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ISOLATION AND IDENTIFICATION OF CYANOBACTERIA AND ITS IMPACT ON SEED GERMINATION POTENTIAL OF MAIZE (*Zea mays* L.) USING SEED GERMINATION EXPERIMENT

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***Abstract*— In this present study, 11 different cyanobacterial cultures were isolated and identified from Galba River of Wolaita Sodo Town, Wolaita region, Ethiopia and identified based on the morphometric characters under microscopic examinations. Among these 11 cyanobacterial isolates 10 isolates were identified as non-heterocystous and one isolate was identified as heterocystous cyanobacteria. The identified non-heterocystous cyanobacterial isolates were *Pseudanabaena* sp. WSU1, *Phormidium* sp. WSU2, *Geitlerinema* sp. WSU3, *Arthrospira* sp. WSU4, *Oscillatoria* sp. WSU5, *Phormidium* sp. WSU6, *Lyngbya* sp. WSU7, *Gloeocapsa* sp. WSU8, *Oscillatoria* sp. WSU9, *Spirulina* sp. WSU10 and the identified heterocystous cyanobacterial isolate was *Calothrix* sp. WSU11. Formulated Aqueous extracts of all these 11 cyanobacterial isolates at three different concentrations such as 1%, 2% and 3% were used for seed germination experiment using *Zea mays* L. (maize) seeds. The non-heterocystous cyanobacterial isolates *Pseudanabaena* sp. WSU1 at 1%, *Phormidium* sp. WSU2 at 2%, *Geitlerinema* sp. WSU3 at 2%, *Lyngbya* sp. WSU7 at 3%, *Spirulina* sp. WSU10 at 2% concentration and heterocystous cyanobacterial isolates *Calothrix* sp. WSU11 at 3% concentration showed significantly higher results in all the morphological and biochemical parameters of *Zea mays* L. (maize) when compared to control. The present study results indicated that the application cyanobacterial aqueous extracts influenced the metabolism of reserve food and promoted the seed germination effectively.**

Keywords— **Cyanobacteria, Aqueous extract, germination, maize**

I. INTRODUCTION

Ethiopia is one of the most populated country in Africa with a population of 102.4 million UNICEF (2017). Even though Ethiopia has faster growing economy in the African continent, it still remains poorest World Bank (2017b). The agriculture sector contributing more towards to the poverty reduction. Agriculture sector is the mainstay of the Ethiopian's economy and therefore this sector regulates the growth of all the other sectors and subsequently the whole national economy of Ethiopia. Crop production alone taking 60% of the agriculture sector's total output whereas livestock 27% and others taking 13%. While the agriculture sector is an important part of the Ethiopian economy, particularly in rural zones where 55 percent of the women and 83 percent men are working in agriculture sector especially involved in crop cultivations CSA and ICF (2016), World Bank (2017b). This agriculture sector is dominated by small-scale growers engaging old-style technology, adopting a low input and low output production system. This low input and low output concept were followed by farmers usually due to the high cost of chemical fertilizers, low availability and side effects of chemical fertilizers in the soil as well as to the crops. So, the requirement of biofertilizer as an alternative to chemical fertilizer is very urgent to improve the productivity of crops in agriculture sector. Biofertilizers are microorganisms applied as live form to enhance the crop productivity and improve the soil health. Different kinds of microorganisms like bacteria, fungi, and algae can be used for the production of biofertilizers (Smith and Read (2008), Lucy et al. (2004), Vessey (2003). Among these various kinds of microorganisms, cyanobacteria placed in a first place.

Cyanobacteria or blue green algae are free living, oxygenic, photosynthetic and nitrogen fixing gram negative prokaryotic microorganisms. Cyanobacteria can produce a different kind

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of compounds and products useful to human being. Application these cyanobacteria influences the plant disease resistant, growth and development. Cyanobacteria can be used as biofertilizer in free living nitrogen fixing form as well as symbiotic association form to cultivate several crops worldwide. In addition, application of these cyanobacterial fertilizers is also used to recover the soil fertility, particularly for the retrieval of alkaline soils making them appropriate for the crop cultivation. Cyanobacteria also known to produce various kinds of plant growth promoting substances like cytokinin, gibberellin, auxin and abscisic acid, vitamins, polypeptides and exopolysaccharides which acts as antifungal and antibacterial properties which give disease resistant to the crops. Cyanobacteria can also mobilize the insoluble inorganic phosphate and influences the plant growth. Cyanobacteria have a selective property to survive in a variety of agronomic and ecological conditions Rai et al. (2019), Manu Arora et al. (2010), Karthikeyan et al. (2007).

