Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Isolation and Identification of Blue Green Algae and Its Plant Growth Promoting Efficacy using Red Kidney Beans (*Phaseolus vulgaris* L.) by Seed Germination Experiment

Krishna Moorthy, S[1] , Aberham Kassahun, Ermias Dereje and Adanech Tomas
Department of Biotechnology, College of Natural and Computational Sciences, Wolaita Sodo University, Wolaita Sodo, Ethiopia
[1]Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Wolaita Sodo, Ethiopia.
.

Abstract
The present study was mainly focused on isolation and identification of fresh water blue green algae (cyanobacteria) and determines its plant growth promoting potential using red kidney beans *Phaseolus vulgaris L.* seed under seed germination experiment. Three cyanobacterial isolates were isolated and identified as *Pseudanabaena* spp. AK-1, *Lyngbya* spp. AK-2 and *Geitlerinema* sp. AK-3 from Kalte river of Wolaita Sodo Town based on the morphometric characters using microscopic examinations. All the isolated and identified cyanobacterial isolates as aqueous formulations at three different concentrations (1%, 2% and 3%) were used to examine the seed germination stimulating potential using red kidney beans. All the three cyanobacterial isolates *Pseudanabaena* spp. AK-1, *Lyngbya* spp. AK-2 and *Geitlerinema* sp. AK-3 in all the three different concentrations showed significantly ($p<0.05$) higher results when compared to control (Treated with only distilled water). But the cyanobacterial isolates *Geitlerinema* sp. AK-3 at 0.3% concentration level showed superior results in both morphological parameters such as seed germination percentage, radicle length, coleoptile length and epicotyl length and biochemical parameters like protein and carbohydrates when compared to other two cyanobacterial isolates and control.

Keywords: Cyanobacteria, Seed germination, Aqueous extract, *Phaseolus vulgaris* L.

Introduction

The human population of our planet is projected to reach ~9.7 billion by 2050, and majority of this increased population would be contributed by developing countries of Asia and Africa (DESA UN, 2015). This increase in global population is associated with increased demand of food security in future. To overcome this challenge, the World Health Organization has suggested a doubling of food production by 2050, while the United Nations have suggested a 50% increase in global food production by 2030. Ethiopia is one of the largest African countries with second more population in Africa (Eshetu Gebre and Alemu Lelago, 2017). Ethiopia, the majority (83.8%) of Ethiopians reside in the rural areas. The majority of these rural peoples are only depending agriculture especially crop production for their regular income.

All the farmers are depending on natural sources and commercially available chemical fertilizers such as Urea and DAP (Di Ammonium Phosphate) to enrich the soil nutrient as well as to stimulate their crop productivity. The continuous and indiscriminate usage of these chemical fertilizers spoils the soil nature which will lead to decreased in crop production. Hence the present study focused to find an alternative solution for the sustainable crop production with sustainable soil health that is biofertilizers concept. Many researches recommended cyanobacteria as a biofertilizer for many vegetable crops e.g. sorghum, maize, lentil, chickpea, barley, sugar beet, and bean (Hegazi *et al.,* 2010 and El-Naggar *et al*., 2014).

Cyanobacteria are a group of extraordinarily diverse Gram-negative prokaryotes that originated 3.5 billion years ago. Their diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, fresh water to marine including hypersaline (salt pans). They are found both free living and as endosymbionts (Thajuddin and Subramanian, 2005). Cyanobacteria are oxygenic, photosynthetic prokaryotic organisms that are distributed worldwide and can inhabit a wide range of habitats including freshwater, marine and terrestrial environments (Pankratova 2006; Tripathi *et al.,* 2007; Nagarajan *et al.,* 2012; Whitton 2012). Cyanobacterial biomass plays an important role in the sustainable agricultural development because they “offer an economically attractive and ecologically sound alternative to chemical fertilizers to improve the soil physicochemical properties like water holding capacity and nutritional status, crop growth and yield (Singh *et al.,* 2016; Osman *et al.,* 2016). In the present study, the blue green microalgae were isolated and identified from Kalte river of Wolaita Sodo Town of Southern Ethiopia and used to determine the seed germination promoting status using red kidney beans *Phaseolus vulgaris L.* seed under seed germination experiment.

Materials and methods

Sample source and sample collection

For the isolation of cyanobacterial cultures, a fresh water and high wet soil samples which having appropriate cyanobacterial colonial growth was collected randomly from different locations of Kalte River, Wolaita Sodo, Southern Ethiopia. The samples for the isolation of cyanobacteria were packed in sterile transparent plastic or glass container with screw cap and brought to the Post Graduate Microbiology Laboratory, Department of Biology, Wolaita Sodo University.

Isolation of Cyanobacteria

All the samples collected from the Kalte river were processed for the isolation of cyanobacteria using BG11 media (Rippka *et al*., 1979). All the samples were serially diluted and inoculated on the petriplates which having sterilized BG11 agar medium and incubated at 25±2ºC with illumination of 1500lux by cool white 40W fluorescent tubes with 16/8hrs light and dark cycle. All the plates were regularly monitored for the cyanobacterial growth and subsequently the cyanobacterial cultures were purified by streak plate method. These entire attempts were aseptically performed in front laminar air flow (Allen and Stanier, 1967; Castenholz, 1992).

