Pak. J. Bot., 53(1): 343-350, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-1(12)

EFFECTS OF CYANOBACTERIUM, *LEPTOLYNGBYA* SP. AND GREEN MICROALGA, *CHLORELLA SOROKINIANA* AS BIOFERTILIZERS ON *IN VITRO* SEED PRIMING AND SEEDLING GROWTH OF SOME ECONOMICALLY IMPORTANT VEGETABLES FROM PAKISTAN

YASMEEN REHMAT[1*], RUKHSANA JABEEN[1], SHAISTA HAMEED[1*], MUHMMAD EJAZ[2] AND [3]MANZOOR IQBAL KHATTAK

[1]Sardar Bahadur Khan Women's University, Quetta, Pakistan
[2]PARC-Balochistan Agricultural Research and Development Center, Quetta
[3]Department of Chemistry, Balochistan University, Quetta
**Corresponding author's email:Yrehmat87@yahoo.com, shaistahameed2013@yahoo.com*

Abstract

The cyanobacterium, *Leptolyngbya* sp. and green microalga, *Chlorella sorokiniana* as biofertilizers, play an important role in agriculture development. Due to a lack of information concerning microalgae as biofertilizers in crop production, the present research aimed to evaluate the possibility of increasing growth using microalgae as biofertilizers on seed germination of four commercially important vegetables i.e., radish (*Raphanus sativus*) subsp. *sativus*, spinach (*Spinacia oleracea*), turnip (*Brassica rapa*) subsp. *rapa* and fenugreek (*Trigonella foenum-graecum*). In the current study the fresh biomass of cyanobacterium, *Leptolyngbya* sp. and green microalga, *C. sorokiniana* was used as bio-fertilizers to note the effect on seed germination of four vegetables. The *In vitro* effects of bio-fetilizers were also noted on the different growth parameters i.e., germination percentage, days to germination (50%), plumule length, radical length, fresh weight and dry weight using Randomize Complete Designed (RCD) with factorial arrangement. The results compared with control showed an improved germination percentage using *Leptolyngbya* sp. (83.17%) and *C. sorokiniana* (80.47%). Both strains exhibited early germination (4.50 days) while *C. sorokiniana* showed the maximum plumule (33.88cm), radical length (4.46cm), fresh (1.38g) and dry weight (0.0708g) associated with *Leptolyngbya* sp. The treatment with *B. rapa* seed germination was 98.67% and took 1.78 days to germinate. The lowest germination (34.44%) was recorded in *S. oleracea* and took 6.56 days to germinate. After applying *Leptolyngbya* sp. on *B. rapa* and *T. foenum-graecum* seeds indicated the highest seed germination (99.67%). While *B. rapa* seeds germinated after one day while primed with *C. sorokiniana*. The maximum increase over check of germination percentage was observed in *Leptolyngbya* sp. (11.63%) and *C. sorokiniana* (8.60%). Both strains improved early germination (3.78%), plumule length (33.88%), radical length (60.31%), fresh weight (7.97%) and dry weight (5.79%).

Key words: *Raphanus sativus*, *Spinacia oleracea*, *Brassica rapa* and *Trigonella foenum-graecum*, vegetable seeds, growth parameters.

Introduction

The increase in world population is affecting on agriculture and availability of food. There is a need to face such challenges without environmental damage to meet future food requirements. To minimize the use of agrochemicals, it is necessary to use biofertilizers, which do not damage the soil fertility, increase eutrophication, normal fauna and flora of soil (Chagnon *et al*., 2014).

In agriculture and horticulture, bad seed germination performance plays an important effect on germination, which leads to a great financial loss and reduced the yield of a particular crop. While priming of seeds can increase vigorous germination uniformity. The treatment of seed priming leads to well establishment and germination in various crops such as wheat, rice, canola, maize (Ghiyasi *et al*., 2008 a, b; Basra *et al*., 2005).

A total cropped area of Pakistan described for the year 2010-11, was 19.9 million ha. Out of this 65.8% were food crops, 24.2% cash crops, 6.7% pulses and 3.3% edible oil seeds. Vegetables constitute a primary component of the cropping pattern but the increasing burden on food and cash crops has limited the area under vegetables about 0.62 million ha, which is 3.1% of the total cropped area. Vegetables fit well in most farming systems due to shorter maturity period (Khokhar, 2014). Vegetable crops are very important due to their higher yield potential, higher return, high nutritional value and suitability for small landholding farmers. Vegetables provide proteins, minerals and vitamins required for human nutrition (Khokhar, 2014). Vegetables make up a major percentage of the diet of humans in many parts of the world and play a significant role in human nutrition, especially as sources of phyto-nutriceuticals, vitamins (C, A, B1, B6, B9, E), minerals, dietary fiber and phytochemicals (Wargovich, 2000; Liua *et al*., 2001; Dias & Ryder, 2011).

