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SYNERGISTIC EFFECT OF *Bacillus* spp. AND *Aspergillus* spp. AS BIOFERTILIZERS SPRAY ON NUTRIENT UPTAKE AND GROWTH ENHANCEMENT IN LETTUCE (*Lactuca sativa*) AND RADISH (*Raphanus sativus*): IMPACT OF BIOFERTILIZER ON SOIL FERTILITY AND SUSTAINABLE AGRICULTURE

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ABSTRACT

Excessive use of chemical fertilizers has led to soil deterioration, water pollution, and the loss of biodiversity, hence requiring eco-friendly alternatives. The concept of biofertilizer emerges as an alternative approach for improving soil fertility and agricultural productivity. The objective of this study was to isolate Phosphate Solubilizing Bacteria (PSB), Phosphate Solubilizing Fungi (PSF), and Nitrogen-Fixing Bacteria (NFB), following the development of biofertilizers sprays that offer a promising solution by improving nutrient availability and promoting plant growth through microbial interactions while minimizing chemical fertilizers dependence. Soil associated with open dump sites and compost sites in the Karadiyana was selected to isolate the PSB, PSF, and NFB strains. Genotypic identification, analysis of antagonistic effect, and nutrient solubilization efficiency were performed for each isolate. A microbial consortium was prepared to consist of four *Bacillus* spp. and two *Aspergillus* spp. and incorporated into a fertilizers spray, which was applied to the leaves of Lettuce (*Lactuca sativa*) and Radish (*Raphanus sativus*) grown in compost, coir dust, and soil medium. Plants were measured for various growth parameters after 30 days of seed germination. Growth parameters, including shoot length, root length, wet weight, dry weight, seed germination time, leaf area, leaf length, leaf width, and the number of leaves of Lettuce plants, were significantly influenced by the application of fertilizer spray compared to control pots ($p < 0.05$). Lettuce and radishes showed shoot lengths of 16.97 ± 1.35 cm and 23.23 ± 0.49 cm, root lengths of 9.27 ± 0.32 cm and 15.57 ± 1.00 cm, leaf areas of 84.10 ± 19.24 cm² and 87.98 ± 33.11 cm², and no leaves were 10.0 ± 0.58 and 8.0 ± 0.52 for lettuce leaves and radish, respectively. This study demonstrates the potential of utilizing selected *Bacillus* spp. and *Aspergillus* spp. consortia as eco-friendly biofertilizers to replace chemical fertilizers. The microbial formulations significantly improved plant growth, offering a sustainable solution for agricultural productivity.

Keywords: Agricultural productivity, *Aspergillus* spp., *Bacillus* spp., Biofertilizer, Plant growth promotion

INTRODUCTION

The global population has exceeded 7 billion today from 1.6 billion in 1900, and is expected to reach an estimated 9 billion by 2050 (**Soumare *et al.*, 2020**). This upcoming population increase will demand a significant rise in agricultural yields. Applying inorganic fertilizers represented one of the most effective approaches to improving productivity in global agriculture (**Hirel *et al.*, 2011; Mueller *et al.*, 2012; Liu *et al.*, 2016**). In contrast, the extensive use of chemical fertilizers was accompanied by massive ecological perturbations at a global scale (**Yang and Fang, 2015; Bishnoi, 2018, Jayathilake *et al.*, 2025**), as well as disrupted soil homeostasis in many regions of the world due to overexploitation processes (**Zheng *et al.*, 2019**). They generate soil degradation, water pollution, biodiversity loss, and greenhouse gas emissions. As a result, minimizing the burden of inorganic fertilizers and promoting sustainable agricultural practices have gained significant attention. Researchers are increasingly exploring alternative approaches to mitigate environmental pollution and improve agricultural productivity and sustainability in light of the growing global population (**Ajmal *et al.*, 2018; Jayathilake *et al.*, 2024**). Nowadays, biofertilizer are one of the best options in alternative approaches; they act as an excellent solution to decrease synthetic nitrogen and phosphorus fertilizers and their contaminant effects on our environment in agriculture.

Biofertilizers, available in various formulations, including peat, liquid, granules, freeze-dried powders, and potting pellets, play a crucial role in sustainable agriculture (**Bashan *et al.*, 2014; Behl *et al.*, 2024**). Biofertilizers help improve nutrient availability, mobilizing necessary nutrients from the soil, leading to plant growth and enhancing productivity. Moreover, bio-fertilizers strengthen the structure and stability of soil and help improve water availability and aeration. They also promote a diverse microbial community that enhances biological activity to support plant health and robust defence mechanisms against pathogens and environmental pressures. Thus, biofertilizer integration in agricultural systems will decrease dependency on chemical fertilizers, which can result in many adverse effects on the environment (**Thadiyan *et al.*, 2024; Yadav and Yadav, 2024**).

