Study the Effect of the Terrestrial Cyanobacterium *Nostoc commune* Aqueous Extract on Seed Germination and Seedling Growth of Rice

Mohammad Hossein Abedi Firoozjaei[1], Seyedeh Batool Hassani[1*], Ehsan Nazifi[2], Somayeh Keypour[3]

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Abstract

Seed priming has a significant effect on seed germination and improves the establishment of crops. In this study, rice seeds (*Oryza sativa* L. cv. *Shiroodi*) were primed by different concentrations (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 g/l) of *Nostoc commune* Vauch aqueous extract. Furthermore, seedling growth, seed germination rate and percentage were examined. The results showed that *N. commune* extract had a significant positive effect on increasing the percent and rate of seed germination. Indeed, the length of roots and shoots in seedlings obtained from primed seeds with *N. commune* extract increased comparing to those of the seedlings from primed seeds with water. Additionally, the highest seed germination percentage and seedling length was obtained by 0.025 g/l *N. commune* extract. Based on the results, seed germination and seedling growth was not affected positively by seed priming with *N. commune* extract at concentrations more than 0.1 g/l. Therefore, sowing rice seeds with a low concentration of *N. commune* extract can improve seed germination and seedling establishment.

Keywords: Seed Priming, *Oryza sativa*, *Nostoc commune*, Germination rate, Germination percentage

Introduction

Rice (*Oryza sativa* L.) has global importance, especially in Asian countries. Due to its importance to human life, the year 2004 is designated as the international year of rice by the United Nations (Gnanamanickam, 2009). It was reported that rice seeds affected on the cultures, diets, and economies of thousands of people worldwide (Van Nguyen and Ferrero, 2006).

As previous works demonstrated, increasing seed germination rate and initial seedling establishment are crucial factors affecting rice production and yield (Jongdee et al., 2002). Seed germination is a crucial stage in the development of plants. There is a direct relationship between the seed germination stage and proper plant production and yield

1-Department of Plant Science and Biotechnology, Faculty of Biological Sciences and Technology, Shahid Beheshti University, Tehran, Iran.
2-Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran.
3-Department of Biology, Farhangian University, Tehran, Iran.
*E-mail address: b_hassani@sbu.ac.ir

(Murungu et al., 2003; Sawan et al., 2009). Seed priming with biological compounds is one of the valuable techniques to accelerate seed germination and improve the early establishment of crops (Lee and Kim 1999; Farooq et al., 2006, 2007). Beneficial effects of seed priming with algae was analysed for several crops such as rice, barley, cucumber, tomato, cotton, berseem clover, corn, and lettuce (Seifikalhor et al., 2019; Shariatmadari et al., 2013; Thajuddin and Subramanian, 2005; Younesi et al., 2019; Rezaee et al., 2019; Soltani et al., 2019). Previous researches indicated that priming the seeds with cyanobacteria increases seed germination and seedling growth in crops and medicinal plants (Shariatmadari et al., 2015; Seifikalhor et al., 2019; Chookalaii et al., 2020).

Cyanobacteria are the first photosynthetic organisms that produced oxygen in photosynthetic pigments (Whitton and Potts, 2012; Liberton et al., 2013). Indeed, cyanobacteria have a unique potential to increase plant productivity in various environmental conditions (Chatzissavvidis and Therios, 2014; Umesha et al., 2018). Water-retaining capacity by cyanobacteria jelly structure, nitrogen fixation, plant growth stimulants production such as auxin, gibberellin, and cytokinin, vitamins, and amino acids are the significant features that improve plant growth (Hashtroudi et al., 2013; Saadatnia and Riahi, 2009).

It seems that considering the positive physiological effects of seed priming with cyanobacteria the concentration and type of extract is required to be optimazed for each species. The present study investigates the efficacy of different concentrations of *Nostoc commune* water extract on seed germination and seedling growth of rice.

Material and methods

Plant material

Seeds of *Oryza sativa* cv. *Shiroodi* obtained from International Rice Research Institute in Amol (IRRI). It is noteworthy to mention that *Shiroodi* cultivar has the highest level of cultivation in the northern regions of Iran.

