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Original Article

Effect of *Chlorella vulgaris* as a biofertilizer on germination of tomato and cucumber seeds

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Abstract: Although inorganic fertilizers are known to raise environmental and health problems, the current agricultural practices are heavily dependent on the application of synthetic fertilizers and pesticides. In this study, we examined the effect of *Chlorella vulgaris* strain on germination of tomato and cucumber seeds. Seeds were germinated in culture medium containing algal strain and grown for 3, 6, 9 and 12 days to study its effect on growth parameters. As results, *C. vulgaris* suspension increased the seed growth compared to those of the control (sterilized culture medium) of seed germination. The best treatments were 0.17 and 0.25 g/L of algal suspension for the root and shoot lengths of tomato and cucumber seeds, respectively.

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Introduction

As the global food demand increases, agriculture sectors have been increasingly using chemical fertilizer. Inorganic fertilizers are rich in chemical substances, such as nitrogen, phosphorus and potassium. The excess uses of chemical fertilizers in agriculture are costly and also have various harmful effects on both living organisms and environment (Santos et al., 2012). 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. Moreover, groundwater contamination could lead to gastric cancer goiter, metabolic disorder, birth malformations, hypertension and livestock poisoning (Khandare, 2013; Youssef and Eissa, 2014). In this regard, organic fertilizers and biofertilizers have become alternative sources.

Biofertilizers are eco-friendly, cost effective and renewable source of plant nutrients to supplement and replace the chemical fertilizers for sustainable agriculture (Raja, 2013). 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 (Goel et al., 1999; Sinha et al. 2010). When biofertilizers are used continuously for many years, parental inoculums become sufficient for further multiplication (Youssef and Eissa 2014), hence they participate in nutrient cycling and benefit crop productivity (Singh et al., 2011). 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 (Gaur, 2010), (6) no adverse effects to ecosystem and (7) longer shelf life (Sahoo et al., 2014).

The main microorganisms used in biofertilizers are *Azotobacter*, *Azospirillium*, cyanobacteria, *Azolla*, phosphate solubilizing microorganisms, mycorrhizae, *Sinorhizobium* and plant growth promoting *Rhizobacteria* (Hegde et al., 1999; Youssef and Eissa 2014). Algal biomass contains macronutrients as well as micronutrients, growth regulators, polyamines, natural enzymes, carbohydrates, proteins, amino acids, and vitamins implemented for improving

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vegetative growth (Abd El Moniem and Abd-Allah, 2008; El-Fouly et al., 1992; Mahmoud and Amara, 2000; Shaaban and Mobarak, 2000). In addition, algal biomass increases the yield due to the presence of vitamins, auxins and gibberellins. The algal varieties tested as biofertilizers primarily belong to the branch of blue-green algae (Cyanophyta) and green algae (Chlorophyta). It has been shown in many studies that green algae can (1) add organic matter, (2) synthesize and liberate amino acids, vitamins and auxins, (3) reduce oxidizable matter content of the soil, (4) provide oxygen to the submerged rhizosphere, (5) improve salinity and buffer the pH, (6) solubilize phosphate, and (7) increase the fertilizer use efficiency of crop plants (Faheed and Abd-El Fattah, 2008; Abd El Moniem and Abd-Allah, 2008; Bileva, 2013; Dubey and Dubey, 2010; Grzesik and Romanowska-Duda, 2015; Vig et al., 2012).

The aim of this work was to study the effect of *Chlorella vulgaris* strain on germination of tomato and cucumber seeds and to determine any potential application of *C. vulgaris* microalga as a biofertilizer to improve the yield quality and productivity.

Materials and Methods

Plant material: The experimental plants were seeds of tomato and cucumber. These seeds were purchased from retailer store under Ministry of Food, Agriculture, Light Industry, Mongolia in 2017 and kept at -4°C under dark condition until the experiment.

Algal culture: Microalga strain was obtained from the Culture Collection of Microalgae at Institute of General and Experimental Biology and cultivated using standard medium 04. The final pH of the 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 8:16 light and dark 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. Algal suspensions were collected at 3d, 6th, 9th and 12th days and examined for both cell count and dry biomass yield.

Determination of cell number and biomass content of alga. Growth of *C. vulgaris* 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 (Sirenko, 1975). 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 using following formula:

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

Where a is total weight of weighing bottle containing dried biomass (g), b=weight of the weighing bottle (g) and Y=sample volume taken (mL). The data were given as mg/g algae mass.

Treatment of tomato and cucumber seeds. Seeds were surface sterilized with 30% sodium hypochlorite for 8 min, then rinsed with distilled water several times before germination. The seeds of tomato and cucumber 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 tomato and cucumber 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.

Statistical 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 algal strain was followed after 12 days. The cell counts and dry weight were recorded at 3, 6, 9, and 12 days. As shown in Table 1, the dry biomass yields were 0.06, 0.12, 0.17 and 0.25 g/L at day 3, 6, 9 and 12, respectively. Meanwhile, the

Table 1. Concentrations of algal suspension used in this experiment.