Identification of cyanobacteria

Identification of cyanobacterial cultures were done microscopically based on morphological observation, 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 heterocysts and its repetition, presence of akintes and its type, the nature of cell wall, presence or absence gas vacuoles, as well as pigment color was taken in consideration according to Desikachary, (1959), Iyengar and Desikachary (1981), Komárek and Anagnostidis (1989), Cornberg and Komárek (2004), Komárek (2005), Komárek and Anagnostidis, (2005), Komárek and Hauer, 2013 and Khare *et al.* (2014),

Maintenance of isolated cyanobacterial cultures

The isolated cyanobacterial strains were sequentially assigned with reference numbers having its own uniqueness and deposited to fresh water cyanobacterial and microalgal repository of Biology Department, Wolaita Sodo University. The unialgal cyanobacterial strains maintained in repository were sub-cultured for every 3-4 months depending on the culture conditions.

Mass Cultivation of cyanobacteria under laboratory condition

The purified cyanobacterial isolates were picked from the culture plates and inoculated in to 1000ml conical flasks containing sterilized BG11 media aseptically. The inoculated conical flasks were incubated under 1500lux (Philips cool-white light, 16hrs light 8hrs dark cycle) and at 25±2ºCtemperature in culture room (Rippka *et al*., 1979). The cyanobacterial cultures were harvested after 15-20days of incubation and aqueous extract was prepared for the seed germination experiment.

Seed germination experiment using plate method

The red kidney bean (*Phaseolus Vulgaris*) seed were collected from local market. Seeds were surface sterilized with 70% ethanol or 0.1 % HgCl2 for 3 min. Ten surface sterilized seeds were placed on petri plates covered with filter paper and moistened with 10 ml of the aqueous extract of cyanobacterial isolates in different concentrations like 1% (1gm/100ml), 2% (2gm/100ml) and 3% (3gm/100ml). Petri-dishes containing seeds with 10 ml of distilled water served as a control. The growth parameters including germination percentage, coleoptile length and radicle length were recorded on the 2 days interval up to 8 days after incubating seed at 280C (Pitchai *et al.,* 2010). The Biochemical parameters such as Carbohydrate (Yemm and Willis, 1954) and Protein (Lowry *et al.,* 1951) were analyzed 2 days interval.

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 test at $p<0.05$ level of significance. All the data included in the figures were presented in mean and standard error (±) of mean of three replicates per treatment and repeated three times.

Results

The present research was mainly focused on isolation and identification of cyanobacteria based on the morphological characteristic features; laboratory cultivation of isolated cyanobacterial species for the determination of growth promoting efficiency using red kidney beans *P. vulgaris L.* seed, Wolaita Sodo Region of Ethiopia from February, 2019 to May, 2019.

Isolation and identification of cyanobacteria:

Totally 3 different cyanobacteria were isolated and identified from all the collected samples. All the 3 isolates were marked with isolates number as AK1, AK2 and AK3; here in this numbering system, AK indicates

Aberham and Krishna. The organisms appeared in the enriched culture were morphometrically analyzed and identified using binocular research OLYMPUS MICROSCOPE Model CX21FS1 based on the scheme proposed by Desikachary (1959). All the identified cultures were also compared with published pictures available in the literatures. The morphometric characteristics features of all the isolated cyanobacteria are as follows.

Morphological characters of Pseudanabaena sp. AK-1

The first isolates possessed solitary trichomes, occasionally in fine mats, no sheathes, sometimes sheaths present very rarely, occasionally motile, rarely more than 30 cells. Lacks heterocysts, Not attenuated; Cells typically cylindrical (rarely ± isodiametric), 1–3.5 µm wide, often barrel shaped, usually with conspicuous constrictions at cross walls. End cells may be rounded or pointed, facultative aerotopes.

Morphological characters of Lyngbya spp. AK-2

Trichomes are thick and straight enclosed in firm, rigid sheath. Filaments are usually un-branched, false branching present. Filaments are not constricted at the cross walls. No of heterocysts, motile, Cells are distinctively shorter than wide, cells are 22 *µ*m in wide. Hormogonia present, apical cells usually have a calyptra. Form motile hormogonia. Filaments are not constricted at the cross walls.

