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Fungal-bacterial biofilm mediated solubilization of Eppawala Rock Phosphate (ERP) and its effect on crop enhancement of chili pepper (*Capsicum annuum*)

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Abstract

Eppawala rock phosphate (ERP) has potential as a substitute for Triple Super Phosphate (TSP) due to its phosphorus content, but its low solubility limits its application. Recent research indicates that fungal- bacterial biofilms could enhance ERP's effectiveness by enhancing its solubility. This study evaluated the effectiveness of fungal-bacterial biofilms (FBBs) on ERP for their solubilization efficiency by releasing phosphorus and their impact on crop enhancement of chili pepper (*Capsicum annuum*). Fungi and bacteria were isolated from soil samples, screened for phosphate solubilization and developed FBBs, which were then tested for their phosphate-solubilizing ability by treating the homogenized powdered ERP with FBBs. The best FBB was evaluated for its phosphate-solubilization efficiency and the impact on crop enhancement of chili pepper with different chemical fertilizer (CF) combinations using a pot experiment. The best biofilm, BF3, composed of *Brevibacillus brevis* and *Penicillium polonicum*, showed significantly greater phosphate solubilization capacity ($P < 0.05$), and the highest medium acidity. Fourier Transform Infrared Spectrophotometer (FTIR) analysis of the biofilm-treated and untreated ERP samples showed significant changes in the intensity and positions of the phosphate bands, confirming the phosphate solubilization by the biofilms. Combining FBBs with ERP, especially when pretreated, significantly ($P < 0.05$) enhanced chlorophyll content, fresh weight and number of fruits of chili pepper plants. Biofilm enriched ERP performs better compared to the recommended TSP dosage in chili pepper cultivation under controlled conditions.

Key words: Fungal- bacterial biofilms, Growth promotion, Phosphate fertilizers, Solubilization

1. Introduction

Phosphorus is a critical nutrient for agricultural production, often added to soil through phosphate fertilizers. A part of which is utilized by plants and the rest is rapidly converted into insoluble complexes in the soil [1]. Triple Superphosphate (TSP) is commonly used in vegetable cultivation due to its high solubility, benefiting short-term, fast-growing crops. However, the extensive use of TSP and other chemical fertilizers (CF) has led to significant environmental and health issues. These include soil and water contamination with heavy metals such as Aluminum (Al), Chromium (Cr), Nickel (Ni), Cadmium (Cd), Lead (Pb), and Arsenic (As) [2].

Eppawala Rock Phosphate (ERP) is a less expensive and more ecologically friendly alternative to TSP. Despite its potential benefits, ERP has intrinsic limitations, including limited phosphorus solubility, which hinders its direct usage as a Phosphorus fertilizer, particularly for short-term crops [3]. Numerous studies have been conducted over the years to improve phosphorus solubility in rock phosphates, but these efforts have had limited success [4]. Even though there are chemical techniques to increase the solubility of ERP, biological procedures such as microbial activities are more cost-effective and ecologically friendly than chemical processes for increasing the water-soluble phosphate content of Rock Phosphate. However, microbial phosphorus solubilization is a slow process that cannot enhance the required level of available phosphate in soil within a short period of time, especially for short-term cultivations. According to [5], biofilms are thought to be a potential solution in this scenario since they can quickly convert insoluble phosphorous into a soluble form.

Biofilms are structured microbial communities that adhere to surfaces and are encapsulated within extracellular polymeric substances (EPS) [6]. These biofilms can be engineered using biofilm inoculants, which are composed of two different microorganisms. Typically, one microorganism, often a type of bacteria, colonizes over another microorganism, which can be either bacteria or fungi. The second microorganism provides a biotic surface for the first, enabling the formation of a metabolically enhanced biofilm compared to a single-culture-biofilm. Such biofilm inoculants have significant agricultural applications. They can secrete organic acids such as citric acid, gluconic acid, and oxalic acid by the biofilm-forming microbes, which lower the pH in the vicinity of the rock phosphate, leading to the dissolution of phosphorus [7]. This

conversion makes phosphorus more accessible for plant uptake, enhancing plant growth and nutrient acquisition [8].

The density of biofilms in soil is typically low, yet they exhibit high phosphate solubilization capabilities and promote plant growth [5]. Consequently, utilizing these biofilms as biofertilizer inoculants through artificial formulation can be highly beneficial for agricultural applications [7]. These inoculants can be applied to soil or directly to rock phosphate materials. Field trials have shown that biofilm-forming microbial inoculants can improve crop yields by increasing phosphorus availability from enhanced ERP [9]. This strategy not only enhances the efficiency of ERP as a fertilizer but also reduces the reliance on CF, thus mitigate related environmental pollution. The study focuses on the effective utilization of ERP to replace TSP by enhancing its solubility through the action of fungal-bacterial biofilms and examining their impact on the growth of chili pepper (*Capsicum annuum*). This biotechnological approach leverages the natural capabilities of microorganisms to improve soil fertility and plant health, contributing to sustainable agricultural practices.

