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# Applications of Plant Growth Promoting Bacteria (PGPB) and Vesicular Arbuscular Mycorrhizal (VAM) Fungi on *Aloe barbadensis* Mill., a Medicinally Important Succulent Herb

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## ABSTRACT

**Background:** Application of biofertilizers is an appropriate replacement of harmful chemical fertilizers to fulfil the rising demands of herbal medicine. The main objective of this study was to establish a potent biofertilizer with the help of microbial consortia and observe their effects on the primary growth of *Aloe barbadensis* Mill.

**Methods:** Rhizospheric soil of the plant *Aloe barbadensis* Mill. was taken and isolation, characterization and identification of the vesicular arbuscular mycorrhiza (VAM) fungi, rhizospheric bacteria and the VAM fungal spore associated bacteria was done. To check the microbial effects on morphological parameters of the plant, a total number of eight experimental sets were prepared by using different microbial strains.

**Result:** In our experiment, VAM+ABM1 treated plant set showed the significant responses in plant primary growth which can be used as a potent biofertilizer in more sustainable way.

**Key words:** *Aloe barbadensis* Mill, MHB, VAM.

## INTRODUCTION

At present, uses of chemical fertilizers, pesticides, herbicides, *etc*. reduce the soil fertility and effectiveness of soil (Mushtaq *et al*., 2021). Thus, the use of chemical fertilizer produces a great concern in agriculture in near future.

One alternative way to diminution bad effects of chemical fertilizers is inoculation of soil by biofertilizers, such as vesicular arbuscular mycorrhizal (VAM) fungi and plant-growth-promoting rhizobacteria (PGPR). Mycorrhizal fungi are able to enhance plant growth mainly by increasing the soil available phosphorus and other nutrients (Huey *et al*., 2020).

The group of bacteria that colonize in the roots of higher plant and helps in plant growth are known as Plant Growth Promoting Rhizobacteria (PGPR). They are also known as efficient bio-fertilizers for enhancing the growth of several crops as well as some medicinal plants (Aloo *et al*., 2022).

Some PGPR are known to enhance mycorrhizal fungal growth, by supporting the spore germination and hyphal growth of VAM fungi (Xavier *et al*., 2003). The exchange of nutrients takes place between PGPR and VAM fungi and these bacteria are considered as mycorrhiza helper bacteria (MHB) (Xavier *et al*., 2003).

The plant *Aloe barbadensis* Mill. belongs to family Asphodelaceae is considered as one of the most important medicinal plants worldwide (Chinchilla *et al*., 2013). The *Aloe* gel is, the world's best natural anti-septic use as an effective anti-oxidant and also are recommend as an analgesic, pain killer, anti-asthmatic, hypoglycaemic, liver stimulant, stomach ache (Chinchilla *et al*., 2013).

Due to multiple uses of *Aloe* gel, its demand in our daily life is rising internationally. Keeping these in mind, the endeavour of this study was to isolate and screen the vesicular arbuscular mycorrhizal fungi and rhizospheric bacteria from the roots and rhizospheric soil of the plant *Aloe barbadensis* Mill. and the bacteria which were reside on the spore wall of mycorrhizal fungi and test out their effects on the primary growth of the plant *Aloe barbadensis* Mill. in different microbial combinations so that they can be established as an effective biofertilizer.

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## MATERIALS AND METHODS

A brief experimental design of this research work was shown in Fig 1.

### Collection of root and rhizosphere soil sample

Tertiary roots and roots adhering soil of the plant *Aloe barbadensis* Mill. were collected from our departmental

garden, Department of Botany, The University of Burdwan (Latitude- N 23°14′20.86″, Longitude- E 87°51′45.743).

**Study of root samples for VAM fungal association**

The root samples were prepared following the standard protocol of Phillips and Hayman (1970):

% Mycorrhizal association =

$$\frac{\text{No. of mycorrhiza associated segments}}{\text{Total number of segments scored}} \times 100$$

**Collection and identification of mycorrhizal spores from soil sample**

It was done following the protocol of Gerdemann and Nicolson (1963). The VAM fungal spores were observed under a microscope (Leica model no. DMLB 3000) with a magnification of 100x and identified the VAM fungi using INVAM (Dwiastuti *et al*., 2019).

**Mycorrhizal inoculum production**

The single spore *Funneliformis mosseae*, formerly known as *Glomus mosseae* derived cultures were maintained by subculturing in the pots under the same atmospheric conditions by using the host plant *Zea mays* L. (Ferrol, 2021).

**VAM fungal spore disinfection**

Spores of *Funneliformis mosseae* were collected following the method of Bécard and Fortin (1988).

**Isolation of bacteria from the rhizospheric soil of the plant**

To isolate the bacteria from the rhizospheric soil of the plant *Aloe barbadensis*, soil was collected from our departmental garden which was followed by serial dilution plating technique. One phosphate solubilizing bacterial colony was selected and named as ABB1.

**Isolation of the bacteria from the spore wall of VAM fungi**

Isolation of bacteria from the outer wall of *Funneliformis mosseae* spore was done following the standard protocol (Mayo *et al*., 1986). Two bacterial colonies were selected and named as ABM1 and ABM2.

***In vitro* characterization of plant growth promoting (PGP) traits**

PGP traits like, phosphate solubilization (PS), indole acetic acid (IAA) production, Ammonia ($NH_3$) production, Hydrogen cyanide (HCN) production, Siderophore production of the selected isolates were detected by the universal methods (Dutta *et al*., 2019).

**Molecular Identification and phylogenetic trees construction of the bacterial strains**

The three bacterial strains (ABB1, ABM1 and ABM2) were identified using 16S rDNA sequencing method from NCMR-NCCS, Pune, India.

