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EFFECT OF HETEROCYSTOUS CYANOBACTERIUM *Calothrix parietina* WSU11 ON SEED GERMINATION POTENTIAL USING *Hordeum vulgare* L. (BARLEY) AS AN EXPERIMENTAL CROP

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ABSTRACT

In this present study, a fresh water heterocystous cyanobacterium *Calothrix parietina* WSU11 was obtained from the department of Biology, Wolaita Sodo University, Wolaita Sodo, Ethiopia. The purity of the obtained cyanobacterium was analyzed by streaking plate and microscopically. The purified cyanobacterium *C. parietina*WSU11 was further cultured at mass level under laboratory conditions. The mass cultured *C.parietina*WSU11 was harvested and prepared different concentrations of aqueous extracts like 1%, 2%, 3%, 4% and 5% to determine the seed germinating potential using Barley (*Hordeum vulgare* L.) seeds underseed germination experiments by plat method. All the concentrations of aqueous extracts of *C.parietina* WSU11 showed significantly higher results in seed germination percentage, radical length, coleoptile length and epicotyl length when compared to control. Similarly, the carbohydrates and protein content were highly reduced in the seeds treated with heterocystouscyanobacterial isolates *C.parietina* WSU11at 5% concentration which indicated that this cyanobacterial culture extracts influence the metabolism of reserve food for the germination process. Based on the above said results, the cyanobacterial culture *C. parietina* WSU11at 5% concentration can be used to influence the seed germination capacity of barley seeds.

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INTRODUCTION

Agriculture sector is the backbone of the Ethiopian economy and therefore this sector determines the growth of all the other sectors and, consequently, the whole national economy of Ethiopia. On average, crop production makes up 60% of the sector's outputs whereas livestock accounts for 27% and other areas contribute 13% of the total agricultural value added. The sector is dominated by small-scale farmers who practice rain-fed mixed farming by employing traditional technology, adopting a low input and low output production system. This low input and low output concept was 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 (Lucy *et al.,* 2004; Vessey, 2003).

The excessive use of chemical fertilizers has generated several environmental problems including the greenhouse effect, ozone layer depletion and acidification of water (Eshetu Gebre and Alemu Lelago, 2017). Cyanobacteria or "blue-green algae" are prokaryotes, which mainly receive their nutrients through photosynthetic processes. They are highly adaptable to the environment and can be found in soil, rocks, and most water bodies, ranging from hot springs to the cold water of Antarctic lakes and low-nutrient freshwater environments. As part of the aquatic environment ecology, cyanobacteria play an important role in the ecosystem maintenance. Photosynthesis of bacteria provides oxygen, while nitrogen-fixing cyanobacteria provide atmospheric nitrogen for another organism (Beck *et al*., 2012). Cyanobacteria or blue green algae are one of the major modules of the potential sources of nitrogen fixation and convert it into a bioavailable ammonia form as well as plant growth regulators required for plant growth. These organisms have a unique potential to enhance productivity in a variety of agricultural and ecological situations and they play an important role in building up soil fertility, consequently increasing the yield (Parry and Hawkesford, 2010; Parry *et al.,* 2011). In the soil, the mineral nutrients are dissolved in water and absorbed through a plant's root. However, the amounts of nutrients in soil are always unpredictable and not enough for plants growth. Though most of the nitrogen contribution in the soils is through the *Rhizobium*-legume symbiosis, the traditional use of cyanobacterial biofertilizers makes the significant contributions to crop production. The cyanobacteria can contribute about 20–30 kg N/ha/season as well as organic matter to the soil which is quite significant for the economically weak farmers who are unable to invest on costly chemical nitrogen fertilizer (Issa *et al.,* 2014). An additional benefit of using cyanobacteria as biofertilizer is the ability to secrete bioactive substances such as auxin, gibberellins, cytokinins, vitamins, polypeptide, amino acid, which promote plant growth and development. They also improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen, available phosphorus, etc. The use of cyanobacterial as of biofertilizer is a viable alternative to improve soil quality and crop production. In addition, cyanobacteria as biofertilizer have the advantages of lower cost, reduced production of greenhouse gasses, such as oxides of nitrogen and carbon dioxide by 30%, which results in less pollution of the environment (Pisciotta *et al.,* 2010). Hence in the present study, an attempt has been made on the effect of fresh water heterocystous cyanobacterium *C. parietina* WSU11 on seed germination potential using *Hordeum vulgare* L. (Barley) as an experimental crop.