2. Materials and Methods

2.1 Materials

Soil samples and ERP samples were collected from Eppawala (E080.172', N80.405') Sri Lanka. Nutrient agar (NA) and czapek dox agar (CZA) were used as the initial isolation media for bacteria and fungi respectively. Pikovskayas (PVK) medium was used to isolate phosphate solubilizing microorganisms. Yeast mannitol broth (YMB) was used to prepare biofilms.

2.2 Isolation of microorganisms and preparation of pure cultures

The soil samples were serially diluted with sterilized distilled water (10-fold) and 100 µL of each "dilution" was plated on NA and CZA amended with sterile gentamycin (50 mg mL^{-1}) respectively. Plates were incubated at 30 °C for 24 h, prepared pure cultures and they were stored at 4 °C until being used. The microscopic and macroscopic characterization (hyphae and spores) were done for the initial identification.

2.3 Preparation and characterization of ERP samples

The collected ERP stones were washed, dried at room temperature, and crushed using an electric crusher. The crushed ERP was sieved with an electric shaker to have particle sizes of <63 µm. The ERP sample with the particle size of <63 µm was milled using a ball mill at 800 rpm for 30 hours to reduce the particle size further and the particle sizes of ball milled ERP samples were analyzed using the particle size analyzer (Beckman Counter LS 13320). Fourier Transform Infrared Spectrophotometer (FT-IR, Bruker-alpha)) spectrum for the ERP sample with reduced particle size were obtained by the ATR method.

2.4 Screening for the phosphate solubilizing microorganisms

The isolated bacteria and fungi were cultured on PVK agar plates using spot culture method and tested for phosphate solubilization capacity by observing clear phosphate solubilizing zones surrounding colonies after 5-10 days of incubation at 30°C. On the seventh day, the bacterial and fungal colonies and halo zone diameters were measured and used to calculate the Phosphate Solubilization Index (PSI), which is the ratio of the overall diameter to the colony diameter [10]. Measurements were taken in triplicate and the average values were reported.

$$\text{Solubilization Index} = \frac{(\text{Colony diameter} + \text{Halozone diameter})}{\text{Colony diameter}}$$

The selected bacteria and fungi that solubilized phosphate were cultured in 100 mL PVK broth medium in triplicates, and they were then incubated for ten days at room temperature (25 °C ± 2 °C) using a shaking incubator running at 125 rpm. Following incubation, 5 mL of sample from each flask was taken and centrifuged for 30 minutes at 4000 rpm in 15 mL centrifuge tubes. Filter papers were used to collect and filter the supernatant. The pH of the filtrates was measured using a portable pH meter. The amount of the orthophosphate released in filtrates was determined spectrophotometrically at 880 nm according to the standard molybdenum blue method. The working standards of 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0 ppm were prepared by making a 100-ppm stock solution of Potassium dihydrogen phosphate (KH_2PO_4), and the solubilization of phosphate was quantified by matching the absorption of standards with the absorption of samples.

2.5 Effective biofilm formation

To generate biofilms, bacterial and fungal isolates with the highest phosphate-solubilizing ability were combined in permutation according to previous study technique [11]. In brief, bacterial and fungal isolates were cultured in YMB without agar separately and incubated for seven days at room temperature (25 °C ± 2 °C). On day seven, bacteria and fungus were mixed into a single culture and incubated for fourteen days at room temperature (25 °C ± 2 °C) using a shaking incubator. During the incubation, the attachment of bacterial cells to fungal filaments was continuously observed using an optical microscope (model BX43F). The biofilms in which bacterial cells become adhered to fungal filaments were chosen to investigate phosphate solubilization effectiveness in a liquid media.

2.6 Determination of phosphate solubilization by the action of biofilms on ERP

The phosphate solubilization of the selected biofilms were evaluated with ERP sample with reduced particle sizes. The selected biofilms were inoculated in a 100 mL YMB media amended with 1 g of ERP powder in triplicates. The flasks were incubated at room temperature in a laboratory shaker at 125 rpm for 14 days. The individual cultures of the biofilm forming fungi and bacteria were prepared for comparison. The released ortho-phosphate in filtrates which were collected after the centrifugation of the samples, was estimated using a UV spectrophotometer at 880 nm. The pH of the filtrates was measured with a portable pH meter.

