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Zinc Solubilizing Rhizobacteria and Fungi: The Potent Contributors to Plant Growth Promotion and Enhancement of Zinc Content in *Aloe* Gel

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ABSTRACT

Background: Zinc solubilizing bacteria and fungi are possible alternatives for zinc supplementation and translate inorganic form of zinc to available forms for the plants.

Methods: Two phosphates solubilising bacterial (ABB1, ABB2) and fungal (FSD1, FSD2) strains were initially isolated from the rhizospheric soil of the plant and were tested for their plant growth promoting ability and zinc solubilizing capability. *Aloe barbadensis* Mill. plants were inoculated with the microbial strains to assess their effects on plant growth and to quantify the zinc content in leaves using atomic absorption spectroscopy.

Result: In our experiment, zinc content in the leaves of ZnO+ABB1+FSD2 set was found highest than ZnO treated set. Thus, present study plausibly designates the ability of the *Aloe* plants for solubilizing zinc only in presence of the rhizospheric microorganisms which indicates the potential of these microbial strains to use as biofertilizers in more sustainable way.

Key words: *Aloe barbadensis* Mill., Biofertlizer, Zinc.

INTRODUCTION

Zinc (Zn) is an important micronutrient for plants which plays numerous functions in the life cycle of plants (Hirschi, 2008). Plants can uptake zinc as Zn^{2+} and the rest is present in the soil as insoluble form (Kamran *et al.,* 2017). Due to the inaccessibility of zinc in soil, zinc deficiency is one of the most prevalent micronutrient deficiencies found in plants (White and Broadley 2005). It has reported previously that, zinc deficiency in *Aloe* plants leads to their stunted growth and reduced gel content in their leaves (Murillo-Amador *et al.,* 2014).

Various factors negatively affect the Zn availability for plants in the soil, counting low Zn content, microbial communities in the rhizosphere etc. (Saleem *et al.,* 2022). Zn deficiency for plants can be mitigated by totting up Zn compounds to the soil, but the high price related with applying Zn fertilizers faces a considerable burden on poor farmers (Singh *et al.,* 2005). In this situation, the use of beneficial rhizosphere microorganisms (Zinc solubilising bacteria and fungi) (Jamal *et al.,* 2002) could be a viable option to replace the chemical Zn fertilizers.

Plant growth promoting rhizobacteria (PGPR) is multifunction rhizospheric microbes functioning in sustainable agriculture (Wani *et al.,* 2008) and some are zinc solubilizers (Kamran *et al.,* 2017). Plant Growth Promoting Fungi (PGPF) maintains plant growth through the production of several significant enzymes and also it solubilizes phosphate, produces plant hormones (Glick, 2014).

The demand of herbal medicine is increasing day by day and among herbal medicines, *Aloe barbadensis* Mill. is one of the most important medicinal herbs considered worldwide (Glick, 2014). The plant *Aloe* belongs to the family *Asphodelaceae* is the world's best natural anti-septic, anti-microbial, anti-diabetic, uterine stimulant (Tiwary *et al.,* 2018). The gel can be used effectively in the general treatment of skins and leg ulcers (Lakshmi and Sekhar 2018). Due to multiple uses of *Aloe* gel, its demand in our daily life has been increasing day by day internationally.

Keeping this, in view the experiment was designed to isolate, screen and identify the zinc solubilizing bacteria and fungi collected from the rhizospheric soil of the plant and also checked their plant growth promoting (PGP) traits like, Phosphate solubilisation, IAA production *etc.* and zinc solubilizing capability and evaluated their response on primary growth and zinc content of the plants, so that they could be established as an up-coming bio-inoculants for biofertilization.

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MATERIALS AND METHODS

Isolation of bacteria and fungi from the rhizospheric soil of the plant *Aloe barbadensis* Mill.

Isolation of bacterial and fungal strains were done after collecting the rhizospheric soil of plant from department of Botany, The University of Burdwan (23.2222° N, 87.8771° E) which was followed by serial dilution technique (Pikovskaya, 1948). Discrete bacterial colonies appeared on the plates at 10^{-8} dilution and two colonies with distinct morphologies were selected for pure culture. After pure culturing, the bacterial colonies were named as ABB1 and ABB2. For fungal isolation, same method was followed. After performing the pure culture, the fungal strains were named as FSD1 and FSD2.