Morphological characters of Geitlerinema sp. AK-3

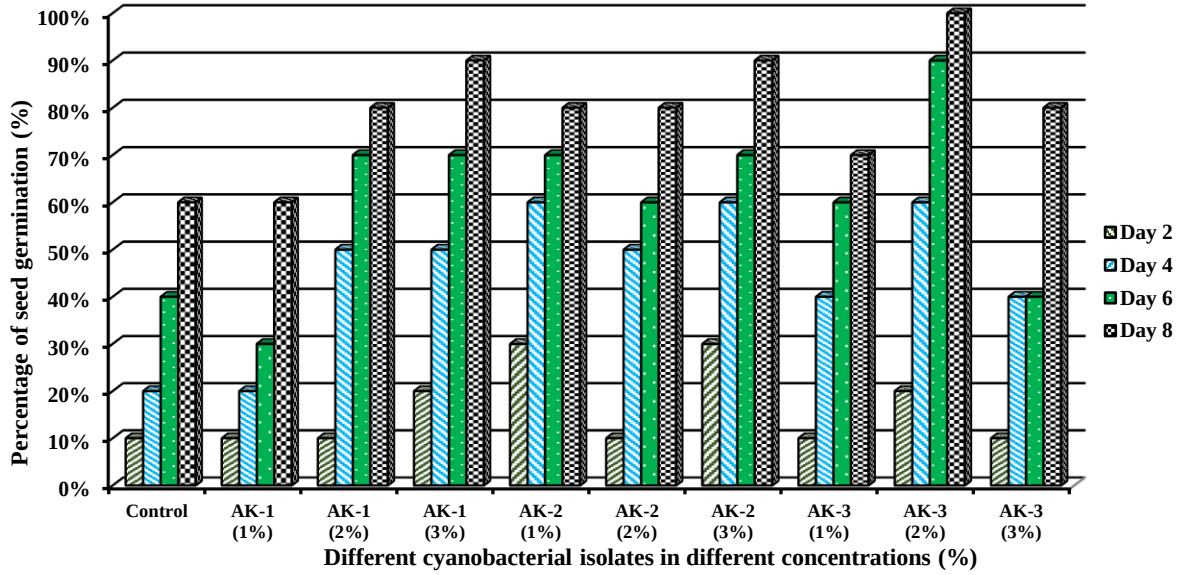
Filaments straight, rarely curved, not constricted septum, translucent; cells longer than wide (4.2× 1.9 µm); provided with one or two beads in the division of cells; apical cell rounded. End cells are cylindrical apical cell with rounded apex. Highly motile with intensive gliding motility, but also rotation and waving.

Determination of Growth promoting efficiency of cyanobacterial isolates on Phaseolus vulgaris L. using seed germination experiment by plate method

The present study was undertaken to evaluate the germination potential ability of cyanobacterial isolates at different concentrations (1%, 2% and 3%) on an economically important *P. vulgaris* L. seed by seed germination experiment using petriplate method. All the data related to seed germination experiment was collected at every 2 days interval up to 8th days. The morphological parameters such as percentage of seed germination, radicle length, coleoptile length, and epicotyl length and biochemical parameters such as protein and carbohydrate content were also quantified and presented as follows.

Morphological parameters

The results of morphological parameters such as percentage of seed germination, radicle length, coleoptile length and epicotyl length obtained from the seed germination experiment are given in the Fig.1-4.



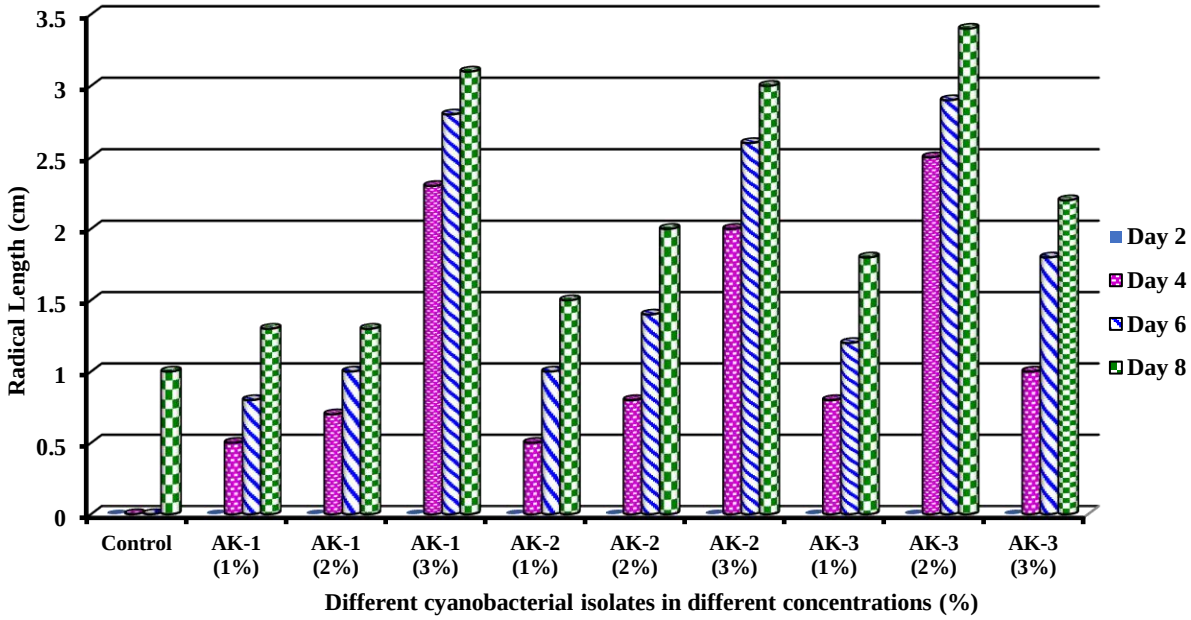
AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 1. Effect of aqueous extract of cyanobacterial isolates on percentage of seed germination of *Phaseolus vulgaris* L. under seed germination experiment (8th day)