2.7 Molecular identification of the biofilm components showing the highest phosphate solubilization

Genomic DNA of bacterial isolates was extracted using the ZR Bacterial DNA KitTM per the manufacturer's instructions (Zymo Research California USA). Using the universal primers AGAGTTTGATCCTGGCTCAG 3′ for the forward primer and GGTTACCTTGTTACGACT 3′ for the reverse, the 16S rRNA was amplified using polymerase chain reaction (PCR) [12]. Fungal isolates' genomic DNA was extracted using the protocol outlined in a prior work [13]. Fungal DNA was amplified using ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [14], and sequencing was carried out for the polymerase chain reaction (PCR) purified products. The amplified products were sent for sequencing to the Macrogen Sequencing facility in Korea, and the sequences were compared using pairwise alignment with the sequence of reference strains using the NCBI BLAST software.

2.8 SEM characterization of ERP samples

The SEM (JEOL, JX-8100) images for the ERP powder before and after the treatment with biofilm were taken to study the characteristic changes in surface morphology.

2.9 Pot experiment using chili pepper

A greenhouse pot experiment was carried out in *Agriculture Research Center, Bibile, Sri Lanka.* The good quality chili seeds were used to make a nursery for 2 to 3 weeks prior to the plantation. The required amount of ERP powder with reduced particle size was prepared for the pot experiment. A part of the ERP powder was treated with the effective biofilm and with their individual cultures in YMB media for 10 days in a laboratory shaker at the speed of 125 rpm. A required volume of pure biofilm and the selected individual cultures were prepared for the pot trials. The 8 kg pots were prepared with the addition of recommended amounts of basal CF mixture (Urea and Muriate of Potash) for the pot experiment with chili (36 pots) using the soil collected from the relevant field lands. Total of 12 treatments were carried out including the control experiment with 5 replicates in each. The selected treatments were; F3 fungal culture only (T1), B2 bacterial culture only (T2), biofilm only (T3), ERP with F3 (T4), ERP with B2 (T5), ERP with biofilm (T6), ERP- pretreated F3 (T7), ERP-pretreated B2 (T8), ERP-pretreated biofilm (T9), phosphorous as ERP (T10), phosphorous as TSP (T11), Control- no treatment (T12).
The selected healthy chili plantlets were used for pot trials. Three plantlets were planted in each pot. The recommended dosage of basal media (Urea and MOP) was applied for all pots before planting. 200 mL of 10-fold diluted biofilm and individual cultures were applied for the relevant treatments (T1- T6). Further, biofilm and individual cultures treated ERP were applied for the treatments T7- T9. The pots were maintained well with good water supply, and proper fertilizer and pesticide application. The pots were monitored regularly for growth parameters. During the study, the maximum temperature inside the greenhouse was about 34 °C and the minimum was 23 °C. The fruits were harvested after three months and the parameters such as average fresh weight of fruit (FW) per plant, the average number of fruits (NF) per plant, average length of the plant at day 60 from planting the plantlets (LP) and average chlorophyll content (CH) of 5th leaf from the apical bud of the plant at day 30 from planting the plantlets were obtained.

2.10 Data analysis

Pot experiment was done in five replications. The statistical data analysis was performed on all collected data using the Analysis of Variance (Two-way ANOVA) model in Minitab 17 Statistical Software. The mean values of plant parameters were compared on a treatment basis using Tukey´s simultaneous test at a 95% confidence interval. To determine the correlations, Pearson´s correlation coefficients were computed for the selected variables. Sigma Plot 10.0 was used to plot bar graphs.

3. Results and discussion

3.1 Isolation and screening of phosphate solubilizing microorganisms

Initially twelve bacteria and eight fungi were isolated from the ERP and soil samples. The bacteria and fungi producing a clear halo-zone with a solubilizing index (SI) greater than 1.5 were identified as phosphate-solubilizing microorganisms [15]. Out of all the isolates, eight bacterial isolates (B1-B8) and five fungal isolates (F1-F5) were identified as phosphate solubilizing microorganisms since they showed higher SI values. Phosphate solubilization capacity of the isolated bacteria and fungi was measured in terms of generated orthophosphate concentration and graphically visualized the Figure 1. Out of all the bacterial isolates significantly higher ($P < 0.05$) phosphate solubilization capacities were recorded in bacterial isolates B8 and B2 (Fig. 1 a). However, significant difference was not observed between bacterial isolate B8 and B2 in phosphate solubilization capacity. Bacterial isolates B4 and B5 showed moderate phosphate solubilization capacity. Out