Screening of isolated bacterial and fungal colonies for plant growth promoting properties

To determine phosphate solubilization ability, the isolated bacterial strains (ABB1 and ABB2) and fungal strains (FSD1 and FSD2) were streaked into Pikovskaya agar medium (Pikovskaya, 1948) and incubated at 28°C for 3 days (Dutta *et al.,* 2019). After 3 days, the microbial colonies produced the clear halo zone and were considered as phosphate solubilizing bacteria and fungi. IAA, Ammonia, hydrogen cyanide, siderophore production and nitrogen fixation tests were performed by using the standard protocol of Dutta *et al.* (2019).

Optimization of media for zinc solubilization in *in vitro* condition

Zinc solubilizing ability of both the bacterial strains (ABB1 and ABB2) and fungal strains (FSD1 and FSD2) were tested in three different types of agar media *viz.* Potato Dextrose Agar (PDA) media, Pikovskaya's (PKV) Agar media and Nutrient Agar (NA) media. Among them, NA media was selected based on proper zone formation and the growth of isolates.

Effect of various Zinc sources like zinc sulphate ($ZnSO_4$), zinc oxide (ZnO), zinc chloride ($ZnCl_2$), zinc phosphate $Zn_3(PO4)_2$ and zinc carbonate ($ZnCO_3$) were studied in Nutrient Agar medium. The bacterial and fungal isolates were checked for solubilization activity in NA medium amended with different Zinc source. It was found that maximum zone of solubilization was in Zinc oxide (ZnO) and was selected as the optimum concentration of zinc source.

Effect of different concentrations of zinc oxide on the efficiency of zinc solubilization

Different concentrations (0.1%, 0.2%,0.3%, 0.4% and 0.5%) of Zinc Oxide (ZnO) were added in the NA medium. After that, the medium was inoculated by using bacterial and fungal strains and allowed to grow for 14 days (Fasim *et al.,* 2002). The zone of solubilization was observed and measured in centimetre (cm).

Selection of best performing strain

Based on the results of phosphate solubilization, plant growth promoting traits and zinc solubilization, one bacterial strain (ABB1) and one fungal strain (FSD2) were selected for further studies.

Molecular identification of the bacterial and fungal strains

The species-level identification of bacterial and fungal strains was done following 16S rDNA sequencing and 18S rDNA sequencing respectively. For rDNA sequencing, genomic DNA was isolated by phenol/chloroform extraction method (Sambrook *et al.,* 1989) followed by PCR amplification of the desired 16S rDNA and 18S rDNA sequence. Identification of the bacterial and fungal strains were done by Ez-Taxon search.

Construction of phylogenetic tree of the microbial strains

The phylogenetic tree of these two above mentioned strains were constructed based on neighbor-joining (NJ) method (Saitou *et al.,* 1987). The trees were generated using MEGA X software (Kumar *et al.,* 2016). The bacterial (ABB1) and fungal (FSD2) strains were sent to NCCS, Pune for molecular identification and the sequences were deposited to NCBI to obtain gene bank accession numbers.

Pot experiment

To analyse the effect of zinc solubilizing bacteria and fungi on the primary growth of the plant *Aloe barbadensis* Mill., pot experiment was performed from July 2022 to July 2023. In total, eight sets (Control, ZnO, ABB1, FSD2, ZnO+ABB1, ZnO+FSD2, ABB1+FSD2, ZnO+ABB1+FSD2) with 5 replicates each. The pots were maintained in our departmental garden and were uprooted after 360 days of inoculation for further studies.

Analysis of physicochemical properties of soil

Various soil parameters like available Phosphorus, Nitrogen, Potassium, Zinc (Patle *et al.,* 2019; Nguyen *et al.,* 2019) of the soil were checked along with pH, electrical conductivity after one year of inoculation by the microorganisms.

Morphological growth parameters

During the experiment, morphological growth parameters like root length (cm), leaf length (cm) *etc.* were selected for recording the growth response in the inoculated and control plants.

Estimation of chlorophyll-a, chlorophyll-b and total chlorophyll

The amount of Chlorophyll content was calculated by using the following formula (Arnon, 1949).