Maize (*Zea mays* L.) is one of the important cereal crops cultivated worldwide and maize act as a staple food crop especially in developing countries like Ethiopia Kandil (2013). In the world cereal production, corn is placed as the 3rd major cereal crop after rice and wheat Zamir et al. (2013). White maize is one the major food crop in Ethiopia after tef (*Eragrostis tef).* Ethiopia ranked fifth largest producer of maize in Africa and 94% of the crop production is covered by smallholder farmers Mitiku Woldesenbet and Asnakech Haileyesus (2016). All the farmers are depending on chemical fertilizers for maize cultivation. Due to continuous usage and over dosages of chemical fertilizers adversely affect the soil properties which declining the yield directly. So many studies outside Ethiopia have been studied related to plant growth promoting efficiency of cyanobacterial cultures using different crops but very rare in Ethiopia. Therefore, the present study has been majorly focussed on cyanobacterial isolation and identification to evaluate its impact on seed germination potential of maize (*Zea mays* L.) using seed germination experiment.

II. MATERIALS AND METHODS

A. Sample source and sample collection–

Soil and Water samples were randomly collected from Galba river of Wolaita Sodo Town Southern Ethiopia from the month of December 2017 to January 2018. Samples of visually exposed cyanobacterial growth on underwater plus exposed soils, aquatic plants and pebbles were collected in polythene bags containing native water and brought to the Post Graduate Microbiology Laboratory, Department of Biology, Wolaita Sodo University, Wolaita Sodo, Ethiopia for further process. All the samples were processed within 48 h of their collection.

B. Isolation of cyanobacteria–

BG-11 medium was used for isolation of cyanobacteria. 1 g of the soil / cyanobacterial samples will be transferred to sterile 100 ml of BG-11 medium (with nitrate source for non-nitrogen fixing and nitrate-free for nitrogen fixing cyanobacteria) in 250 ml conical flasks. All samples were incubated in growth chamber at 25±2ºC with illumination of 1500lux by cool white 40W fluorescent tubes. The flasks were regularly monitored for the cyanobacterial growth. After 10-15 days of incubation, when visible algal growth appeared 2-3 wet mounts from each flask will be prepared and observed microscopically using binocular research OLYMPUS MICROSCOPE Model CX21FS1. After the microscopic observation, it was streaked on BG-11 agar media with nitrogen source for non heterocystous forms and without nitrogen source for heterocystous forms. Inoculated plates were incubated in the culture room which was maintained at 25±2°C illuminated with cool white 40W fluorescent tubes at an irradiance of 1500lux with 16/8hrs light and dark cycle. The culture rack was fitted with photoperiodic automatic model timer coupled with room temperature controller to provide alternative light and dark phases. The plates were observed regularly for cyanobacterial growth and isolated filaments of cyanobacteria. Isolation of the cyanobacterial strains was done by randomly picking different types of colonies developed on the BG11 agar media and was examined under binocular research microscope. If growth of heterotrophic bacteria or any other mixed culture was observed under any of the condition after incubation, cyanobacterial colonies were continuously cultured onto fresh BG11 agar medium until uni-cyanobacterial cultures obtained. The entire process was performed aseptically Rippka et al. (1979), Allen and Stanier (1967), Castenholz (1992), Krishna Moorthy et al. (2019). After the isolation of pure algal cultures, the identification was done by the following methods

C. 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, presence of aikinites 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), Komárek and Hauer (2013) and Khare et al. (2014), Krishna Moorthy et al. (2019).

D. Maintenance of isolated cyanobacterial cultures–

Most widely used method for laboratory maintenance of cultures is by storing them in agar slants. This was done by inoculating pure cultures into a nutrient agar medium which solidified in sterile tubes. All the unialgal strains were maintained at a temperature of 19±1ºC under 1500lux of cool white 40W fluorescent tubes. All the strains were maintained in BG-11 agar slants and broth medium. The isolated cyanobacterial cultures were sequentially assigned with reference numbers having its own uniqueness and deposited to fresh water cyanobacterial and microalgae repository of

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Biology Department, Wolaita Sodo University. The isolated and identified cyanobacterial cultures maintained in repository will be sub cultured for every 3-4 months depending on the culture conditions Krishna Moorthy et al. (2019).

E. 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 culture room Rippka et al. (1979). The mass cultured cyanobacterial isolated were harvested after 20-25days of incubation and used for the preparation of aqueous extract Krishna Moorthy et al. (2019).