MATERIALS AND METHODS

Sample source and sample collection: A fresh water heterocystous cyanobacterial culture *Calothrix parietina* WSU11 was obtained from the germplasm collections of Department of Biology, College of Natural and computational Sciences, Wolaita Sodo University, Wolaita Sodo, Ethiopia.

Mass Cultivation of cyanobacteria under laboratory condition: The heterocyst forming cyanobacterium *Calothrix parietina* WSU11 was 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±2^{o}Ctemperature 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 by plate method: The Barley seeds were collected from local market. Seeds were surface sterilized with 70% ethanol or 0.1 % $HgCl_2$ for 3 min. 10 viable seeds in each plat was tested for each cyanobacterial aqueous extract. Seeds, without cyanobacterial 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 culture *Calothrix parietina* WSU11 in different concentrations like 1% (1gm/100ml), 2% (2gm/100ml), 3% (3gm/100ml), 4% (4gm/100ml) and 5% (5gm/100ml). Petri-dishes containing seeds with 10 ml of distilled water served as a control. The growth parameters including germination percentage, radicle length, coleoptile length and epicotyl length were recorded on the 2 days interval up to 8 days after incubating seed at 28^{0}C (Pitchai *et al.,* 2010; Krishna Moorthy *et al.*, 2019). The Biochemical parameters such as Carbohydrate (Yemm and Willis, 1954) and Protein (Lowry *et al.,* 1951) were also 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 the effect of heterocystous fresh water cyanobacterium *Calothrixparietina* WSU11 for the determination of plant growth promoting efficiency using *Hordeum vulgare* L. (Barley) as an experimental crop under seed germination experiment, Wolaita Sodo, SNNPR. The entire research work was carried out in the Post Graduate Research Laboratory, Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Wolaita Sodo, Ethiopia. The present research findings are written as follows.

Effect of *Calothrix parietinaWSU11*on seed germination experiment by plate method using *Hordeum vulgare*L. as experimental crop: In this experiment, aqueous extracts of the cyanobacterial culture *Calothrix parietina*WSU11 was prepared in different concentrations like 1%, 2%, 3%, 4% and 5%. 10ml of each concentration was inoculated into the petriplates containing 10 number of barely seeds and incubated under fluorescent light at room temperature. All the data related to seed germination experiment was collected at every 2 days interval up to 8th days. During this incubation periods, 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 was also quantified. All the results collected from seed germination experiments are as follows.

Morphological parameters

Changes in the Percentage of Seed germination: The changes in the percentage of seed germination of *Hordeum vulgare* L. seed by different concentrations of aqueous extracts of *Calothrix parietina*WSU11showed in the Figure1.The seed germination percentage increased progressively throughout the period in all plates inoculated with different concentrations of cyanobacterial culture extracts of *C.parietina*WSU11. All concentrations of aqueous extracts (1%, 2%, 3%, 4% and 5%) of *C. parietina*WSU11showed significantly higher level of seed germination percentage when compared to control (Only distilled water). The seeds treated with only distilled water showed only 70% of germination even on 8th day of incubation. The cyanobacterial aqueous extracts 3% and 4% concentration showed 100% of seed germination on 8th day of incubation while the other concentrations like 1% and 2%

showed only 90% percentage of seed germination. The aqueous extracts at 5% level of concentration showed 100% of seed germination even at 6th day of incubation. The best result in case of seed germination percentage was found to be in the concentration level of 5% aqueous extracts followed by 4%, 3%, 2% and 1% of aqueous extracts of cyanobacterium *C.parietina*WSU11 (Figure 1).

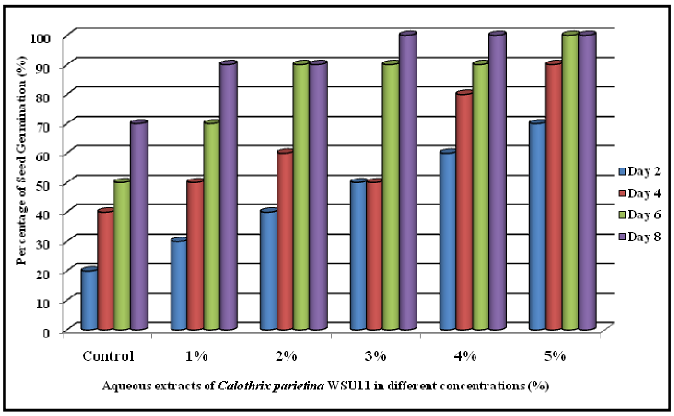

Figure 1. Effect of aqueous extract of *Calothrix parietina* WSU11 on percentage of seed germination of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