Estimation of total carbohydrate and total protein

Estimation of total carbohydrate and total protein content of the plants were also done following standard protocols (Khan *et al.,* 2019).

Estimation of zinc content using atomic absorption spectroscopy

Estimation of zinc content using Atomic Absorption Spectroscopy was done following the standard protocol (Jepkoech, 2013).

Statistical analysis

Every set in our present study was done in five replicates and standard errors (SE) were calculated by using the mean. SE represented as error bars in figures and ± in tables. Differences among groups were determined by one-way analysis of variance (ANOVA) by Microsoft Excel 2010. Different letters above the bars represented significant differences at $p<0.05$ by F-test.

RESULTS AND DISCUSSION

Isolation of bacteria and fungi from the rhizospheric soil of the plant *Aloe barbadensis* Mill.

Two bacterial and fungal colonies were isolated based on the production of clear halo zones around their colonies and after performing pure culture, they were designated as ABB1 and ABB2 and fungal colonies as FSD1 and FSD2. Based on gram staining and cell morphology, the bacterial isolates were characterized according to Bergey's manual of determinative bacteriology (Table 1). The morphological and microscopic characterization of two fungal colonies were also done (Table 2).

Screening of bacterial and fungal colonies for plant growth promoting properties

In the qualitative test of Phosphate solubilization, both the bacterial colonies (ABB1 and ABB2) and fungal colonies (FSD1 and FSD2) gave the positive results (Table 3).

In the qualitative assessment of IAA production, all the four microbial strains changed the colour of the medium from pink to reddish after the addition of Salkowski's reagent which indicates their ability to produce IAA (Table 3).

In the test of Ammonia production and nitrogen fixation, all the bacterial and fungal strains showed positive results (Table 3). Both ABB1 and FSD2 have the ability to produce siderophore as the colour of the medium changed from greenish blue to yellow (Table 3). The strains ABB2 and FSD1 were found negative for HCN production as colour change in filter paper was not observed after 72 hours of incubation whereas the strains ABB1 and FSD2 changed the colour of the filter paper which indicates their ability to produce HCN (Table 3). Plant growth promoting traits like Phosphate solubilization, IAA production HCN production and Siderophore production of *Aspergillus terreus* had been reported previously by Dey *et al.* (2019).

Table 1: Morphological and Microscopic characters of PGPR isolates.

Isolates	Motility	Shape	Gram Stain	Colour	Surface	Margin	Pigmentation
ABB1	Motile	Bacilli	+	White	Smooth	Rough	Not showing
ABB2	Motile	Bacilli	+	Cream	Shiny	Wavy	Not showing

Table 2: Morphological and microscopic characters of fungal isolates.

Isolates	Mycelia	Growth rate	Colour	Hyphae	Conidia	Conidiophore
FSD1	Flat with high white colour sporulation	Moderate	White	Septate, hyaline, branched, long hyphae with width1.2-2.8 (1.76) μm.	Hyaline, circular to oval, single or in chains, diameter: 22.5-63.03 (42.69) μm.	Septate with round shaped, width: 22.5-63.03 (42.69) μm. vesicles width: 17.1-23.5 (21.34) μm.
FSD2	Flat with high green colour sporulation	Moderate	Green	Septate, hyaline, branched, long hyphae with width 1.4-3.4 (2.26) μm.	Hyaline, oval, mainly in chains, diameter: 31.5-71.03 (48.45) μm.	Septate with flask shaped, width: 31.5-71.03 (48.45) μm. vesicles width: 22.1-27.1 (24.63) μm.

Table 3: Qualitative study of plant growth promoting traits of bacterial (ABB1 and ABB2) and fungal (FSD1 and FSD2) isolates.

Tests performed	ABB1	ABB2	FSD1	FSD2
Phosphate Solubilization	++	+	+	++
IAA production	++	+	+	++
Ammonia production	+	+	+	+
HCN production	+	-	-	+
Nitrogen fixing Ability	+	+	+	+
Siderophore producing ability	+	-	-	+

The result of the qualitative tests done for the isolated PGPFs. '+' sign indicated that the test is positive, '++'signs mean more positive. '- 'sign indicated that the test is negative. All data are the mean value of five replicates.