Preparation of N. commune extract

Naturally, growing colonies of *N. commune* collected from the soil in Babolsar, Mazandaran, Iran (36°42'47"N, 52°41'25"E) during September 2020. Then the colonies were washed under tap water to remove the soil and air-dried at room temperature in the shade. Then, the sample was grounded using an electrical blender. Next, the *N. commune* powder (0.5 gr) was suspended in autoclaved distilled water (100 ml) and placed on a shaker at room temperature overnight. Also, the mixture was sonicated for 30 minutes for achieving better extraction. After that, the sonicated mixture was centrifuged at 4000 rpm for 5 minutes at room temperature. Finally, the supernatant was filtered through Whatman filter paper No. 1 and a clear extract obtained.

Seed sterilization

The seeds incubated in distilled water containing 2 drops of detergent per liter for sterilizing in 10 minutes. Then, the seeds were soaked in 1% sodium hypochlorite for 10 minutes. Finally, the seeds were washed twice with autoclaved distilled water for 2 minutes.

Seed priming, treatments, and cultivation conditions

N. commune extract was diluted to eight concentrations; 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 g/l for treating the rice seeds. Ten seeds treated with 5 ml of each concentration in separate Petri dishes, each with three replications. All the Petri dishes incubated under sterile laboratory conditions at 25 ± 2 °C. Indeed, cultures were placed at laboratory conditions for 3 days in the dark. Finally, cultures kept in a photoperiod of 16 h light with 5500 lux /8h darkness for seven days to study the growth rate of seedlings.

Measurement of rate and percentage of seed germination

Germinated seeds (seeds with root emerged) were counted daily and seed germination rate was calculated by the following equation (Agrawal, 1991)

growth rate∑ = The number of germinated seeds at (n) day/ (n) day (No. 1)

Where n is the number of the day which germinated seeds counted.

The percentage of seed germination was calculated using the following equation.

percentage of seed germination = (number of germinated seeds at third day / total number of seeds) × 100 (No. 2)

Measurement of seed growth parameters

Seedlings transferred to 16 h light/8 h dark conditions within three days of germination in the dark. After seven days the length of seedling, shoot, and root measured by ImageJ software (version 1.44P; US National Institutes of Health, Bethesda, Maryland, USA) (Collins, 2007).

Statistical analysis

The experiments conducted with completely randomized designs arrangedat least three replications. Statistical analysis performed using SPSS software (version 18). Microsoft Excel 2016 software used to create charts. The data presented as the mean ± standard error. Significance of differences between the data obtained from One-Way ANOVA analysis followed by Duncan test ($p<0.05$).

Results

The effect of N. commune extract on rice seed germination

Although the results indicated that different concentrations of *N. commune* extract had a significant effect on the germination percentage of rice seeds, no significant effect on seed germination was reported (Table 1).

Furthermore, comparison of germination percentages showed that only 0.025 g/l of *N. commune* extract increase significantly seed germination percentage (Figs 1 and 2). While, the highest values of germination (93%) occurred when the extraction applied in 0.025 g/l only 70% of untreated seeds germinated. Although an increase and decrease in seed germination percentage observed at 0.05, 0.1 g/l and 0.2, 0.4 g/l of *N. commune* extract, respectively that it was not significant (Figs 1 and 2).

However, the highest germination rate of rice seeds (approximately 10 germinated seeds per day) was obtained in primed seeds with 0.025 g/l *N. commune* extract. Indeed, negative effect of higher concentrations of *N. commune* extraction (0.3 and 0.5 g/l) on seed germination rate is shown in Figure 3.

The effect of N. commune extract on rice seedling growth

The results of rice seedling growth indicated the significant effect of *N. commune* extract concentration on the length of seedling, root, and shoot of rice seedling (Table 2).

Further, the highest seedling length was

Table 1. One-way analysis of variance (ANOVA); effect of different concentrations of *N. commune* water extract on percentage and germination rate of rice seed (p <0.05)

		Sum of Squares	df	Mean Square	F	Sig.
Percentage of seed germination	Between Groups	2429.167	7	347.024	4.899	.004
	Within Groups	1133.333	16	70.833		
	Total	3562.500	23			
rate of seed germination	Between Groups	25.685	7	3.669	1.655	.191
	Within Groups	35.481	16	2.218		
	Total	61.167	23			

