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EFFECT OF CHLORELLA AS A BIOFERTILIZER ON GERMINATION OF WHEAT AND BARLEY GRAINS

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Abstract: *Most of the agriculture sectors have been dependent heavily on inorganic chemical-based fertilizers, which are harmful for both human health and environment. The utilization of beneficial microbes as a biofertilizer has become major concern in industrial agriculture for their potential role in food safety and sustainable crop production. In this study, we examined the effect of Chlorella on germination of barley and wheat grains. Seeds were germinated in culture medium containing microalga (Chlorella sp. 56) and grown for 3, 6, 9 and 12 days in order to study its effect on growth parameters. In conclusion, Chlorella suspension increased the seed growth compared to those of control (sterilized culture medium) of seed germination. The best treatments were 0.06 g/L and 0.23 g/L of algal suspension for the root and shoot lengths of barley and wheat seeds, respectively.*

Keyboards*: Chlorella; biofertilizer; germination; barley and wheat grains;*

INTRODUCTION

In order to meet the food demands of a growing world population, agriculture sectors have been increasingly using chemical fertilizer. Chemical fertilizers are mainly a mixture of substances, such as nitrogen, phosphorus and potassium. The excess uses of chemical fertilizers in agriculture are costly and also have various harmful effects [1]. For instance, residual chemicals reach to water bodies through rainwater and cause eutrophication in water bodies. It can also reduce water holding capacity, soil fertility and disparity in soil nutrients, which could further led to unfavorable condition for living microorganisms in soil [2]. In this regard, organic fertilizers and biofertilizers have become alternative sources.

As compared to chemical fertilizers, biofertilizers are eco-friendly and cost effective. Biofertilizers contain various microorganisms that provide all kinds of micro and macro-elements via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth promoting substances, production of antibiotics and biodegradation of organic matter in the soil [3], [4]. When biofertilizers are used continuously for many years, parental inoculums become sufficient for further multiplication [2], hence they participate in nutrient cycling and benefit crop productivity [5]. Main benefits of biofertilizers are 1) cheap source of nutrients; 2) suppliers of microelements; 3) suppliers of organic matter; 4) counteracting negative impact of chemical fertilizers; 5) secretion of growth hormone [6]; 6) no adverse effects to ecosystem and 7) longer shelf life [7]Azotobacter species were selected via phenotypic, biochemical and molecular characterisations from different rice fields. Acetylene reduction assay of Azotobacter spp. showed that Azotobacter vinelandii (Az3.

Certain species of microorganisms are widely used in biofertilizers. Among them, benefiting the crop production are Azotobacter, Azospirillium, Acetobacter, cyanobacteria, Azolla, phosphate solubilizing microorganisms, mycorrhizae, Sinorhizobium and plant growth promoting Rhizobacteria [2], [8]. For instance, Some strains of Azotobacter, Azospirillium, Phosphobacter and Rhizobacter can provide significant amount of nitrogen and increase the plant height, number of leaves, stem diameter percentage of seed filling and its dry weight in Helianthus annus [9]. Similar results were also observed in roots of rice [10] this element is usually supplied to the rice crop as the commercially available fertilizer urea. But unfortunately a substantial amount of the urea-N is lost through different mechanisms causing environmental pollution problems. Utilization of biological N fixation (BNF. In last few decades, some researchers conducted studies on potential uses of green algae as biofertilizers [11]–[16]. Dry biomasses of green algae contain high percentage of macronutrients, considerable amount of micronutrients and amino acids [17], [18].

The aim of this work was to study the effect of Chlorella sp. 56 strain on germination of barley and wheat grains and to determine any potential application of Chlorella microalga as a biofertilizer in order to improve the yield quality and productivity and avoid environmental pollution.

MATERIALS AND METHODS

Plant material. The experimental plants used in this study were seeds of barley and wheat. The wheat seeds were grown in the soil of State farm of Mongolia, and were collected by “Altan Taria” Co.Ltd in September, 2014. While the barley seeds were collected from Altai, Khovd aimag in 2010 and kept at -4°C under dark condition until the experiment.

