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Isolation and identification of some cyanobacteria and their plant growth promoting effect on wheat (*Triticum aestivum* L.), Ethiopia

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Abstract

Cyanobacteria are gram negative photosynthetic prokaryotic microorganisms otherwise known as Blue Green Algae (BGA). Numerous cyanobacteria were isolated and identified worldwide and most of them are known to possess the ability to discharge plant growth promoting substances as well as fixing atmospheric nitrogen. Therefore, the present study mainly focussed on evaluating the plant growth promoting activity of cyanobacterial isolates using wheat as an experimental crop by seed germination and pot experiments. In the present study, five different cyanobacterial species were isolated and identified as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3, *Rivularia* sp. KA4 and *Lyngbya* sp. KA5 based on the morphometric characters using microscopic investigations. The heterocystous cyanobacterium *Rivularia* sp. KA4 at 0.3% aqueous concentration showed significantly ($p<0.05$) highest results in the morphological parameters as well as in the biochemical parameters under seed germinations experiment. The same heterocystous cyanobacterium *Rivularia* sp. KA4 at 2g dried application significantly ($p<0.05$) boosted the morphological growth parameters (plant height, number of leaves, leaf length, leaf width, number of roots, root length, shoot fresh and dry weight) and biochemical parameters (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) of the experimental crop under pot experiment when compared to all other cyanobacterial species, chemical fertilizer treatment, and control. Therefore, the heterocystous cyanobacterial isolate *Rivularia* sp. KA4 at 3% aqueous extracts can be used to do the pretreatment of wheat seeds and the same heterocystous cyanobacterium *Rivularia* sp. KA4 can also be used as biofertilizers in both dried as well as liquid form for the cultivation of *T. aestivum*.

Keywords: Cyanobacteria, seed germination, pot experiment, wheat, chemical fertilizer

Introduction

Ethiopia's agriculture sector is involving substantial dissimilarity in crops cultivation across the country's different regions and agro-ecologies. This agriculture sector is dominated by small scale farmers. The small-scale farmers are cultivating 96 percentage of the total area under crop, determined more than 90 percent of agriculture output and 97 percentages of food crops. Agriculture sector is one of the important sectors in Ethiopia which acts as a vital role in the food security as well as providing regular income for nearly 85% of its people (ATA, 2013/14). Wheat (*Triticum aestivum* L.) is one of the common and the most important food crops worldwide (Akililu et al., 2015). It has acted a key role in feeding a hungry world and enlightening the global food security (Mengistu and Belay, 2016). Ethiopia is the second-largest producers of wheat after South Africa in Sub-Saharan Africa (CSA, 2010). Wheat is one of the most important staple food crops of Ethiopia in terms of crop production and consumption. In terms of total dietary calories and protein intake, wheat is the 2^{nd} most important food crops in the country next to maize (FAO, 2014).

Fertilizers usage has progressed vigorously from about 3,500 tons consumption level during 1970s seasons and further the consumption level increased up to 450,000 tons in 2008 cropping season in Ethiopia (World Bank, 2008). Several study reports carried out at different locations of Ethiopia indicated that the indiscriminate applications of chemical fertilizers have adverse effects on soil health which leads to unsustainable yield (Yasin, 2015). Thus, to reduce and eliminate the adverse effects of chemically synthesized chemical fertilizers on the soil health; currently a new agriculture practice has been established and known as organic farming, sustainable agriculture or organic agriculture (Keeney and Follet, 1991).

Microbial fertilizers or biofertilizers are one of the most essential components of organic agriculture, play an important role in the sustainable soil fertility with eco-friendly way at cost effective manner. Different kinds of microorganisms such as bacteria, fungi and algae can be utilized for the production of biofertilizers (Smith and Read, 2008; Lucy et al., 2004; Vessey, 2003). Among these various kinds of microorganisms, algae especially cyanobacteria placed in the first place. Cyanobacteria (Bluegreen-Algae) are gram-negative, nitrogen-fixing, oxygenic, photosynthetic prokaryotic, aquatic microorganisms with wide range of diversities (Olsen, 2006). Cyanobacteria play a vital role in buildup and maintenance of soil fertility, consequently increasing growth and yield as a natural biofertilizer (Song et al., 2005). Therefore, the present study has initiated to isolate and identify cyanobacteria and its plant growth-promoting effects using wheat (*Triticum aestivum* L.).

Materials and methods

Sample collection and isolation

Cyanobacterial samples were randomly collected from in and around Wolaita Sodo University, Southern Ethiopia from the month of November 2018 to January 2019. All the collected samples were put in the sterile transparent glass containers with a screw capped and brought to the Microbiology Laboratory, Department of Biology, Wolaita Sodo University for further study. All the samples were processed within 48 h of collection (Krishna and Kibrom, 2019). The BG-11 medium (the common cyanobacterial medium) was used for isolation of cyanobacteria (Rippka et al., 1979). Cyanobacterial cultures were isolated and purified by using serial dilution, spread plate and streak plate techniques (Krishna et al., 2019; Castenholz, 1992).

Identification of cyanobacteria

All the purified cultures were identified by microscopically based on morphometric observation like the length and the width of the vegetative cells also the width of the sheath, type of spores, presence or absence of hormogonia, presence or absence of spores and its position, number of heterocyst and its repetition, the nature of cell wall, presence of akinetes, presence or absence gas vacuoles, as well as pigment color was taken in consideration according to Krishna et al. (2019); Krishna and Kibrom (2019); Khare et al. (2014); Komárek and Hauer (2013)and Desikachary (1959).