For better identification and their systematic position, the phylogenetic trees of the strains were constructed based on neighbor-joining method (Saitou and Nei, 1987) using MEGAX software (Kumar *et al*., 2018).

**Cell load optimization for VAM and bacterial strains for application on the plant**

Post imbibed seeds of the plants were treated with different concentrations ($10^6$ - $10^9$ CFU $mL^{-1}$) of bacteria (ABB1, ABM1 and ABM2). After germination, seeds were treated with mycorrhizal fungi (number of spores $mL^{-1}$). Then the seed germination percentage and the seedling survival percentage were calculated by using the formula of Pattanaik *et al.* (2015).

**Pot experiment**

Eight experimental sets were prepared, including CONTROL set and seven microbial treated sets (VAM, ABB1, ABM1, ABM2, VAM+ABB1, VAM+ABM1, VAM+ABM2). The experimental pots were maintained in the net house where temperature (27-33°C). After 360 days of inoculation morphological and biochemical parameters of the plants were measured.

**Analysis of physicochemical properties of soil**

Various soil parameters like available phosphorus, nitrogen, potassium, pH and electrical conductivity of the soil were checked after inoculating by the microorganisms (Patle *et al*., 2019).

**Study of morphological parameters of the plant**

Morphological growth parameters like, root length, leaf length, fresh root weight, fresh leaf weight, fresh gel weight and fresh rind weight were recorded for inoculated and control sets.

**Study of biochemical parameters of the plant**

Estimation of chlorophyll-a, Chlorophyll-b and Total Chlorophyll was done by using the method of Arnon's (Arnon, 1949). Assessment of total carbohydrate and total protein content of the plants were done following standard protocols (Khan *et al*., 2019).

**Statistical analysis**

In our present study, all data were presented as mean of five replicates. Standard errors (SE) represented as error bars in figures and ± in tables. Differences between groups were determined by one-way analysis of variance (ANOVA). Different alphabets represented significant differences at $P<0.05$.

## RESULTS AND DISCUSSION

**Isolation and Identification of VAM fungi from the rhizospheric soil sample**

Among the isolates, the dominant species was *Funneliformis mosseae* (165±6 spores per 10 g of rhizospheric soil of the plants) which was followed by *Glomus fasciculatum* (67±7 spores per 10 g of rhizospheric soil of the plants) and *Rhizophagus irregularis* (58±9 spores per 10 g of

rhizospheric soil of the plants) respectively (Table 1). So, we selected *Funneliformis mosseae* for further studies.

**Isolation of bacteria from the rhizospheric soil of the plant**

One phosphate solubilizing bacterial isolate was selected and named as ABB1.

**Isolation of the bacteria from the spore wall of VAM fungi**

Two phosphate solubilizing bacterial isolates were selected and named as ABM1 and ABM2.

**Table 1:** VAM fungi isolated from the rhizospheric soil of the plant *Aloe barbadensis* Mill.

| Name of vesicular arbuscular mycorrhizal fungi | Number of spores/10 grams of rhizospheric soil of the plant *Aloe barbadensis* Mill. |
|---|---|
| *Funneliformis mosseae* (*Glomus mosseae*) | 165±6[a] |
| *Glomus fasciculatum* | 67±7[b] |
| *Rhizophagus irregularis* | 58±9[c] |

Values in the table followed by different alphabets are significantly different at P<0.05. The values represent the mean±SD (n = 5).

**Morphological characterization of the bacterial strains**

The bacterial isolates were characterized according to Bergey's manual of determinative bacteriology (Table 2). Scanning electron microscopic (SEM) study (Fig 2) showed their morphology.

***In vitro* characterization of PGP traits**

The bacterial strain ABM1 solubilize the phosphate more readily (184.8 µgmL$^{-1}$) than the bacterial isolates ABM2 (162.4 µgmL$^{-1}$) and ABB1 (158.4 µgmL$^{-1}$) respectively (Fig 4). The phosphate solubilization index was highest in strain ABM1 (Fig 5). Quantitative assay showed maximum IAA production by the bacterial isolate ABM1 (224.5 µgmL$^{-1}$) which was followed by ABB1(206 µgmL$^{-1}$) and ABM2 (201 µgmL$^{-1}$) (Fig 4). All the bacterial isolates except ABM2 showed positive result for $NH_3$ production, siderophore assay and HCN production (Table 3) (Fig 3). PGPR plays a significant role in sustainable agriculture through the enhancement of plant growth via different processes like biological nitrogen fixation, phosphate solubilization, siderophore production and phytohormone synthesis (Riaz *et al*., 2021). The practice of PGPR is possibly increased in sustainable farming for its eco-friendly and capable nature (Riaz *et al*., 2021).