F. Seed germination experiment using plate method–

The Maize seeds (*Zea mays* L.) were collected from local market. Seeds were surface sterilized with 70% ethanol or 0.1 % HgCl2 for 3 min. Ten viable seeds in each plat was tested for each cyanobacterial aqueous extract (non-nitrogen fixers and nitrogen fixers). Seeds, without algal extract were served as control. Each Petri dish contain ten surface sterilized seeds was placed on 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 as well the biochemical parameters such as carbohydrate and protein content were also recorded on the 2 days interval up to 8 days after incubating seed at 28^{0}C Krishna Moorthy et al. (2019), Pitchai et al. (2010).

G. Statistical Analysis–

The data related morphological and metabolic parameters were exposed to one-way analysis of variance (ANOVA) technique (Origin pro software package 8.0) and mean separations were adjusted by the Multiple Comparison test. Mean were compared by using Fisher's LSD test at $p<0.05$ level of significance. All the data included in the results were presented in mean and standard error (±) of mean of three replicates per treatment and repeated three times.

III. RESULTS AND DISCUSSIONS

The present research was mainly focused on the collection of cyanobacterial samples, isolation and purification of different cyanobacterial species, identification of cyanobacteria based on the morphological characteristic features, cultivation of isolated cyanobacterial cultures under laboratory conditions and its impact on seed germination potential of maize (*Zea mays* L.) crops, Wolaita Sodo, Southern Region of Ethiopia from December, 2017 to May, 2018. The results are as follows.

A. Isolation and identification of cyanobacteria

Isolation of cyanobacteria from soil, aquatic and cyanobacterial samples of Galba River was the first study conducted in study area of Wolaita Sodo Town, Southern Ethiopia. From all the collected samples, total of 11 cyanobacterial species were isolated and identified based on the morphometric characteristic's features using microscope (Table 1). Filamentous and non-heterocystous group of cyanobacteria are predominant and heterocystous were least. Among 11 isolated cyanobacteria species, 10 non-heterocystous cyanobacterial isolates were identified and named as *Pseudanabaena* sp. WSU1, *Phormidium* sp. WSU2, *Geitlerinema* sp. WSU3, *Arthrospira* sp. WSU4, *Oscillatoria* sp. WSU5, *Phormidium* sp. WSU6, *Lyngbya* sp. WSU7, *Gloeocapsa* sp. WSU8, *Oscillatoria* sp. WSU9, *Spirulina* sp. WSU10 and only one was identified as heterocystous cyanobacterium *Calothrix* sp. WSU11. The cyanobacterial identification process of current study is similar to the study conducted by Desikachary (1959), Gomont (1982), Komárek and Anagnosti-dis (1998 and 2005). Similarly, Stanier et al. (1978), Castenholz and Waterbury (1989) used morphological, physiological and ecological characteristics for the cyanobacterial identifications. The heterocystous and non-heterocystous cyanobacterial isolates were identified based on the morphological characters using microscope in 10x, 40x magnifications by Mayur Gahlout (2017), Krishna Moorthy et al. (2019).

Isolate No.	Morphometric characters	Identified cyanobacteria
WSU1	Solitary trichomes, no sheathes, occasional motile, less than 30 cells, lack of heterocyst, apical cells are not attenuated, cells typically cylindrical with cross walls, 1–3.5 µm wide, end cells are rounded	*Pseudanabaena* sp. WSU1
WSU2	Bright blue-green trichomes attenuated at the ends, hooked. Cells shorter than wide, 3.9-7.1 µm wide, 1.3-3.8 µm long, no cross walls, granular cell content. Conical apical cells without calyptra.	*Phormidium* sp. WSU2
WSU3	Straight filaments with rarely curved, not constricted septum, cells longer than wide (3.8 - 8.2 × 1.6 -2.4 µm). One or two beads in the septum; apical cell is cylindrical with rounded apex. Severe gliding motility with waving and rotation.	*Geitlerinema* sp. WSU3
WSU4	Non motile solitary filaments with coiled structure, 22 µm in distance between the coils, no sheaths. Cells are longer than wide (1.7 µm in width), presence of granules, absence of aerotopes, rounded apical cells.	*Arthrospira* sp. WSU4
WSU5	Unbranched, straight and long filaments without sheath, lack of akinetes and heterocyst. Cells are shorter than wide and arranged in a vertical row with conical apical cell. Filaments are motile with gliding movement.	*Oscillatoria* sp. WSU5
WSU6	Filaments are solitary, flexuous, curved with sheath, highly motile, attenuated apex. Cells wider than long (2.6 - 3.7 × 5.5 - 7.0 µm), presence of granules; rounded apical cell with calyptra.	*Phormidium* sp. WSU6
WSU7	Filaments are blue green, unbranched, un-tapered with cells shorter than wide, lack of heterocyst, presence of mucilaginous sheath and presence hormogonia. These hormogonia released to develop into a new filament.	*Lyngbya* sp. WSU7
WSU8	Singles cells but mostly arranged in pairs. Spherical, hemispherical, oval to slightly elongated cells are found with granular content, presence of sheath with blue to violet.	*Gloeocapsa* sp. WSU8
WSU9	Straight un branched long filaments without mucous sheath, lack of heterocyst, equal diameter throughout whole length. Vertical cell arrangement with conical apical cells. Highly motile with gliding movement.	*Oscillatoria* sp. WSU9
WSU10	Coiled motile filament. Individual trichomes comprised of single cells that spiral down its entire length.	*Spirulina* sp. WSU10
WSU11	Calothrix was identified by its tapering trichomes with terminal heterocyst. The lower part of the trichome was surrounded by colorless sheath. Unbranched filaments with 12µm wide, up to 120 µm long, cells are shorter than wide, Trichome are straight sometimes bent, with a distinct hair at the end.	*Calothrix* sp. WSU11

