Effect of *Ulothrix subconstricta* on Seed Germination of *Allium cepa* L. Var. Regal F1 and Malbec

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KEYWORDS

Bio-fertilizer
Cost-effective
Germination rate
Onion seeds
Seedling growth
Viability of seeds

ABSTRACT

Algae are photosynthetic organisms that are found in mostly marine and freshwater habitats. *Allium cepa* (*A. cepa*) is perennial herb and a rich source of dietary fibre. Algae can be used as a bio-fertilizer for *A. cepa* seeds germination. The aim of using algae as bio-fertilizers is to provide an eco-friendly, natural fertilizer and protect against the harmful effects of chemical fertilizers. An experiment was conducted in the Phycology lab at Government College University (GCU), Lahore, to study the impact of freshwater algae on the seed germination of *A. cepa* varieties, Regal F1 and Malbec. Seeds were collected from the Seed Certification Department in Lahore, Punjab, and algae were obtained from the GCU botanical garden. The algae were identified under a microscope and used in powdered form to prepare different algal concentrations (10%, 50%, and 100%) to assess their impact on seedling growth, with water serving as the control. The seedlings were observed every three days under favourable conditions for a total of 15 days, measuring parameters such as shoot length, root length, number of leaves, and leaf length. The total germination rate for Regal F1 was 95.10%, while Malbec exhibited the germination rate of 94.40%. Statistical analysis was performed using Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) test for further confirmation of results. The findings indicate that maximum growth (in terms of shoot length, root length, and leaf length) occurred at the 10% algal concentration, while minimum growth occurred at 100% algal extract concentration as confirmed by the LSD test. In the future, using algae as bio-fertilizers can help create a more sustainable environment, improve soil health, reduce pressure on farmers due to low expense and protect from chemical fertilizers.

1. Introduction

Allium cepa L. (onion) is one of the most widely cultivated vegetable crops globally, valued for its nutritional, medicinal, and economic importance, and belongs to the Amaryllidaceae family (Chakraborty *et al*., 2022). Its seed germination rate and uniformity are critical factors for successful crop establishment, particularly under suboptimal environmental conditions. Variability among onion cultivars in germination response highlights the importance of identifying effective natural treatments to enhance early seedling performance (Reed *et al*, 2022).

Seed germination is a critical phase in the plant life cycle, determining the successful establishment, growth, and productivity of crops (Bewley *et al*., 2013). The germination process is influenced by various internal and external factors, including temperature, light, water availability, and biotic interactions (Nonogaki *et al*., 2010). Recent research has focused on the use of natural bio-stimulants derived from algae to improve seed germination, seedling vigor, and overall crop performance (Ronga *et al*., 2019). Algae are known to produce a wide range of bioactive compounds, such as phytohormones, amino acids, polysaccharides, vitamins, and trace elements that can positively influence plant metabolism and development (Dineshkumar *et al*., 2020)

Green algae (Chlorophyta) are among the most studied groups for their potential agricultural applications. Species belonging to genera such as *Chlorella*, *Ulva* and *Ulothrix* have demonstrated bio-fertilizing and growth-promoting properties when applied to seeds or soils (Kholssi *et al*., 2022). Microalgae directly affect essential

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ecosystem services. It is economically feasible for the farmers and have a great potential applicability in agriculture as it is very cost effective for producers and easily affordable for them. When the biomass or microalgae byproducts are used in the fields then the plants show remarkable growth. Micronutrients and macronutrients are in larger amount in the microalgae (Shaaban, 2001). Algal extracts also act as bio stimulants, boosting seed germination, crop quality, and overall yield (Sengar *et al*., 2010; Enan *et al*., 2016). The genus *Ulothrix*, a filamentous green alga commonly found in freshwater habitats, is known to contain chlorophyll pigments and micronutrients beneficial for plant growth (Mariam *et al*., 2025).