Mass cultivation of cyanobacteria under laboratory condition

All the purified cyanobacterial isolates were selected from the culture plates and transferred to 1000ml capacity culture flasks containing sterilized BG11 media aseptically. The inoculated conical flasks were then incubated under 1500lux (16hrs light 8hrs dark cycle) and at 25±2^{o}C in the culture room (Rippka et al., 1979). The mass cultured cyanobacterial isolated were harvested after 20-25days of incubation and used for the seed germination and pot experiments (Krishna et al., 2019).

Seed germination experiment using plate method

The experimental crop wheat (*Triticum aestivum* L.) seeds were purchased from local market. All seeds were surface sterilized with 70% ethanol for 1-2 minutes before inoculation by cyanobacterial aqueous extracts. Ten (10) numbers of surface-sterilized seeds were placed in each Petri dish covered by filter paper. Ten (10) ml of each cyanobacterial aqueous extract at different concentrations like 1% (1gm/100ml), 2% (2gm/100ml) and 3% (3gm/100ml) were inoculated to the appropriate Petri dishes (Krishna and Kibrome, 2019). Wheat seeds treated with 10 ml of distilled water was served as control. The growth parameters including germination percentage (%), radicles length, coleoptiles length and epicotyls length were recorded at 2 days interval up to 8^{th} day of incubation at $28^{0}C$. The biochemical parameters such as carbohydrate and protein content of the experimental seeds were also analyzed at 2 days interval up to 8^{th} day (Krishna et al., 2019; Pitchai et al., 2010).

Pot Experiments

The second phase of the experiment was studying the effect of isolated cyanobacteria cultures on the growth parameters of Wheat (*Triticum aestivum* L.) under the pot experiment. Five numbers of healthy seeds of *T. aestivum* were disseminated at seeding depth of 2-3cm to the 3 Liter capacity pots. The pot experiments were treated with five different species of cyanobacterial isolates as liquid and dried form. For liquid extract preparation 2.0g of each fresh cyanobacterial culture was homogenized with 100ml of distilled water and inoculated to the appropriate pots. For dried application, 2.0g of each cyanobacterial isolate was harvested and dried under shade for 5-7days. Further the dried cultures were powdered by using mortar & pestle and inoculated to the pots at 15days intervals. 2.0gm of Di-Ammonium Phosphate (DAP) was added to the selected pots for comparative purposes at 15 days interval. Pot without any cyanobacterial cultures inoculation and chemical fertilizer treatment was served as a control. The morphological parameters including plant height, leaf length, leaf width, number of leaves, number of roots, root length, shoot length; dry weight and fresh weight of shoot **(**Francis and Berhanu, 2017; Muluneh and Zinabu, 2013), and the biochemical parameters such as chlorophyll *a*, chlorophyll *b*, total chlorophyll (Arnon, 1949) and carotenoid (Siegelman and Kycia, 1978) contents were recorded on 30 DAP (Days After Planting).

Statistical analysis

The measurements of growth and biochemical parameters were subjected to one-way analysis of variance (ANOVA) technique (Origin pro software package 7.0) and mean separations were adjusted by the Multiple Comparison test. Means were compared by using Fisher's LSD test at $P<0.05$ level of significance. All the data included in the figures were presented in

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mean and standard error (±) of a mean of three replicates per treatment and repeated three times.

Results

Isolation and identification of cyanobacteria

Totally, 5 different cyanobacterial cultures were isolated from the samples. All the five isolates were identified and named as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3, *Rivularia* sp. KA4 and *Lyngbya* sp. KA5 based on the morphometric characteristic's features in the table-1 using microscopic analysis. Among these five different isolates, only one was identified as heterocystous, filamentous blue-green algae *Rivularia* sp. KA4 and the other four isolates were identified as non-heterocystous filamentous algae (Table 1).

Growth promoting efficiency of cyanobacterial isolates on *Triticum aestivum* L. using seed germination experiment by plate method.

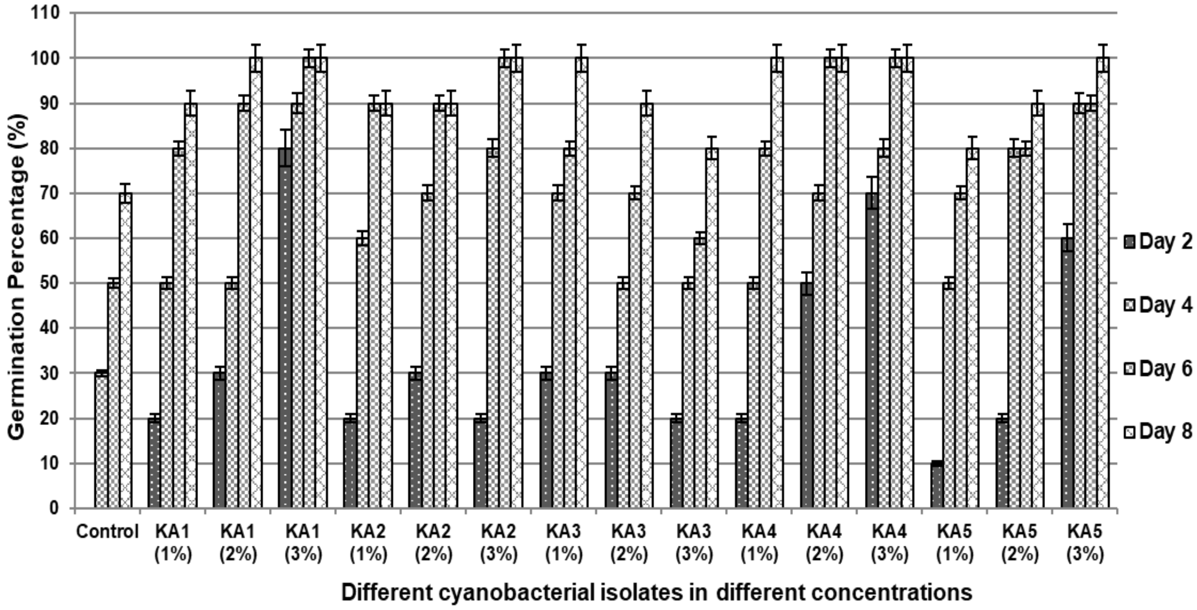
Morphological parameters:

It is clearly indicated that the changes in the morphological parameters of germinated *T. aestivum* seeds by different concentrations of aqueous extracts of all the cyanobacterial isolates are shown in the Figure 1, Figure 2, Figure 3, and Figure 4. The results in figure 1 shows that the seed germination percentage increased progressively throughout the period in all plates inoculated with different cyanobacterial isolates as aqueous extracts at different concentrations except control. The non-heterocystous cyanobacterial isolates such as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2 and heterocystous cyanobacterial isolate *Rivularia* sp. KA4 showed 100% of seed germination surprisingly at 6^{th} day of incubation with respective concentrations of 3%, 3% and 2% while the other cyanobacterial isolates such as *Phormidium acutum* KA3 and *Lyngbya* sp. KA5 showed 100% of seed germination only at 8^{th} day of incubation (Figure 1).

Table 1 Morphometric characteristics features of cyanobacterial isolates under microscope

Isolate No.	Morphometric characters	Identified As
KA1	Found solitary trichomes, straight as well as curved cells. Cylindrical cells were found to be with cross walls, non-granulated septa without sheathes. Cells were occasional motile, less than 30 cells, lack of heterocyst, apical cells were not attenuated. Cells size 2µm in diameter with 4µm length.	*Pseudanabaena galeata*
KA2	Trichomes were solitary, straight equal diameter throughout the whole length, not constricted, not attenuated to the apices, often granular, absence of mucous sheath. Shorter than wide cells were found to be with motile. Sizes of the cells were found to be with 10µm in diameter and 3µm length. Apical cells were rounded and convex.	*Oscillatoria perornata*
KA3	Trichome were solitary, almost straight and briefly attenuated at the ends, not constricted at the cross wall. Motile cells were found to be with 4µm length and 7µm wide. Found conical epical cells without calyptra. Cells were granulated with yellow colored sheath.	*Phormidium acutum*
KA4	Thallus was unbranched, filamentous, blue-green in color, filaments were tapered from base to tail. Trichomes were mostly ending in a hair. Cells were 3µm in length and 2µm in wide. Found terminal heterocyst. Cells were motile with gelatinous sheath. Presence of hormogonia and absence of akinetes.	*Rivularia* sp.
KA5	Trichomes were thick and straight enclosed infirm with rigid sheath. Filaments were un-branched found to be with false branching. Filaments were not constricted at the cross walls. Filaments were motile without heterocyst. Cells were distinctively shorter than wide, cells were 22 μm in wide. Found motile hormogonia and apical cell with calyptra.	*Lyngbya* sp.