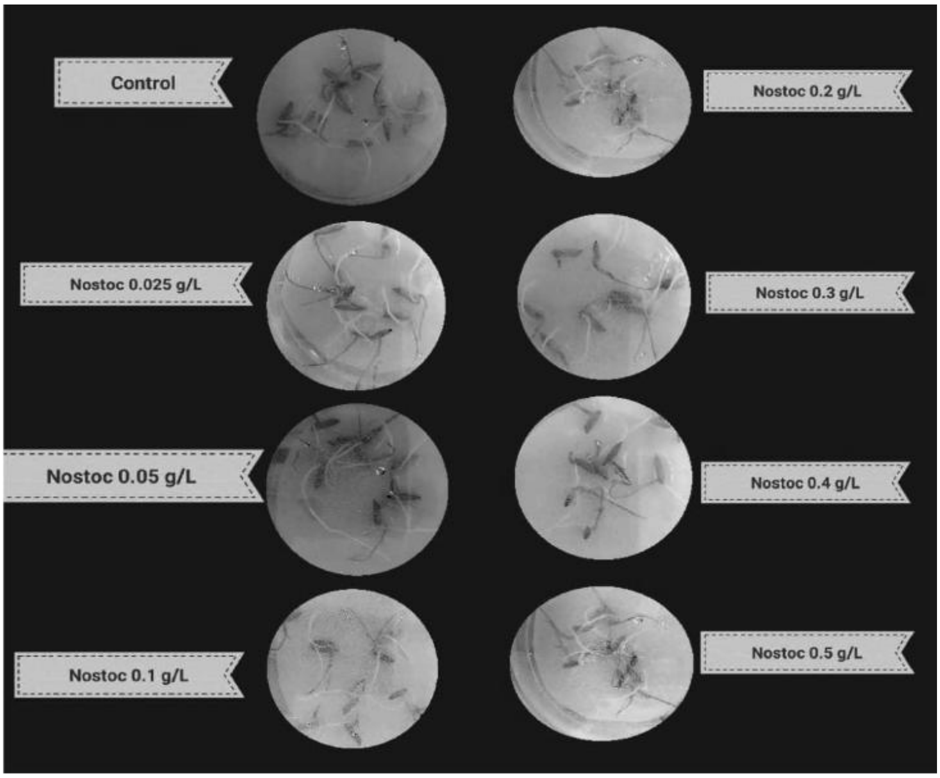

Fig. 1. Effect of different concentrations of *N. commune* water extracts on rice seed germination

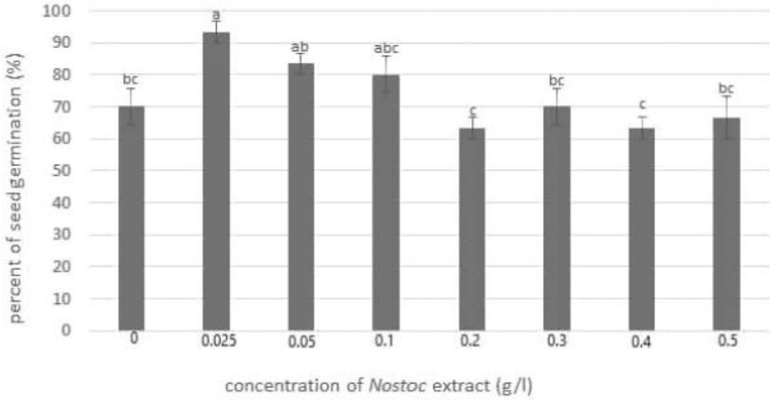

Fig. 2. The effect of different concentrations of *N. commune* extract on the germination percentage of rice seed. The bars are the means of three repetitions ± standard error. Letters indicate a significant difference between the means at the probability level of P <0.05

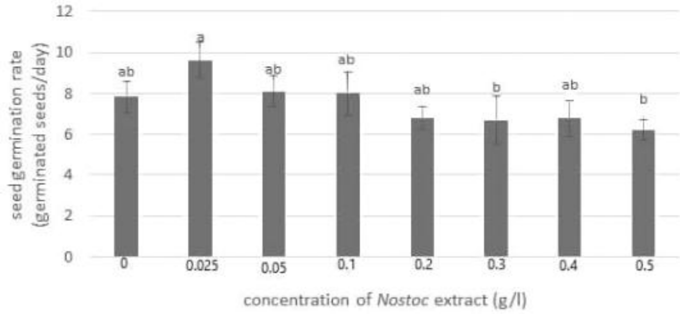

Fig. 3. The effect of different concentrations of *N. commune* water extract on rice seed germination rate. The bars are the means of three repetitions ± standard error. Letters indicate a significant difference between the means at the probability level of P <0.05.

observed in seeds treated with 0.025 g/l *N. commune* extract (Figure 4). However, seedling height under treatments of 0.05 and 0.1 g/l of *N. commune* extract was significantly increased; higher concentration of *N. commune* extraction (0.5 g/l) had no affect on seedling height (Figure 4).