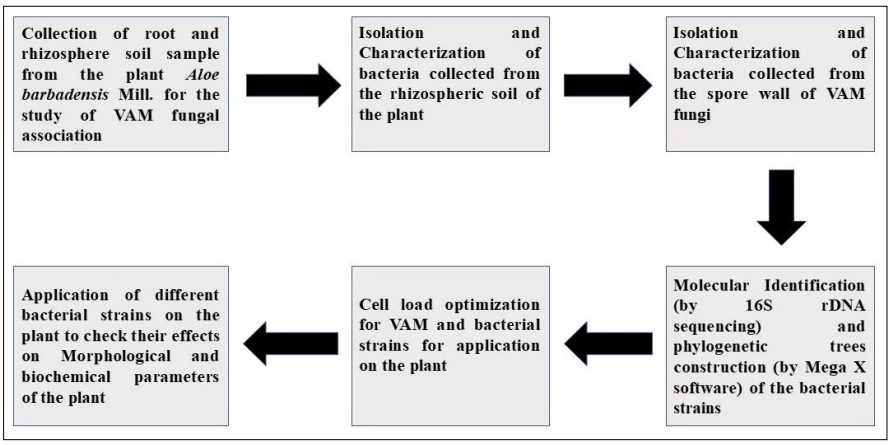



**Fig 1:** Complete flowchart to explain this research work briefly.

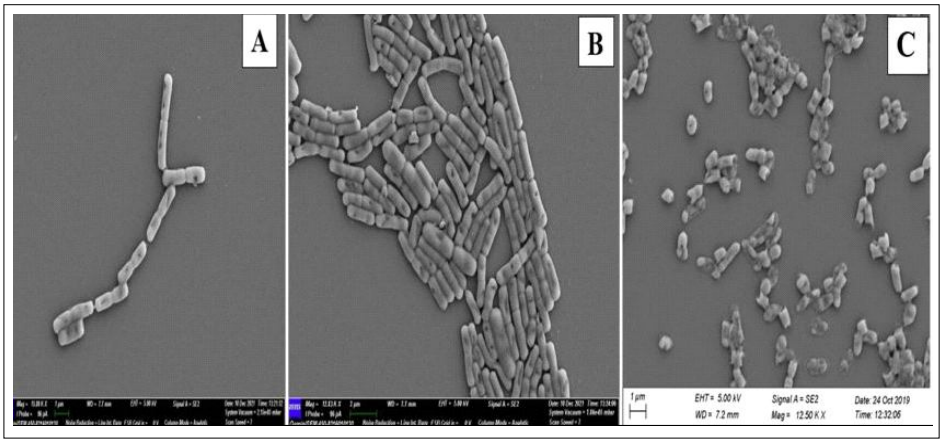
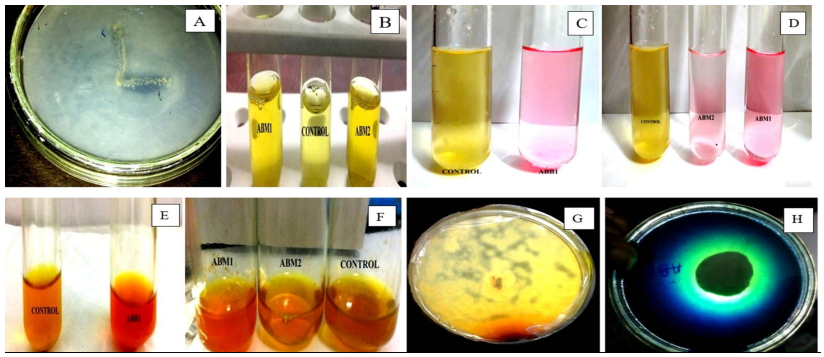
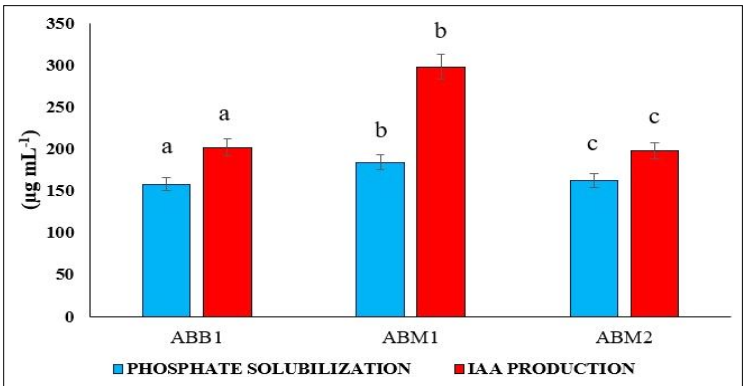



**Fig 2:** Scanning electron microscopic images of the bacterial strain.



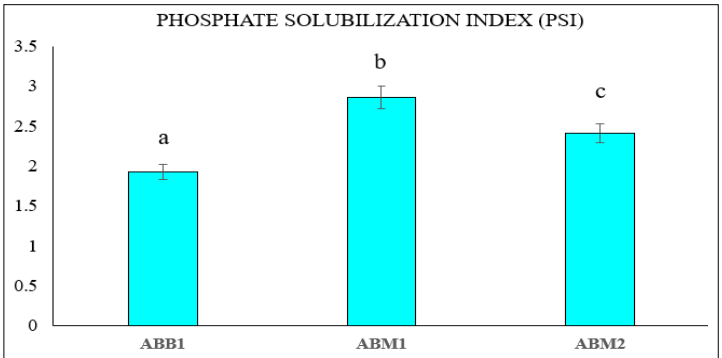

A, B); Phosphate solubilization, C, D); IAA production, E, F); Ammonia production, G); HCN production, H); Siderophore production.

**Fig 3:** Some important PGP traits by the bacterial strains.




Different alphabets above the bar indicate statistically significant differences between groups (mean±SD, n =5, oneway ANOVA, p<0.05).

**Fig 4:** Quantitative estimation of Phosphate solubilization and IAA production by the bacterial strains.




Different alphabets above the bar indicate statistically significant differences between groups (mean±SD, n=5, one way ANOVA, p< 0.05).

**Fig 5:** Phosphate solubilizing index (PSI) by the bacterial strains.

**Table 2:** Morphological characterization of bacterial isolates isolated from rhizospheric soil (ABB1) and outer wall of VAM fungi (ABM1 and ABM2) of the plant *Aloe barbadensis* Mill.

| Isolates | Colour | Shape | Gram stain | Motility | Margin | Pigmentation |
|---|---|---|---|---|---|---|
| ABB1 | White | Round | + | Motile | Smooth | None |
| ABM1 | White | Round | + | Motile | Smooth | None |
| ABM2 | White | Round | _ | Motile | Irregular | None |

### Molecular Identification and construction of phylogenetic tree for the bacterial isolates

NCMR-NCCS, Pune identified the isolates ABB1, ABM1 and ABM2 as *Bacillus cereus* ATCC 14579(T), *Bacillus tequilensis* KCTC 13622(T) and *Enterobacter chuandaensis* 090028(T) (Table 4) respectively.