Uniformity in germination and healthy seed is the basis for agriculture production and a better crop stand especially in the case of vegetables. Vegetable growers need an accurate prediction of the emergence, more uniformity and crop stand (Pervez *et al.*, 2009). Crops lacking a good stand and uniformity expression reduced efficacy of other agronomic practices and usually such inputs can never reimburse for the negative impact of a poor stand. Healthy and vigorous seedlings, which can strive and survive adverse abiotic and biotic stresses, are much more significant. Prompt and unvarying germination and emergence of seeds, regular and vigorous seedlings are central determinants of positive stand establishment and ultimately increased yield (Cantliffe, 2001).

The establishment of a uniform crop stand can be obtained by vital seeds and genetic improvement, as well as improved seeding techniques. Seed priming is a technique of seed enrichment that improves seed performance by quick and uniform germination, standard and healthy seedlings, which results in a faster and higher

rate of germination and emergence in different crops (Farooq *et al.*, 2008). It also supports seedlings to grow under biotic or abiotic stressed environments (Ashraf & Foolad, 2005; Khan *et al.*, 2009). Such seed treatments result in synchronized emergence and uniform stand establishment leading to improved yield. The microalgae (*Acutodesmus dimorph* and *Chlorella vulgaris;* Garcia-Gonzalez, 2016; Sommerfeld, 2016 and Shaaban, 2001) and cyanobacteria (*Nostoc ellipsosporum, Anabaena oryzae* and *Synechococcus* sp.; Kumar & Kaur, 2014; Adam, 1999) show great diversity worldwide and have the ability to improve the development and growth of the plant releasing various biologically active compounds.

The beneficial effects of cyanobacterial inoculation were also reported on some other crops such as barley, oats, tomato, radish, cotton, sugarcane, maize, chili and lettuce (Thajuddin & Subramanian, 2005). *Phormidium* sp. has a rich source of biologically active elements and most promising group of cyanobacteria capable of producing bioactive compounds (Fish & Codd, 1994). The effect of alga on pre-soaking rice seeds with blue green algal (BGA) cultures or extracts enhances the germination and promotes the growth of roots and shoots and increases the weight and protein content of the grain (Svircev *et al*., 1997). The cyanobacterium, *Phormidium* sp. has growth promoting substances such as auxins, amino acids, sugars and vitamins (vitamin B12, folic acid, nicotinic acid and pantothenic acid), which have beneficial effects on growth of different plants (Misra & Kaushik, 1989a &b). Hussain & Hasnain (2011) and Mazhar *et al*., (2013) have reported that the wheat plants that were inoculated with *Anabaena* sp. have significantly enhanced the seed germination, shoot length, tillering number of lateral roots, spike length, grain weight, protein content, micronutrients and endogenous phytohormone.

Materials and Methods

The present study was conducted at Sardar Bahadur Khan Women's University (SBKWU), Balochistan, Pakistan. The seed of vegetables i.e. radish (*Raphanus sativus*) subsp. *sativus,* spinach (*Spinacia oleracea*), turnip (*Brassica rapa*) subsp. *rapa* and fenugreek (*Trigonella foenum-graecum*) were collected from Agriculture Research Institute (ARI), Quetta, Pakistan. The strains of cyanobacterium, *Leptolyngbya* sp. and green microalga, *C. sorokiniana* were used as bio-fertilizers, isolated from a freshwater pond, located at SBKWU. Both strains were mass cultured in microalgal culture laboratory, SBKWU.

Isolation, identification and mass culturing of cyanobacterium, *Leptolyngbya* sp. and green microalga, *Chlorella sorokiniana*: The natural samples of algal mats and scums were collected from freshwater pond. The both strains were isolated using standard techniques i.e. streaking (Hoshaw & Rosowski, 1973), capillary (Rippka, 1988) and Serial Dilution Culture (SDC; Andersen & Throndsen, 2003) methods. The axenic strains of culture of *Leptolyngbya* sp. and *C. sorokiniana* were obtained using antibiotic, streptomycin (60 mg L^{-1}; Padgett *et al*., 1985). The *Leptolyngbya* sp. was identified by the taxonomic keys of Komarek and Anagnostidis (2005) and Desikachary, (1959). While *C. sorokiniana* was identified using Bellinger & Sigee (2015) and Vuuren *et al*., (2006).