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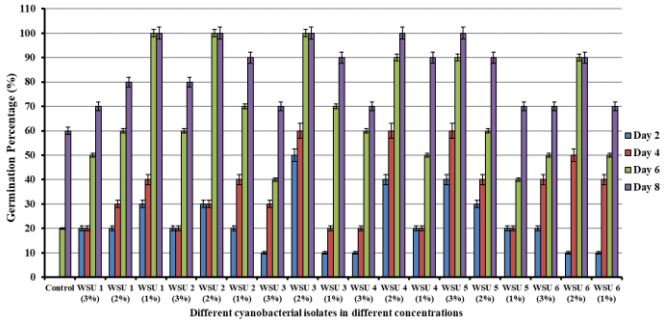
Table. 1. Morphometric characteristics features of cyanobacterial isolates under microscope (40x and 100x oil immersion)

B. Impact cyanobacterial isolates on morphological parameters

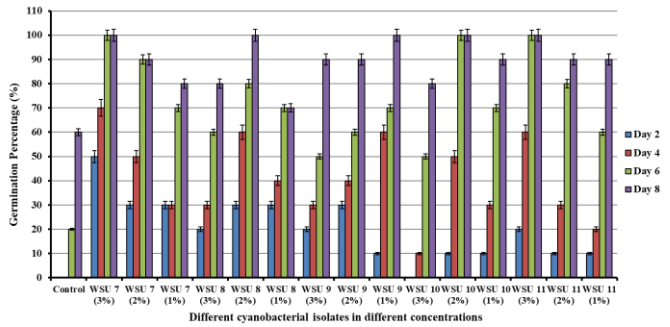
The impact in the percentage of seed germination of *Zea mays* L. seed by different concentrations of aqueous extracts of all the cyanobacterial isolates are showed in the Fig. 1a and Fig.1b. The results in the Fig. 1a and Fig.1b. shows that the seed germination percentage increased progressively throughout the period in all plates inoculated with different cyanobacterial isolates except control. The plates inoculated with cyanobacterial isolates *Pseudanabaena* sp. WSU1, *Phormidium* sp. WSU2, *Geitlerinema* sp. WSU3, *Lyngbya* sp. WSU7, *Spirulina* sp. WSU10 and *Calothrix* sp. WSU11 (Heterocystous) reached maximum of 100% in the 6th day of incubation at respective concentrations of 1%, 2%, 2%, 3%, 2%, and 3% of aqueous extracts. The cyanobacterial isolates *Arthrospira* sp. WSU4, *Oscillatoria* sp. WSU5, *Gloeocapsa* sp. WSU8 and *Oscillatoria* sp. WSU9 reached 100% level of seed germination only in the 8th day of incubation while the cyanobacterial isolate *Phormidium* sp. WSU6 showed maximum of 90% level of seed germination even in the 8th day.



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU1 – *Pseudanabaena* sp. WSU1; WSU2 – *Phormidium* sp. WSU2; WSU3 – *Geitlerinema* sp. WSU3; WSU4 – *Arthrospira* sp. WSU4; WSU5 – *Oscillatoria* sp. WSU5; WSU6 – *Phormidium* sp. WSU6; WSU – Wolaita Sodo University