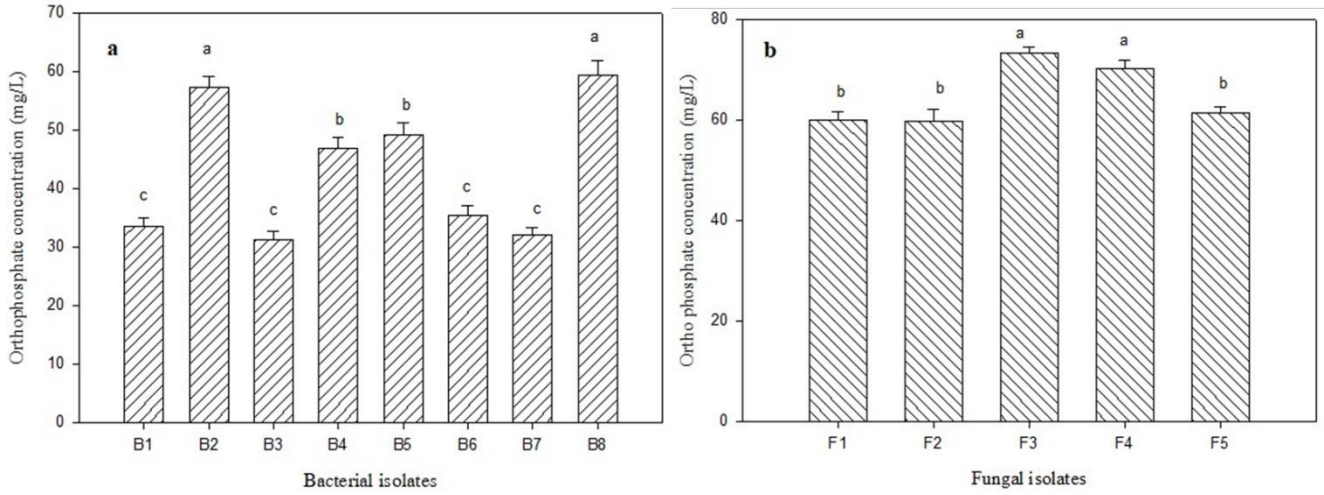


Fig. 1 – Generated orthophosphate concentration in determining the phosphate solubilization capacity of the a) bacterial and b) fungal isolates

of all fungal isolates significantly the highest ($P < 0.05$) phosphate solubilization capacities were recorded in fungal isolates F3 and F4 (Fig. 1 b) and the highest was recorded in F3. However, significant difference was not observed between the aforementioned two fungal isolates in phosphate solubilization capacity. It was observed that the generated orthophosphate concentration in all fungal isolates were higher than that of bacterial isolates. This confirms that the phosphate solubilization capacity of the isolated fungi were higher than the bacterial isolates. Soil microbes are essential to several functional activities in the soil, such as the decomposition of organic matter, soil turnover, and Phosphate release [16]. The majority of the Phosphate that plants take up in natural terrestrial plant environments comes from phosphate-solubilizing microorganisms (PSM). Microbial enzymes are in charge of the mineralization and hydrolysis of organic Phosphate, whereas low molecular weight organic acids or protons are released during the dissolution (mobilization) of inorganic Phosphate by PSM [17]. PSM are highly metabolically active organisms that often accumulate near the rhizosphere of plants due to the availability of nutrients generated by root exudates.

Phosphate-solubilizing bacteria constitute 1–50% of soil PSM, while phosphate-solubilizing fungi make up 0.1–0.5% [18]. The most prevalent bacterial genera include *Bacillus* and *Pseudomonas*, and the notable fungal genera are *Penicillium* and *Aspergillus*. The population of the phosphate solubilizing bacteria is influenced by the chemical and physical characteristics of the soil, the amount of phosphate present, and the presence of organic matter [19]. PSM isolation is necessary for the long-term use of phosphates. The National Botanical Research Institute Phosphate (NBRIP) medium and PVK medium are the two main culture media that are currently being utilized for the isolation procedure [18]. PSM solubilize phosphate surrounding their colonies to generate a clean zone, or halo zone, which reflects their primary role [7]. To obtain pure solitary microbial colonies, isolated halo zone producing microorganisms can be selected and purified on solid media for many rounds [20]. According the current study, the fungal (F3 and F4) and bacterial isolates (B2, B4, B5 and B8) showing the highest and the moderate phosphate solubilization capacity were selected to develop biofilms.

3.2 Formation of microbial consortia

The fungal filaments' surface allowed bacterial cells to accommodate (Fig. 2a and b). The degree to which the bacteria adhered to the fungal filament surface differed depending on the type of microbial partner. The development of biofilm with any other bacterial isolate was not aided by the fungal isolates F1 and F5. Attachments were seen in FBBs with four bipartite and one tripartite association compared to other FBBs. The combination of *Penicillium polonicum* (F3) and *Brevibacillus brevis* (B2) exhibited the highest attachment strength (Fig. 2). Entangled, connected bacterial cells were seen in the FBB of *B. brevis* with *P. polonicum*, indicating compacted, thick fungal mycelial entrapment. SEM revealed that the extracellular matrix of the biofilm arranged around the *P. polonicum* hyphae and bacterial cells attached to them.