The phylogenetic trees of the bacterial strains ABB1, ABM1 and ABM2 were done in Mega X software (Fig 6A-C). The analysis involved 9 nucleotide sequences for strain ABB1 (Fig 6A), 16 nucleotide sequences for strain ABM1 (Fig 6B), 14 nucleotide sequences for strain ABM2 (Fig 6C).

**Table 3:** Some important PGP traits of the bacterial isolates.

| Characters | Bacterial strains | | |
|---|---|---|---|
| | ABB1 | ABM1 | ABM2 |
| Phosphate solubilization | ++ | ++ | +++ |
| IAA production | ++ | ++ | +++ |
| Ammonia production | + | ++ | - |
| HCN production | + | ++ | - |
| Siderophore production | + | +++ | - |

All ambiguous positions were removed for each sequence pair. There were a total of 1485 positions in the final dataset for ABB1, 1658 for ABM1 and 1613 for ABM2 (Fig 6A-C).

### Effects of bacterial strains on spore germination and hyphal growth of VAM fungi under *In vitro* condition

After applying three bacterial strains (ABB1, ABM1 and ABM2) on sterilized VAM fungal (*Funneliformis mosseae*) spores, it was found that ABM1 showed more promising result than the other two strains (ABB1 and ABM2). The strain ABM1, isolated from the spore wall of VAM fungi showed highest VAM spore germination percentage (Fig 7) and it also significantly increased the length of the hyphae after VAM spore germination (Fig 8, 9).

On the other hand, the rhizobacteria (ABB1) did not increase the VAM spore germination significantly (Fig 7) along with hyphal length after VAM spore germination (Fig 8, 9).

### Cell load optimization for VAM and bacterial strains

For cell load optimization of three bacterial strains (ABB1, ABM1 and ABM2), we have used different concentrations of bacterial suspension on the post-imbibed seeds of the

**Table 4:** Molecular identification of bacterial strains ABB1, ABM1 and ABM2 by 16S rDNA sequencing.

| Strains 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 |
| ABM1 | *Bacillus tequilensis* KCTC 13622 (T) | 99.71 | MCC 4174 | MT 636542 |
| ABM2 | *Enterobacter chuandaensis* 090028 (T) | 99.56 | MCC 4178 | MT 636544 |

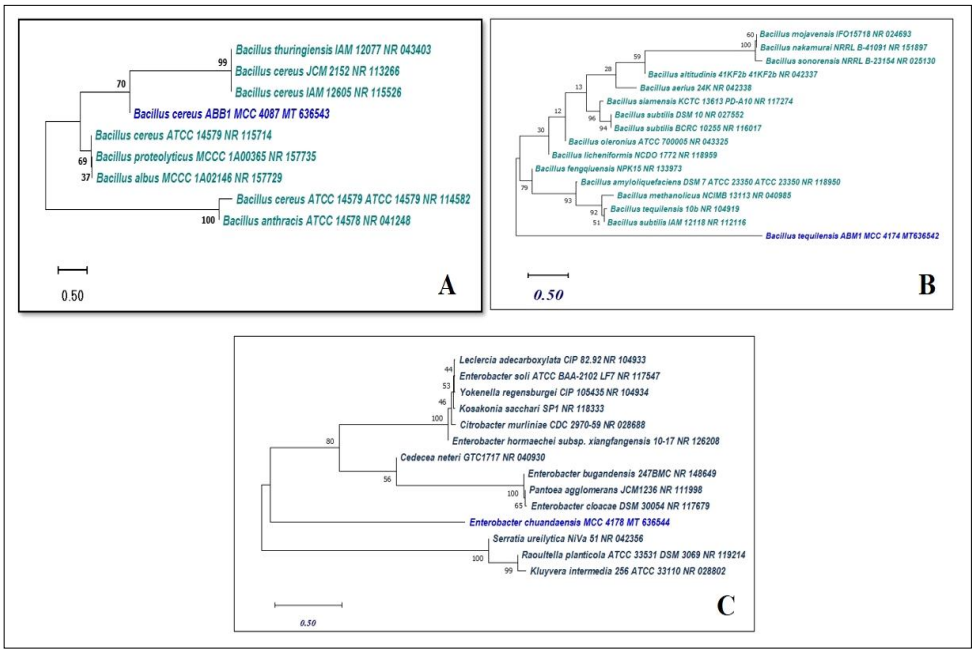



**Fig 6:** Phylogenetic trees showing the position of *Bacillus cereus.* (A): *Bacillus tequilensis;* (B): and *Enterobacter chuandaensis;* (C): Which were identified by 16SrDNA gene sequencing. Bootstrap values of 1000 replications expressed as percentages are given at branch points. Bar 0.50 substitutions per site. The trees were generated using MEGA X software.

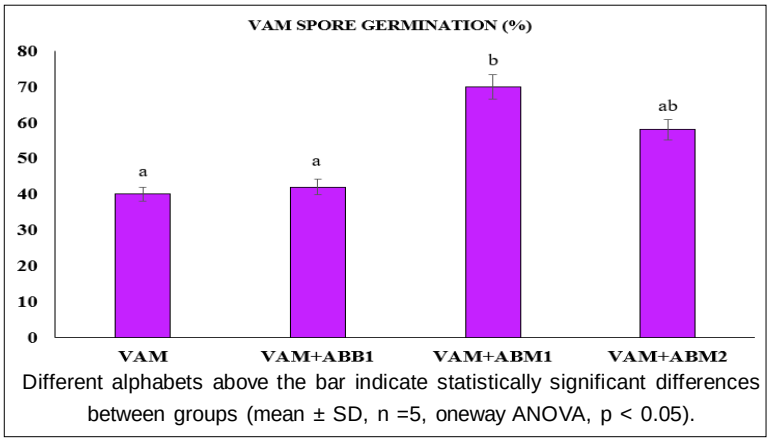



**Fig 7:** VAM fungi spore germination percentage either in presence of bacteria (VAM+ABB1, VAM+ABM1 and VAM+ABM2) or absence of bacteria (VAM).