The primary growth and development of plants demand some essential nutrients. Among these, nitrogen and phosphorus remain two of the most critical macronutrients. Nitrogen is mainly taken up by plants in the forms of NO_3^- and NH_4^+, while phosphorus is taken up as orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) (**Sinha and Tandon, 2020**). These fertilizers are vital in improving plant nutrient uptake through different mechanisms, including microbial-plant root interactions and microbial interactions with the soil environment (**Shahwar *et al.*, 2023; Khan *et al.*, 2023**). Different mechanisms performed by some strains of microbes, like PSB, PSF, and NFB convert the insoluble forms of nutrients into bioavailable forms. Organic acids produced by the PSB and PSF strains solubilize bound phosphorus in the soil for its availability to plant roots. Similarly, atmospheric nitrogen can be fixed as ammonia by NFB, which then becomes readily assimilable by plants. This enhanced availability of nutrients due to biofertilizers is directly reflected in plants' increased growth and productivity (**Yang *et al.*, 2023**). Thus, the present study aimed to develop a biofertilizer spray to enhance plant growth and productivity while reducing dependency on chemical fertilizers, promoting sustainable agricultural practices.

MATERIAL AND METHODS

Soil sample collection

Soil samples were collected from the open dump and compost sites in Karadiyana (6°48′ 51.8″ N, 79° 54′ 17.0″ E) for the isolation of NFB, PSB, and PSF. Each sample was collected into clean, sterile bags, sealed, and transferred to the laboratory.

Analysis of physiochemical parameters of soil samples

Soil pH, Electrical Conductivity (EC), total soil carbon, soil organic nitrogen, and soil available phosphorus were determined following standard protocols and methods. (**Pal *et al.*, 2009; AOAC, 2000**).

Isolation of phosphate-solubilizing bacteria and fungi

Phosphate-solubilizing bacterial and fungi strains were isolated using Pikovskaya's Agar medium. A weight of 10g of each soil sample was homogenized in 90 ml sterilized saline and shaken at 120 rpm for 60 min. A series of 10-fold dilutions of the suspension were prepared for each sample. For bacteria isolation, 100µl of each dilution and 200 µL of each dilution were spread directly onto the surface of Pikovskaya Agar medium plates for the isolation of bacteria and fungi strains respectively (**Ha and Chu, 2020; Doilom *et al*., 2020**).

Isolation of free-living NFB

Free-living NFB strains were isolated using Ashby medium agar medium. A series of 10-fold dilutions of the suspension were prepared for each sample. 100µl of each concentration was spread directly onto the surface of Ashby medium plates. Plates were incubated at 30ºC for 3 days. The nitrogen-fixing strains were identified using colony characteristics (**Ha and Chu, 2020**).

Genotypic identification fungal isolates and bacterial isolates

The DNA extraction was performed from the mycelium grown on PDA at 25°C for 7 days using the ZR Fungal/Bacterial DNA MiniPrep™ kit per the manufacturer's protocol. The DNA extraction of isolated bacterial strains was performed using the Wizard Genomic DNA Purification Kit according to the manufacturer's protocol. The extracted DNA samples were subsequently sequenced at Macrogen, Korea.

Antagonistic effect of isolated bacteria

The method described by **Kaur *et al* (2022)** was followed to determine the antagonistic effect of isolated bacteria. The cross-streak method was followed. Sterile nutrient agar plates were prepared and allowed to solidify. A single streak of the test bacterium was lined across the center of each agar plate. The plates were incubated at 37°C for 24 hours to allow the initial growth of the test bacterium. After incubation, the isolated bacterial strains were streaked perpendicular to the original streak of the test bacterium on the same plates. The plates were then incubated at 37°C for 24-48 hours. Following incubation, the plates were examined for inhibition zones along the intersection lines of the bacterial streaks, indicating antagonistic activity. The presence of inhibition zones was recorded to assess the antagonistic effect of the isolated bacteria against the test bacterium.

Antagonistic effect of isolated fungi

The antagonistic effect of isolated fungi was investigated using the cross method. Two species of fungi, each with a 5 mm diameter mycelial disc from a 7-day-old culture, were selected. Sterile Petri dishes containing PDA were prepared. A mycelial disc of the first fungal species was placed at one edge of the Petri dish, and a mycelial disc of the second fungal species was placed at the opposite edge of the same Petri dish. The Petri dishes were sealed and incubated at 25°C for 7 days to allow the fungi to grow towards each other. Following incubation, the Petri dishes were observed for the formation of inhibition zones or changes in growth patterns at the intersection points. These observations were recorded to assess the antagonistic effect between the isolated fungal species (**Mahmoud *et al*., 2015**).