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



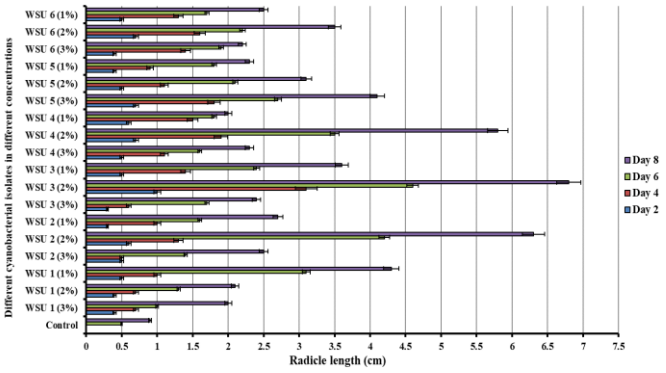
Data are the means of three replicates and Error bars represent the standard errors of the means

WSU7 – *Lyngbya* sp. WSU7; WSU8 – *Gloeocapsa* sp. WSU8; WSU9 – *Oscillatoria* sp. WSU9; WSU10 – *Spirulina* sp. WSU10; WSU11 – *Calothrix* sp. WSU11; WSU – Wolaita Sodo University

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

The percentage of seed germination in control reached maximum of 60% even in the 8th day of incubation while all the cyanobacterial isolates in all concentrations showed significantly higher level of seed germination percentage than control treatment. The cause for this great response is naturally cyanobacteria can release the plant growth regulators (PGR) like cytokinin, auxins and gibberellins. This PGR directly involved in the seed germination and increased the percentage of seed germination. This result was highly supported by Osman *et al.* (2010), who reported that the cyanobacteria play a main role in the in the seed germination by releasing phytohormones like gibberellins, auxins and cytokinin. Similarly, the different concentrations (1% - 10%) of cyanobacterial extracts encouraged the earlier germination of *Pisum sativum* L. seeds than control which treated with only distilled water Gayathri et al. (2017). Similar to the present study results was obtained by Mayur et al. (2017) who reported that the application of cyanobacterial cultures such as *Gloeocapsa* spp., *Oscillatoria* spp., *Aphanocapsa* spp., *Closterium* spp., *Rivularia* spp., *Nostoc* spp., and *Anabaena* spp., showed great impact on the germination status of wheat as well as mung.

The changes in the radicle length are showed in the Fig. 2a and Fig.2b. The highest radicle length (7.1± 0.437cm) was observed in the petriplates inoculated with 3% concentration of heterocystous cyanobacterial isolates *Calothrix* sp. WSU11 and the lowest radicle length (0.9± 0.212cm) was observed in the control plates inoculated with distilled water on 8th day.



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU1 – *Pseudanabaena* sp. WSU1; WSU2 – *Phormidium* sp. WSU2; WSU3 – *Geitlerinema* sp. WSU3; WSU4 – *Arthrospira* sp. WSU4; WSU5 – *Oscillatoria* sp. WSU5; WSU6 – *Phormidium* sp. WSU6; WSU – Wolaita Sodo University

Fig. 2a. Effect of aqueous extract of cyanobacterial isolates on radicle length of *Zea mays* L. under seed germination experiment (8th day)

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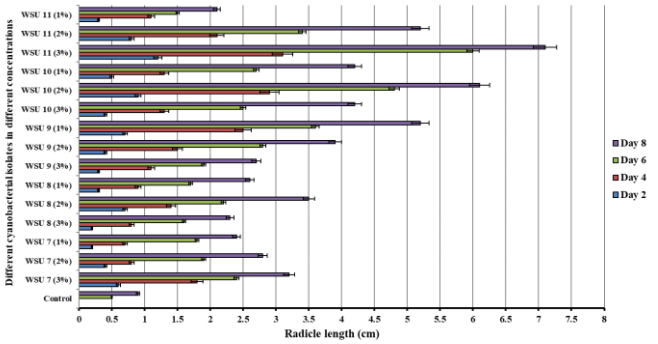


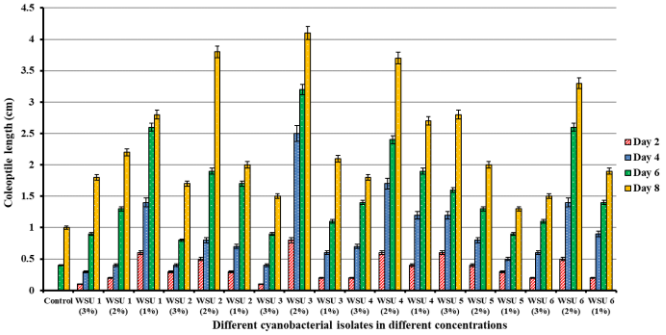
Data are the means of three replicates and Error bars represent the standard errors of the means

WSU7 – *Lyngbya* sp. WSU7; WSU8 – *Gloeocapsa* sp. WSU8; WSU9 – *Oscillatoria* sp. WSU9; WSU10 – *Spirulina* sp. WSU10; WSU11 – *Calothrix* sp. WSU11; WSU – Wolaita Sodo University

