1. Introduction

Biological soil crust (BSC) is one of the major components of the sacred grove forests. The cyanobacterial species–rich in Biological soil crust, which is beneficial for fixing atmospheric nitrogen (N) in the soil, retain moisture, soil adherence and preventing erosion by extracellular polymorphic substances (Xie et al., 2007). These biological soil crusts have flourished in monsoon season and it becomes dryly wrinkled in the summer season. In living conditions, biological soil crusts have flourished in monsoon season and it

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Biological soil crusts (BSC) (Cyanobacteria) play an important role in the soil nitrogen fixation and soil stabilization. However, limited researches were carried out about the diversity and distribution of BSC in sacred forests. The study aims to identify the distribution of cyanobacteria in biological soil crusts from different sacred groves of Ariyalur and Pudukottai, Tamil Nadu. We identified following microbes of Microcoleus, Scytonema, Anabaena and Nostoc in biological soil crust. A surface experiment was conducted for the efficacy of biological soil crusts on crops seedling growths.

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Phosphorus solubilizing cyanobacteria play an important role in nitrogen mobilization and phosphorus solubilization for the benefit of plant growth (Adessi et al., 2018).

Most of the crop soil has a natural population of filamentous cyanobacteria members which provide favorable nutrient availability (Weber et al., 2015), and moisture for seed germination and plant growth. Abundance and diversity of cyanobacteria in sacred grove soil BSCs have been reported. These all features indicate that the biological soil crust directly involved in plant growth and spread the plant vegetation in sacred groves forest (Vinoth et al., 2017). The cyanobacteria dominated Biological soil crust chosen for the study had formerly shown many plant-growth-promoting hormones like indole 3-acetic acid, IAA; indole 3-propionic acid, IPA and indole 3-butyric acid, IBA. These BSCs cyanobacteria are good P-solubilizer and N-fixers in a live condition and rich amounts of nutrients consisted of dry nature (Obana et al., 2007).

Soil inoculant rich in cyanobacteria members significantly increases the yield of many crop plants in the cultivated field. The cyanobacterium in the form of biological soil crust is a good beneficiary for the growth of crop plant and soil restoration. An important genus such as Nostoc and Anabaena has been used as a biofertilizer for crop cultivation (Shariatmadari et al., 2013).

Vigna mungo (L.) (Black gram) belongs to the Fabaceae family is a chief pulse crop cultivated in more than 50 countries. Black gram has a high amount of proteins, potassium, calcium and amino acids. Black gram has amino acids found in cereals which play a vital role in the diets of Nepalese and Indian people (Nitin et al., 2012). India is the biggest black gram, making country, but few works have been recognized. The 0.5 L of the BSC cyanobacteria sample was taken and transferred to the sterile testing vials sealed with rubber cork.

The percentage of air in the head phase of each vial was exchanged with pure acetylene gas (99.8%) using an airtight syringe. Then the vials were kept for 30 min to allow the reduction of acetylene gas to ethylene. After 30 min a 5 µL gas mixture was aspirated for gas chromatography analysis (Turner and Gibson, 1980).

2.4. Biological soil crusts as biofertilizer

Plastic pots of uniform size (height-10 cm, dia-10 cm) were filled with the required amount of garden soil for plant growth. A uniform and known quantity of powdered biological soil crusts were added to the surface of the soil in the pots. Pots without biological soil crusts served as control. All the pots were sprayed with water regularly for three days. On the fourth day, 20 healthy seeds of V. mungo, were sown in each pot. The seed was procured from the Tamil Nadu, Government Agriculture Department, Tiruchirappalli, India. The pots were kept in the garden and watered regularly. Plant growth parameters were analyzed on the 14th day after sowing seeds.

2.5. Seed germination

The percentage of seed germination was analyzed in triplicates of 100 seeds using pot culture as defined in ISTA guidelines. During the germination test was performed at atmospheric temperature. In the germination test, germination counts were documented for normal seedlings on the fifth and the eighth day. The percentage of seed germination (G%) was determined by the following formula.

\[ G\% = \frac{100 \times A}{N} \]

\( A = \) Number of seeds found germinated  
\( N = \) Total number of seeds used in the germination test.

2.6. Root length of seedlings

The length of the main root measurement was taken from root tip to stem of the plant at last harvest point, in centimeters (cm).

2.7. Shoot length of seedlings

The length of the main stem measurement was taken from shoot collar to tip of the plant at last harvest point, in centimeters (cm).

2.8. Seedling fresh weight

After harvest, the fresh weight of V. mungo (Shoots and roots) was evaluated in a gram (g).