The use of bio-fertilizers and organic fertilizers has become popular. In sustainable agriculture, bio-fertilizers can be used in place of or in addition to chemical fertilizers since they are inexpensive, renewable, and ecologically friendly sources of plant nutrients. In order to fix nitrogen, mineralize phosphate and potassium release plant growth stimulants, produce antibiotics, breakdown organic detritus in soil, whereas[WU1] fertilizers employ microorganisms (Abdel-Raouf *et al*., 2012). Long-term, continuous use of bio-fertilizers makes parental inocula sufficient for further multiplication, enabling them to take part in nutrient cycling and boost crop output (Youssef and Eissa, 2014). Compared to chemical or other manure fertilizers, algae produce less mineral runoff, making them an excellent source of fertilizer (Hameed, 2007; EI-Kouny, 2007[WU2]).

Algae are used for producing the biomass which is applied in the fields as bio-fertilizers and soil conditioner. Bio-fertilizers mainly consist of nitrogen-fixing cyanobacteria, which are utilized in tropical lowland rice cultivation. Eukaryotic, unicellular, green microalgae are used as soil-conditioning agents in many fields (Metting, 1990). Microalgae can directly affect ecosystem services such as reducing greenhouse gas emission in the ecosystem, reclaiming water and recovering additional nutrients from the soil, sequestering CO_2 and in agricultural runoff (Abd Allah, 2022).

The aim of this study is to explore the potential of freshwater algae as a bio-fertilizer and assess its effect on the seed germination of two varieties of *A. cepa* L., namely Regal F1 and Malbec. Algae, being rich in nutrients like nitrogen, phosphorus, and trace elements, are considered a sustainable alternative to chemical fertilizers. The objective of this research is to evaluate how algae-based bio-fertilizers influence the germination rate, growth, and overall health of onion seeds.

2. Materials and Methods

The experiment was conducted in the Phycology lab of the Department of Botany at Government College University, Lahore, to investigate the effects of different concentrations of algal extract on seed germination of *A. cepa*.

2.1 Seed collection

Two varieties of *A. cepa* L. (onion), Regal F1 and Malbec, were selected for the study. Seeds were collected from the Federal Seed Certification Department in Lahore, Punjab, and used in the experiment (Fig. 2a (A)).

2.2 Collection of algae

Freshwater algae were collected from the Botanical Garden of Government College University, Lahore (Fig. 1). Polythene bags were used to transport the algae to the Phycology lab, ensuring the samples remained intact (Fig. 2a (B)).

Fig. 1. Map Showing the Algal Collection site GCU Botanical

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[WU3]***2.3 Preservation and identification of***

For preserving the algae, a 4% formalin solution (Formalin) was prepared by mixing 4mL of formalin with 96mL of distilled water to make a total volume of 100mL. A small portion of the algae was immersed in this solution for 15 min to preserve it. Afterward, a small portion of the preserved algae was placed on a microscope slide, covered with a cover slip, and examined under a compound microscope for identification (Fig 2a (C)).

2.4 Grinding of algal sample

The collected algae were then dried on blotting paper in an arid condition, away from direct light to prevent damage (Fig. 2a (D)). The drying process took about 3 to 4 days. Once dried, the algae were stored in airtight bags (Fig. 2a (E)). The dried algae were then ground into a fine powder using a grinder mixer, which was subsequently used to prepare different concentrations of algal solutions for the experiment.

2.5 Checking viability of seeds

Before initiating the seed germination experiment, the viability of the seeds was checked by soaking them in water for 24 h (Fig. 2a (F)). Seeds that floated on the water's surface were considered non-viable and discarded, while the seeds that sank were deemed viable for the experiment. These viable seeds were then used for germination testing.

2.6 Preparation of algal Extract

The algal extract was prepared by dissolving 12.4g of the dried algal powder into distilled water. The total volume was raised to 250mL by adding distilled water. The flask was sealed with a cotton plug and wrapped in cling film, labeled as "algal extract solution." The prepared solution was then autoclaved at 121°C and 15lb pressure for sterilization. After cooling, the algal extract was ready to be used for making different concentrations (Fig. 2a. (G)).