The percentage of seed germination in control reached maximum of 60% even at 8th day of incubation while all the cyanobacterial isolates (*Pseudanabaena* sp. AK-1, *Lyngbya* sp. AK-2 and *Geitlerinema* sp. AK-3) in all three concentrations showed significantly ($p<0.05$) higher level of seed germination percentage than control

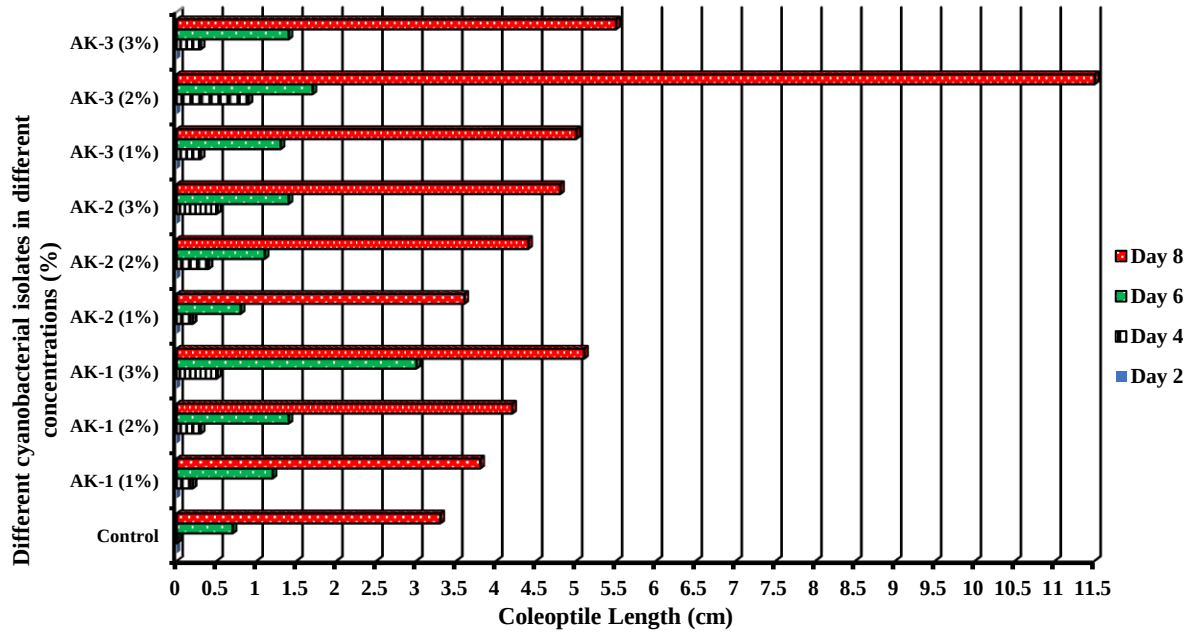
treatment. The cyanobacterial isolates *Geitlerinema* sp. AK-3 reached 100% seed germination in 8th day of incubation at 2% concentration of aqueous extract (Fig.1).

All the cyanobacterial isolates with all concentrations showed significantly ($p<0.05$) higher results in case of radicle length, coleoptile length and epicotyl length over control. The highest radicle length (3.5 ± 0.3 cm), coleoptile length (11±0.5cm) and epicotyl length (4.5±0.3) was observed in the seeds inoculated with *Geitlerinema* sp. AK-3 at 2% concentration level when compared to control and all other cyanobacterial isolates in all concentrations (Fig.2-4).



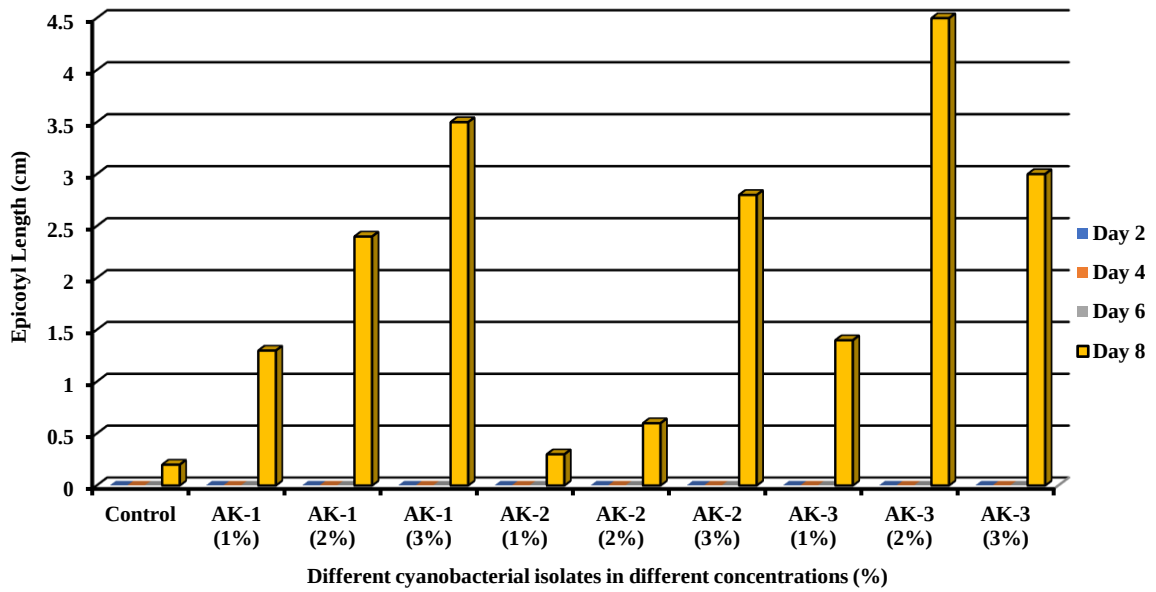
AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 2. Effect of aqueous extract of cyanobacterial isolates on radical length of *Phaseolus vulgaris* L. under seed germination experiment (8th day)



AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 3. Effect of aqueous extract of cyanobacterial isolates on coleoptile length of *Phaseolus vulgaris* L. under seed germination experiment (8th day)



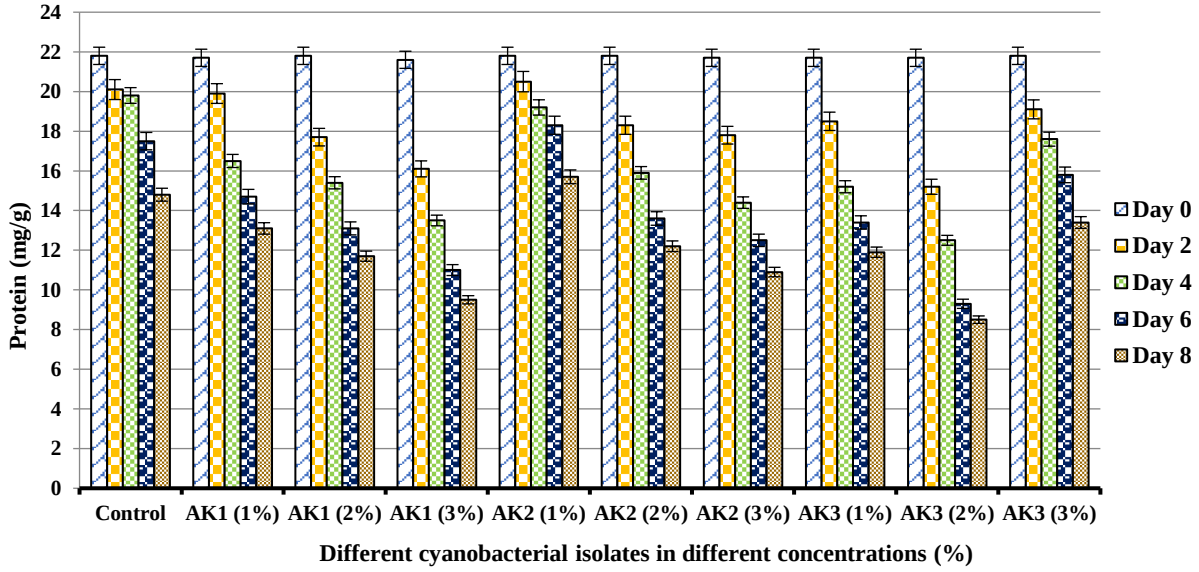
AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 4. Effect of aqueous extract of cyanobacterial isolates on epicotyl length of *Phaseolus vulgaris* L. under seed germination experiment (8th day)

Biochemical parameters

Protein and carbohydrate are the reserve foods in the *Phaseolus vulgaris* L seeds. All the stored forms of reserved foods are hydrolyzed during the germination process. Hence, in the present study about protein and carbohydrate changes in the seed germination experiments are more important.