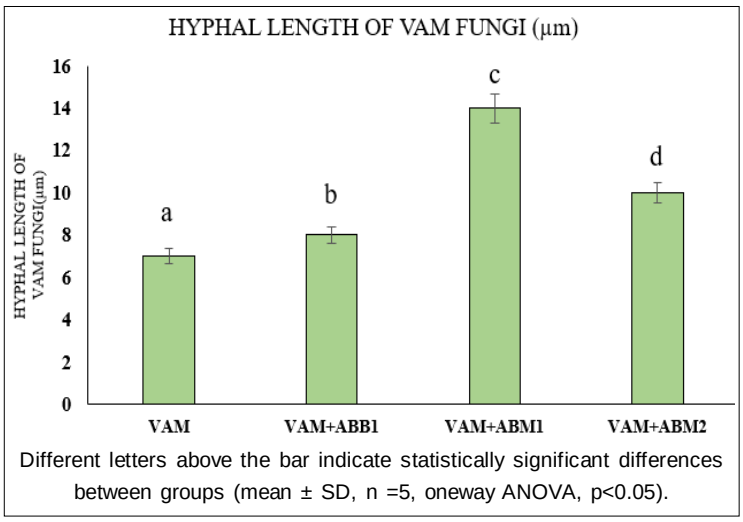



**Fig 8:** Hyphal length of VAM fungi after inoculating sterilized VAM spores by different bacterial strains.

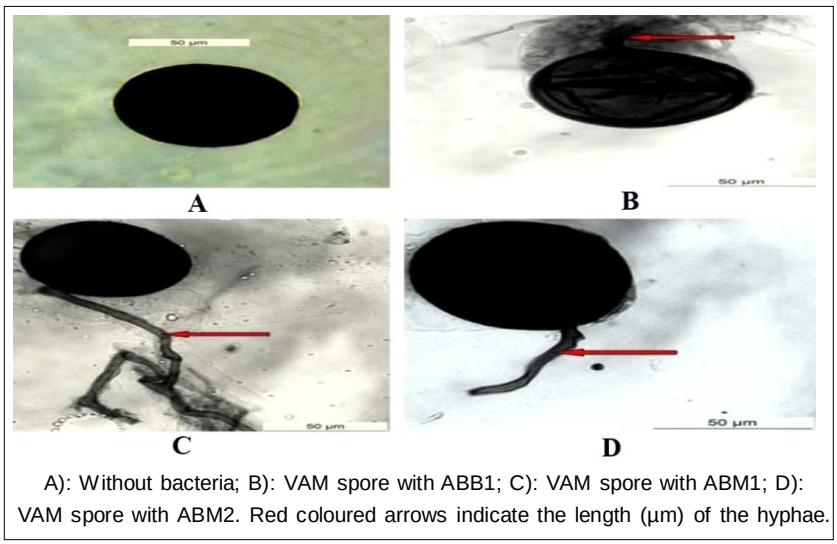



**Fig 9:** Effect of bacterial strains on VAM fungal spore germination.

plant and after that we found that the bacterial suspension ($10^8$ CFUmL$^{-1}$) showed the highest seed germination percentage (Fig 10A) and seedling survival percentage (Fig 10B). Thus, we chose $10^8$ CFUmL$^{-1}$ as optimum concentration of bacteria for the final application procedure.

For cell load optimization of VAM fungal spore, we used different number of VAM fungal spore mL$^{-1}$ on the germinating seedlings of the plant and found that 100 number of VAM spore mL$^{-1}$ of distilled water showed the highest seedling survival percentage and thus, we selected 100 VAM spore mL$^{-1}$ as the optimum concentration of VAM fungi for the final application procedure (Fig 10C).

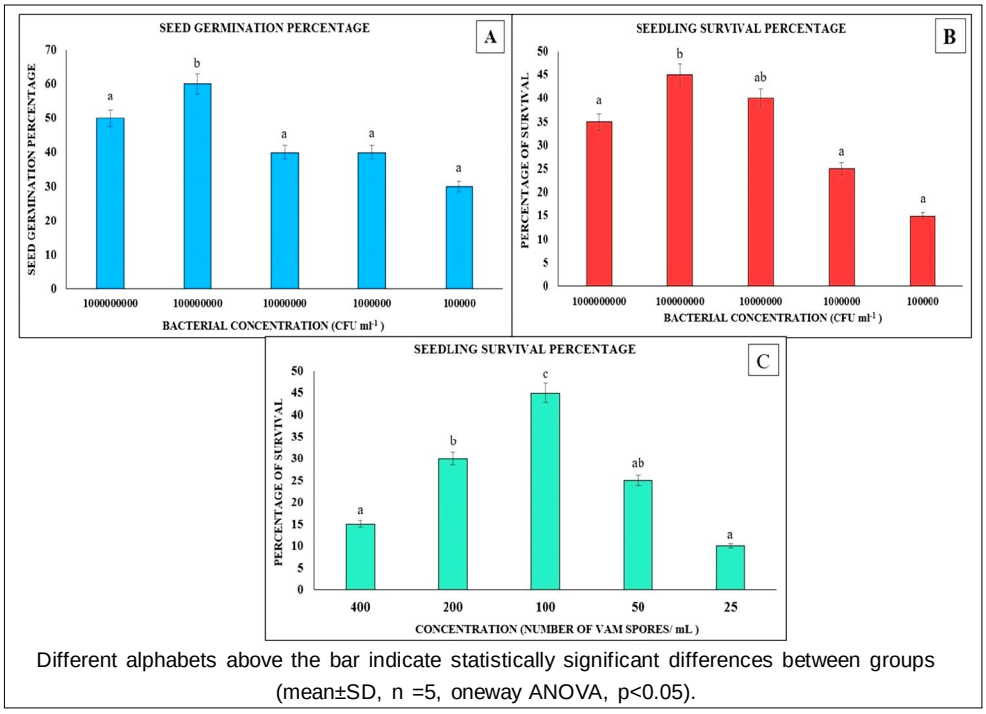
### Study of Physicochemical properties of soil