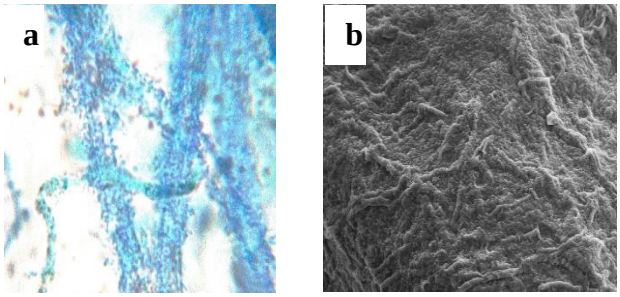


Fig. 2- Colonization of *Brevibacillus brevis* on *Penicillium polonicum* mycelium in the biofilm. Colonization in consortia a) at x 400 magnification with optical microscope. Darkness (x) is due to lactophenol cotton blue stain absorbed by EPS produced by the consortia. B) SEM images for the colonization in consortia (Scale bar = 1 µm,, Magnification = 5 KX).

3.3 Quantitative determination of solubilized phosphate by microbial treatments and the changes in medium pH

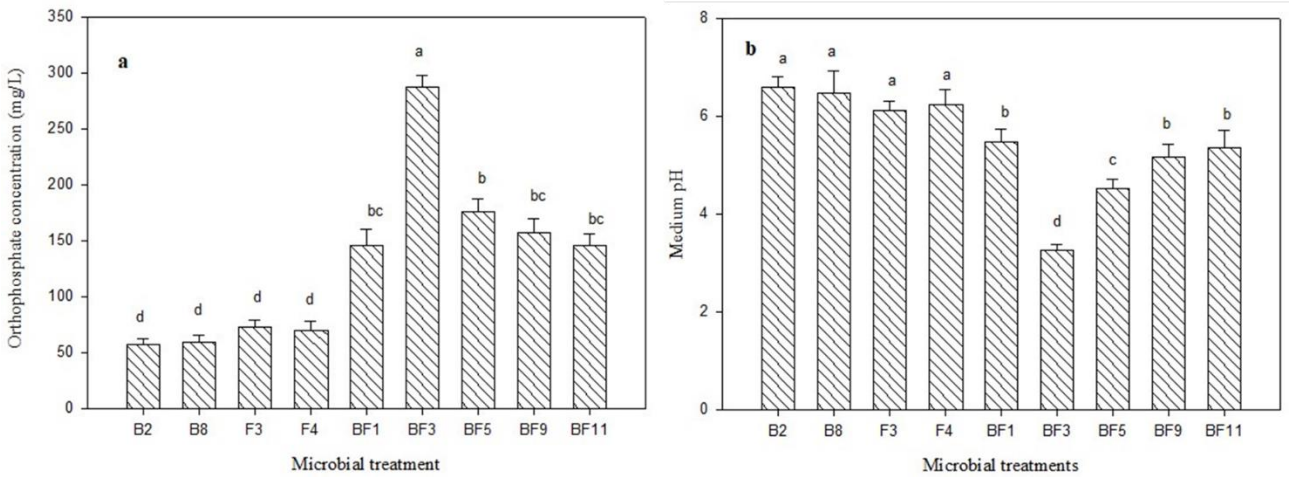


Fig . 3-Microbial responses on orthophosphate concentration and pH reduction. a) Generated orthophosphate concentration for selected microbial treatments b) Medium pH variation with respect to microbial treatment

Out of all microbial treatments significantly high ($P < 0.05$) phosphate solubilization capacity was recorded in the biofilm combination BF3 (one bacterium and one fungus) since it was the combination showed the highest orthophosphate generation (Figure 3a). It was noted that the F3 which showed the highest phosphate solubilization capacity among the individual cultures was one of the components in the responsive biofilm BF3 (*Brevibacillus brevis* on *Penicillium polonicum* filament). Out of all microbial treatments, the significantly lower ($P < 0.05$) pH was recorded in biofilm BF3 (Figure 3b). All biofilm combinations showed significantly higher ($P < 0.05$) phosphate solubilization capacity and lower medium pH over their individual cultures.

Biofilms are common in various environments, but few are known as phosphate solubilizers. The ability of microbes to produce and release organic acids primarily determines the bio solubilization of rock phosphate [1]. During biofilm formation, bacteria attach to surfaces, which stimulates the synthesis of exopolysaccharides. This process enhances the production of organic and inorganic acids, exopolysaccharides, and proton extrusion, thereby increasing phosphorus solubilization [21]. The secretion of organic acids from the metabolic activities of PSM is the key mechanism for phosphate solubilization [7]. In the current study, all biofilms showed high solubilization of phosphates and a decrease in the pH of the broth simultaneously (Figure 3). Organic acids contribute to this process by lowering the pH and chelating cations like Al^{3+}, Fe^{3+}, and Ca^{2+} bound to phosphate ions, thus releasing the phosphate [6]. The acidification of the media and H^+ substitution reactions further aid in converting insoluble phosphates into a soluble form [7]. The significant drop in pH is commonly associated with phosphate solubilization, a mechanism well-documented in various studies involving different microorganisms, including bacteria and fungi [22,23].