Six Erlenmeyer flasks (6x250 mL) containing 100 mL BG-11 growth medium (0‰; Allen & Stanier, 1968 modified by Stanier *et al.,* 1971) were autoclaved for 15 mins at 15 lb of pressure and 120°C (Kawachi & Noël, 2005) in an autoclave (Hirayama, Japan). The next day single and pure filament/cell of *Leptolyngbya* sp. and *Chlorella sorokiniana* was inoculated in triplicates in each pre-labeled flask. All flasks were placed under white continuous fluorescent illumination (2400 lux) at 25°C± 2°C for one month. After obtaining one month old cultures, both strains were further inoculated in 2x10L round flat bottom flasks (1 flask for each strain) for mass culturing. Both flasks were supplied with continuous aeration (Rippka *et al.*, 1979) and kept under cool white fluorescent light for 30 days.

Preparation of biofertilizers, *Leptolyngbya* sp. and *C. sorokiniana* for seed germination treatments: The fresh biomass of both strains was obtained by filtration using an ordinary filter paper. The fresh cells were washed with distilled water (Water still, UK) and homogenized by homogenizer (Wheaton, USA). Four economically important vegetable seeds were selected for the present study i.e. radish (*Raphanus raphanistrum* subsp. *sativus*), spinach (*Spinacia oleracea*), turnip (*Brassica rapa* subsp. *rapa*) and fenugreek (*Trigonella foenum-graecum*). The seeds were sterilized by soaking in 2.5% sodium hypochlorite (Sigma-Aldrich, USA) for 30 mins and then washed several times with distilled water (Ismail & Abo-Hamad, 2017; Essa *et al.*, 2015 and Nezarat & Gholami, 2009). Ten grams of homogenized fresh cells of each strain were suspended in 500 mL distilled water to soak testing seeds (Brahmbhatt & Kalasariya, 2015; Shariatmadari *et al*., 2012 and 2011).

Treatment-seed priming: To evaluate the effect of homogenized live cells of *Leptolyngbya* sp. and *C. sorokiniana* on the performance of the tested seedlings, three seed priming treatments were made:

1. Seeds primed in 1% fresh cells of *Leptolyngbya* sp. for 30 mins (treatments).
2. Seeds primed in 1% fresh cells of *C. sorokiniana* for 30 mins (treatments).
3. Seeds primed in distilled water for 30 mins (control).

Twenty-four glass Petri plates (22.5cm Ø) were autoclaved and prepared for treatments and controls by placing Whatman filter discs (22cm Ø). The air-dried seeds of all vegetables were soaked in the microalgal solutions for 30 mins, separately. The seeds without green microalgal and cyanobacterial solutions used as control. Ten sterilized seeds of each vegetable were transfered to pre-labeled Petri plates aseptically in laminar flow (ESCO, USA). After each treatment, the seeds were left to grow in Petri plates under white fluorescent light (24 lux) at 25°C for 1 week. During the experiment and till emergence of sprouts, distilled water (5mL/plate) was sprinkled over seeds to provide the moisture to grow. Each treatment was run in triplicate using Completely Randomized Block Design (CRBD) with the

factorial arrangement. After the emergence of sprouts, germination percentage (number of germinated seeds), days to germination (50% of germination of seeds from sowing day), plumule length (from grain to the end portion), radical length (from grain to the end portions), fresh weight (at the end of experiment) and dry weight (after 3 days drying at 130ºC in an oven; Genlab, UK) were noted. All results were expressed as mean ± SD in triplicate. Analysis of Variance (ANOVA), a test for significant differences between means at $p \leq 0.05$, was performed to compare the impact of different treatments on growth and performance of the tested seedlings using SAS 9.0 software.

Results

Germination percentage: During the experiment the germination percentage of vegetable seeds was significantly increased ($p \leq 0.05$) when treated with *Leptolyngbya* sp. and *C. sorokiniana* compared with corresponding control (Table 4). An increase in germination percentage was also observed when seeds treated with *Leptolyngbya* sp. (11.63%) and *C. sorokiniana* (8.6%; Fig. 1a). The statistically higher germination % was observed in vegetable seed treated with *Leptolyngbya* sp. (83.17 %) which was at par with *C. sorokiniana* (80.47%) followed by control treatment (73.50%; Table 1; Fig. 1a). It was apparent that the germination % was higher (98.67%) in *B. rapa* which was at par with the *T. foenum-graecum* (97.44%) followed by the *R. sativus* 85.56%) and *S. oleracea* (34.44%; Table 2; Fig. 2b).