Fig. 2b. Effect of aqueous extract of cyanobacterial isolates on radicle length of *Zea mays* L. under seed germination experiment (8th day)

The radicle length of 6.8± 0.237cm was observed in the plates inoculated with *Geitlerinema* sp. WSU3 non-heterocystous cyanobacterial isolate at 2% concentration followed by *Phormidium* sp. WSU2 at 2%, *Spirulina* sp. WSU10 at 2%, *Arthrospira* sp. WSU4 at 2, *Oscillatoria* sp. WSU9 at 1%, *Pseudanabaena* sp. WSU1 at 1%, *Oscillatoria* sp. WSU5 at 3%, *Gloeocapsa* sp. WSU8 at 2%, *Phormidium* sp. WSU6 at 2%, and *Lyngbya* sp. WSU7 at 3% concentration with respective radicle length of 6.3±0.157cm, 6.1±0.411cm, 5.8±0.137cm, 5.2± 0.200cm, 4.3±0.421cm, 4.1±0.331m, 3.5± 0.437cm, 3.5±0.202cm and 3.2± 0.137cm (Fig. 2a and Fig.2b). The treatment with heterocystous cyanobacterial isolates WSU11 with 3% level of concentration showed significantly higher in radicle length than control and all other cyanobacterial treatment with all other concentrations on 8th day of incubation. The changes in the radicle length of maize seed was stimulated by the presence of phytohormones in the aqueous extracts of cyanobacterial isolates. This result was supported by several other similar studies done by Osman et al. (2010) reported that several species of cyanobacteria play serious role in the germination process by releasing bioactive elements such as auxins, gibberellins, cytokinin, vitamins, amino acids, and peptides etc. Similarly, the study done by Krishna Moorthy et al. (2019) who reported that the three cyanobacterial isolates such as *Pseudanabaena* spp. AK-1, *Lyngbya* spp.AK-2 and *Geitlerinema* spp. AK-3 showed best results in case of radicle length, coleoptile length and epicotyl length of rend beans when compared control seeds treated with only distilled water.

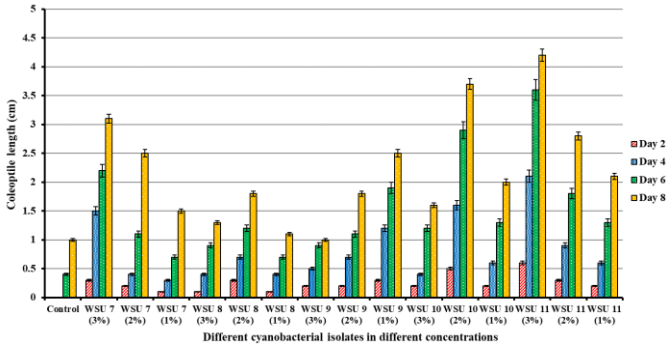
The changes in the percentage of coleoptile length of *Zea mays* L. seed by different concentrations of aqueous extracts of all the cyanobacterial isolates are showed in the Fig. 3a and Fig.3b. The very least coleoptile length (1± 0.437cm cm) of *Z. mays* L. seed was observed in the control when compared to all other treatments with all concentrations even at 2nd, 4th, 6th and 8th day of incubation. The coleoptile length (4.2± 0.135cm) of *Z. mays* L. seed treated with aqueous extract of heterocystous cyanobacterial isolate *Calothrix* sp. WSU11 at 3% concentration showed significantly higher result when compared to coleoptile length (1.0± 0.437cm) of control and all other non-heterocystous cyanobacterial isolates *Pseudanabaena* sp. WSU1, *Phormidium* sp. WSU2, *Arthrospira* sp. WSU4, *Oscillatoria* sp. WSU5, *Phormidium* sp. WSU6, *Lyngbya* sp. WSU7, *Gloeocapsa* sp. WSU8, *Oscillatoria* sp. WSU9 and *Spirulina* sp. WSU10 in all concentrations on 8th day with respective values of 2.8±0.037cm, 3.8 ±0.421cm, 3.7 ± 0.207cm, 2.8 ± 0.175cm, 3.3 ± 0.227cm, 3.1± 0.332cm, 1.8 ± 0.234cm, 2.5±0.421cm and 3.7±0.230cm (Fig. 3a and Fig.3b.). Current study result was similar to the study done by Gayathri et al. (2017) who reported that the extracts of *Scytonema bohneri* (80%), *Dolichospermum spiroides* (70%), *Aphanothece stagnina* (66.6%), *Calothrix* sp. MBDU 901 (66.6%) and *Nostoc microscopicum* (56.6%) showed higher response in case of seed germination, radicle and plumule length than control (53.3%). Similarly, the applications of cyanobacterial cultures *Pseudanabaena* spp. AK-1, *Lyngbya* spp. AK-2 and *Geitlerinema* spp. AK-3 in different concentrations (1%, 2%, 3%) showed significant impact on the coleoptile length of *Phaseolus vulgaris* L. seeds Krishna Moorthy et al. (2019).