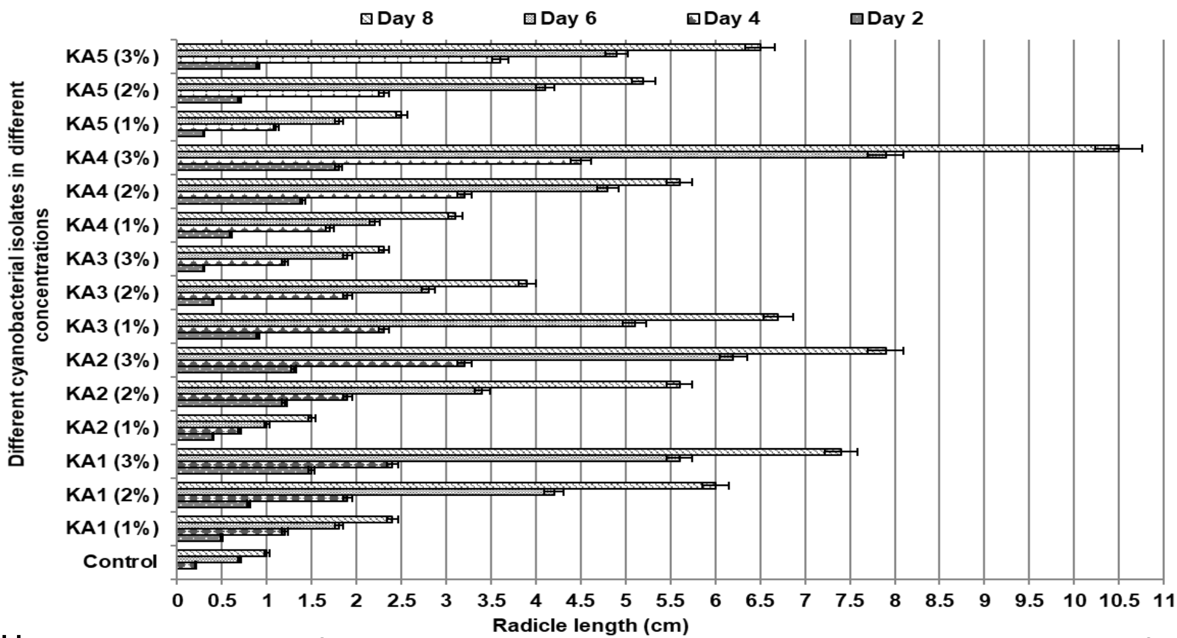
Key: KA=Krishna and Abera



⌗ Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

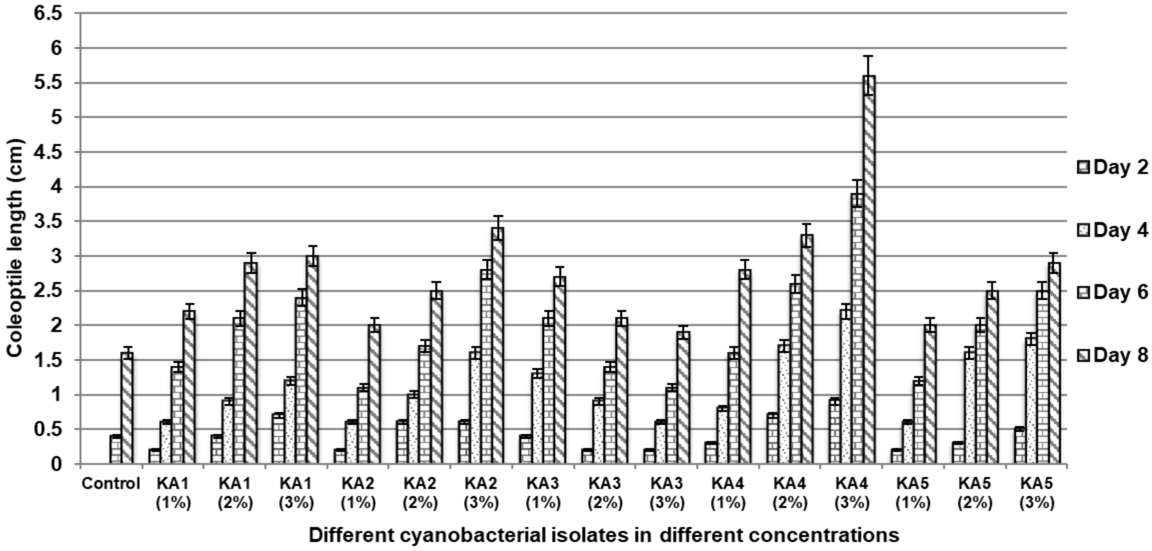
Figure 1. Effect of aqueous extract of cyanobacterial isolates on the percentage (%) of seed germination of wheat (*Triticum aestivum* L.) (8th day)



⌗ Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

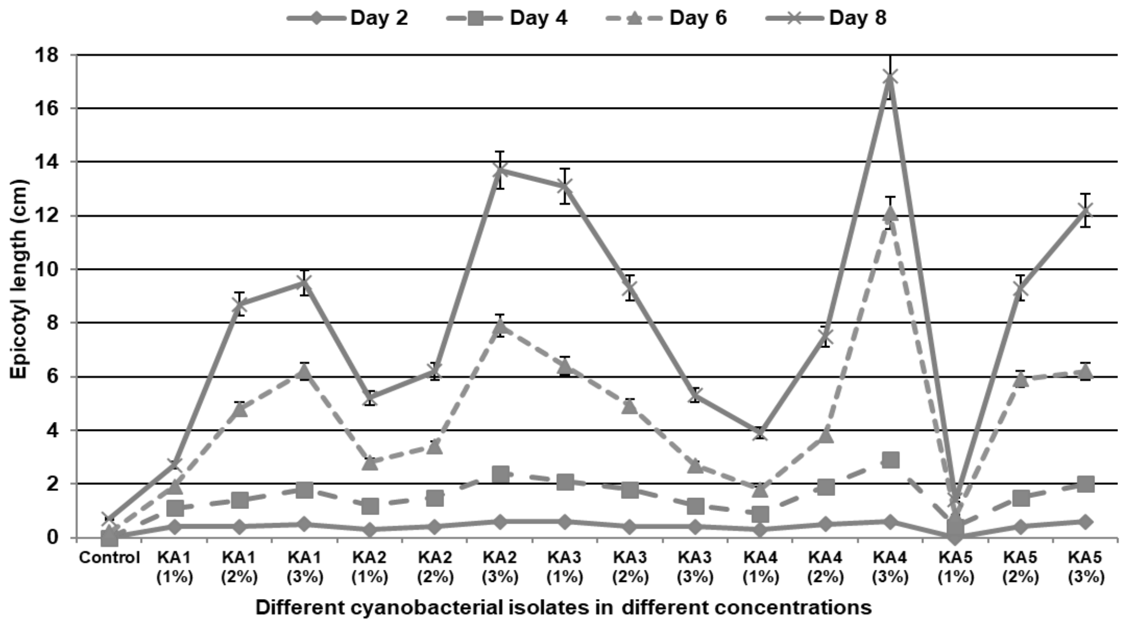
Figure 2. Effect of aqueous extract of cyanobacterial isolates on the radicle length of wheat (*Triticum aestivum* L.) (8th day)



- Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

Figure 3. Effect of aqueous extract of cyanobacterial isolates on the coleoptile length of wheat (*Triticum aestivum* L.) (8th day)



- Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

Figure 4. Effect of aqueous extract of cyanobacterial isolates on the epicotyl length of wheat (*Triticum aestivum* L.) (8th day)