The study of interaction between treatment and vegetable seed germination % is presented in Table 3. The *B. rapa* seed treated with *Leptolyngbya* sp. showed significantly higher germination (99.67%) and control with *T. foenum-graecum* (99.33%). The *C. sorokiniana* also represented significantly higher germination with *B. rapa* (98.33%), control with *B. rapa* (98.00%), *Leptolyngbya* sp. with *R. sativus* (96.67%), *C. sorokiniana* with *R. sativus* (93.33%) and *C. sorokiniana* with *T. foenum-graecum* (93.33%). While *R. sativus* seed treatment represented higher germination with control (66.67%), *S. oleracea* seed treated with *Leptolyngbya* sp. (36.67%), *S. oleracea* seed treated with *C. sorokiniana* (36.67%) and *S. oleracea* seed treated with control (30%), respectively (Fig. 2C-D).

Days to germination: Early germination of vegetables was observed in *C. sorokiniana* (4.50 days) and late in control treatment (Table 1; Figs. 1b and 2b). The seed treated with *C. sorokiniana* was found 3.78% days earlier and seed treated with *Leptolyngbya* sp. was 1.97% days earlier than non-treated seeds (Table 4; Fig. 1b). Table 2 shows significant differences of days to germination (50%) among the treatment means of vegetables. *B. rapa* seeds germinated after 1.78 days followed by *T. foenum-graecum* 4.67 days, *R. sativus* 5.33 days and *S. oleracea* trail germinated after 6.56 days (Fig. 2c). Significant difference was found among the treatment means of vegetables and vegetable seed days to germination (50%; Table 3). The prompt germination (after 1 day) was found in *B. rapa* seed treated with *C. sorokiniana* which was at par with *B. rapa* seed treated with control (2 days) and *Leptolyngbya* sp. (2.33 days) followed by *T. foenum-graecum* seeds treated with control (4 days) and *Leptolyngbya* sp. (4 days). The *S. oleracea* seeds were treated with *Leptolyngbya* sp., control and *C. sorokiniana*. (statistically at par) took maximum days to germinate which was 6.67 days, 6.67 days and 6.33 days, correspondingly (Fig. 2C and D).

Plumule length: The plumule length observations showed a significant difference among the treatment means (Table 1). *C. sorokiniana* produced maximum plumule length (1.83cm) which was at par with the treatment *Leptolyngbya* sp. (1.60cm) followed by the control treatment (1.21cm). *R. sativus* represented the longest plumule length (2.31cm) (Fig. 1A), which was at par with the *T. foenum-graecum* (1.82cm) and *B. rapa* (1.77cm) than *S. oleracea* (0.28cm; Table 2; Fig. 2C-D). The results also indicated a significant difference among treatment means of interaction among treatments and vegetable plumule length (Table 3). The plumule length over check was 24.38% and 33.88% when seeds were treated by *Leptolyngbya* sp. and *Chlorella* sp., respectively (Table 4).

Table 1. Influence of bio-fertilizers (*Leptolyngbya* sp. and *C. sorokiniana*.) on growth parameters of vegetable seeds.

S. No.	Treatment	Germination (%)	Days to germination (50%)	Plumule length (cm)	Radical length (cm)	Fresh weight (g)	Dry weight (g)
1.	*Leptolyngbya* sp.	83.17a	4.58a	1.60ab	3.00b	1.33a	0.0702a
2.	*C. sorokiniana*	80.47a	4.50a	1.83a	4.46a	1.38a	0.0708a
3.	Control	73.50b	4.67a	1.21b	1.77c	1.27a	0.0667a
LSD value (0.05)		6.77	0.67	0.61	0.86	0.13	0.04

Values within the same column followed by the same letters are not significantly different, using LSD Range Test at 5% level

Table 2. Effect of bio-fertilizers (*Leptolyngbya* sp. and *C. sorokiniana*) on vegetable seeds and their growth parameters.

S. No.	Treatment	Germination (%)	Days to germination (50%)	Plumule length (cm)	Radical length (cm)	Fresh weight (g)	Dry weight (g)
1	*R. sativus*	85.56b	5.33b	2.31a	5.15a	1.29a	0.07a
2	*S. oleracea*	34.44c	6.56a	0.28b	0.78b	1.32a	0.09a
3	*B. rapa*	98.67a	1.78 c	1.77a	5.54a	1.35a	0.09a
4	*T. foenum-graecum*	97.44a	4.67b	1.82a	0.83b	1.35a	0.09a
LSD value (0.05)		7.82	0.78	0.70	0.99	0.15	0.04

Values within the same column followed by the same letters are not significantly different, using LSD Range Test at 5% level

Table 3. Effect of interaction between bio-fertilizers (*Leptolyngbya* sp. and *C. sorokiniana*) on growth parameters.