2.7 Preparation of different treatments

To prepare the different concentrations for testing, the algal extract was divided into 10%, 50%, and 100% solutions. For the 10% solution, 10mL of the algal extract was mixed with 90mL of distilled water, and the flask was labeled accordingly. Similarly, for the 50% concentration, 50mL of the algal extract was mixed with 50mL of distilled water. The 100% concentration was used as is, without adding any distilled water, as it contained the pure algal extract.

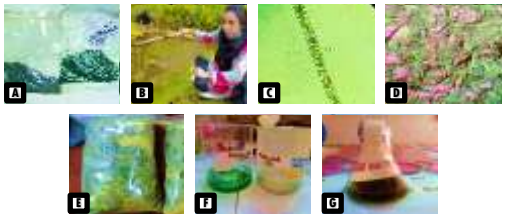

Fig. 2a. Some photographs during experiment (A) Seeds of *A. cepa* (Regal F1 and Malbec) collected from the Seed Certification Department, Punjab, Lahore (B) Collection of algae from the Botanical Garden of Government College University, Lahore (C) *U. subconstricta* was identified under the microscope (D) Drying the freshwater algae by placing it on the blotting paper € Placed the algal samples in polythene bags for the Grinding (F) Soaking of seeds in order to check the viability (G) Preparation of algal extract

2.8 Experimental setup

For the seed germination experiment, a total of 24 petri plates were used, with 12 plates designated for the Regal F1 variety and 12 for the Malbec variety. The petri plates were thoroughly washed with alcohol to prevent contamination. The plates were then divided into different groups: three petri plates for the control (no algal extract), three petri plates for the 10% algal extract, three petri plates for the 50% algal extract, and three petri plates for the 100% algal extract (Fig. 2b (H)). This procedure was repeated for both onion varieties, and the petri plates were labeled according to their respective treatments using paper tape and markers.

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Each petri plate was prepared by placing six viable seeds in it, ensuring they were evenly spaced (Fig. 2b (I-K)). The plates were covered with their lids and wrapped with cling film to prevent contamination. They were then placed in the Phycology lab under controlled conditions to observe seed germination over time (Fig. 2b (L)).

Fig. 2b. Some photographs during experiment (H) Set the apparatus according to the instruction and place the seeds at a remarkable distance and label everything using paper tape and marker (I-K) Pouring the different concentration of algal extract in different petri-plates named as 10%, 50% and 100% and place the seeds in these petri-plates (L) Germination of onion seedling.

2.9 Readings and Measurements

The readings and measurements were taken after 3 days respectively for 15 days.

2.10 Statistical analysis

The statistical analysis was carried out using SPSS version 16. The different groups were compared using a two-way ANOVA LSD. The values reported are the means and standard deviations (mean ± SD) of three replicates. $P < 0.05$ was considered statistically significant in this study.

3. Results

Algal extract was utilized as a bio-fertilizer for studying the seed germination of onion. Different parameters were studied such as shoot length, root length, total height, leaf number, number of adventitious roots and germination percentage of both onion seed varieties (Table 1).

The total germination rate of the variety Regal F1 is 95.10% and the Variety Malbec shows germination of 94.40% in the whole experiment at different concentration of *U. subconstricta*.

Table 1. Germination percentage of varieties of seed

Varieties	Germination percentage
Variety Regal F1	95.10%
Variety Malbec	94.40%

3.1 Effect of different concentrations of algal extract on various parameters of onion seedlings

The relationship between mean shoot length and algal extract concentrations is shown in Fig.3. According to this graph the Variety Regal F has shown maximum shoot length on the 10% concentration which is about 2.28cm and the minimum value is at 100% concentration which is 1.04cm. Similarly the variety Malbec has maximum shoot length at the 10% which is 2.16cm and the minimum shoot length of Malbec is at 100% which is 1.07cm.

A two-way ANOVA was conducted to examine the effects of *U. subconstricta* extract concentration and variety type (Regal F1 and Malbec) on the shoot length of *A. cepa* L. The results revealed a significant statistical effect of *U. subconstricta* extract concentration on shoot length ($p = 0.00$), whereas variety type had no significant effect on plant growth ($p = 0.81$). Due to significant differences among concentration levels, a LSD test was conducted to compare the concentration levels to the control.