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU1 – *Pseudanabaena* sp. WSU1; WSU2 – *Phormidium* sp. WSU2; WSU3 – *Geitlerinema* sp. WSU3; WSU4 – *Arthrospira* sp. WSU4; WSU5 – *Oscillatoria* sp. WSU5; WSU6 – *Phormidium* sp. WSU6; WSU – Wolaita Sodo University

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



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU7 – *Lyngbya* sp. WSU7; WSU8 – *Gloeocapsa* sp. WSU8; WSU9 – *Oscillatoria* sp. WSU9; WSU10 – *Spirulina* sp. WSU10; WSU11 – *Calothrix* sp. WSU11; WSU – Wolaita Sodo University

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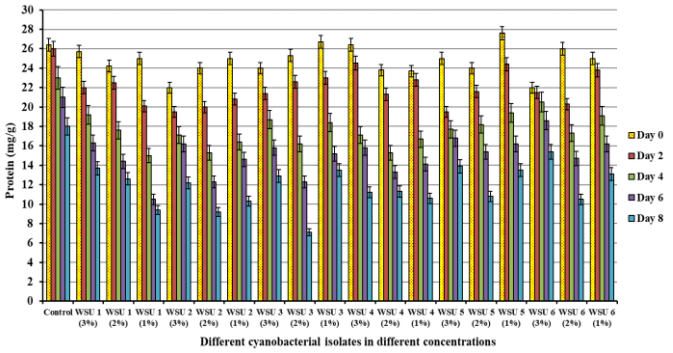
Fig. 3b. Effect of aqueous extract of cyanobacterial isolates on coleoptile length of *Zea mays* L. under seed germination experiment (8th day)

C. Impact of cyanobacterial isolates on the biochemical parameters

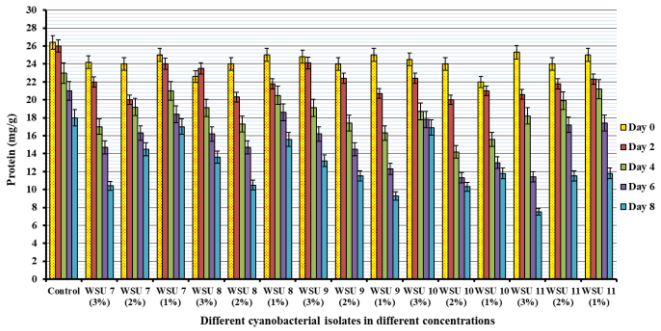
Protein is one of the reserve foods in the *Z. mays* L. seeds. All the stored forms of reserved foods are hydrolysed during the germination process. Hence, the study about protein changes in the seed germination experiments is more important. Here in this present research, protein changes in seeds from all the experiments were analysed properly and presented in the forms of Fig. 4a and Fig.4b. The protein contents of *Z. mays* L. seeds in the control treatment was not decreased much from 0th day to 8th day. The maximum level of protein reduction was observed in the seeds treated with *Geitlerinema* sp. WSU3 at 2% level of concentration followed by *Calothrix* sp. WSU11 at 3%, *Phormidium* sp. WSU2 at 2%, *Oscillatoria* sp. WSU9 at 1%, and *Pseudanabaena* sp. WSU1 at 1% which was significantly higher than control and all the cyanobacterial aqueous extracts (Fig. 4a and Fig.4b).



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU1 – *Pseudanabaena* sp. WSU1; WSU2 – *Phormidium* sp. WSU2; WSU3 – *Geitlerinema* sp. WSU3; WSU4 – *Arthrospira* sp. WSU4; WSU5 – *Oscillatoria* sp. WSU5; WSU6 – *Phormidium* sp. WSU6; WSU – Wolaita Sodo University

4a. Effect of aqueous extract of cyanobacterial isolates on protein content of *Zea mays* L. under seed germination experiment (8th day)