Algal culture. Microalga (Chlorella sp. 56) strain was obtained from the Culture Collection of Microalgae at Institute of General and Experimental Biology, MAS and was cultivated using standard medium 04. The final pH of this medium was 6.8, after being autoclaved. The culture was grown with a light intensity of 8 Klux provided by cool white fluorescent lamps and a temperature of 25±2°C under illumination regime of 6:18 dark and light cycle for a week. Filtered air was let to bubble in the culture vessels to provide aeration and agitation.

Effect of culture media after growth of algal strain on seed germination. In this experiment, algal suspensions were collected at 3d, 6th, 9th and 12th days and were examined for both cell count and dry biomass yield.

Determination of cell number and biomass content of alga. Growth of Chlorella sp. 56 strain was measured in terms of cell number and dry weight biomass. Cell concentrations were counted using a hemocytometer. Data were given as cell per mL. The determination of dry biomass yield was performed using Vladimirova’s method [19]. The culture suspensions were mixed well prior to the sampling. 5 mL of samples were collected in weighing bottles thrice weekly. The bottles were dried at 105°C oven until the weight of the bottles become constant. The dry biomass yield was determined by using following formula:

$$DW\ (g/L) = (a - b)/Y * 200$$

Where: a – the total weight of weighing bottle containing dried biomass (g)

b – weight of the weighing bottle (g)

Y – sample volume taken (mL)

The data were given as mg/g algae mass.

Treatment of barley and wheat seeds. Seeds were surface sterilized with 30% sodium hypochlorite for 8 min, then rinsed with distilled water several times before germination. The seeds of wheat and barley were placed in petri dishes containing 3 mL of sterilized culture medium as a control. 2 ml of algal suspension was collected after growing the algal strain for 3, 6, 9 and 12 days, and added to each petri dish containing barley and wheat seeds. Petri dishes were maintained in thermostat at temperature of 18±2°C under the light regime of 8:16 light and dark for a week. At the end of the experiment, lengths of shoots and roots per plant were determined.

Statistic analysis. All experimental analyses were performed in triplicate and the mean values were calculated. The data were subjected to analysis of variance and Student's t-test and F-test were used to assess differences between means.

RESULTS

Growth parameters (cell counts and dry biomass yield) of alga. The growth of Chlorella sp. 56 strain was followed after 12 days. The cell counts and dry weight were recorded at 3, 6, 9, and 12 days.

Table 1.

Concentrations of algal suspension used in this experiment.

Days	3	6	9	12
Dry biomass (g/L)	0.06	0.14	0.23	0.33
Cell counts (cells/ml)	$1.5*10^6$	$5.3*10^6$	$6.8*10^6$	$11.8*10^6$

As shown in Table 1, the dry biomass yields were 0.06 g/L, 0.14 g/L, 0.23 g/L and 0.33 g/L at day 3, 6, 9 and 12, respectively. Meanwhile, the cell counts were 1.5, 5.3, 6.8 and 11.8 million cell/mL in respective algal suspensions.

Seed germination and seedling growth of barley and wheat plants.

The results of growth parameters obtained for the germination of barley and wheat seeds subjected to culture media after growth of Chlorella sp. 56 strain for 3, 6, 9, and 12 days treatments are given in Figure 1 and 2.