Different soil parameters were checked after treatment and found that the physicochemical properties of soil significantly increased in all the treated sets than that of uninoculated control set (Table 5). The soil, inoculated with VAM+ABM1 showed highest increase in available phosphorus, potassium and nitrogen than other experimental sets (Table 5). Gupta *et al*. (2012) found the simultaneous increase in soil available Phosphorus after application of Plant Growth Promoting Rhizobacteria in *Aloe barbadensis* Mill. Phosphate solubilizing rhizobacteria produces organic acids which improve Phosphate availability chemically and other growth substances hence stimulating plant growth (Uzma *et al*., 2022).

**Table 5:** Physicochemical properties of soil after treatment.

| Treatments | pH | EC (dS m$^{-1}$) | Available nitrogen (kg ha$^{-1}$) | Available phosphorus (kg ha$^{-1}$) | Available potassium (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| Control | 6.34±0.8$^{a}$ | 0.32±0.03$^{a}$ | 220.43±2.6$^{a}$ | 52.87±1.3$^{a}$ | 304.7±3.7$^{a}$ |
| VAM | 6.56±0.65$^{b}$ | 0.30±0.09$^{b}$ | 221.76±2.1$^{a}$ | 56.7±1.6$^{b}$ | 311.4±3.6$^{b}$ |
| ABB1 | 6.53±0.53$^{b}$ | 0.33±0.08$^{c}$ | 224.6±2.5$^{a}$ | 58.9±1.8$^{c}$ | 316.6±3.1$^{c}$ |
| ABM1 | 6.66±0.55$^{c}$ | 0.31±0.09$^{b}$ | 237.65±1.8$^{b}$ | 61.6±1.6$^{d}$ | 319.9±2.9$^{d}$ |
| ABM2 | 6.54±0.57$^{b}$ | 0.28±0.09$^{d}$ | 225.6±2.0$^{a}$ | 59.8±1.6$^{c}$ | 312.6±3.2$^{b}$ |
| VAM+ABB1 | 6.87±0.51$^{d}$ | 0.26±0.04$^{e}$ | 243.8±2.1$^{c}$ | 59.76±1.9$^{c}$ | 311.7±3.5$^{b}$ |
| VAM+ABM1 | 6.98±0.45$^{e}$ | 0.25±0.05$^{e}$ | 277.7±2.3$^{d}$ | 64.9±1.4$^{e}$ | 326.8±3.9$^{e}$ |
| VAM+ABM2 | 6.76±0.66$^{cd}$ | 0.27±0.03$^{d}$ | 265.6±1.9$^{cd}$ | 63.8±1.5$^{e}$ | 318.9±3.2$^{d}$ |

Values in the same column followed by different alphabets indicating significant difference at P≤0.05. The values represent the mean±SD (n = 5).




Different alphabets above the bar indicate statistically significant differences between groups (mean±SD, n =5, oneway ANOVA, p<0.05).

**Fig 10:** A): Seed germination percentage; B): Seedling survival percentage of the plant *Aloe barbadensis* Mill. after inoculation by different concentration of bacterial strains (ABB1, ABM1 and ABM2); C): Seedling survival percentage of the plant after inoculation by VAM spores.

### Morphological parameters of the plant

The results of the plant experiment showed that all the microbial inoculated sets considerably increased the morphological parameters like root length, leaf length, fresh root weight, fresh leaf weight, fresh gel weight and fresh rind weight of the plant *Aloe barbadensis* than uninoculated Control set (Table 6, 7). The overall morphological growth was found highest in the VAM+ABM1 treated set where the leaf length increased up to 137.9%, root length 158.19%, fresh leaf weight 184.12%, fresh gel weight 212.73% times than uninoculated control set. Among Unipartite interactions, the overall morphological growth was found highest in ABM1 treated set and lowest in VAM treated set (Table 6, 7). The same trend was noticed in case of dry leaf weight, dry gel weight and dry rind weight (Table 8). Pandey *et al.* (2009) reported augmentation of morphological growth was occurred in *Aloe barbadensis* Mill. after dual inoculation with *Glomus mosseae* and *Azotobacter*. Gupta *et al*. (2012) reported that after applying the phosphate solubilizing bacteria on the plant *Aloe barbadensis* Mill., the leaf length, root length and fresh gel weight of the plant increased by 39.5%, 31.1% and 143% respectively. Mamatha *et al*. (2002) also reported that increase in morphological parameters of *Morus alba* L. were noticed after inoculating with *Glomus fasciculatum* and *Bacillus coagulans*. Among unipartite sets, the highest growth was found in ABM1 treated set and lowest was found in VAM treated set. Vafadar *et al*. (2014) also reported that the morphological parameters of the plant *Stevia rebaudiana* increased more in *Azotobacter chroococcum* treated set from *Glomus intraradices* treated set.