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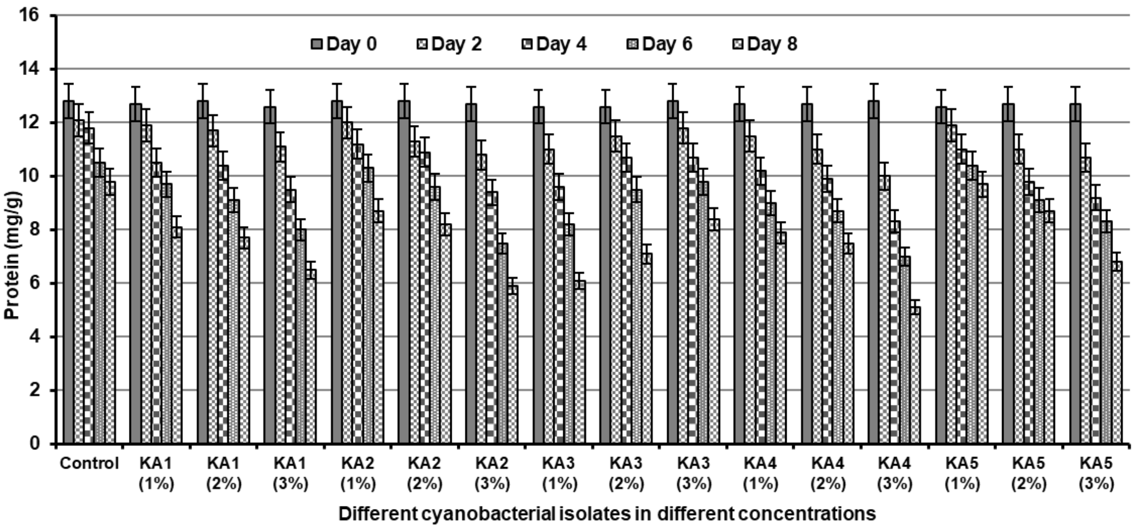
The other morphological parameters such as radicle length, coleoptile length and epicotyl length were significantly (P<0.05) influenced by the treatment of all the five cyanobacterial isolates in all the concentrations (1%, 2% and 3%) when compared to control on the 8th day of incubation. The maximum improvement in case of radicle length, coleoptile length and epicotyl length were found to be in the treatment of heterocystous cyanobacterial isolate *Rivularia* sp. KA4 at 3% concentration level followed by *Oscillatoria perornata* KA2 at 3% concentration on the 8th day of incubation (Figure 2, Figure 3, and Figure 4).

Biochemical parameters:

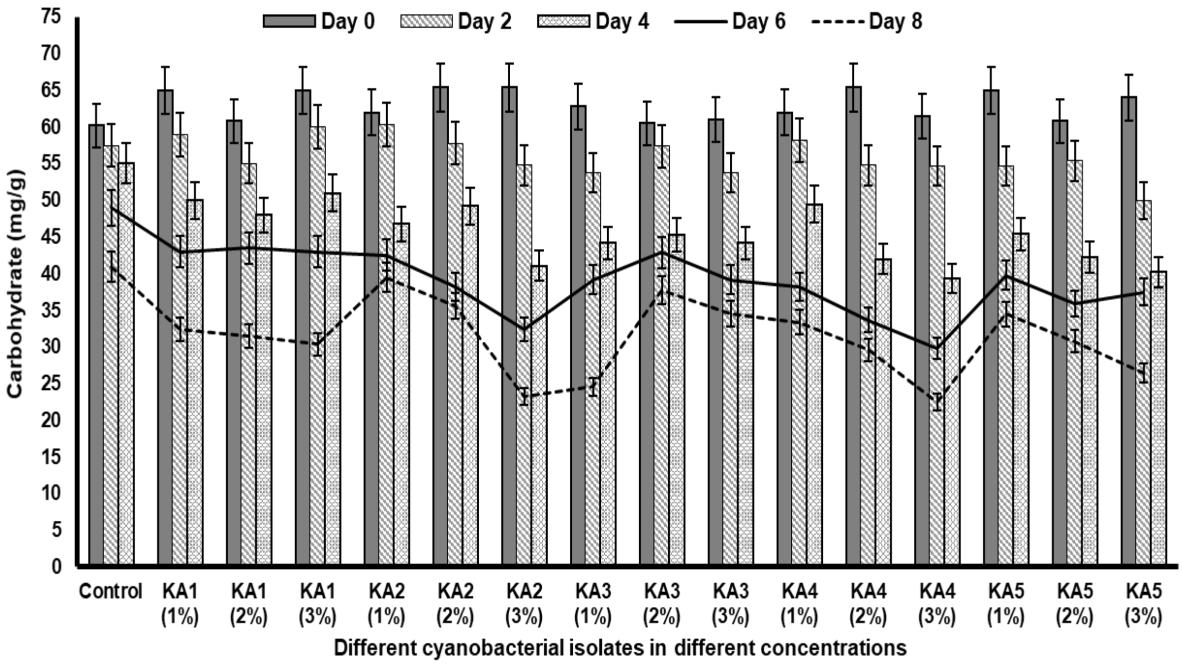
The changes in the protein and carbohydrate content in the control was too less when compared to all the cyanobacterial cultures treatment even at the 8th day of incubation. The maximum amount of protein and carbohydrate reduction was observed in the treatment of *Rivularia* sp.KA4 at 3% followed by *Phormidium acutum* KA3 at 1% and *Oscillatoria perornata* KA2 at 3% level of concentrations which was significantly (P<0.05) higher than control and the other two cyanobacterial isolates treatments on the 8th day of incubation (Figure 5 and 6).



- Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

Figure 5. Effect of aqueous extract of cyanobacterial isolates on the protein content of wheat (*Triticum aestivum* L.) (8th day)



⌘ Data are the mean of three replicates and error bars represent the standard errors of the means

KA1 – *Pseudanabaena galeata*; KA2 – *Oscillatoria perornata*; KA3 – *Phormidium acutum*; KA4 – *Rivularia* sp.; KA5 – *Lyngbya* sp.; KA – KRISHNA ABERA

Figure 6. Effect of aqueous extract of cyanobacterial isolates on the carbohydrates content of wheat (*Triticum aestivum* L.) (8^{th} day)

Growth promoting efficiency of cyanobacterial isolates on *Triticum aestivum L.* under pot experiment

Analysis of morphological parameters

The results in Tables (2a), (2b), (3a), and (3b) reveal that the morphological parameters of the experimental crop *Triticum aestivum* as affected by different kinds of treatments like control, chemical fertilizer (DAP - Di Ammonium Phosphate), cyanobacterial isolates such as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3, *Rivularia* sp. KA4 and *Lyngbya* sp. KA5 in liquid form as well as in dried form of application. The morphological parameters such as plant height (30.43±0.23), number of leaves (4.67±0.42), leaf length (23.8±0.30) and leaf width (0.80±0.058), root length (10.60±0.17), number of roots (8.30±0.33), shoot fresh (1.39±0.067) and dry weight (0.263±0.023) of experimental plant pots treated with liquid form of cyanobacterial isolates especially heterocystous cyanobacterium *Rivularia* sp. KA4 showed significantly higher results when compared to control, chemical fertilizer (DAP) and all other cyanobacterial isolates treatment (Tables 2a and 2b).