There was a significant increase in root length at concentrations of 0.025-0.2 g/l *N. commune* extraction. The results showed that concentrations higher than 0.2 g/l *N. commune* water extract had no positive effect on root length (Figure 5).

Although the highest shoot length was obtained in seedlings treated with 0.025 g/l of extract, the result showed no significant increase comparing to the control, or a negative effect at high concentration of *N. com-*

Table 2. One-way analysis of variance (ANOVA) of *N. commune* water extract concentrations on rice seedling growth parameters at p <0.05

		Sum of Squares	df	Mean Square	F	Sig.
Seedling height	Between Groups	11.132	7	1.590	6.414	.001
	Within Groups	3.967	16	.248		
	Total	15.099	23			
Shoot height	Between Groups	4.642	7	.663	5.698	.002
	Within Groups	1.862	16	.116		
	Total	6.505	23			
Root height	Between Groups	2.262	7	.323	4.478	.006
	Within Groups	1.154	16	.072		
	Total	3.416	23			

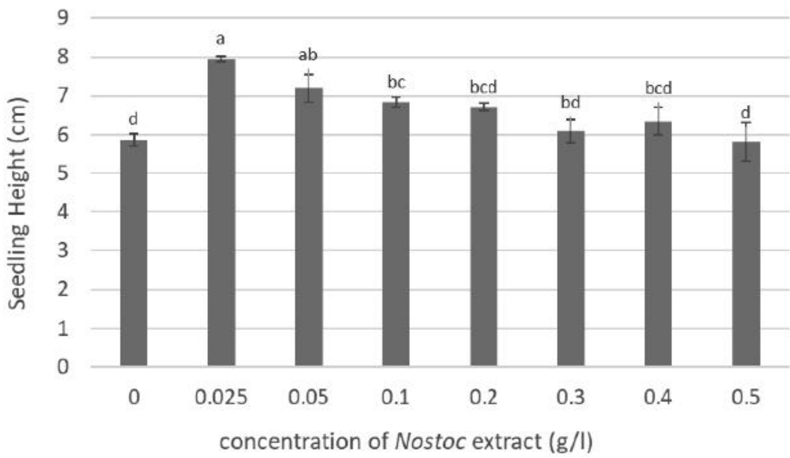

Fig. 4. Effect of different concentrations of *N. commune* extract on rice seedling height. The bars are the means of three repetitions ± standard error. Letters indicate a significant difference between the means at the probability level of P <0.05

mune extract on shoot growth. The shoot height was significantly reduced at 0.5 g/l *N. commune* extract (Figure 6).

Discussion

The percentage and rate of seed germination improvement in crops leads to seedling establishment, plant growth, and a strong root system (Murungu et al., 2003). In addition, increasing the seed germination and production of healthier seedlings provide a better chance for the plant to withstand the environmental stresses. Besides, these factors allow the farmers to introduce more resistant cultivars for agriculture (Conrath et al., 2001; Kalefetoğlu et al., 2009; Borges et

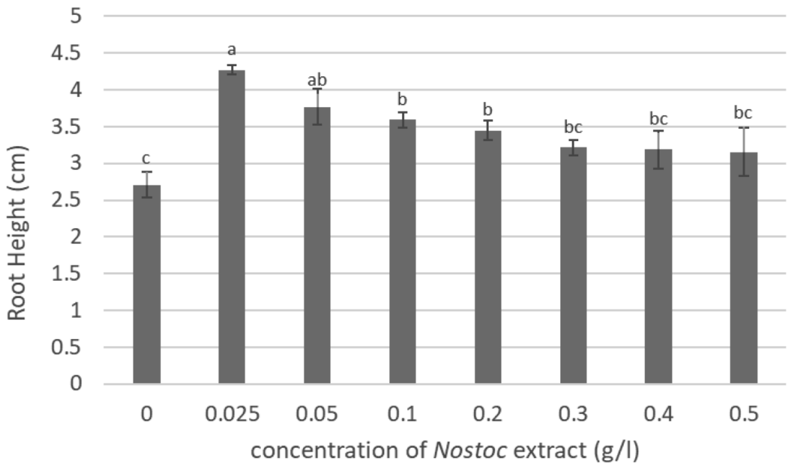

Fig. 5. Effect of different concentrations of *N. commune* water extract on rice root length. The bars are the means of three repetitions ± standard error. Letters indicate a significant difference between the means at the probability level of P <0.05