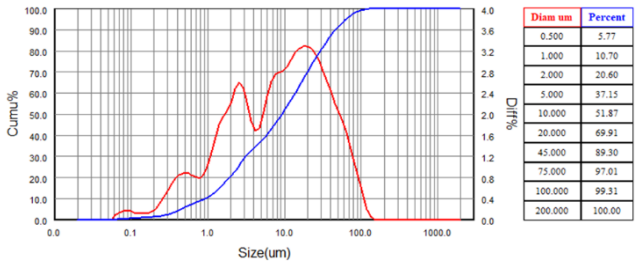
Phosphate solubilizing microorganisms (PSMs) in biofilm mode can enhance the solubility of ERP, as supported by previous research [24]. This study corroborates that fungal-bacterial biofilms exhibit higher phosphate solubilization compared to their individual cultures (Figure 3a), likely due to the presence of PSMs in the biofilms. Consistent with earlier findings, biofilm combinations are more effective at solubilizing mineral phosphate in ERP than individual cultures. Specifically, the *P. ostreatus* – *B. elkanii* SEMIA 5019 biofilm significantly outperformed *P. ostreatus* alone in phosphate release [24]. The soluble phosphorus content varies with the type of biofilm (Figure 3a) due to the diverse acids secreted by bacterial and fungal biofilms, which dissolve various forms of phosphorus in soil [25, 26]. Utilizing biofilm-enriched natural fertilizers offers an eco-friendly alternative to chemical phosphate fertilizers, potentially enhancing crop yields [27]. Consequently, the biofilm with the highest phosphorus solubilization capacity in this study (BF3) was further tested for its efficiency on ERP in a pot experiment with chili plants under greenhouse conditions.

3.4 Characterization and Optimization Physical Status of ERP

3.4.1 Particle size analysis of ERP sample

The particle size analysis confirmed that the particle sizes of ERP were in micrometer range and approximately 50% of the sample had less than 9.237 µm size.

3.4.2 FT-IR Characterization of ERP, and ERP after treated with the responsive biofilm



Diam um	Percent
0.500	5.77
1.000	10.70
2.000	20.60
5.000	37.15
10.000	51.87
20.000	69.91
45.000	89.30
75.000	97.01
100.000	99.31
200.000	100.00

Fig. 4- Particle size analysis for ERP sample with reduced particle size

The FT-IR spectra of ERP sample before and after solubilization treatment illustrated within the section of 3500- 500 cm^{-1} in Figure 5. It was noticed that the process had a notable and significant effect on the intensity and positions of the vibrational bands [28]. Vanishing of some peaks had occurred after the treatment. After the solubilization process the intensities of OH stretching at 3276 cm^{-1}, phosphate bands at 1030 cm^{-1} and 602 cm^{-1}, were reduced. The peak observed at *1642 cm* $^{-1}$ is assigned due to the stretching vibrations of P-O-H groups. The disappearance of these peaks confirms the involvement of the peaks in phosphate solubilization [29]. The disappearance of the bands related to carbonate situated at 1542 cm^{-1} and –OH hydroxyl carbonate group at 1414 cm^{-1} implied that carbonate substitution induces vacancies at the OH sites, and it is assumed that the treatment was responsible of the total decomposition of carbonate bands and the decrement of intensities [29].

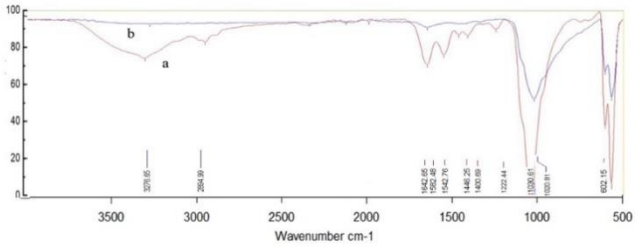


Fig. 5- a) FT-IR spectrum for ERP, b) FT-IR spectrum for treated ERP

3.4.3 SEM characterization of ERP and biofilm-treated ERP

The SEM micrographs of ERP showed significant surface morphology changes after microbial treatment (Figure 6 a & b). Untreated ERP particles had smooth surfaces with clusters of regular, and sharp-edged particles (Figure 6a). After microbial treatment, a rough structure formed on the ERP surfaces (Figure 6b), which appeared markedly corroded with asymmetrical holes which were shown in orange arrow on the ERP surface, likely due to microbial secretions like acids compounds and leaching of phosphatize [30]. The treated ERP particles displayed irregular, round structures without sharp edges and significant deformation, indicating solubilization by microbial action. This aligns with previous findings that microbial colonization leads to weathering of feldspars with apatite inclusions [31]. As such, the heavy colonization and fungal– rhizobial biofilm formation on the ERP particles have evidently enhanced the process of microbial weathering of the ERP.