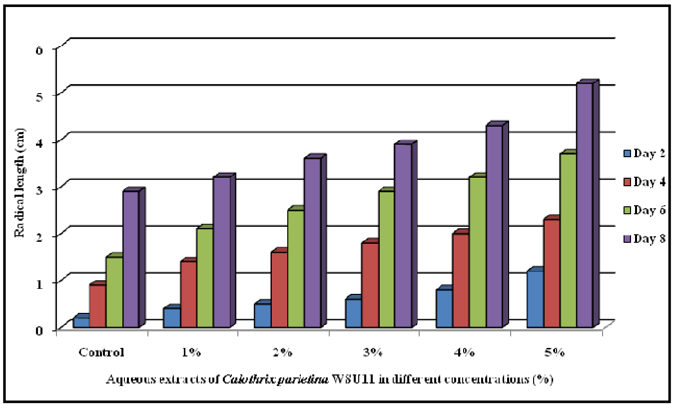

Figure 2. Effect of aqueous extract of *Calothrix parietina* WSU11 on radical length of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

The changes in the radicle length are showed in the figure 2.The cyanobacterial culture of *C.parietina*WSU11in all the five concentrations of aqueous extracts (1%, 2%, 3% 4% and 5%) showed significantly higher results when compared to control. The highest radicle length was observed in the plates inoculated with *C.parietina*WSU11at 5% concentration level followed by 4%, 3%, 2% and 1% concentration level of aqueous extracts. The very least level of radical length was observed in the plates treated by distilled water that is control. The cyanobacterial isolates *C.parietina*WSU11showed maximum length of coleoptile at 5% concentration of aqueous extracts when compared to *C.parietina*WSU11with rest of the other concentrations such as 4%, 3%, 2% and 1%. Similarly, the cyanobacterial isolate *C.parietina*WSU11showed higher results in coleoptile length at 4% concentration of aqueous extracts when compared to *C.parietina*WSU11at 3% concentration with rest of the other concentrations 2% and 1% (Figure 3). The Fig. 4 shows that the effects of cyanobacterium *C. parietina*WSU11at different concentrations on epicotyl length ofbarley seed under seed germination experiment. The control treatment showed very least in length of epicotyl (0.2±0.1cm) even on the last day (8th day) incubation. The cyanobacterial aqueous extracts at different concentrations such as 1%, 2%, 3%, 4% and 5% level of concentrations showed significantly higher results when compared to control in total period of incubation. The maximum results in case of epicotyl length (4.5±0.3) was observed in the plates treated with *C. parietina*WSU11at 5% level of concentrations which was significantly ($p<0.05$) different when compares control and all other cyanobacterial aqueous extracts concentrations (Figure 4).

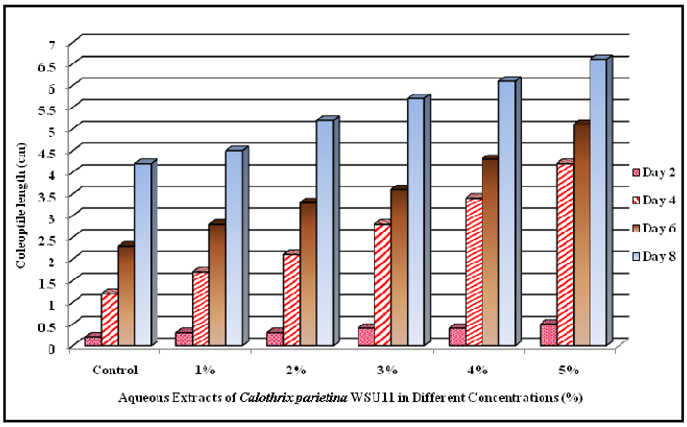

Figure 3. Effect of aqueous extract of *Calothrix parietina* WSU11 on coleoptile length of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