Zinc solubilising ability

On the ZnO medium, maximum zone was observed for the bacterial isolate ABB1 (Fig 1A) and FSD2 (Fig 1B). On $Zn_3(PO_4)_2$ and $ZnCl_2$ medium, both the bacterial strains and fungal strains showed very little zone of solubilization. Again, the fungal strains solubilized zinc on $ZnSO_4$ supplemented medium very minutely (Fig 1B) whereas very little zone was observed for the bacterial strains in $ZnSo_4$ (Fig 1A).

Thus, from the above results, it was found that zinc oxide (ZnO) was maximally solubilized by both the bacterial strains and fungal strains as compared to other zinc salts, hence ZnO was selected for zinc supplementation in further plant experiments. The maximum zinc (ZnO) solubilization was found in 0.1% ZnO by both the bacterial strains (Fig 2A) as well as the fungal strains (Fig 2B) than that of other ZnO concentrations. Zinc mobilization by zinc solubilizing bacteria (Bacillus aryabhattai in wheat) had previously reported by Ramesh *et al.* (2014).

Selection of best performing strain

On the basis of phosphate solubilization, plant growth promoting traits and zinc solubilization, one bacterial strain (ABB1) and one fungal strain (FSD2) was selected as the best performing strain and they were used for further studies.

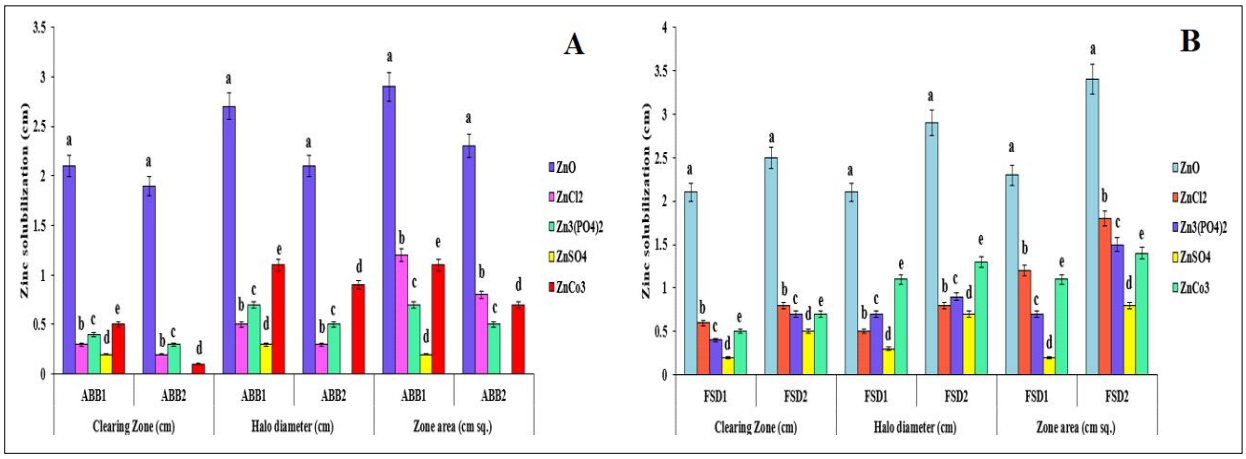

Fig 1: Zinc solubilization by the bacterial strains (ABB1 and ABB2) (A) and fungal strains (FSD1 and FSD2) (B) on different zinc salts.