2.9. Seedling dry weight

After harvest, the dry weight of V. mungo (Shoots and roots) was evaluated in gram (g) at 70 °C for 48 h after drying in an oven until a constant weight was reached.

2.10. Seed vigor index

The seed vigor index was studied using the succeeding formula.

\[ SVI = \text{Germination percentage} \times \text{Seedling length} \]

2.11. Absolute growth rate (AGR)

AGR was calculated by using the below-mentioned formula

\[ AGR = \frac{h_2 - h_1}{t_2 - t_1} \text{cm day}^{-1} \]

Where that \( h_1 \) and \( h_2 \) are the height of the plant at the time of \( t_1 \) and \( t_2 \).
2.12. Relative growth rate (RGR)

RGR was calculated by using the formula given below

\[ RGR = \frac{W_2 - W_1}{t_2 - t_1} \text{gg}^{-1}\text{d}^{-1} \]

Where that W1 and W2 are the dry weight of whole-plant at the time of t1 and t2 respectively.

2.13. Estimation of biochemical parameters of plants

The biochemical parameter such as total chlorophyll (Mackinney, 1941), sugar (Sadasivam and Manickam, 1996) and protein (Lowry et al., 1951) were analyzed.

2.14. Statistical analysis

The statistical analysis was performed with the SPSS-16 version. The outcomes are presented as mean ± SE (Standard error) and data from the different biological soil crust inoculant and control were related by Duncan’s multiple range test (p < 0.05). Graphs were plotted using Microsoft Office Excel 2010 to represent morphological and biochemical parameters.

3. Results

Sacred grove forests are the most important biome, which has been conserved the plants for many years. Sacred grove forests are protected ecosystems that signify a promising site for the evolution of numerous microorganisms like cyanobacteria in the form of biological soil crust.

In the present study, typical soil samples were obtained from the sacred grove forest in the Ariyalur and Pudukottai area of Tamil Nadu. In total, 3 types of biological soil crust were observed from the selected sacred grove forest (Fig. 1).

3.1. Total species inventory of cyanobacteria in BSC from Ariyalur district

A maximum of 79 species of cyanobacteria representing 13 genera was identified in the BSC collected from the sacred groves of Ariyalur, in which three species were members of Chroococcaceae, 42 Oscillatoriaceae, seven Nostocaceae, and 25 Scytonemtaceae. A total of 72 species of cyanobacteria representing 18 genera was identified in the BSCs from Pudukkotai district sacred groves, of which eight species belonged to the members of Chroococcaceae, 48 Oscillatoriaceae, three Microchaetaceae, four Nostocaceae, eight Scytonemtaceae and one Stigonemataceae (Fig. 2 & Table 1).

Table 1 shows the cyanobacteria genera in BSC collected from Ariyalur and Pudukottai district, Tamil Nadu.

The efficacy of the Biological soil crust as bioinoculant for V. mungo plant growth was tried. Comparative analysis disclosed that there is a remarkable variance between inoculated pot and controls pot, mainly in morphological as well as biological parameters such as total chlorophyll, protein, and carbohydrate of V. mungo.

![Image](image_url)

Fig. 1. Appearance of biological soil crusts in sacred groves forest. A) Blackish crusts in the dry soil of Ariyalur, B) Blackish crusts in the dry soil of Pudukkotai, C) Green coloured crusts in the soil of Ariyalur, D) Green coloured crusts in the soil of Pudukkotai, E) Blackish-brown crusts in the soil of Ariyalur, F) Blackish-brown crusts in the soil of Pudukkotai.)
3.2. Plant growth promoters in BSCs

The HPLC chromatograms of biological soil crust samples raised in optimal conditions are shown in Fig. 3A. The fluorescence detector equipped HPLC revealed the existence of three endogenous auxins comprising IAA, IBA, and IPA in biological soil crusts collected both from Ariyalur and Pudukkottai districts. The HPLC chromatogram for the standard auxin is shown in Fig. 3B.

3.3. Acetylene reduction assay

Nitrogenase activity was higher in BSCs obtained from the Pudukkottai district and lesser in Ariyalur district. The value of nitrogenase activity was 55.007 nmol. ml⁻¹h⁻¹ in BSCs from Pudukkottai and 45.020 nmol. ml⁻¹h⁻¹ in Ariyalur (Fig. 4).

3.4. Effect of biological soil crusts on plant growth

The comparative analysis showed that there is a significant difference between biological soil crust inoculated pot culture and controls, exclusively in vegetative growth features (Fig. 5). A twofold increase in plant root length over the control documented in *V. mungo* inoculated with biological soil crusts collected both from Ariyalur and Pudukkottai districts (Fig. 6.1 A). However, a small development in shoot size than the control was noticed in *V. mungo* inoculated with biological soil crusts from Ariyalur but, the shoot length of *V. mungo* inoculated with the BSC from Pudukkottai district was on par with the control (Fig. 6.1B).