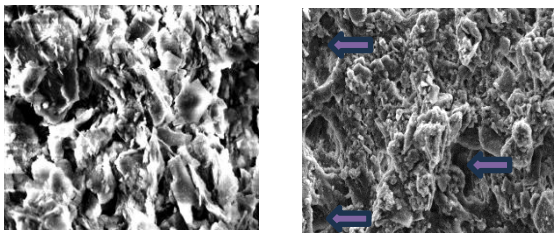


Fig. 6- a) ERP before the treatment, b) ERP after the treatment

3.5 Molecular identification of microbial components in the responsive biofilm

GenBank search revealed that the isolates had high sequence similarity to the species B2- *Brevibacillus brevis* (NR041524.1) and F3- *Penicillium polonicum* (MT529240.1), among the nucleotide sequences available in the NCBI database.

3.6 Influence of inoculants on growth of the chili plants

In the pot experiment with chili plants, variable responses were observed with different treatments involving microbial inoculants, ERP, and CF (Figure 7 a-d). The application of microbial inoculant showed promoting effects on chili plant compared to non-inoculated soil. Out of all the treatments, treatment T9 where the ERP was pretreated with FBB showed significantly ($P < 0.05$) the highest average FW and NF whereas treatment T6 which was the direct soil inoculation of the FBB combined with ERP showed the second highest FW and NF (Fig. 7 a and b). Combining FBB with ERP (especially pretreated) significantly enhanced both FW and NF. FBB pretreatment (T9) outperforms simple FBB and ERP application (T6) but both are effective.

The solubilization of phosphorus by soil microorganisms is crucial for maintaining soil fertility and promoting sustainable agriculture. These microorganisms improve phosphorus availability, enhancing plant growth, crop yields, and reducing the need for chemical fertilizers. Scientific studies have shown that combining mineral phosphates with phosphate-solubilizing FBB increases crop productivity and phosphorus circulation [32, 33]. Colonization of the hyphae of the soil fungus *Penicillum* sp. by *Bradyrhizobium elkani* SEMIA 5019 produced a biofilm that significantly enhanced P mobilization from an ERP [9]. Phosphate-solubilizing bacteria adhering to rock phosphate