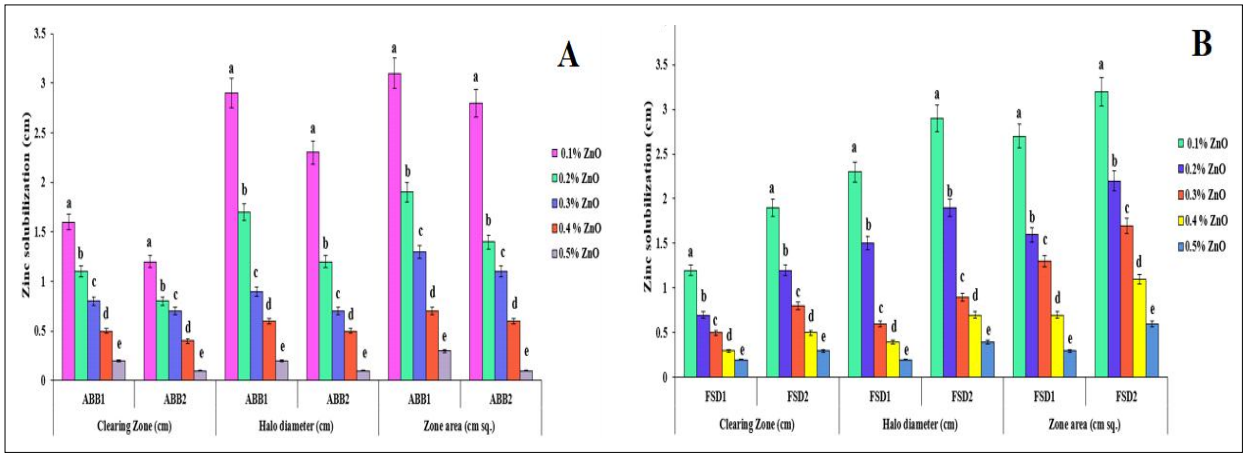

Fig 2: Zinc solubilization by the bacterial strains (ABB1 and ABB2) (A) and fungal strains (FSD1 and FSD2) (B) on different ZnO concentrations.

Table 4: Molecular Identification of ABB1 and FSD2 strains by 16S rDNA and 18S rDNA sequencing respectively.

Strain Name	Closest similarity [EzTaxon seq. acc. no.]	Pairwise similarity (%)	Strain sequence accession number	NCBI sequence accession number
ABB1	*Bacillus cereus* ATCC 14579(T)	99.91	MCC 4087	MT 636543
FSD2	*Aspergillus terreus* AB008409.1	99.00	MCC 1819	MT 636881

Molecular identification of bacterial (ABB1) and fungal (FSD2) strain

After deposition of the bacterial and fungal strain to NCCS, Pune, India for molecular identification, we found that the bacterial strain ABB1 shows 99.91% pairwise similarity with the bacterial strain *Bacillus cereus* ATCC 14579(T) (Table 4). The fungal strain FSD2 shows 99.00% pairwise similarity with the fungal strain *Aspergillus terreus*AB008409.1 (Table 4).

Construction of phylogenetic tree

Construction of phylogenetic tree of the bacterial and fungal isolate was done by using the Neighbor-Joining method (Saitou *et al.*, 1987). The optimal tree with the sum of branch length = 0.00539059 was shown for ABB1 (Fig 3A) and 0.00867904 for FSD2 (Fig 3B). The analysis involved 11 nucleotide sequences for 'ABB1' and 21 nucleotide sequences for 'FSD2'. There were a total of 1485 positions for 'ABB1' and '1618' positions for FSD2 in the final dataset.

Analysis of physicochemical properties of soil

The pH of the soil increased significantly in all the treated set than control set (Table 5). The soil of the plants treated with ZnO+ABB1+FSD2 had highest pH value. But significantly, the EC (dS m^{-1}) of the soil decreased after applying the microorganisms (Table 5). Available phosphorus (kg ha^{-1}), potassium (kg ha^{-1}) and nitrogen (kg ha^{-1}) content of all treated soil significantly increased from uninoculated control set (Table 5). The soil which was inoculated with ZnO+ABB1+FSD2 showed highest increase in available phosphorus (32.02%), potassium (9.25%) and nitrogen (25.02%) than uninoculated control set.

The Zinc content of the soil was checked and it was found that the available zinc content (mg kg^{-1}) in the soil of all the treated sets were increased from uninoculated control set (Table 5). The highest available zinc content was found in ZnO+ABB1+FSD2 treated set.

Morphological parameters of the plant

The results of the plant experiment showed that all the microbial inoculated sets considerably increased the root length and leaf length of the plant from that of Control set (Table 6). The root length (cm) and leaf length (cm) of the plants were found maximum in 'ZnO+ABB1+FSD2' treated set (Table 6).