Table 2a. Effect of liquid form of cyanobacterial isolates on the morphological parameters of wheat (*Triticum aestivum*) under pot experiment on 30 DAPS (Days after planting)

	Morphological parameters			
Treatments	Plant height (cm	No. Of leaves (nos.)	Leaf length (cm)	Leaf width (cm)
Control	15.56±0.38	2±0.30	12±0.28	0.40±0.058
Chemical fertilizer (DAP)	23.63±0.32*	2.66±0.31	18.5±0.37*	0.50±0.057
Pseudanabaena galeata KA1	26.66±0.49*a	2.72±0.33	19.3±0.23*a	0.53±0.033*
Oscillatoria perornata KA2	28±0.23*a	3.66±0.38*a	20.6±0.24*a	0.66±0.030*
Phormidium acutum KA3	27.83±0.14*a	3.30±0.40*	21.7±0.20*a	0.57±0.066*
Rivularia sp. KA4	30.43±0.23*a	4.67±0.42*a	23.8±0.30*a	0.80±0.058*a
Lyngbya sp. KA5	27.33±0.17*a	3.34±0.33*	19.86±0.12*a	0.47±0.036

Values are the mean of three replicates ± SEM.

* - Indicates significance results over control (P<0.05)

a – Significance results over chemical (P<0.05)

Table 2b. Effect of liquid form of cyanobacterial isolates on the morphological parameters of wheat (*Triticum aestivum* L.) under pot experiment on 30 DAPS (Days after planting)

	Morphological parameters			
Treatments	Root Length (cm)	No. of Roots (Nos.)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)
Control	5.56±0.69	3.30±0.33	0.17±0.009	0.020±0.005
Chemical fertilizer (DAP)	7.93±0.20*	5.33±0.30*	0.32±0.014*	0.036±0.003
Pseudanabaena galeata KA1	9.86±0.26*a	5.70±0.67*	0.49±0.020*a	0.073±0.009*a
Oscillatoria perornata KA2	7.60±0.15*	6.66±0.69*	0.58±0.015*a	0.090±0.006*a
Phormidium acutum KA3	8.60±0.10*	5.33±0.32*	0.43±0.021*a	0.076±0.007*a
Rivularia sp. KA4	10.60±0.17*a	8.30±0.33*a	1.39±0.067*a	0.263±0.023*a
Lyngbya sp. KA5	7.40±0.15*	5.70±0.30*	0.48±0.024*a	0.073±0.008*a

Values are the mean of three replicates ± SEM.

* - Indicates significance results over control (P<0.05)

a – Significance results over chemical (P<0.05)

In case of dried form of cyanobacterial application, the pots treated with *Rivularia* sp. KA4 (heterocystous) resulted significant improvement in plant height (33.9±0.25), number of leaves (5.30±0.33), leaf length (25.80±0.49) and leaf width (0.83±0.033), root length (12.53±0.20), number of roots (11.66±0.67), shoot fresh (1.46±0.024) and dry weight (0.336±0.020) when compared to all other cyanobacterial isolates *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3 and *Lyngbya* sp. KA5 and chemical fertilizer treatment, and control (Tables 3a and 3b). The superior results were found to be in the pots treated with *Rivularia* sp.KA4 (heterocystous) as dried form in overall morphological parameter when compared to all other cyanobacterial isolate applied in dried form as well as liquid form, chemical fertilizer (DAP - Di Ammonium Phosphate) treatment, and control.

Table 3a. Effect of cyanobacterial isolates as dried form on the morphological parameters of wheat (*Triticum aestivum* L.) under pot experiment on 30 DAPS (Days after planting)

Treatments	Morphological parameters Plant height (cm	No. Of leaves (nos.)	Leaf length (cm)	Leaf width (cm)
Control	15.56±0.38	2±0.30	12±0.28	0.40±0.058
Chemical fertilizer (DAP)	23.63±0.32*****	2.66±0.31	18.5±0.37*	0.50±0.057
Pseudanabaena galeata KA1	27.73±0.15*a	3.77±0.30*a	19.43±0.29*a	0.60±0.057*
Oscillatoria perornata KA2	29.06±0.24*a	4.33±0.29*a	22.83±0.12*a	0.74±0.035*
Phormidium acutum KA3	28.4±0.26*a	3.67±0.31*a	21.60±0.33*a	0.70±0.042*a
Rivularia sp. KA4	33.9±0.25*a	5.30±0.33*a	25.80±0.49*a	0.83±0.033*a
Lyngbya sp. KA5	26.8±0.17*a	3.33±0.37*	18.06±0.30*	0.42±0.038

Values are the mean of three replicates ± SEM.