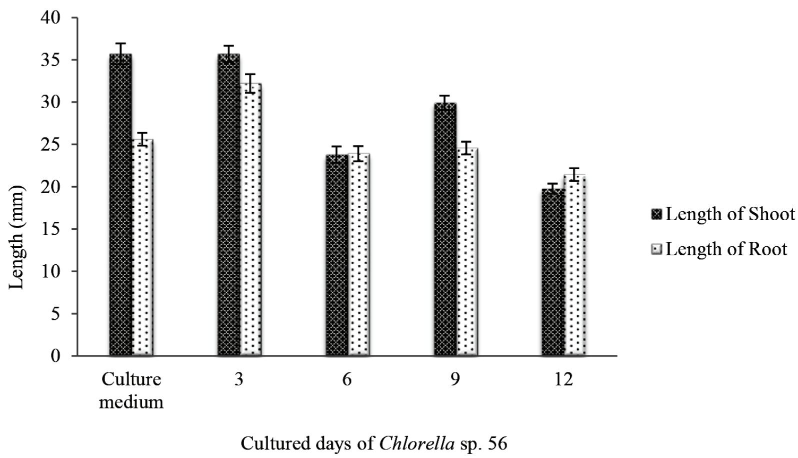

Figure 1. Effect of culture medium containing Chlorella sp. 56 grown for 3, 6, 9 and 12 days on growth parameters of seeds germination of barley plant.

As shown in Figure 1, the growth of barley shoot at day 3 was close to that of control. Also, the lengths of both shoot and root were shorter than that of control at days 6, 9 and 12. However, the length of root at day 3 was 25% longer than that of control.

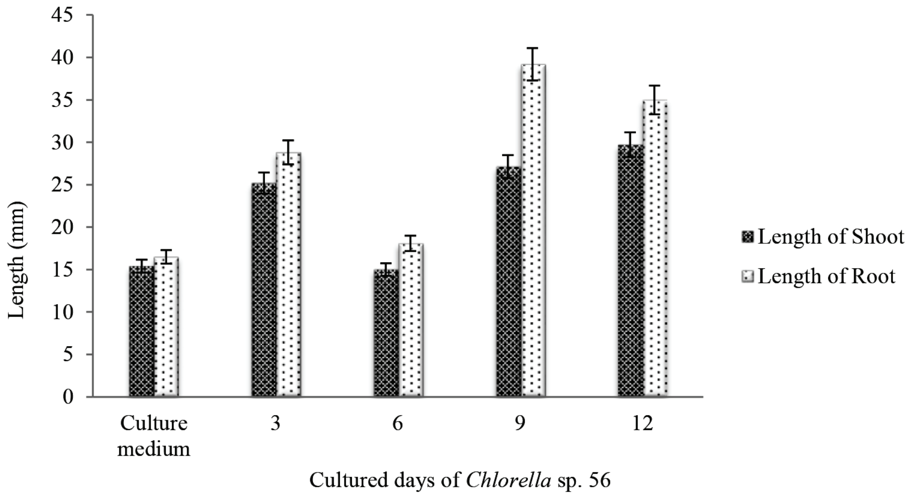

Figure 2. Effect of culture medium containing Chlorella sp. 56 grown for 3, 6, 9 and 12 days on growth parameters of seeds germination of wheat plant.

It is shown in Figure 2, that the lengths of both wheat shoot and root were much longer at days 3, 9 and 12 as compared to control. The lengths of shoot and root were close to that of control at day 6. The highest length of shoot was obtained at day 12, which was 1.9 times higher than that of control. However, the highest length of root was observed at day 9 and it was 2.4 times higher than that of control.

DISCUSSION

The application of microalgal suspension led to increase significantly the wheat seed germination under lab condition. This idea was previously confirmed by Abd El-Bake, (2008) who found that, splaying wheat cultivated with microalgae extracts obtained from Chlorella sp. led to keep good growth and yield of wheat compared to those received recommended dose of chemical nitrogen. Similar trends were found by Adam (1999) [20]. Furthermore, increase in lengths of shoot and root resulted from the floating of plant seeds in algal suspension. The stimulatory effects of alga as bio-fertilizer on some growth parameters of lettuce was obtained in the study results by Rani and Sathiamoorthy (1997) [21]. Similar results were also obtained by Mahmoud and Amara (2000), in which all treatments significantly increased plant growth parameters compared to untreated plant [22]. Moreover, enhancement in the growth parameter leads to improved crop productivity [22]–[26].

CONCLUSIONS

It can be concluded that Chlorella suspension can enhance the germinations of wheat and barley seeds. Chlorella sp. 56 algal suspensions of 0.06 g/L and 0.23 g/L can improve the root and shoot lengths of barley and wheat seeds, respectively.

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