**Table 6:** Root length, leaf length of the plants after inoculating with mycorrhizal fungi and bacterial strains.

| Treatments | Root length (cm) | Leaf length (cm) |
|---|---|---|
| Control | 12.2±0.3$^{a}$ | 24.5±0.6$^{a}$ |
| VAM | 14.6±0.6$^{b}$ | 26.7±0.9$^{b}$ |
| ABB1 | 15.5±0.4$^{b}$ | 27.4±0.8$^{b}$ |
| ABM1 | 18.2±0.5$^{c}$ | 32.6±0.8$^{c}$ |
| ABM2 | 16.3±0.5$^{b}$ | 28.6±1.5$^{b}$ |
| VAM+ABB1 | 17.7±0.3$^{c}$ | 31.1±0.5$^{c}$ |
| VAM+ABM1 | 19.3±0.8$^{d}$ | 33.8±0.7$^{d}$ |
| VAM+ABM2 | 18.5±0.5$^{c}$ | 32.2±1.3$^{c}$ |

Values in the same column followed by different alphabets indicating significant difference at P≤0.05. The values represent the mean± SD (n = 5).

In terms of mycorrhizal root colonization percentage (Fig 11), the highest root colonization by the VAM fungal species was found in VAM+ABM1 treated set, that is, mycorrhizal association within the roots become enhanced when we inoculated the plant roots with *Funneliformis mosseae* with *Bacillus tequilensis*. Mycorrhizal association was found lowest in VAM treated set.

### Estimation of biochemical parameters of the plant

In the present study, highest chlorophyll content was found in VAM+ABM1 treated set (Table 9). In the un-inoculated

**Table 7:** Fresh root weight, fresh leaf weight, fresh gel weight and fresh rind weight of the plants after inoculating with mycorrhizal fungi and bacterial strains.

| Treatments | Fresh root weight (g) | Fresh leaf weight (g) | Fresh gel weight (g) | Fresh rind weight (g) |
|---|---|---|---|---|
| Control | 8.17±0.23$^{a}$ | 109.76±1.7$^{a}$ | 45.87±0.8$^{a}$ | 62.5±0.7$^{a}$ |
| VAM | 8.87±0.21$^{b}$ | 116.7±2.2$^{b}$ | 53.43±0.6$^{b}$ | 61.65±0.9$^{b}$ |
| ABB1 | 9.76±0.29$^{c}$ | 134.9±2.5$^{c}$ | 61.56±0.9$^{c}$ | 71.54±1.2$^{c}$ |
| ABM1 | 11.54±0.32$^{d}$ | 176.7±2.3$^{d}$ | 81.67±1.2$^{d}$ | 90.53±1.5$^{d}$ |
| ABM2 | 10.11±0.66$^{c}$ | 143.7±2.9$^{c}$ | 65.89±1.2$^{c}$ | 75.45±1.3$^{c}$ |
| VAM+ABB1 | 12.71±0.57$^{d}$ | 156.9±2.1$^{cd}$ | 73.86±1.1$^{cd}$ | 80.56±1.4$^{c}$ |
| VAM+ABM1 | 14.01±0.86$^{e}$ | 202.1±3.6$^{e}$ | 97.58±1.7$^{e}$ | 101.92±2.1$^{e}$ |
| VAM+ABM2 | 13.45±0.81$^{d}$ | 187.6±2.7$^{de}$ | 88.52±1.1$^{de}$ | 95.45±1.8$^{d}$ |

Values in the same column followed by different letters indicating significant difference at P≤0.05. The values represent the mean±SD (n = 5).

**Table 8:** Dry root weight, dry leaf weight, dry gel weight and dry rind weight after inoculating with mycorrhizal fungi and bacterial strains.

| Treatments | Dry root weight (g) | Dry leaf weight (g) | Dry gel weight (g) | Dry rind weight (g) |
|---|---|---|---|---|
| Control | 0.04±0.002$^{a}$ | 1.87±0.06$^{a}$ | 0.65±0.01$^{a}$ | 1.07±0.02$^{a}$ |
| VAM | 0.07±0.002$^{b}$ | 1.95±0.06$^{b}$ | 0.73±0.02$^{b}$ | 1.21±0.03$^{b}$ |
| ABB1 | 0.09±0.006$^{b}$ | 1.98±0.07$^{b}$ | 0.76±0.02$^{b}$ | 1.20±0.05$^{b}$ |
| ABM1 | 0.11±0.004$^{c}$ | 2.23±0.08$^{c}$ | 0.92±0.01$^{c}$ | 1.28±0.06$^{c}$ |
| ABM2 | 0.10±0.005$^{bc}$ | 2.14±0.05$^{bc}$ | 0.87±0.009$^{bc}$ | 1.23±0.05$^{bc}$ |
| VAM+ABB1 | 0.11±0.008$^{c}$ | 2.08±0.09$^{bc}$ | 0.82±0.05$^{bc}$ | 1.24±0.07$^{bc}$ |
| VAM+ABM1 | 0.15±0.006$^{d}$ | 2.87±0.05$^{d}$ | 1.32±0.08$^{d}$ | 1.45±0.04$^{d}$ |
| VAM+ABM2 | 0.13±0.007$^{cd}$ | 2.51±0.09$^{cd}$ | 1.16±0.05$^{cd}$ | 1.28±0.08$^{cd}$ |

Values in the same column followed by different letters indicating significant difference at P≤0.05. The values represent the mean±SD (n = 5).