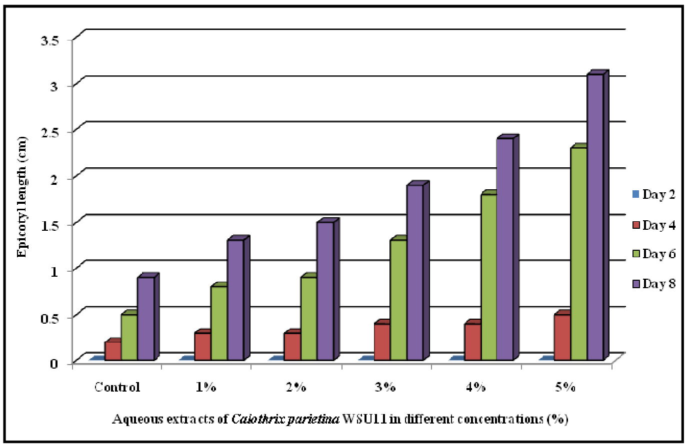

Figure 4. Effect of aqueous extract of *Calothrix parietina* WSU11 on epicotyl length of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

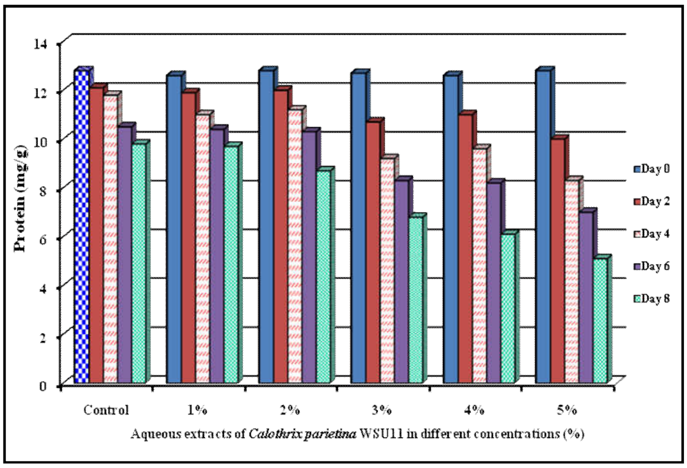

Figure 5. Effect of aqueous extract of *Calothrix parietina* WSU11 on protein content of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

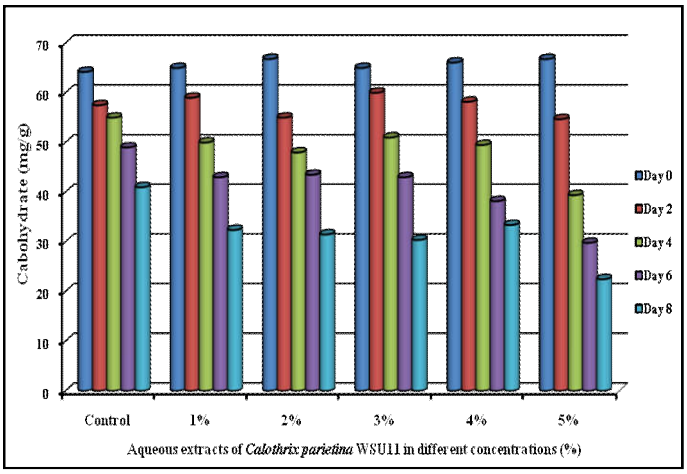

Figure 6. Effect of aqueous extract of *Calothrix parietina* WSU11 on carbohydrate content of *Hordeum vulgare* L. (Barley) under seed germination experiment (8th day)

Biochemical parameters: Protein is one of the reserve foods in the *Hordeum vulgare* L. All the stored forms of reserved foods are hydrolyzed during the germination process. Hence, the study about protein changes in the seed germination experiments is more important. Here in this present project, seeds from all the experiments were analyzed properly and presented in the forms of figures in this result section. Figure 5 shows clearly about the changes of protein content of all the treatments including control from 0th day to 8th day. The protein contents of *H. vulgare*L. seeds in the control treatment was not decreased in high level from 0th day to 8th day. The cyanobacterium *Calothrix parietinaWSU11*in five different concentrations (1%, 2%, 3%, 4% and 5%) showed significant changes in the protein content when compared to control. The maximum level of protein reduction was observed in the seeds treated with *C. parietinaWSU11*at 5% level of concentration when compared all other concentrations and control. The changes in the carbohydrate content of control were significantly ($p<0.05$) 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 *C. parietinaWSU11*at 5% level of concentrations which is significantly higher than control and all other concentrations (Figure 6).