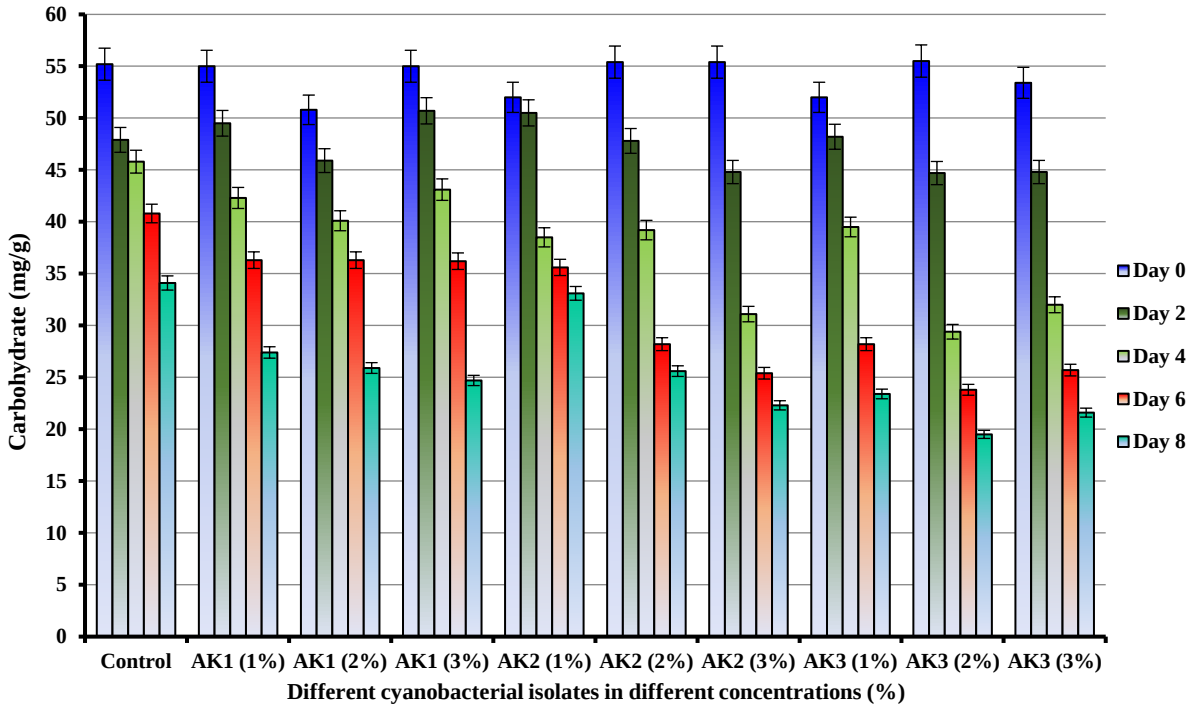
Fig.5 showed clearly about the changes of protein content of all the treatments including control from 0th day to 8th day. The protein contents of *Phaseolus vulgaris* L. seeds in the control treatment was not decreased in high level from 0th day to 8th day. The cyanobacterial isolates *Pseudanabaena* sp. AK-1, *Lyngbya* sp. AK-2 and *Geitlerinema* sp.AK-3 in all three concentrations (1%, 2% and 3%) showed significant changes in protein content when compared to control. The maximum level of protein reduction was observed in the seeds treated with *Geitlerinema* sp. AK-3 at 2% level of concentration when compared other concentrations of same cyanobacterial isolates AK-3, control and all other cyanobacterial isolates in all concentrations.



AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 5. Effect of aqueous extract of cyanobacterial isolates on protein content of *Phaseolus vulgaris* L. under seed germination experiment (8th day)

Carbohydrates content of all the treatments were analyzed properly and displayed in the form of Graph (Fig. 6). The changes in the carbohydrate content of control were significantly lesser than all other treatments in all the concentrations even at the 8th day incubation. The maximum amount of carbohydrate reduction was observed in the treatment of AK-1 (*Geitlerinema* sp.) at 2% level of concentrations which is significantly higher than control and all other treatments in all concentration.



AK-1 – *Pseudanabaena* sp. AK-2 – *Lyngbya* sp.AK-3 – *Geitlerinema* sp. AK-3; AK-Aberham and Krishna

Fig. 6. Effect of aqueous extract of cyanobacterial isolates on carbohydrate content of *Phaseolus vulgaris* L. under seed germination experiment (8th day)

Discussion

Isolation and identification

From the samples collected at different sites of Kalte River of Wolaita Sodo Town, total of 3 cyanobacterial species were isolated and identified based on the morphometric characteristic's features using microscope such as morphology of cells and filaments, shape of the terminal cells, presence or absence of sheaths, gas vacuoles, motile hormogonia, nitrogen-fixing heterocysts and resting akinetes/spores. The cyanobacterial identification process of current study is highly supported by Desikachary, (1959), Iyengar and Desikachary (1981), Komárek and Anagnostidis (1989), Cornberg and Komárek (2004), Komárek (2005), Komárek and Anagnostidis, (2005), Komárek and Hauer, (2013), Khare *et al.* (2014) and Mayur Gahlout (2017).

Effects of isolated cyanobacteria on Morphological parameters of P. vulgaris L seeds

At present study, 3 cyanobacterial species were identified and primarily screened for the plant growth promoting efficiency on *Phaseolus vulgaris* L. seed germination assay using plate method with three different concentrations of aqueous extracts such as 1%, 2% and 3%. All the cyanobacterial isolates showed significant response in the percentage (%) of seed germination in all different concentrations of aqueous extracts when compared to control. The reason for this great response is naturally cyanobacteria having the potential to release the plant growth hormones like auxins, cytokinins and gibberellins. These plant growth hormones directly involved in the seed germination and increased the percentage of seed germination. This result was highly supported by Gayathri *et al.* (2017) reported that the cyanobacterial extracts in different concentrations (1% and 10%) stimulated the germination of *Pisum sativum* L. seeds earlier (within 3 days) than seeds treated with only water (Control). Similarly, Mayur *et al.* (2017) reported that the cyanobacterial isolates *Rivularia* spp., *Nostoc* spp., *Oscillatoria* spp., *Closterium* spp., *Gloeothece* spp., *Anabaena* spp., *Aphanocapsa* spp. and *Gloeocapsa* spp. showed positive effects on the seed germination rate of mung as well as wheat.