Similarly, all the microbial inoculated sets considerably increased the morphological growth (Table 7) of the plant from that of Control set. The plants which were inoculated with ABB1+FSD2 showed greater increase in morphological

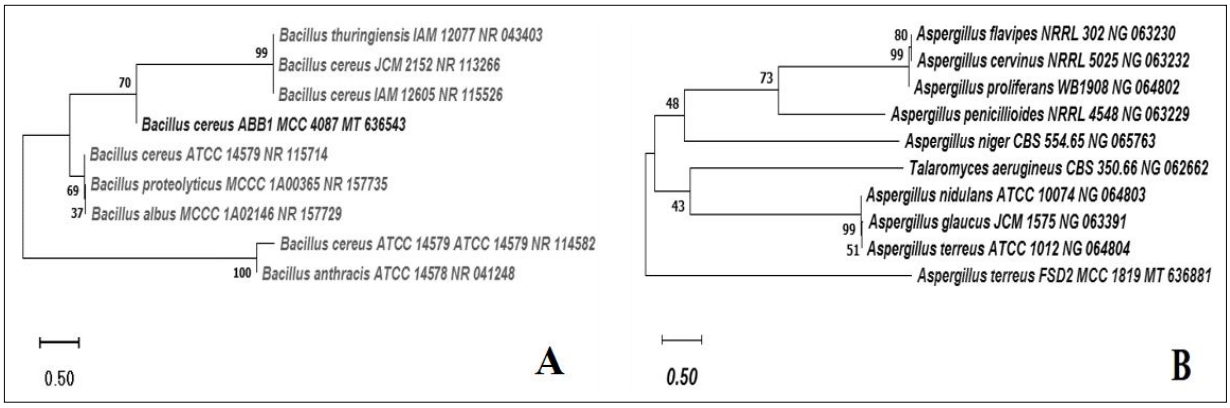

Fig 3: Phylogenetic tree of the bacterial strain 'ABB1' (A) and fungal strain 'FSD2' (B).

Table 5: Physico chemical properties of soil after treating with different microbial strains.

Treatments	pH	EC (dS m^{-1})	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Available potassium (kg ha^{-1})	Available zinc (mg kg^{-1})
Control	6.34±0.09^{a}	0.32±0.03^{a}	220.43±2.6^{a}	52.87±1.3^{a}	304.7±3.7^{a}	5.58±0.02^{a}
ZnO	6.56±0.1^{b}	0.30±0.09^{b}	221.76±2.1^{a}	56.7±1.6^{b}	311.4±3.6^{b}	6.50±0.07^{b}
ABB1	6.53±0.09^{b}	0.33±0.08^{c}	224.6±2.5^{a}	58.9±1.8^{c}	316.6±3.1^{c}	8.90±0.06^{c}
FSD2	6.66±0.11^{c}	0.31±0.09^{b}	237.65±1.8^{b}	61.6±1.6^{d}	319.9±2.9^{d}	11.70±0.08^{d}
ZnO+ABB1	6.54±0.12^{b}	0.28±0.09^{d}	235.6±2.0^{b}	59.8±1.6^{c}	317.6±3.2^{c}	12.02±0.09^{d}
ZnO+FSD2	6.87±0.13^{d}	0.26±0.04^{e}	258.9±2.1^{c}	64.7±1.9^{e}	328.7±3.5^{e}	13.18±0.14^{e}
ABB1+FSD2	6.58±0.1^{b}	0.29±0.05^{d}	247.7±2.3^{bc}	62.9±1.4^{d}	323.8±3.9^{de}	12.42±0.13^{de}
ZnO+ABB1+FSD2	6.96±0.13^{e}	0.27±0.03^{d}	275.6±1.9^{d}	69.8±1.5^{f}	332.9±3.9^{f}	13.68±0.09^{f}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p < 0.05$.

growth than 0.1% ZnO treated set (Table 7). The same trend was noticed in case of dry leaf weight, dry gel weight and dry rind weight (Table 8). Gupta *et al.* (2012) reported that after applying the phosphate solubilizing bacteria on the plant *Aloe barbadensis* Mill., the morphological growth of the plant increased significantly. Naziya *et al.* (2019) also reported that the growth of *Capsicum annuum* L. was increased after applying with plant growth promoting fungi.

Biochemical parameters of the plant

In case of chlorophyll-a and chlorophyll-b (Table 9) content of the plants, all the sets which were inoculated with microbial strains significantly increased the chlorophyll content of the plants from control set. The chlorophyll a and b content were increased maximum in ZnO+ABB1+FSD2 treated set (chlorophyll-a increased upto 50.09% and chlorophyll-b increased upto 116.52% compared to control set). The same trend was noticed in the case of total chlorophyll content also (Table 9).