DISCUSSION

Effect of *Calothrix parietina WSU11* on morphological parameters of *Hordeum vulgare* L. Seeds: At present study, the freshwater heterocyst cyanobacterium *Calothrix parietina* WSU11 was obtained from the department of Biology, Wolaita Sodo University and screened for the plant growth promoting efficiency on *Hordeum vulgare L.* using seed germination assay by plate method with five different concentrations of aqueous extracts such as 1%, 2%, 3% 4% and 5%. All the concentrations of cyanobacterial aqueous extracts showed significant response in the percentage (%) of seed germination in all different concentrations of aqueous extracts when compared to control. The 100% percentage of seed germination was observed in the 5% concentration level even at the 6th day incubation. The higher concentration of aqueous extracts given higher amount of plant growth regulators like auxins, cytokinins and gibberellins which stimulated the seeds germination faster when compared to all other concentrations and control. This result was highly supported by Krishna Moorthy *et al.* (2019) reported that 3 cyanobacterial isolates such as *Pseudanabaena* spp.AK-1, *Lyngbya* spp.AK-2 and *Geitlerinema* sp. AK-3 showed significantly higher results in the percentage of seed germination when compared control.Godlewska *et al.* (2019) also reported that the application of different concentrations of blue-green microalga *Spirulina platensis* filtrate stimulate the growth parameters significantly when compared to control. Similarly, 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). Our results are similar to Mayur *et al.* (2017) who reported that the cyanobacterial isolates *Oscillatoria* spp., *Closterium* spp., *Anabaena* spp., *Rivularia* spp., *Nostoc* spp., *Gloeothece* spp., *Aphanocapsa* spp. and *Gloeocapsa* spp., showed positive effects on the seed germination rate of mung as well as wheat. Similarly, Anand *et al.* (2015) who reported that the Seeds when soaked in heterocystous cyanobacterial cultures like *Aulosira prolifica*, *Stigone madendroideum*, *Nostoc muscurum* and *Tolypothrix tenuis* exhibited 80-90% germination and in *Nostoc calcicola* and *Anabaena oryzae* 90% germination. exhibited 70 to 90% germination. The cyanobacterium *C.parietina* WSU11 culture extracts at different concentrations such as 1%, 2%, 3%, 4% and 5% showed significantly higher results in case of radicle, coleoptile and epicotyl length when compared control where seeds were treated with only distilled water alone. The reason for this great response in the radicle and coleoptile length 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 Krishna Moorthy *et al.* (2019) reported that 3 cyanobacterial isolates such as *Pseudanabaena* spp.AK-1, *Lyngbya* spp.AK-2 and *Geitlerinema* sp. AK-3 showed significantly higher results in case of radicle length, coleoptile length and epicotyl length when compared control. Present study was supported by Gayathri *et al.* (2017) who reported that the cyanobacterial isolates *Scytonemabohneri* 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 great responsein case of radicle, plumule and germination rate when compared to control (53.3%). Similarly, The applications of *Microcystis aeruginosa* MKR 0105, *Anabaena* sp. CC 7120, and*Chlorella* sp. monocultures to the conditioned corn grainsand roots significantly increased the number of the germinatedgrains, dynamics, and mean time of germination andaccelerated growth of seedlings, exhibited by faster elongationof roots and leaves and enlarged their fresh and dry biomass (Mieczyslaw Grzesik and ZdzislawaRomanowska-Duda, 2014).

Effects of isolated cyanobacteria on Biochemical parameters of *P. vulgaris* L seeds: The protein and carbohydrate contents of *H. vulgare 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 *C. parietina* WSU11at 5% level of concentration followed by 4% and 3% concentration of aqueous extraction. Protein and carbohydrate are the reserve food materials in barley 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. 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 0^{th} day to 8^{th} 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. Similarly, Bewley and Black (1985). Shutov and Vaintraub (1987), Mayer and Poljakoff – Mayber (1989) and Salisbury and Ross (1991) 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

The seed germination study results directed that the heterocystes cyanobacterium *Calothrix parietina* WSU11 showed more active in the seed germination in aqueous extracts formulated at different concentrations than control. Based on the overall seed germination experiment results, it has concluded that the heterocystes cyanobacterial *calothrix parietina* WSU11 can be used at 5% aqueous extract concentration can be used as effective liquid biofertilizers for the pretreatment of seeds.

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