S. No.	Treatment	Germination (%)	St. Dev ± (σn-1)	Days to germination (50%)	St. Dev ± (σn-1)	Plumule length (cm)	St. Dev ± (σn-1)	Radical length (cm)	St. Dev ± (σn-1)	Fresh weight (g)	St. Dev ± (σn-1)	Dry weight (g)	St. Dev ± (σn-1)
1.	*Leptolyngbya* sp. x *R. sativus*	96.67a	5.77	5.33abc	0.4	2.41a	0.4	4.96b	0.125	1.07b	0.45	0.07abc	0.04
2.	*Leptolyngbya* sp. x *S. oleracea*	36.67c	11.55	6.67a	0.577	0.30d	0.146	0.52c	0.3009	1.30ab	0.057	0.07abc	0.02
3.	*Leptolyngbya* sp. x *B. rapa*	99.67a	0.577	2.33d	0.577	1.87ab	0.41	5.75b	0.257	1.35a	0.0289	0.08abc	0.0057
4.	*Leptolyngbya* sp. x *T. foenum graecum*	99.67a	0.577	4.00c	0	1.83ab	0.431	0.79c	0.271	1.38a	0.0551	0.06abc	0.044
5.	*C. sorokiniana*. x *R. sativus*	93.33a	11.54	4.67 bc	0.588	2.59a	0.588	9.01a	1.79	1.46a	0.219	0.06abc	0.032
6.	*C. sorokiniana* x *S. oleracea*	36.67c	11.55	6.33a	0.577	0.39cd	0.09	1.34c	0.129	1.33a	0	0.09a	0.0519
7.	*C. sorokiniana* x *B. rapa*	98.33a	2.886	1.00d	0	2.27ab	0.02	6.25b	2.684	1.38a	0.0416	0.06abc	0.1044
8.	*C. sorokiniana* x *T. foenum-graecum*	93.33a	5.774	6.00ab	1	2.05ab	0.933	1.22c	0.282	1.34a	0.024	0.10a	0.02
9.	Control x *R. sativus*	66.67b	5.77	6.00ab	0.208	1.93ab	0.208	1.48c	0.54	1.35a	0.219	0.08ab	0.006
10.	Control x *S. oleracea*	30.00c	17.32	6.67a	0.577	0.14d	0.1	0.48c	0.4202	1.33a	0	0.10a	0.0153
11.	Control x *B. rapa*	98.00a	3.464	2.00d	0	1.17bcd	0.17	4.63b	0.703	1.33ab	0.0058	0.06abc	0
12.	Control x *T. foenum-graecum*	99.33a	1.155	4.00c	0	1.58abc	0.345	0.47c	0.098	1.33a	0.015	0.10a	0.02
		13.55		**1.34**		**1.22**		**1.72**		**0.26**		**0.7**	

Values within the same column followed by the same letters are not significantly different, using LSD Range Test at 5% level

Radical length: The data presented in Table 1 indicates the significant differences among the treatment means. The seeds treated with *C. sorokiniana* indicated an increase in radical length (4.46cm) followed by *Leptolyngbya* sp. (3cm) and non-treated seed 1.77cm (Fig. 1b). *B. rapa* significantly enhanced radical length 5.54cm which was at par with the *R. sativus* 5.15cm (Fig. 1A) followed by *T. foenum-graecum* 0.83cm and *S. oleracea* 0.78cm, correspondingly (Figs. 2C and 2D).

The effect of biofertilizer treatments on vegetable seeds was indicated significant differences among the treatment mean (Table 3). The *R. sativus* seed treated with *C. sorokiniana* significantly had maximum radical length 9.01cm while *B. rapa* seeds treated by *C. sorokiniana* 6.25cm, *B. rapa* seed treated with *Leptolyngbya* sp. 5.75cm, *R. sativus* seed treated with *Leptolyngbya* sp. 4.96cm and *B. rapa* non treated seeds with 4.63cm. The radical length (0.47cm) of non-treated seed of *T. foenum-graecum* was at the trailed (Table 3). The radical length was increased by 41% over check the seed treated with *Leptolyngbya* sp. and 60.31% radical length was improved by seed treated with *C. sorokiniana* (Table 4).