Days	3	6	9	12
Dry biomass (g/L)	0.06	0.12	0.17	0.25
Cell counts (cells/ml)	$26.6*10^6$	$50.1*10^6$	$77.2*10^6$	$109.8*10^6$

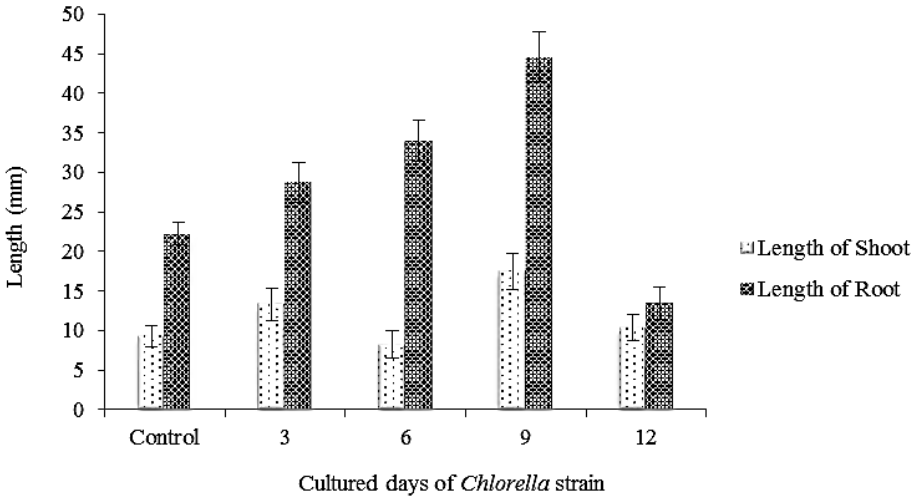

Figure 1. Effect of culture medium containing *Chlorella* grown for 3, 6, 9 and 12 days on growth parameters of seed germination of tomato.

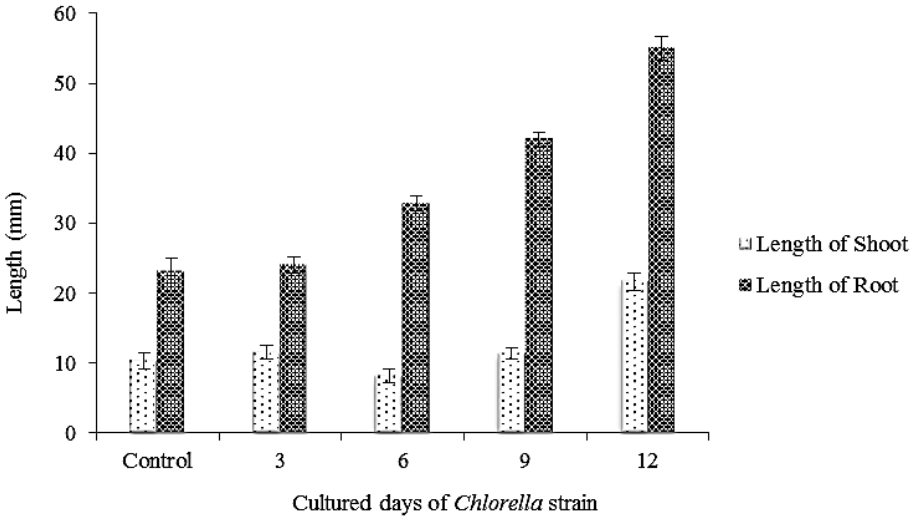

Figure 2. Effect of culture medium containing *Chlorella* grown for 3, 6, 9 and 12 days on growth parameters of seed germination of cucumber.

cell counts were 26.6, 50.1, 77.2 and 109.8 million cell/mL in respective algal suspensions.

Seed germination and seedling growth of tomato and cucumber seeds: The results of growth parameters obtained for the germination of tomato and cucumber seeds subjected to culture media after growth of *Chlorella* strain for 3, 6, 9, and 12 days treatments are given in Figures 1 and 2.

The lengths of shoot and root of tomato were highest at day 9, which were 17.4 and 44.6 mm, respectively (Fig. 1). As compared to the control, the growths of roots gradually increased by 29.1, 52.8, 100% at days 3, 6 and 9, respectively. However, the length of root was 40% shorter than that of control at day 12. The lengths of shoot at days 6 and 12 were close to that of control. The lengths of shoots at day 3 and 9 were higher than that of control by 43.3 and 87.9%, respectively.

The lengths of cucumber shoot and root were 2.1 and 2.4 times longer than that of control at day 12,

which were 21.6 and 55 mm, respectively (Fig. 2). The lengths of shoot were close to that of control at day 3, 6, and 9. The length of root was close to that of control at day 3. However, the lengths of roots were 41.5 and 80.8% higher than the control at days 6 and 9.

Discussions

Tomato and cucumber are considered as the most important and common vegetable plants in many countries. Many studies have been conducted on tomato to develop bio-stimulants, which can improve lateral and longitudinal root formation, roots nutrient uptake, increasing total volume and vigor of the root system. Among them, *Chlorella* microalga was also tested for the tomato bio-stimulant. In our study, the result showed the significant growth of shoot and root in tomato plant. The similar results were also observed in other researchers' studies. The application of *C. vulgaris* was tested on tomato plant, which showed strong stimulating effect on plant growth while inhibiting the development of *Meloidogyne arenaria* nematode parasite (Choleva et al., 2005). It was also observed in Garcia-Senin's (2013) study that the use of irrigation water with *C. pyrenoidosa* and *Chlorella* sp. cultures favored the production of tomato plants with special attention in poor soils. Author also concluded that their results could lead to a lower environmental impact and a cost-effective tomato crop production. Moreover, several ocean algae extracts were tested on tomato seedlings and their results also showed the enhanced seed germination, plant growth, and germination rate (Hernández-Herrera et al., 2014; Shariatmadari et al., 2011). The similar phenomenon was also observed in cucumber plant. The results showed the growth of root in cucumber plant was increasing as the concentration of microalgal suspension increases. In the study of Abd Elhafiz et al. (2015), *Chlorella* sp. cultures were shown to enhance the germination of cucumber seeds.

Conclusion

It can be concluded that *C. vulgaris* suspension can enhance the germinations of tomato and cucumber seeds. Algal suspensions of 0.17 and 0.25 g/L can improve the root and shoot lengths of tomato and cucumber seeds, respectively.

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