* - Indicates significance results over control (P<0.05)

a – Significance results over chemical (P<0.05)

Table 3b. Effect of cyanobacterial isolates as dried form on the morphological parameters of wheat (*Triticum aestivum*) under pot experiment on 30 DAPS (Days after planting)

	Morphological parameters			
Treatments	Root Length (cm)	No. of Roots (Nos.)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)
Control	5.56±0.69	3.30±0.33	0.17±0.009	0.020±0.005
Chemical fertilizer (DAP)	7.93±0.20*	5.33±0.30*	0.32±0.014*	0.036±0.003
Pseudanabaena galeata KA1	7.70±0.25*	7.30±0.33*a	0.54±0.020*a	0.083±0.003*a
Oscillatoria perornata KA2	7.86±0.20*	7.33±0.66*a	0.72±0.021*a	0.130±0.006*a
Phormidium acutum KA3	7.50±0.17*	6.00±0.57*	0.56±0.020*a	0.113±0.001*a
Rivularia sp. KA4	12.53±0.20*a	11.66±0.67*a	1.46±0.024*a	0.336±0.020*a
Lyngbya sp. KA5	7.53±0.22*	6.00±0.50*	0.36±0.026*	0.076±0.009*a

Values are the mean of three replicates ± SEM.

* - Indicates significance results over control ($P<0.05$)

a – Significance results over chemical ($P<0.05$)

Analysis of biochemical parameters

The experimental crop *Triticum aestivum* plant was treated with different cyanobacterial isolates such as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3, *Rivularia* sp. KA4 and *Lyngbya* sp. KA5 as liquid form as well as in dried form and examined for the changes occurred in the photosynthetic pigment contents and compared with control and chemicals fertilizer (DAP - Di Ammonium Phosphate) treatment (Table 4 and Table 5). Based on the results in Table 4, the pots treated with chemical fertilizers, the cyanobacterial isolates in liquid form significantly increased the photosynthetic pigment content of the experimental crop *T. aestivum* over control. The pots treated with heterocystous cyanobacterial isolates *Rivularia* sp. (KA4) showed significantly the best results followed by the other cyanobacterial isolates *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3 and *Lyngbya* sp. KA5 when compared to control and chemical fertilizers (DAP).

Table 4. Effect of liquid form of cyanobacterial isolates on the biochemical parameters of wheat (*Triticum aestivum* L.) under pot experiment on 30 DAPS (Days after planting)

Treatments	Biochemical parameters			
	Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
Control	0.61 ± 0.014	0.30± 0.08	0.91 ± 0.012	0.12 ± 0.017
Chemical fertilizer (DAP)	0.78 ± 0.023*	0.39 ± 0.011*	1.11 ± 0.020*	0.22 ± 0.018*
Pseudanabaena galeata KA1	1.00 ± 0.029*a	0.44 ± 0.021*a	1.34 ± 0.035*a	0.23 ± 0.021*
Oscillatoria perornata KA2	1.09 ± 0.026*a	0.51± 0.015*a	1.60 ± 0.032*a	0.30 ± 0.023*a
Phormidium acutum KA3	1.05 ± 0.012*a	0.49 ± 0.020*a	1.42 ± 0.039*a	0.26 ± 0.020*
Rivularia sp. KA4	1.73 ± 0.016*a	0.75 ± 0.021*a	2.46 ± 0.023*a	0.48 ± 0.026*a
Lyngbya sp. KA5	0.73 ± 0.026*	0.30 ± 0.020*	1.08 ± 0.043*	0.22 ± 0.023*

Values are the mean of three replicates ± SEM.

* - Indicates significance results over control (P<0.05)

a – Significance results over chemical (P<0.05)

The Table (5) shows that the application of the dried form of the heterocystous cyanobacterial isolates *Rivularia* sp. (KA4) significantly increased the photosynthetic pigment contents when compared to the all other cyanobacterial isolates in dried form, control, and chemicals fertilizer treatment. The cyanobacterial isolate *Rivularia* sp. (KA4) in dried form showed superior results in overall biochemical aspects when compared to liquid and dried form cyanobacterial isolates, chemical fertilizer (DAP - Di Ammonium Phosphate) treatment, and control.

Table 5. Effect of dried form of cyanobacterial isolates on the biochemical parameters of wheat (*Triticum aestivum* L.) under pot experiment on 30 DAPS (Days after planting)

Treatments	Biochemical parameters Chlorophyll *a* (mg/g)	Chlorophyll *b* (mg/g)	Total Chlorophyll (mg/g)	Carotenoids (mg/g)
Control	0.61 ± 0.014	0.30 ± 0.08	0.91 ± 0.012	0.12 ± 0.017
Chemical fertilizer (DAP)	0.78 ± 0.023*	0.39 ± 0.011*	1.11 ± 0.020*	0.22 ± 0.018*
Pseudanabaena galeata KA1	1.11 ± 0.018*a	0.54 ± 0.021*a	1.54 ± 0.034*a	0.26 ± 0.021*
Oscillatoria perornata KA2	1.21 ± 0.017*a	0.60 ± 0.012*a	1.81 ± 0.029*a	0.32 ± 0.023*a
Phormidium acutum KA3	1.17 ± 0.012*a	0.58 ± 0.008*a	1.63 ± 0.037*a	0.29 ± 0.020*a
Rivularia sp. KA4	1.91 ± 0.020*a	0.91 ± 0.023*a	2.76 ± 0.049*a	0.51 ± 0.026*a
Lyngbya sp. KA5	0.85 ± 0.023*a	0.41 ± 0.015*	1.22 ± 0.037*a	0.25 ± 0.023*

Values are the mean of three replicates ± SEM.
* - Indicates significance results over control (P<0.05)
a – Significance results over chemical (P<0.05)

Discussion

In the present study, totally five different cyanobacterial cultures were isolated from the samples collected around Wolaita Sodo University and identified as *Pseudanabaena galeata* KA1, *Oscillatoria perornata* KA2, *Phormidium acutum* KA3, *Rivularia* sp. KA4 and *Lyngbya* sp. KA5 based on the phenotypic characters rather than genotypic characters, such as the morphology of cells and filaments, the shape of the terminal cells, presence or absence of sheaths, gas vacuoles, motile hormogonia, and nitrogen-fixing heterocyst. The cyanobacterial identification process of the current study is highly supported by Komárek and Anagnostidis (2005); Komárek and Anagnosti-dis (1998); Gomont (1982) and Desikachary

(1959). Similar to the present study, cyanobacteria were isolated and identified based on the morphometric characteristic's features using microscope by Krishna et al. (2019) and Mayur et al. (2017).