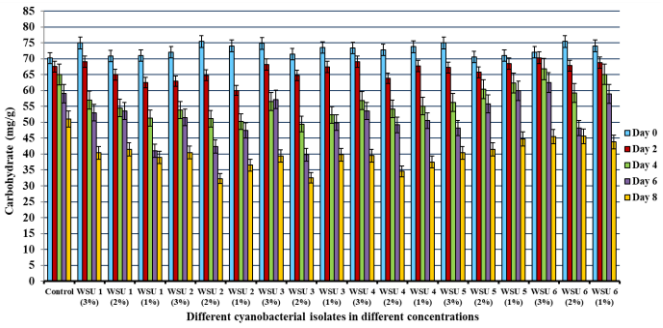
Data are the means of three replicates and Error bars represent the standard errors of the means

WSU7 – *Lyngbya* sp. WSU7; WSU8 – *Gloeocapsa* sp. WSU8; WSU9 – *Oscillatoria* sp. WSU9; WSU10 – *Spirulina* sp. WSU10; WSU11 – *Calothrix* sp. WSU11; WSU – Wolaita Sodo University

4b. Effect of aqueous extract of cyanobacterial isolates on protein content of *Zea mays* L. under seed germination experiment (8th day)

The changes in the carbohydrate content of control was 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 WSU3 (*Geitlerinema* sp. WSU3) and WSU2 (*Phormidium* sp. WSU2) at 2% level of concentrations which is significantly higher than control and all other treatments in all concentrations except WSU11 (*Calothrix* sp. WSU11) at 3% and WSU2 (*Phormidium* sp. WSU2) at 1% concentrations on 8th day of incubation (Fig. 5a and Fig. 5b). 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. The present study is supported by Salisbury and Ross (1991) who reported that the seed germination stimulated many metabolic activities such as secretion and activation of hydrolytic enzymes which caused in breaking down of stored carbohydrates, proteins and other stored materials into available simple form for embryo development.

Similar to the present study results, the research done by Krishna Moorthy *et al.* (2019) showed 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



Data are the means of three replicates and Error bars represent the standard errors of the means

WSU1 – *Pseudanabaena* sp. WSU1; WSU2 – *Phormidium* sp. WSU2; WSU3 – *Geitlerinema* sp. WSU3; WSU4 – *Arthrospira* sp. WSU4; WSU5 – *Oscillatoria* sp. WSU5; WSU6 – *Phormidium* sp. WSU6; WSU – Wolaita Sodo University

Fig. 5a. Effect of aqueous extract of cyanobacterial isolates on carbohydrate content of *Zea mays* L. under seed germination experiment (8th day)

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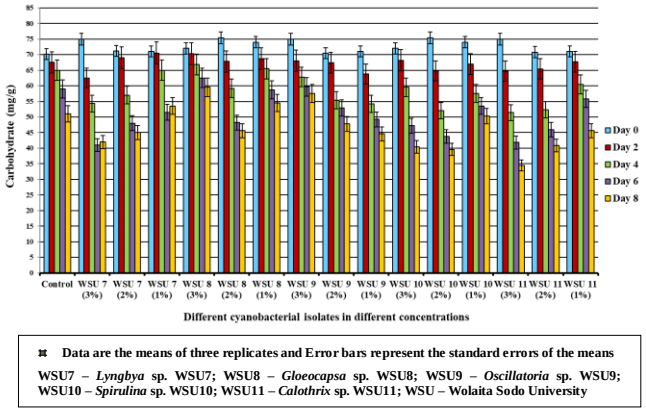

Fig. 5b. Effect of aqueous extract of cyanobacterial isolates on carbohydrate content of *Zea may*s L. under seed germination experiment (8th day)

IV. CONCLUSION

Based on the above said results, the non-heterocystous cyanobacteria are more dominant than heterocystous cyanobacteria in the Galba river of Wolaita Sodo Town Southern Ethiopia. The non-heterocystous cyanobacterial isolates *Geitlerinema* sp. WSU3 at 2% concentration and heterocystous cyanobacterial isolates *Calothrix* sp. WSU11 at 3% concentration showed immediate response and reached 100% of germination, faster development in the radicle and coleoptile length even on the 6th day of incubation. Similarly, the stored food materials such as protein and carbohydrate content were hydrolyzed maximum in the seeds treated by non-heterocystous cyanobacterial isolate *Geitlerinema* sp. WSU3 at 2% concentration and heterocystous cyanobacterial isolates *Calothrix* sp. WSU11 at 3% concentration. Therefore, the present study has been concluded that the application of non-heterocystous cyanobacterium *Geitlerinema* sp. WSU3 at 2% concentration and heterocystous cyanobacterial isolates *Calothrix* sp. WSU11 at 3% concentration can be used as effective liquid fertilizers for the pretreatment of *Zea may*s L. seed.

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