There is no significant difference between the control and the 10% concentration of *U. subconstricta* extract. But the 50% and 100% concentration show significant difference from the control at $\alpha=0.05$. Basically, LSD is performed to determine which concentration shows significance from the control.

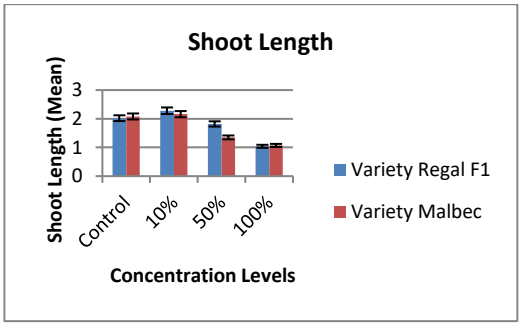

Fig. 3. Graphical representation of different concentrations (Control, 10%, 50% and 100%) of *U. subconstricta* on the shoot length of *A. cepa* L.

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The relationship between root number and varying concentration of algal extract is shown in Fig. 4. *A. cepa* L. variety Regal F1 has a maximum root number at the 10% concentration level which is 1.89cm while the minimum root number is 1.17cm at the 100% concentration. The Variety Malbec show maximum root number is at the control as well as 10% concentration which is about 1.89cm and its lowest value is 1.17cm on 100% concentration.

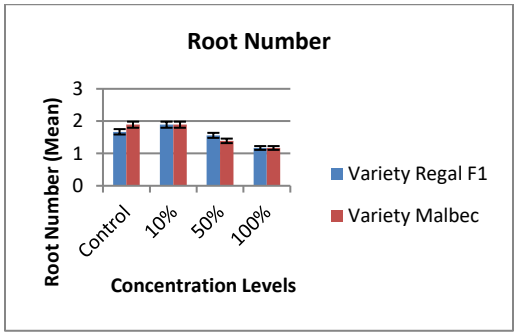
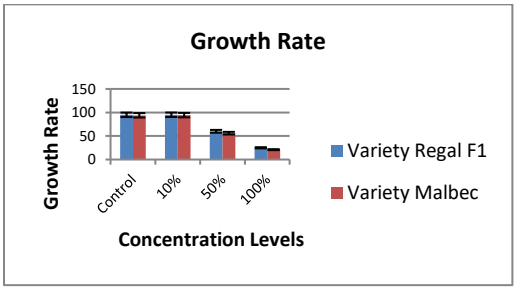


Fig. 4. Graphical representation of *Ulothrix subsconstricta* extract of different concentrations (Control, 10%, 50%, 100%) on the root number of onion seedling.

Two-way ANOVA to analyze the effect of *U. subsconstricta* extract concentration and variety type (Malbec and Regal F1) on root number of *A. cepa* L. was used. There is no significant difference in the variety type because the p=0.894 but the concentration level shows a remarkable significant difference p=0.00 with the control. So, as there is a significant difference is present in the concentration level, we apply the LSD test for further analysis.

There is a significant difference between control and 50% and between control and 100%. When we apply the LSD, it shows a significant difference between the control and 50% and 100% concentration at α=0.05 but there is no significant difference between the control and 10% concentration. It explains that they have a great impact on the plant growth rate[WU4] (Fig.



[WU5] **Fig. 5.** Graphical representation of *Ulothrix subsconstricta* of different concentrations (Control, 10%, 50%, 100%) on the growth rate of onion seedling

The relationship between mean root length and varying concentration of algal extract is shown in Fig. 6. The Regal F1 has maximum root length at 10% concentration which is about 1.03cm while the minimum root length is at 100% concentration which is 0.27cm. Malbec variety shows maximum root length at 10% concentration which is 0.75cm, and its minimum root length is seen on the 100% concentration which is 0.34cm.

Two-way ANOVA to analyze the effect of algal extract concentration and variety type (Regal F1 and Malbec) on root length of *A. cepa* L. was used. There is a significant difference in the variety type p=0.00 and also in the concentration levels p=0.00. As there is a significant difference, the LSD test is used to compare the control and concentration levels and variety type.