Among all the inoculated sets, ZnO+ABB1+FSD2 showed highest carbohydrate content (increased upto 48.84% than control set). The same trend was noticed in the case of total Protein (Table 9) also where maximum protein content was found in ZnO+ABB1+FSD2 treated set (increased upto 26.98% than control set).

Estimation of zinc content using atomic absorption spectroscopy

After atomic absorption spectroscopic study, significant increase in zinc content in the leaves and roots of all the inoculated plants were noticed as compared to control set. ZnO+ABB1+FSD2 treated set showed maximum increase in zinc content (increased up to 199.47% in leaves) as compared to control set. The zinc content in the leaves of ABB1+FSD2 treated set was found higher (increased upto 144.83%) than ZnO treated set (Table 10). But the zinc content in the roots of ZnO treated set was found higher than ABB1+FSD2 treated set, which probably suggest that the bacterial and fungal genera has successfully transported the zinc from roots towards the leaves of the plant by various mechanisms and our result corroborates with the findings of Kamran *et al.* (2017). It has been reported previously that bacterial and

Table 6: Effects of bacterial and fungal strain on root and leaf length of the plant *Aloe barbadensis* Mill.

Treatments	Root length (cm)	Leaf length (cm)
Control	11.8±0.1^{a}	21.6±0.3^{a}
ZnO	11.8±0.5^{b}	22.3±0.4^{b}
ABB1	12.3±0.4^{b}	23.4±0.1^{b}
FSD2	13.2±0.6^{c}	25.2±0.7^{c}
ZnO+ABB1	13.9±0.5^{c}	23.6±0.8^{b}
ZnO+FSD2	14.5±0.6^{d}	25.7±0.4^{c}
ABB1+FSD2	14.2±0.5^{cd}	24.8±0.6^{bc}
ZnO+ABB1+FSD2	15.6±0.7^{e}	26.1±1.1^{d}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p < 0.05$.

Table 7: Effects of bacterial and fungal strain on fresh root weight (g), fresh leaf weight (g), fresh gel weight (g) and fresh rind weight (g) of the plant *Aloe barbadensis* Mill.

Treatments	Fresh root weight (g)	Fresh leaf weight (g)	Fresh gel weight (g)	Fresh rind weight (g)
Control	7.53±0.12^{a}	104.76±1.1^{a}	42.87±0.9^{a}	60.8±0.8^{a}
ZnO	7.59±0.17^{a}	116.7±1.7^{b}	50.66±0.5^{a}	63.12±0.3^{a}
ABB1	8.11±0.19^{b}	135.6±2.1^{c}	62.44±0.8^{b}	70.76±1.9^{b}
FSD2	10.01±0.15^{c}	162.7±2.8^{d}	77.57±1.1^{c}	82.11±1.7^{c}
ZnO+ABB1	9.8±0.31^{c}	142.7±2.7^{c}	67.91±1.3^{bc}	72.66±1.8^{b}
ZnO+FSD2	12.04±0.41^{d}	185.9±2.9^{e}	90.87±1.6^{d}	93.54±1.9^{d}
ABB1+FSD2	11.09±0.32^{cd}	176.1±3.1^{de}	85.45±1.9^{cd}	90.87±1.5^{d}
ZnO+ABB1+FSD2	13.43±0.51^{e}	191.6±3.2^{f}	93.76±1.3^{e}	96.72±1.9^{e}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p < 0.05$.

Table 8: Effects of bacterial and fungal strain on dry root weight (g), dry leaf weight (g), dry gel weight (g) and dry rind weight (g) of the plant *Aloe barbadensis* Mill.