Fresh weight: During the study it was observed that non-significant difference among the treatment means (Table 1). The seed treated with *C. sorokiniana* produced maximum fresh weight (1.38g) of vegetable and minimum (1.27g) was observed in non-treated seeds. Non-significant difference was recorded among the vegetables in relation to fresh weight (Table 2). *B. rapa* and *T. foenum-graecum* produced the highest fresh weight (1.35g each). The lowest fresh weight 1.29g was recorded in *R. sativus.* The significant difference among the treatment means for interaction between treatment and vegetables is indicated in Table 4. *C. sorokiniana* and *R. sativus* produced maximum fresh weight (1.46g) which was at par with the interaction between *Leptolyngbya* sp. and *T. foenum-graecum* (1.38g), *C. sorokiniana* with *B. rapa* (1.38g). *Leptolyngbya* sp. showed interaction with *B. rapa* (1.35g), non-treated seed with *R. sativus* (1.35g), *C. sorokiniana* with *T. foenum-graecum* (1.34g), non-treated seed with *T. foenum-graecum* (1.33g), *C. sorokiniana* with *S. oleracea* (1.33g), control with *S. oleracea* (1.33g), control with *B. rapa* (1.33g) and *Leptolyngbya* sp. with *S. oleracea* (1.30g) followed by *Leptolyngbya* sp. with *R. sativus* (1.07g, Fig. 1a).The seeds were soaked in *Leptolyngbya* sp. an *C. sorokiniana* had increased fresh weight 4.51% and 7.97% over check, respectively (Table 4).

Dry weight: There was non-significant difference was found among the treatment means of dry weight (Table 1). The highest dry weight was observed when seeds were treated with *C. sorokiniana* (0.07g) and slightest dry weight was recorded in untreated seeds (0.06g). Non-Significant difference was found among treatment means of vegetables for dry weight (Table 2). The maximum dry weight (0.09g) was observed in *S. oleracea*, *B. rapa* and *T. foenum-graecum* and the minimum in *R. sativus* (0.07g). Table 3 shows a significant difference among treatment means. The higher dry weight (0.10g) was observed in untreated seeds of *S. oleracea* and the lowest dry weight was observed in untreated seeds of *B. rapa* (Figs. 1A and B). The dry weight increased over check (5.79%). The highest dry weight produced by seeds, treated with *C. sorokiniana* and *Leptolyngbya* sp. An increased dry weight of vegetable was 4.99% over checked (Table 4).

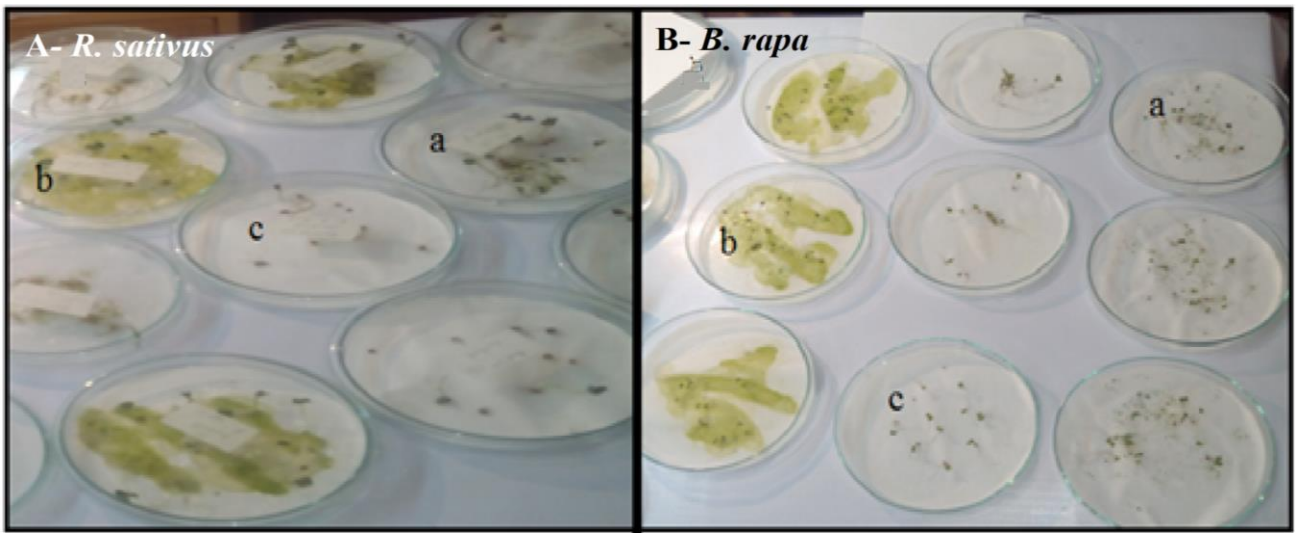

Fig. 1. The treatment of seeds of *R. sativus* (A) and *B. rapa* (B) with biofertilizers
(a. *Leptolyngbya* sp., b. *C. sorokiniana*, c. Control)

