.164±0.004^{a}	0.059±0.012^{a}
	0.5%	0.174±0.005^{a}	0.133±0.007^{a}	0.041±0.003^{a}
	1%	0.207±0.02^{a}	0.164±0.015^{a}	0.042±0.005^{a}
	1.5%	0.178±0.01^{a}	0.140±0.008^{a}	0.037±0.008^{a}
Ceratophyllum desmersum	Control	0.224±0.01^{b}	0.164±0.004^{c}	0.059±0.012^{a}
	0.5%	0.188±0.00^{c}	0.165±0.000^{c}	0.022±0.000^{b}
	1%	0.245±0.00^{a}	0.193±0.000^{b}	0.051±0.000^{a}
	1.5%	0.244±0.00^{a}	0.224±0.000^{a}	0.019±0.000^{bc}

As shown in (Table 6), the highest germination % for barely seeds was 26.6% and 23.3% for *Chara* sp. and *Cladophora glomerata* at 0.5% dosage. While it reaches 47.57% for *Ceratophyllum desmersum* at 1.5% dosage. *Cladophora glomerata* had the highest inhibition percentage 90% at 1% dosage compared with the other two species. No germination index, plumule and radical elongation velocity were observed for all studied plant residues and treatments. Some algal allelochemicals can serve as allelopathic agents, reducing seed germination. These chemicals can interfere with the normal physiological processes of germination. Algal residues can have an impact on the availability of nutrients in the soil. Some allelochemicals can alter nutrient concentrations in soil by either directly affecting nutrient release or indirectly influencing microbial activity [28].

4. Conclusion

The allelopathic effects of lower plants via plant growth characteristics and barely seed germination were assessed by using different doses. From the results, algal residues of *Chara* sp. led to increased shoot weight, and number of spikes. *Cladophora glomerata* residues enhance the growth of shoot weight, number of spike, spike length, and weight of seeds. While *Ceratophyllum desmersum* residue decreases the width of the flag leaf, shoot weight and weight of 100 seeds. Algal residues have very effective role in seed germination inhibition, with observation of plumule and radical elongation velocity. Generally, *Ceratophyllum desmersum* residues have an effective allelopathic response to growth characteristics and chlorophyll content than the other two studied plants.

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التأثيرات الأليلوباثية للنباتات الواطئة على إنبات الشعير (*Hordeum vulgare* L.) وبعض خصائص النمو

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الخلاصة:

يهدف البحث الحالي إلى دراسة تأثير المخلفات النباتية الواطئة لكل من (*Cladophora glomerata*, *Chara* sp. و*Ceratophyllum demersum*) وبثلاث معاملات 0.5%، 1%، و1.5% كعامل أليلوباثي على إنبات الشعير (*Hordeum vulgare* L.) ،خصائص نموه ،محتوى الكلوروفيل ومعدل النمو النسبي (RGR). إحصائيًا ، تم استخدام تصميم عشوائي الكامل (CRD) وبأربعة مكررات ، وتم استخدام اختبار Duncan لمقارنة المعدلات. أظهرت النتائج أن استخدام *Chara* sp. بتركيز 1.5٪ أدت إلى زيادة معنوية ($P\leq0.05$) في وزن الجزء الخضري وعدد السنابل والوزن الكلي للبذرة ، بينما زاد طول السنبلة معنوياً بجرعة 1٪. استخدام بقايا *Cladophora* sp. بجرعة 1.5٪ ادت الى زيادة معنوية ($P\leq0.05$) في طول النبات ، ووزن النبتة ، وطول السنبلة ، وعدد السنابل ، والوزن الكلي للبذرة مقارنة بالنبات المقارنة ، كما أدى إلى زيادة معنوية ($P\leq0.05$) في طول ورقة العلم عند جرعة 1٪. بينما أدت تراكيز *Ceratophyllum demersum* عند 1٪ و 1.5٪ إلى زيادة معنوية ($P\leq0.05$) لمحتوى الكلوروفيل الكلي والكلوروفيل أ و RGR. وقد لوحظت أعلى نسبة تثبيط عند تركيز 1% من بقايا *Cladophora glomerata*.