Treatment	Dry root weight (g)	Dry leaf weight (g)	Dry gel weight (g)	Dry rind weight (g)
Control	0.03±0.003^{a}	1.65±0.03^{a}	0.76±0.02^{a}	0.87±0.03^{a}
ZnO	0.05±0.001^{b}	1.87±0.04^{b}	0.77±0.03^{a}	1.05±0.05^{b}
ABB1	0.07±0.005^{b}	1.92±0.09^{b}	0.84±0.02^{b}	1.04±0.04^{b}
FSD2	0.12±0.006^{c}	2.69±0.04^{c}	1.09±0.04^{c}	1.49±0.05^{c}
ZnO+ABB1	0.11±0.006^{bc}	2.34±0.06^{bc}	0.95±0.02^{b}	1.37±0.07^{bc}
ZnO+FSD2	0.14±0.009^{d}	2.88±0.07^{d}	1.21±0.04^{d}	1.56±0.09^{c}
ABB1+FSD2	0.13±0.008^{cd}	2.54±0.02^{c}	1.02±0.07^{c}	1.46±0.06^{c}
ZnO+ABB1+FSD2	0.16±0.009^{e}	2.97±0.04^{e}	1.29±0.08^{e}	1.62±0.07^{d}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p < 0.05$.

Table 9: Effects of bacterial and fungal strain on of the plant *Aloe barbadensis* Mill. different letters above the biochemical parameters of the plant.

Treatment	Chlorophyll-a (mg g^{-1} FW)	Chlorophyll-b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Total carbohydrate (mg g^{-1} DW)	Total protein (mg g^{-1} DW)
Control	10.22±0.23^{a}	2.3±0.07^{a}	12.25±0.56^{a}	16.87±0.12^{a}	14.12±0.03^{a}
ZnO	11.1±0.38^{b}	2.86±0.07^{b}	13.96±0.82^{b}	17.94±0.16^{b}	14.32±0.06^{a}
ABB1	12.28±0.32^{c}	3.15±0.12^{c}	15.42±0.43^{c}	18.56±0.12^{c}	14.98±0.04^{b}
FSD2	13.8±0.66^{d}	3.32±0.15^{d}	17.1±0.79^{d}	22.75±0.15^{d}	16.45±0.06^{c}
ZnO+ABB1	12.3±0.44^{c}	3.54±0.18^{d}	15.84±0.92^{d}	18.58±0.18^{c}	14.97±0.07^{b}
ZnO+FSD2	14.7±0.85^{e}	4.65±0.16^{e}	19.34±0.79^{e}	23.51±0.21^{e}	17.02±0.09^{d}
ABB1+FSD2	13.44±0.78^{d}	4.32±0.19^{de}	17.76±0.81^{d}	22.44±0.16^{d}	15.23±0.04^{bc}
ZnO+ABB1+FSD2	15.34±0.73^{e}	4.98±0.18^{f}	20.32±0.97^{f}	25.11±0.25^{f}	17.93±0.08^{e}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p < 0.05$.

Table 10: Effects of bacterial and fungal strain on zinc content in (mg kg^{-1}) the leaves and roots of the plant *Aloe barbadensis* Mill.

Treatments	Zinc content in leaves (mg kg^{-1})	Zinc content in roots (mg kg^{-1})
Control	3.78±0.9^{a}	5.43±1.5^{a}
ZnO	3.97±1.2^{b}	15.54±2.7^{b}
ABB1	4.76±1.7^{c}	9.98±1.7^{ab}
FSD2	7.5±1.76^{d}	9.95±2.4^{ab}
ZnO+ABB1	8.32±1.5^{d}	9.03±2.2^{ab}
ZnO+FSD2	9.96±1.8^{e}	8.87±2.9^{ab}
ABB1+FSD2	9.72±1.7^{e}	8.09±2.7^{a}
ZnO+ABB1+FSD2	11.32±2.1^{f}	7.09±3.3^{a}

Different alphabets above each value indicate statistically significant according to the least significant difference (LSD) test at $p<0.05$.

fungal genera colonize in the rhizospheric soil as well as inside the plant roots which significantly solubilize minerals and make them available for the plants (Walia *et al.,* 2017).

CONCLUSION

The nutrients content in the soil were significantly increased after applying the microorganisms. The results indicate that the zinc content in the leaves were boosted in all the treated sets than uninoculated control set. Thus, the study offers that the bacterial (*Bacillus cereus*) as well as fungal strain (*Aspergillus terreus*) are one of the major soil-biological components that need to be considered for mitigating micronutrient deficiencies in the plants and also for upregulating soil nutrients content which indicates the potential of these strains to be used as biofertilizers in more sustainable way. But intense study of this microbial combination is required to progress agricultural productivity in more sustainable ways.

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