All the 3 cyanobacterial isolates (*Pseudanabaena* spp.AK-1, *Lyngbya* spp.AK-2 and *Geitlerinema* sp. AK-3) showed significantly higher results in case of radicle, coleoptile and epicotyl length when compared control where seeds were treated with only distilled water alone. Present study was supported by Gayathri *et al.* (2017) who reported that the cyanobacterial isolates *Scytonema bohneri* MBDU 104 (80%), *Aphanothece stagnina* MBDU 803 (66.6%), *Calothrix* sp. MBDU 901(66.6%), Nostoc microscopicum MBDU 102 (56.6%) and Dolichospermum spiroides MBDU 903 (70%) showed comparatively increasing responses (radicle, plumule and total seedling responses) than control (53.3%)

Effects of isolated cyanobacteria on Biochemical parameters of P. vulgaris L seeds

The protein and carbohydrate contents of *Phaseolus vulgaris* L seeds in the control treatment was not decreased in high level from 0th day to 8th day. The maximum level of protein and carbohydrate reduction was observed in the seeds treated with *Geitlerinema* sp. AK-3 at 2% concentration level of concentration followed by *Pseudanabaena* spp. AK-1 at 3% concentration and *Lyngbya* spp.AK-2 at 3% concentration. Protein and carbohydrate are the reserve food materials in the *Phaseolus vulgaris* L seeds. On seed hydration, the seeds containing protein and carbohydrates acted as energy sources. So, during the seed germination all these protein and carbohydrate based reserved food materials may be hydrolyzed by hydrolytic enzymes and converted in to simple available form for embryo uptake. So during the seed starts to germinate, the protein and carbohydrates level will be reduced automatically. This result was highly supported by Bewley and Black (1985). Shutov and Vaintraub (1987), Mayer and Poljakoff – Mayber (1989) and Salisbury and Ross (1991) and who are all reported that the seed received hydration, separate intercellular bodies of seed stored carbohydrates, proteins, lipid and phosphate act as energy source and carbon skeleton. Seed imbibition triggered many metabolic processes such as activation or freshly synthesis of hydrolytic enzymes which resulted in hydrolysis of stored starch, lipid, protein hemicellulose, polyphosphates and other storage materials into simple available form for embryo uptake.

Conclusions

Based on the findings of isolation and identification studies, the non-heterocystes cyanobacterial species are more dominant in Kalte River of Wolaita Sodo Town, Southern Ethiopia. In the seed germination study the cyanobacterial isolates *Pseudanabaena* spp.AK-1 at 3%, *Lyngbya* spp.AK-2 at 3% and *Geitlerinema* sp. AK-3 at 2% concentration active more in the *P. vulgaris* L. seed germination when compared to control. The cyanobacterial isolate *Geitlerinema* sp. AK-3 at 2% concentration showed superior results in all morphological and biochemical aspects of seed germination experiments when compared to all other cyanobacterial isolates and control on 8th day incubation. Based on the above said findings, it has concluded that the cyanobacterial isolates *Geitlerinema* sp. AK-3 at 2% can be used as aqueous extracts to do pretreatment of *P. vulgaris* L. seeds to enhance the seed germination.

Acknowledgements

The authors would like to gratefully acknowledge the Post Graduate Microbiology Laboratory, Department of Biology, Wolaita Sodo University for providing us the facility to do the research successfully.

References

Allen, M.M. and R.Y. Stanier. 1967 Selective isolation of Blue-Green algae from water and soil. *J. gen. Microbiol.* **5I**: 203-209.

Bewley, J.D., and M. Black. 1985 Seeds: Physiology of Development and Germination. New York: Plenum Press.

Castenholz, R.W. 1992 Species usage, concept, and evolution in the cyanobacteria (blue-green algae). *Journal of Phycology.* **28**: 737–745.

Cornberg, G. and J. Komárek. 2004 Some Nostocalean Cyanoprokaryota from lentic habitats of Eastern and South Africa. *Nova Hedwigia.* **78 :**71 - 106.

Desikachary, T.V. 1959. Cyanophyta Indian Benemann, J. R. 1979.Production of nitrogen fertilizer with nitrogen-fixing blue-green algae. **Enzyme Microb.Techol. 1**: 83-90.

El-Naggar, A.H. Mohamed El-Anwar, H. Osman Mostafa M. and El Gheda. 2014 Cyanobacteria mediated promotion of growth and some metabolic activities of barley seedlings. *Egyptian Journal Experimental Biology.* **10**(1): 63-73.

Eshetu Gebre and Alemu Lelago. 2017 Effect of Cyanobacterial Biofertilizer on Soil Quality in Kale (*Brassica Oleracea*L.) Crop Growing Field at Ziway Area, Ethiopia. *Food Science and Quality Management.* 61: 41-46.