Seedling fresh weight (Fig. 6.2A) and dry weight (Fig. 6.2B) improved by twofold over the control in *V. mungo* inoculated with biological soil crusts of Ariyalur and Pudukkottai district. The seedling fresh weight and dry weight of *V. mungo* on the 14th day of inoculation are shown in Fig. 6.2C. The seedling fresh weight and dry weight of *V. mungo* inoculated with biological soil crusts from Ariyalur and Pudukkottai districts were significantly higher than the control (Fig. 6.2C).
day as affected by BSC from Ariyalur (A) and Pudukkottai (B) districts.

The absolute growth rate increased by more than three times over the control in *V. mungo* inoculated with BSCs collected from Ariyalur sacred forest while a significant increase in the absolute growth rate over the control was recorded with BSCs from Pudukkottai sacred grooves (Fig. 6.3, A). The relative growth rate increased by three times over the control in *V. mungo* inoculated...
with BSCs collected from both Ariyalur and Pudukkottai district sacred forests (Fig. 6.3.B).

The seedling vigor index increased threefold and twofold over the control in *V. mungo* inoculated respectively, with biological soil crusts collected from Ariyalur and Pudukkottai district (Fig. 6.3.C). Chlorophyll *a* content got doubled over the control in *V. mungo* inoculated with biological soil crusts collected from Ariyalur while, chlorophyll *b* and total chlorophyll showed a slight increase over the respective controls (Fig. 6.4.A).

Similarly, total protein content revealed a slight increase over the control as seen with chl. *b* and total chlorophyll, while the total sugar content exhibited a twofold increase over the control in *V. mungo* inoculated with biological soil crusts from Ariyalur (Fig. 6.4.A). In the case of Pudukkottai, *V. mungo* inoculated with biological crusts recorded a little increase of Chl. *a* content over the control. Total chlorophyll and chl. *b* in biological crust inoculated seedlings were more or less similar to their respective controls (Fig. 6.4.B) whereas the total sugar content increased by more than two times over the control (Fig. 6.4.B).

Comparative analysis displayed that there is a substantial difference between BSC's inoculated plants and control. The vegetative growth parameters, especially the length of the plant and its weight were analyzed. A progressive effect of PGP (Plant growth promoters) on plant weight and the length was previously reported (Zahir et al., 2000). The outcomes of the present study disclosed that the growth parameters, for example, root length and

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### 4. Discussion

There are some rare reports about the study of biological soil crust of cyanobacteria in sacred groves forest Ariyalur and Pudukkottai area of Tamil Nadu, India. Though *V. mungo* is the chief crop it was cultivated in the country. Representative soil samples were collected from sacred groves forest cultivation.

Among the biological soil crust collected, three types of crust (Blackish, Greenish, and Pinkish) were documented. Blackish and Pinkish color biological soil crust is found to be the dominant type in most sacred grove forests, whereas greenish were limited to sacred grove forests of Ariyalur and Pudukottai. Parallel results were stated by Vinoth et al. (2017) in the sacred grove forest of Ariyalur and Pudukottai, India. Soil crust cyanobacteria affected by solar radiation in drought conditions and plain soil produces photo protecting pigments (Vinoth et al., 2017). The black and pink soil crust in the sacred forest is due to the abundance of UV absorbing compounds Mycosporine-like amino acids and Scytonemin (Catzenholz and Garcia-Pichel, 2000). This cyanobacterial crust has an impact on the growth of plants by releasing plant growth hormones and promoting the fertility of the soil.

Further, this study, disclosed that there is a substantial difference between the BSC's inoculated plants and control. The vegetative growth parameters, especially the length of the plant and its weight were analyzed. A progressive effect of PGP (Plant growth promoters) on plant weight and the length was previously reported (Zahir et al., 2000). The outcomes of the present study disclosed that the growth parameters, for example, root length and
weight, enlarged considerably, which increase uptake of nutrients and water from Biological soil crust. In the present study, biological soil crusts analyzed for the presence of growth-promoting substances with HPLC showed the presence of phytohormones such as IAA, IBA and IPA. Auxins are generally used to initiate root, particularly IBA, used as plant hormones for induction of root formation. Previously, multiple plant seedlings with IBA reported an increase in lateral root development (Khavar and Özcan, 2002; Mobli and Baninasab, 2009). Li et al. (2009) have also shown that phytohormones in rice seedlings can stimulate absorption and root elongation. Our results showed that improved rooting can be influenced by plant hormones like IAA and IBA.