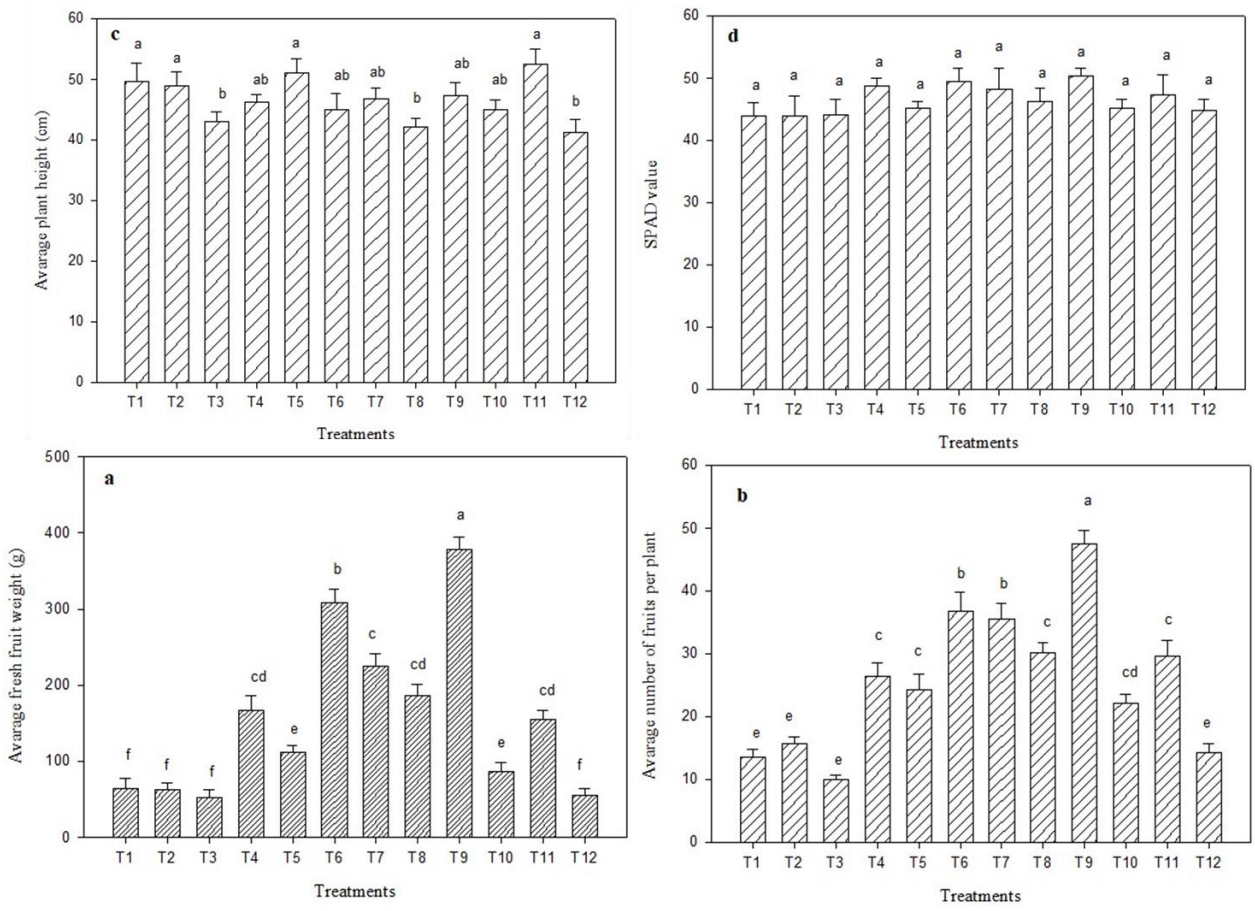


Fig. 7- Different responses of chili plant for different microbial, ERP and CF treatments. The effect of different treatments on a) Fresh weight of chili fruit, b) number of chili fruits per plant, c) plant height, d) Chlorophyll content of the 5th leaf as SPAD value. Treatments- F3 fungal culture only (T1), B2 bacterial culture only (T2), biofilm only (T3), ERP with F3 (T4), ERP with B2 (T5), ERP with biofilm (T6), ERP- pretreated F3 (T7), ERP-pretreated B2 (T8), ERP-pretreated biofilm (T9), phosphorous as ERP (T10), phosphorous as TSP (T11), Control- no treatment (T12).

can synergize with fungi, forming biofilms that stimulate fungal exudate production and offer potential agricultural applications. A review of interactions between bacteria and both saprophytic and mycorrhizal fungi [34], illustrates that soil bacteria can adhere to fungal cells, stimulate fungal exudates production, and also form a biofilm along the fungal hyphae. The resulting biofilms can be used for potential applications in the field [35].

The biofilm-only treatment (T3) resulted in the lowest FW and NF, even lower than the control treatment (T12) which did not receive phosphorus or microbial treatments (Figure 7 a and b). This may be due to the over-absorption of nutrients by biofilm microorganisms, leading to nutrient unavailability for the plants. Biofilm alone can negatively impact plant growth and yield due to competition for nutrients between microorganisms and the plants. The triple superphosphate (TSP) treatment (T11) outperformed the ERP only treatment (T10) in terms of FW and NF, highlighting TSP's superior ability to promote vegetative growth and yield. Low solubility of ERP results in reduced effectiveness. Figure 7c shows that TSP treatment (T11) enhanced plant length more than other treatments, confirming its role in promoting vegetative growth. The highest chlorophyll content was observed in treatments combining biofilm and ERP (T9 and T6), indicating better plant health and potential for higher photosynthetic activity (Fig. 7 d), though there was no significant difference between T9 and T6. This finding aligns with previous studies showing that biofilm formation on plant roots enhances photosynthesis and leaf growth. A previous study confirmed that the co-inoculation with 50% recommended fertilizers and biofilm biofertilizers increases leaf growth [36]. Inoculation of biofilm with *Pseudomonas plecoglossicida* and *Bacillus licheniformis*, significantly improved photosynthetic pigments (10–67%), in sunflower [37]. In addition, [38] reported that the bacterial biofilm with *Pseudomonas azotoforman* effectively enhanced photosynthetic pigment efficiency.

4. Conclusions

This study was conducted to test the potential of biofilm-enriched ERP to replace the TSP in chili cultivation. The overall results conclude that the biofilm enriched ERP performed better in comparison to the DOA recommended TSP dosage on P supply to the chili cultivation under

controlled conditions. All the biofilms applied treatments (*B. brevis* on *P. polonicum*) except biofilm only treatment significantly contributed to enhanced Fruit fresh weight, fruit number and chlorophyll content compared to other treatments. Thus, further studies are required to evaluate the performance of the biofilm combination with *B. brevis* and *P. polonicum* to use as an alternative for TSP in chili cultivation under field conditions.

Conflicts of Interest

Author declared that there's no conflict of interest.

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