There is a significant difference between control and all other concentrations. When we compare the means of concentrations and LSDα, they show a significant difference at α=0.05. This statistical study proves that it has a great effect on the plant germination rate.

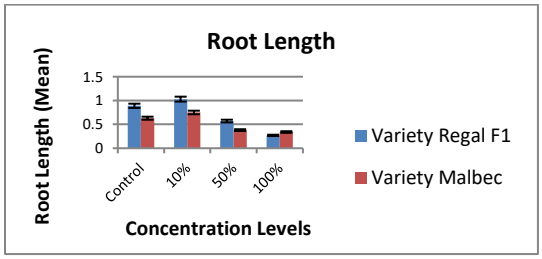


Fig. 6. Graphical representation of different concentrations (Control, 10%, 50%, 100%) of *U. subconstricta* on the root length of *A. cepa* L.

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The relationship between leaf number and varying concentration of algal extract is shown in Fig. 7. *A. cepa* L. variety Regal F1 showed maximum leaf number on the control and 10% concentration solution which is about 1.00cm, but the minimum value is on the 100% concentration which is 0.72cm. The Malbec variety also shows maximum value on the control and 10% concentration solution which is 1.00cm and its minimum value is also the same on the 50% and 100% concentration that is 0.78cm.

There is no significant difference in the variety at p=0.776 but the concentration levels show a significant difference from the control at p=0.00. As there is a significant difference in the concentration levels so we apply the LSD test for further analysis and determine which concentration show significant difference from the control.

Control and 10% concentration show that there is no significant difference between these, showing the same growth at α=0.05 but at the same time the 100% and 50% concentrations show a significant difference with the control at α=0.05 error margin. This analysis explains to us that a significant difference has a great influence on the plant germination rate.

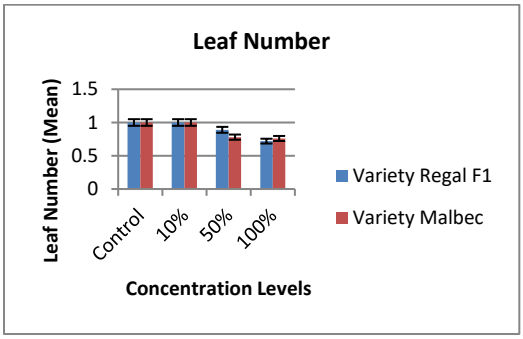

Fig. 7. Graphical representation of varying concentrations of *U. subconstricta* on the leaf number of *A. cepa* L.

The relationship between leaf length mean and varying concentration of algal extract is shown in Fig. 8. *A. cepa* L. variety Regal F1 has shown maximum leaf length at the 10% concentration which is about 3.31cm, and the minimum value is 1.71cm at the 100% concentration. The variety Malbec has a maximum value at the control which is 3.17cm and 1.68cm is the lowest value at the 100% concentration.

Two-way ANOVA to analyze the effect of *U. subconstricta* extract concentration and variety type (Regal F1 and Malbec) on leaf length of *A. cepa* L. was used. There is no significant difference between the variety type because the p=0.161 but a significant difference is present in the concentration levels by p=0.00. As there is a significant difference is present in the concentration level so we apply other test known as LSD, in order to know which concentration shows a significant difference from the control.

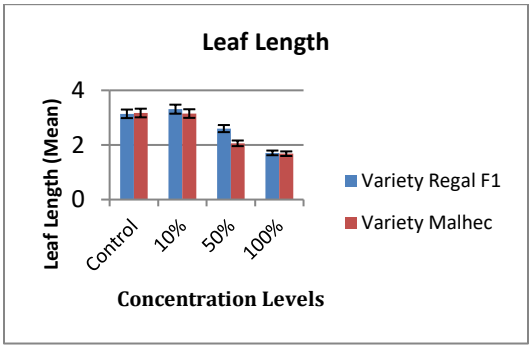

Fig. 8. Graphical representation of different concentrations (Control, 10%, 50% and 100%) of *U. subconstricta* on the leaf length of *A. cepa* L.