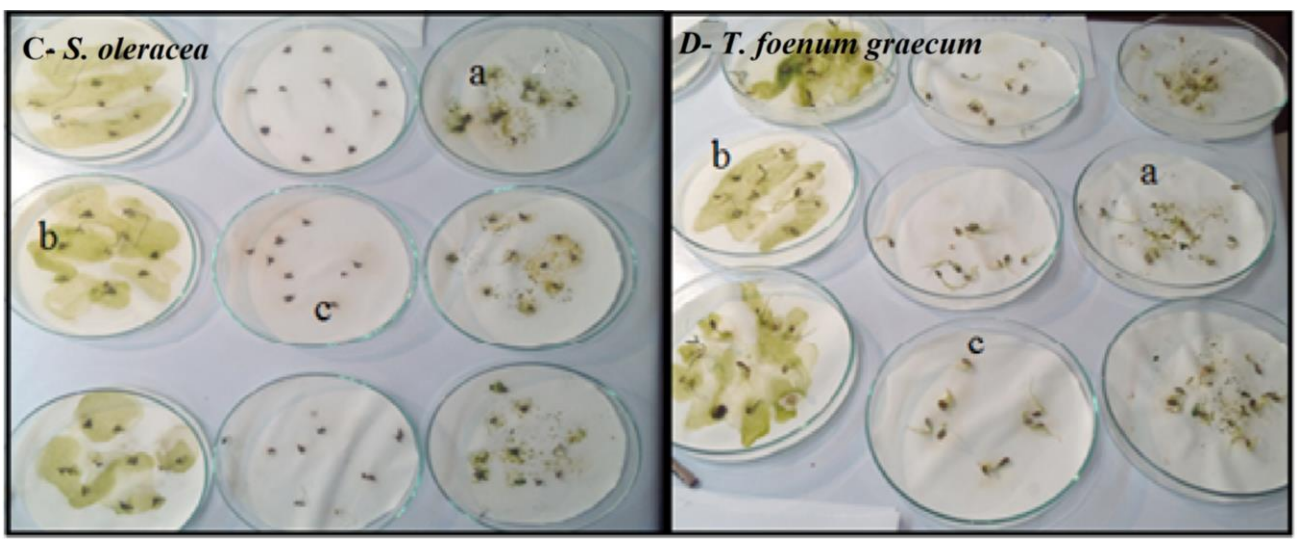

Fig. 2. The treatment of seeds of *S. oleracea* (C) and *T. foenum- graecum* (D) with biofertilizers
(a. *Leptolyngbya* sp., b. *C. sorokiniana*, c. Control)

Table 4. The effect of bio-fertilizers (*Leptolyngbya* sp. and *C. sorokiniana*) on increase or decrease over check (%) of vegetable seed growth parameters.

S. No.	Treatment	Increase or decrease over check (%)					
		Germination	Days to germination (50%)	Plumule length	Radical length	Fresh weight	Dry weight
1.	*Leptolyngbya* sp.	11.63	-1.97	24.38	41.00	4.51	4.99
2.	*C. sorokiniana*	8.60	-3.78	33.88	60.31	7.97	5.79

Discussion

The germination is a vivacious phase in the life cycle of plants, which aids the embryo to sustain the period between seed maturation and seedling establishment. There are other factors affect seed germination such as salinity, temperature, moisture and light intensity (Gorai *et al*., 2011). In the currecnt study the highest germination percentage was observed among the treatments. *Leptolyngbya* sp. produced significantly maximum germination percentage while compared with other tested vegetable seeds (*R. sativus, S. oleracea, B. rapa* and *T. foenum-graecum*) while *B. rapa* showed the higher germination percentage.

The biofertilizer (*Leptolyngbya* sp. and *C. sorokiniana*.) cultures have an amount of soluble carbohydrates, proteins and phosphorus (Faheed & Fattah, 2008; Talbot & De la Noüe, 1993). The seed germination and growth parameters were stimulated in the presence of these supernatant compounds (Karthikeyan *et al*., 2009; Xu *et al*., 2013). The vegetables, *B. rapa* and *T. foenum-graecum* seeds, treated with *Leptolyngbya* sp. supernatant showed a maximum germination percentage. Wael (2016) reported that the pre-soaking of wheat seed in algal extracts showed a significant increase in germination % ranged from 12-25% when compared with non-treated seeds (water-soaked). Ashraf *et al*., (2015) reported that the