Gayathri Manickam, Sumathy Shunmugam, Nooruddin Thajuddin, Gangatharan Muralitharan. 2017. Phytohormones and free volatile fatty acids from cyanobacterial biomass wet extract (BWE) elicit plant growth promotion. *Algal Research.* **26**: 56–64.

Hegazi, A.Z. Mostafa, Soha S. M. Mostafa. and Hamdino M. I. Ahmed. 2010 Influence of different cyanobacterial application methods on growth and seed production of common bean under various levels of mineral nitrogen fertilization. *Nature and Science.* **8**(11): 183-194.

Iyengar, M.O.P. and T.V. Desikachary. 1981 *Volvocales*. pp. 1-532. New Delhi: Indian Council of Agricultural Research.

Khare, P., A. Singh, C. Prabha and S. Kumari. 2014. Study of cyanobacterial biodiversity in rice fields of Central Bihar. In: 3rd World conference on applied sciences, Engineering and Technology, 27-29 September 2014, Kathmandu, Nepal.

Koma´rek, J. and T. Hauer. 2013 CyanoDB.cz – The on-line databaseof cyanobacterial genera. University of South Bohemia & Institute ofBotany AS CR. http://www.cyanodb.cz

Komárek, J. 2005 Phenotype diversity of the heterocystous Cyanoprokaryotic genus *Anabaenopsis*. *Czech Phycology.* **5 :** 1 - 35.

Komárek, J. and K. Anagnostidis. 1989 Modern approach to the classification system of cyanophytes. 4 Nostocales. *Archiv für Hydrobiologie. Algological Studies.* **56 :** 247-345.

Komárek, J. and K. Anagnostidis. 2005 *Cyanoprokaryota*. 2. *Oscillatoriales*. – In: Büdel B., Krienitz L., Gärtner G. & Schagerl M. (eds), Süsswasserflora von Mitteleuropa 19/2, p. 759, Elsevier/Spektrum, Heidelberg.

Lowry, O. H., Rosebrough, L. Farr and R. L. Randall. 1951 Protein measurement with Folin-phenol reagent*. J. Biol. Chem.* **193:** 265-275.

Mayer, A.M., and A. Poljakoff-Mayber. 1989 The Germination of Seeds. 4th ed. Oxford: Pergamon Press.

Mayur Gahlout, Hiren Prajapati, Poonam Chauahan, Rathod Himita kumari and Juhikapatel. 2017 Isolation, Identification and Evaluation of Seed Germination Efficiency of Cyanobacterial Isolates.*International Journal of Research and Scientific Innovation* (IJRSI). **4**(4): 64-68

Nagarajan, M., V. Maruthanayagam and M.A. Sundararaman. 2012 A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites. *J Appl Toxicol.* **32**: 153–185.

Osman, M.E.H., A.M. Abo-Shady and M.M.F. El-Nagar. 2016 Cyanobacterial *Arthrospira* (*Spirulina platensis*) as safener against harmful effects of fusilade herbicide on faba bean plant. *Rend Fis Acc. Lincei*. **27**: 455–462

Pankratova, E.M. 2006. Functioning of cyanobacteria in soil ecosystems. *Eurasian Soil Sci.* **39**: 118-127.

Pitchai Palaniappan., P. Malliga, K. Manian, S. Sivaramakrishnan, S. Madhaiyan and S.A. Tongmin. 2010 Plant growth promontory effect on cow pea (*Vigna unguiculata*) using coir pith aqueous extract formulation of *Cyanobacterium Phormidium*. *Am.-Eur. J. Agric. Environ.* **8(**2): 178-184.

Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman and R.Y. Stanier. 1979 Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol*. **111**: 1 - 61.

Salisbury, F.B. and C.W. Ross. 1991 Plant Physiology. 4th ed. Belmont, California, USA: Wadsworth Publishing Company.

Shutov, A.D., and I.A. Vaintraub. 1987 Degradation of storage proteins in germinating seeds. *Phytochemistry.* **26**:1557-1566.

Singh, J.S., A. Kumar, A.N. Rai and D.P. Singh. 2016 Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front Microbiol.* **7**: 529.

Thajuddin, N. and G. Subramanian. 2005 Cyanobacterial biodiversity and potential applications in biotechnology. *Current Science.* **89**: 47–57.

Tripathi, S.N., I.K. Chung and J.A. Lee. 2007 Diversity and characteristics of terrestrial cyanobacteria near Gimhae city. *Korea. J. Plant Biol.* **50**: 50-59

UN DESA. 2015 'World Populations Prospects: The 2015 Revision' reported by United Nations, Department of Economics and Social Affairs (UN - DESA).

Whitton, B.A. 2012 Ecology of Cyanobacteria II: Their Diversity in Space and Time. Dordrecht, New York, London: Springer.

Yemm, E. W. and A. J. Willis. 1954 The estimation of carbohydrates in plant extracts by Anthrone. *J. Biol. Chem*. **57:** 508–514.