Under seed germination experiment, the heterocystous cyanobacterial isolates *Rivularia* sp. (AB4) at 3% concentration level showed significant effects in the aspects of morphological parameters such as seed germination percentage, radicle length, coleoptile length and epicotyl length and biochemical parameters like protein and carbohydrates when compared to all other cyanobacterial isolates and control. The reason for this great response is naturally cyanobacteria having the potential to release the plant growth hormones like auxins, cytokinin and gibberellin. These plant growth hormones directly involved in the seed germination and increased the percentage of seed germination. Similarly, Osman et al. (2010) have reported that the cyanobacteria play a major role in the seed germination by secreting phytohormones like auxins, cytokinin and gibberellins. The present study results were well supported by Mayur et al. (2017) who reported that the cyanobacterial isolates *Rivularia* sp., *Nostoc* sp., *Oscillatoria* sp., *Closterium* sp., *Gloeothece* sp., *Anabaena* sp., *Aphanocapsa* sp. and *Gloeocapsa* sp. showed positive effects on the seed germination rate of mung as well as wheat. Similarly, Krishna et al. (2019) and Krishna and Kibrome (2019) have reported that the cyanobacterial isolates significantly influenced the morphological parameters such as seed germination percentage, radicle length, coleoptile length and epicotyl length of maize seeds when compared control seeds treated with only distilled water.

The biochemical parameters like protein and carbohydrate changes in the present study was similar to the study of Salisbury and Rose (1991) who have reported that the during the seed germination the intercellular bodies of seed stored carbohydrates, proteins, lipid and phosphate acts as an energy source. Similar to the present study results, the protein and carbohydrate content of *Phaseolus vulgaris* L seeds were decreased by the treatment of different concentrations of cyanobacterial aqueous extracts during seed germination (Krishna et al., 2019).

A Pot experiment was conducted to study the effect of cyanobacterial isolates in both liquid form as well as the dried form on *T.* aestivum L. as an experimental crop and compared with chemical fertilizer and control. The very best result in morphological parameters (plant height, number of leaves, leaf length, leaf width, number of roots, root length, shoot fresh and dry weight) and biochemical parameters (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid) of *T. aestivum* L. was found to be in the pots treated with heterocystous cyanobacterial isolate *Rivularia* sp. (AB4) in dried as well as liquid form when compared to control, chemical fertilizers and all other cyanobacterial treatments in both liquid as well as dried form. The application cyanobacteria can increase the soil organic carbon by photosynthetic nature and may due to the ability of phytohormones production like Auxins and cytokinin which help plants to reach higher results. Another one reason, *Rivularia* sp. (AB4) is a heterocystous cyanobacteria which can fix atmospheric nitrogen and can stimulate the plants growth.

Similarly, Mulat et al. (2013) have reported that cyanobacterial bio-fertilizer treatments, applied in either dry or liquid form, consistently increased SOC. The study coincides with the study of Francis and Berhanu, (2016) who have reported that the plants showed better in growth parameters (fresh shoot and root weight, dry shoot, root weight, leaf area, and number of branches) with application of cyanobacteria bio-fertilizers than with urea fertilizer and compost, thus indicating the potential of cyanobacteria biofertilizer as having a positive effect on soil fertility and yield and nutritional quality of cultivated vegetables such as tomato plants. Similar to the present study, the liquid forms of cyanobacterial inoculants showed significantly higher results in the morphological parameters such as plant height, shoot fresh weight, number of leaves, leaf area and shoot dry weight than the dried form of cyanobacterial inoculum, urea (chemical fertilizer) and control (Krishna and Kibrom, 2019; Eshetu, 2017).

Conclusion

The heterocystous cyanobacterial isolate Rivularia sp. (KA4) at 3% concentration have shown that the highest performance in terms of morphological and biochemical parameters of T. aestivum L. when compared to all other cyanobacterial isolates such as such as Pseudanabaena galeata (KA1), Oscillatoria perornata (KA2), Phormidium acutum (KA3) and Lyngbya sp. (KA5), and control on 8th day incubation under seed germination experiment. The maximum improvement in terms of morphological and biochemical parameters of T. aestivum L. were found to be in the treatment of dried and liquid form of heterocystous cyanobacterium Rivularia sp. (AB4) when compared to the non-heterocystous cyanobacteria such as Pseudanabaena galeata (KA1), Oscillatoria perornata (KA2), Phormidium acutum (KA3) and Lyngbya sp. (KA5), chemical fertilizer (Di Ammonium Phosphate) treatment, and control. Thus, it can be concluded that the heterocystous cyanobacterial isolate Rivularia sp. KA4 at 3% aqueous extracts can be used to do the pretreatment of wheat seeds and the same heterocystous cyanobacterium Rivularia sp. KA4 can also be used as biofertilizers in both dry as well as liquid form for the cultivation of wheat (T. aestivum L.). The heterocystous cyanobacterium Rivularia sp. (KA4) can be used as alternatives to the chemical fertilizers for the cultivation of T. aestivum L. after the field conformation studies.

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