The result of LSD proves that there is no significant difference between the control and 10% concentration at α=0.05 but at the same time 50% and 100% concentration show a remarkable significant difference with the control at α=0.05. This explains us to that the germination of plants is highly influenced.

4. Discussion

The cultivation and use of onions is widespread. They are generally served cooked, as a vegetable, or as a component of a prepared culinary food, but they can also be consumed raw or used to produce pickles or sauces. They have a strong flavor when chopped and contain several chemicals that could irritate the eyes.

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Dineshkumar *et al*. 2020 studied the soil treated with microalgae and containing cow dung had the highest availability of micro- and macronutrients, whereas the control plant soil had the lowest mineral content. When the plants were first sprayed with cow dung plus *Spirulina* and subsequently with cow dung plus *vulgaris*, the factors, such as plant height, leaf weight per plant, fresh weight per plant, leaf numbers per plant and dry weight were showed higher growth than they did for the control plants. In the current study, it was discovered that plants with cow dung plus *S. platensis* or cow dung plus *C. vulgaris* application shows higher values for growth parameters such as plant height, number of leaves per plant, fresh weight per plant, leaf weight per plant and dry weight than control plants. Plants that were clearly marked as having Platensis treatment indicated that the onions had a larger yield and size which increases market value. Micro-algal bio-fertilizers can replace chemical fertilizers because they are easily renewable and cost-effective sources (Abdel-Raouf *et al*., 2012). *S. platensis* and cow dung mixed bio-fertilizer treatment showed higher growth parameters, yield attributes, anti-nutritional composition, bio-chemical composition and minerals than the other treatments. In the same manner when *A. cepa* (Regal F1 and Malbec) is treated with freshwater algae (*Ulothrix*) there is considerable increase in the growth parameters such as shoot length, root length, leaf number, and leaf length as compared to the control because it fulfills the nutrient requirements for germination at a specific concentration.

Mahmoud *et al*., 2007 studied and their experiment was conducted in Upper Egypt in order to enhance the productivity, growth and quality of onion plants that were grown in the loamy soil by applying the algae spray. They obtained the maximum yield at 4.0g/L concentration. When they applied algae extract with the nitrogen/fed to the soil, the total growth of the onion plant was (16.21 and 20.74%) as compared to the control. In the same way when the Regal F1 and Malbec were treated with algal extract, they showed maximum germination at 10% concentration as compared to the control. The total germination rate of the variety Regal F1 was 95.10% and the variety Malbec shows germination of 94.40% in the whole experiment at different concentration of algae. Economically, algae spray is considered as the best. Moreover, it will prevent the plants from the harmful effects of chemical fertilizers.

5. Conclusion

Freshwater algae prove to be an effective bio-fertilizer for plant growth, with optimal results at a 10% concentration. The experiment demonstrated that algae are cost-effective and environmental friendly, offering a viable alternative to chemical fertilizers. The total germination rate of the variety Regal F1 is 95.10% and the Variety Malbec show germination of 94.40% at a 10% concentration of algal extract. They show maximum shoot length, root length, leaf number, and leaf length in both varieties as compared to the control. For Regal F1, the shoot length is 2.28 cm, root length 1.03 cm, leaf length 3.31 cm, and leaf number 1 are the maximum values observed at 10% concentration. Similarly, Malbec variety shows maximum growth at 10% which is shoot length 2.16cm, root length 0.75cm, leaf length 3.17cm, and leaf number 1. The minimum growth occurred at 100% concentration even though some seeds did not show any growth. Variety Regal F1 show minimum germination of 100% with shoot length 1.04cm, root length 0.34cm, leaf length 1.71cm and leaf number 0.72. In the same manner, Malbec shows minimum growth at 100% such as shoot length 1.07cm, root length 0.27cm, leaf length 1.68cm and leaf number 0.78. Overall, the use of freshwater algae can significantly improve crop yield, benefiting farmers while being safe

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and sustainable agriculture by capturing and storing carbon dioxide from the atmosphere, which can help mitigate climate change.

6. Ethical Considerations & Conflict of Interest

All authors confirm originality and no potential conflict of interest was reported.

7. Acknowledgement

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