**Table 9:** Chlorophyll content, total carbohydrate and total protein content of the plants after inoculating with microbial strains.

| Treatments | 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 | 12.82±0.24$^{a}$ | 2.2±0.09$^{a}$ | 14.88±0.6$^{a}$ | 17.65±0.22$^{a}$ | 17.83±0.09$^{a}$ |
| VAM | 14.1±0.58$^{b}$ | 3.05±0.08$^{b}$ | 17.69±0.79$^{b}$ | 19.54±0.25$^{b}$ | 17.89±0.13$^{a}$ |
| ABB1 | 14.28±0.82$^{b}$ | 3.87±0.18$^{b}$ | 18.07±0.53$^{c}$ | 22.7±0.32$^{c}$ | 18.46±0.07$^{b}$ |
| ABM1 | 15.8±0.66$^{c}$ | 4.46±0.11$^{c}$ | 20.09±0.90$^{d}$ | 25.75±0.29$^{d}$ | 18.985±0.1$^{c}$ |
| ABM2 | 15.3±0.36$^{bc}$ | 4.18±0.2$^{bc}$ | 19.24±1.0$^{cd}$ | 24.98±0.34$^{cd}$ | 18.753±0.12$^{bc}$ |
| VAM+ABB1 | 17.7±0.75$^{d}$ | 6.44±0.17$^{d}$ | 23.74±0.7$^{e}$ | 27.87±0.23$^{e}$ | 19.283±0.18$^{d}$ |
| VAM+ABM1 | 20.44±0.98$^{e}$ | 7.08±0.16$^{e}$ | 26.09±0.86$^{f}$ | 28.98±0.24$^{f}$ | 20.636±0.14$^{e}$ |
| VAM+ABM2 | 18.34±0.79$^{de}$ | 6.68±0.16$^{d}$ | 25.94±0.92$^{ef}$ | 26.71±0.21$^{de}$ | 19.714±0.09$^{de}$ |

Values in the same column followed by different alphabets indicating significant difference at P≤0.05.
The values represent the mean±SD (n = 5).

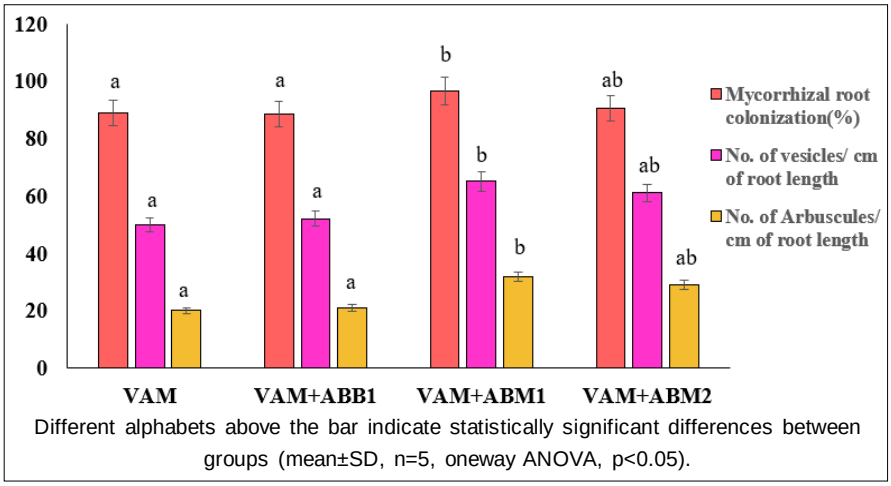



**Fig 11:** Mycorrhizal root colonization (%), number of vesicles and arbuscules per cm of root length (cm) of the plants, after inoculating with mycorrhizal fungi and bacterial strains.

control set, the chlorophyll-a, chlorophyll-b and total chlorophyll content were 12.82±0.24 mg $g^{-1}$ FW, 2.2±0.09 mg $g^{-1}$ FW and 14.88±0.6 mg $g^{-1}$ FW respectively (Table 9). But the chlorophyll content was significantly increased in all the treated sets than uninoculated control set. In the VAM+ABM1 treated set, the highest chlorophyll content was found. The chlorophyll-a, chlorophyll-b and total chlorophyll content were increased up to159.43%, 321.81% and 175.33% in VAM+ABM1 than uninoculated control set (Table 9).

In case of total Carbohydrate content of the plants, all the inoculated sets showed the highest carbohydrate content from that of control set (Table 9). The highest carbohydrate content was found in VAM+ABM1 treated set. In this treated set, the carbohydrate content of the plants increased upto 164.19% than uninoculated control set. The total Protein content in all the treated sets were significantly ($p<0.05$) increased than uninoculated control set (Table 9). The total protein was increased upto 115.73% in VAM+ABM1 treated set than control set. Khan *et al*.(2019) also reported that the PGPR+PGRs treatment produced significantly higher sugar content than the uninoculated set. The increase in sugar content of the treated sets helped to maintain a healthy photosynthetic system which demonstrates a significantly increased in morphological growth rate compared to the uninoculated control set.

## CONCLUSION

From our experiment, we found that the overall morphological growth of the plant was highest in the VAM+ABM1 treated set. In terms of mycorrhizal colonization percentage, it was highest in VAM+ABM1 treated set and was much lowered in VAM+ABB1 treated set. Moreover, after application of different bacterial strains with mycorrhizal fungi, the nutrient contents in soil were increased in all the treated sets than uninoculated set. In this study, the bacterial strain (ABM1) which was initially isolated from the spore wall of the VAM fungi proved to be quite beneficial and served the function of a potent Mycorrhiza Helper Bacteria (MHB) along with plant growth promotion. Thus, inoculation of *Aloe barbadensis* Mill. with VAM fungi (*Funneliformis mosseae*) in conjugation with Mycorrhiza Helper Bacteria (*Bacillus tequilensis*) proved to be quite beneficial and this microbial combination might be used as a potent biofertilizer in near

future. But intense study of this microbial combination is required to progress agricultural productivity in more sustainable ways.

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### Author contributions

Sikha Dutta provided the main concept of the work. Literature study and lab work were done by Shinjan Dey. Debapriya Choudhury and Chandrama Mukherjee helped in formatting. All authors read and approved the manuscript.

### Declaration

### Ethical approval

Authors declare this manuscript does not include any studies using animal and human beings.

### Consent to publication

All authors read and approved the final manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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