Phormidium sp. has growth promoting substances i.e., auxins and gibberellins. Osman *et al*., (2010), Singh *et al*., (2011) and Hashtroudi *et al*., (2013) isolated different growth encouraging compounds such as gibberellin-like substances, indole-3-butyric acid, indole-3-propionic acid, indole-3-acetic acid and cytokinins from cyanobacterial strains i.e., *Phormidium, Anabaena, Oscillatoria, Chroococcidiopsis, Synechocystis, Anabaenopsis,* and *Cylindrospermum*. Singh (2014) reported that the treated plants prompt the expression of certain genes responsible for the endogenous phytochormone balance, which enhance the photosynthetic activities and regulation of several enzymes. In this study the germination percentage of *S. oleracea* was poor. It was due to a hard seed coat and requirement of high temperature to germinate. Katzman (1999) and Katzman *et al*., (2001) investigated that the dormancy and inhibition in *S. oleracea* due to exogenous and endogenous factors. Leslie *et al*., (2001) found out that the germination percentage of *S. oleracea* was progressively declines at temperature >20°C. Dormancy has been ascribed to chemical inhibitors and/or physical properties of the pericarp (outer fruit coat), as pericarp elimination permits germination at high temperatures. The pericarp reduces the germination limiting the amount of oxygen available to the embryo for respiration and physically blocking (hard seed coat) the radicle emergence. In the present study it was also observed that the germination percentage was increased over non-treated seed as observed in *Leptolyngbya* sp. and *C. sorokiniana*. Mieczyslaw *et al.,* (2014) reported that the monocultures of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120 and *Chlorella* sp. increased germination, beneficial to grow and improves metabolic activities of corn seedlings.

It was also observed that the days to germination (50%) indicated non-significant differences among the treatment means. In this study the early germination was recorded in the seeds, pre-soaked in fresh cells of *C. sorokiniana. B. rapa* seeds germinated significantly earlier and took approximately 2 days. The days to germination (50%) over pre-soaked in water was recorded in *C. sorokiniana* and *Leptolyngbya* sp. According to Grzesik *et al.*, (2009) and Badek *et al.*, (2006 & 2007) the corn seeds treated with *Chlorella* sp. germinated earlier because soften seed coat make it permeable and initiated metabolic process as compared to non-treated seeds.

In this study the longer plumule and radical lengths were recorded when seeds treated with *C. sorokiniana*. Mazhar, *et al*., (2013) reported that the wheat seeds treated with *Phormidium* spp., *Nostoc* sp. *Chroococcidiopsis* sp., *Calothrix* sp. and *Anabaena* sp. superficially escalation impact of *Leptolyngbya* sp. It is suggested that *Leptolyngbya* sp. exudates on seed germination and seedling growth parameters of the tested plants due to presence of a wide array of bioactive metabolites that are released by *Leptolyngbya* sp. into surrounding environment. These bioactive metabolites might be directly or indirectly involved in root initiation, cell division and cell enlargement (Prasnna *et al*., 2010). The maximum plumule length was observed in *R. sativus* and *B. rapa* produced longer radical due to the presence of growth promoting substances especially auxins (Serdyuk *et al*., 1992), cytokinins (Stirk *et al*., 1999& 2002), and gibberellins (Serdyuk *et al.,* 1992) in *Leptolyngbya* sp. The *R. sativus* seeds conditioned with *C. sorokiniana* significantly observed longer plumule and radical length. The increased plumule and radical lengths over check were observed in the seed primed with *C. sorokiniana*. The seedlings treated with *Sargassum muticum* extract increased the plumule length (Wael, 2016).

The highest fresh and dry weights were observed in *C. sorokiniana* treatments. The extract of alga, *Laurencia obtusa* showed a significant increase in seedlings' fresh weight (Wael, 2016). The pre-soaked *R. sativus* seed in *C. sorokiniana,* produced maximum fresh and dry weights. Haroun & Hussein, (2003) reported that the seedlings of peas treated with different treatments of *Leptolyngbya* sp., results in an increase in the dry weight. They speculated that the *Leptolyngbya* sp. adds nutrients in environment and increases the uptake of nutrients, which directly propotional to the high dry weight of peas.The release and uptake of nutrients produced by *Leptolyngbya* sp. plays an important role to fix the nitrogen and made available to plants (Kulhlbusch *et al.,* 1991). The *C. sorokiniana* also showed increased fresh and dry weights over checked. The application of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. PCC 7120, *Leptolyngbya* sp. and *Chlorella* sp. on pre-soaked corn grains greatly increased their fresh and dry weights (Mieczyslaw, 2014). It is concluded from the obtained results that the bio-fertilizers, *Leptolyngbya* sp. and *C. sorokiniana* released growth promoting substances (soluble carbohydrates proteins, phosphorus auxins, gibberellins and cytokine) in their surrounding growth media, which enhanced the metabolic activities and increased the nutrient up-take of seedlings, which results in an increase in seed germination percentage and early germination of the vegetables. This leads to uniform stand of the crops and farmers can acquire the maximum outcome of efforts.

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(Received for publication 22 January 2019)