Estimation of the BSC’s N fixation rate revealed that these heterocystous cyanobacteria were capable of fixing atmospheric N in natural conditions which affect the plant growth (Nilsson et al., 2002). Nitrogenase activity was more in the biological crusts collected from the sacred groves of Pudukkottai, despite its lower diversity of heterocystous cyanobacteria than in Ariyalur. The increased acetylene reduction assay activity of Pudukkottai samples would probably be due to bacterial fixers.

To understand the possibility of biological soil crusts as biofertilizer (soil inoculants); the present study was performed with V. mungo. The plant growth parameters such as shoot and root length, fresh and dry weight of seeding, absolute growth rate, relative growth rate, seedling vigor index and biochemical contents such as chlorophyll, protein and sugar were more in V. mungo inoculated with biological soil crusts than their respective controls. This study is supported by the findings of Shariatmadari et al. (2013).

The comparative analysis of pot experiments exhibited a significant difference in morphological and biochemical growth factors, especially root growth parameters between the crusts inoculated pot and controls. Similarly a study by Thiet et al. (2014) revealed that algal biocrusts significantly influenced the growth of Deschampsia flexuosa (height 28.95 cm, vigor 5.29 cm and Morella pensylvanica (height 22.61 cm, vigor 18.36 cm) plants. When compared to controls, algal biocrusts and moss mats tended to increase

Fig. 6.3. Changes in the absolute growth rate (A), relative growth rate (B) and seedling vigor index (C) in Vigna mungo on the 14th day as influenced by BSC from Ariyalur and Pudukkottai districts.
Effect of biological soil crust on the growth of vegetable plants (Mean ± SE) (Pan et al., 1999; Zahiret al., 2000). The results of the current study showed that plants grown under biological soil crusts exhibited the morphological and biochemical parameters in plants grown under control. The means followed by the same letters within a column are not significantly different at p ≤ 0.05. BSC = Biological soil crust.

Table 2
Effect of biological soil crust on the growth of vegetable plants (Mean ± SE).

| Growth parameter | Control | BSC of Ariyalur | BSC of Pudukkottai |
|------------------|---------|-----------------|------------------|
| Shoot length (cm) | 5.5 ± 0.11<sup>a</sup> | 7.33 ± 0.11<sup>a</sup> | 5.86 ± 0.10<sup>b</sup> |
| Root length (cm) | 3.43 ± 0.12<sup>a</sup> | 6.8 ± 0.05<sup>a</sup> | 6.74 ± 0.20<sup>b</sup> |
| Fresh weight (g) | 0.39 ± 0.00<sup>a</sup> | 0.79 ± 0.03<sup>a</sup> | 0.73 ± 0.04<sup>a</sup> |
| Dry weight (g) | 0.04 ± 0.01<sup>a</sup> | 0.09 ± 0.00<sup>a</sup> | 0.1 ± 0.00<sup>b</sup> |
| Chlorophyll a (mg/g) | 10.7 ± 0.01<sup>a</sup> | 19.9 ± 0.00<sup>a</sup> | 14.6 ± 0.03<sup>b</sup> |
| Chlorophyll b (mg/g) | 7.05 ± 0.01<sup>a</sup> | 8.64 ± 0.01<sup>a</sup> | 8.35 ± 0.01<sup>b</sup> |
| Total chlorophyll (mg/g) | 17.8 ± 0.01<sup>a</sup> | 26.6 ± 0.01<sup>a</sup> | 17.0 ± 0.01<sup>c</sup> |
| Total protein (mg/g) | 5.93 ± 0.00<sup>a</sup> | 6.97 ± 0.01<sup>a</sup> | 6.63 ± 0.00<sup>b</sup> |
| Total sugar (mg/g) | 1.03 ± 0.00<sup>a</sup> | 2.56 ± 0.00<sup>a</sup> | 2.24 ± 0.00<sup>b</sup> |

Means followed by the same letters within a column are not significantly different at p ≤ 0.05. BSC = Biological soil crust.

Fig. 6.4. Changes in biochemical contents in Vigna mungo (L) on the 14th day as affected by BSC from Ariyalur (A) and Pudukkottai (B) districts.

5. Conclusion

The biological soil crust had numerous cyanobacteria such as unicellular, heterocystous and non-heterocystous forms. These biological soil crust cyanobacteria act as mediators for the incorporation of organic carbon and nitrogen through PGR secretion and nitrogenase activity, which increases nutrient availability and sustains the soil fertility. The BSC inoculated soil increases the V. mungo plant growth. These results proved that an ecosystem rich with biological soil crusts showed an enhanced population of cyanobacterial species which in turn increased the growth of floral species in the soil.

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