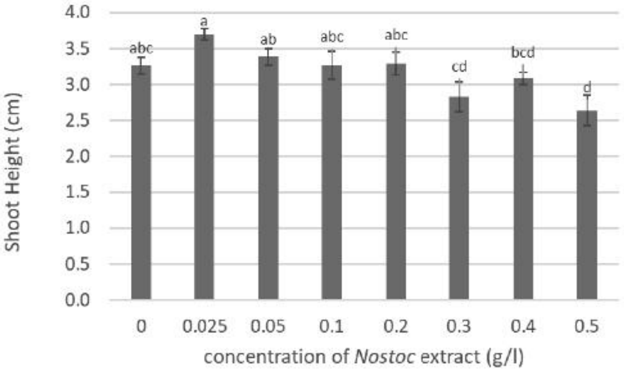

Fig. 6. Effect of different concentrations of *N. commune* extract on rice shoot height. The bars are the means of three repetitions ± standard error. Letters indicate a significant difference between the means at the probability level of P <0.05

al., 2014; Ahmadpour et al., 2016). Several studies focused on increasing the seed germination percentage and improving the initial establishment of seedlings in crops (Seifikalhor et al., 2019; Thajuddin and Subramanian, 2005) and medicinal plants (Chookalaii, et al., 2020) by priming and treating the seeds with biological compounds.

In this study, priming *Shiroodi* rice seeds with *N. commune* extract increased the germination percentage and rate. Previous researches have shown that priming the rice and alfalfa seeds increased the rate and percentage of seed germination (Lou et al., 2004; Basra et al., 2006). Indeed, primed seed performs better metabolic processes of germination, especially the hydrating step and the hydrolytic activity. Further, seed priming increases catalase and superoxide dismutase activity (Yan, 2016; Falahhosseini et al., 2017). In addition, seed priming appears to increase germination by reducing damage to proteins, RNA, and DNA. Following the priming of seeds, the activity of phosphatase, synthesis of RNA, DNA, and cell division increases (Eskandari, 2013).

In the last decade, the study of biotechnology of microalgae and active compounds derived from cyanobacteria is progressed (Dahms et al., 2006). *N. commune* is known to be rich in growth-promoting substances such as vitamins and amino acids (Abed et al., 2009). Furthermore, *Nostoc* sp. can poduce phytohormones, including auxin, gibberellin, and cytokinin (Hashtroudi et al., 2013; Esch, 2014). Cytokinins increase the cell division in seeds and activate the alpha-amylase enzyme, that increas germination rate and percentage (Craigie, 2011). Moreover, an algal extract contains gibberellin and auxin that cause the breaking of seed dormancy, stimulate seedling growth, and proper establishment of seedling in the soil (Yamaguchi and Kamiya, 2001; Gayathri et al., 2017; Tan et al., 2021). However, low or high concentration of auxin reduces the cell division in root, intermediate concentration of auxin have a positive effect (Campanoni and Nick, 2005).

In this study, the positive effect of *N. commune* extract observe at 0.025 g/l concentration (0.025 g/l). It seems that at low concentration of *N. commune* extract the level of auxin is lesser to improve seed germination. Therefore, the concentration of algal extract is crucial to achieve better germination and establishment.

In addition, treated rice seedling growth was improved using low concentration of *N. commune* extract. Similarly, several studies confirmed that cyanobacteria increase seed germination and shoot length in rice (Sinha et al. 1999; Saadatnia and Riahi, 2009). Julia et al. (2020) reported that *Macrocystis pyrifera* extract had a positive effect on seed germination and seedling establishment of lettuce. Also, priming of *Medicago sativa* L. seeds with the extracts of *Oscillatoria* sp. and *Spirogyra* sp. increased seed germination and seedling length (Brahmbhatt et al., 2015). Generally, the higher rate of seed germination and growth rate of rice seedlings provide farmers a greater opportunity for replanting and reduce the germination time. This research performed on *Shiroodi* cultivar, which has a high cultivation area in northern Iran. It seems that it is necessary to conduct similar research on other rice cultivars and other crops to analysis the effect of *N. commune* and other algal extract concentration on seed germination and growth.

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