Antagonistic effects between fungi and bacteria

The method described by **Mahmoud *et al* (2015)** was followed to determine the Antagonistic effects between fungi and bacteria**.** Sterile 5-mm diameter Whatman No. 1 filter paper discs were impregnated with bacterial suspensions (10^7 CFU/mL). These discs were carefully placed 5 mm apart from one edge of a petri dish containing growth medium. The bacterial isolates were incubated at 26 ± 2°C for 24 hours to establish growth. Subsequently, a 5-mm diameter plug from a 7-day-old fungal culture was aseptically placed on the opposite edge of the same petri dish. The plates were further incubated at 26 ± 2°C for 5 days. The presence of inhibition zones was recorded to assess the antagonistic effect between fungi and bacteria.

Determination of the Phosphate Solubilization Index (PSI) of PSF and PSB

The fungal mycelium of each fungal strain was cultured on PDA at 28°C for 7 days. Subsequently, a sterile cork borer (5 mm3) excised mycelial plugs from the periphery of actively growing colonies. These isolated mycelium plugs were then transferred onto petri plates containing PVK agar; similarly, a loopful of pure bacterial inoculum for each bacteria strain was placed in the center of separate PVK agar plates, with uninoculated PVK agar plates serving as controls. On the seventh day of incubation, a comparative measurement of the PSB and PSF solubilization index was conducted by assessing the clear zone and colony diameters in centimeters. The PSI was subsequently determined using the following formula. (**Doilom *et al*., 2020**).

$$\text{PSI} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

Determination of Phosphate Solubilization Efficiency of PSB and PSF

The phosphate solubilization activity test was conducted in 150 mL conical flasks containing 100 mL of PVK broth supplemented with 0.5% TCP (pH = 7). 1 mL of bacterial inoculant (10^7 CFU/mL) was inoculated into prepared broth media, while sterile distilled water served as the control. Each fungal culture was inoculated for PSF strains with 10 mL of spore suspension (10^6 spores/mL), while sterile distilled water was the control. The cultures were then incubated on a rotary shaker at 28°C and 130 rpm for 7 days. Aseptic aliquots of 1.5 mL of culture supernatant were collected on the 2nd, 4th, 6th, and 8th days. Following the centrifugation at 12,000 rpm for 2 minutes to remove suspended solids and mycelial fragments, 0.1 mL of each culture supernatant was taken to estimate the phosphorus released from TCP. The available soluble phosphate in culture supernatants was assessed using the Bray extraction method at 882 nm (**Hui *et al*., 2011; Doilom *et al*., 2020**).

Determination of Ammonia, Nitrite, and Nitrate production

The nitrogen fixation activity of NFB strains was determined using the method described by **Ha and Chu (2020)** with slight modification. Exactly 50 mL of Ashby broth media in sterilized flasks were prepared, and isolated NFB strains were inoculated into each flask separately. The broth media were then kept in a rotary shaker at 160 rpm for a 7-day incubation period. After the 7 days of incubation, broth media's ammonia, nitrite, and nitrate levels were analyzed using standard spectrophotometry methods at 640 nm, 550 nm, and 420 nm, respectively.

Preparation of microbial consortia

A microbial consortium was developed comprising two NFB, two PSB, and two PSF. The bacterial strains were inoculated in nutrient broth media and incubated under optimal conditions until high cell density was achieved. The fungal strains were inoculated on Potato Dextrose Agar (PDA) and incubated at 28-30°C until substantial mycelial growth was observed. Following cultivation, bacterial cultures were centrifuged at 4,000-6,000 rpm for 10-15 minutes to collect the bacterial pellet, which was then resuspended in sterile water to achieve a concentration of 10^8 CFU/mL, with bacterial concentrations assessed at an optical density (OD) of 0.5-0.9 at 595 nm. The fungal mycelium was scraped from the agar surface and homogenized in sterile water to yield a uniform suspension at a concentration of 10^6 spores/mL, with quantification performed using a hemocytometer. Finally, the bacterial and fungal suspensions were combined in a sterile container to ensure that the final concentration of each microbial strain was optimal for effective biofertilization. The prepared consortia were stored at 4°C.

Formation of biofertilizer spray

Biofertilizer spray was developed following the method described by **Dey (2021),** with minor modifications. Glycerol (2% w/v) and Tween 20 (0.5%) were added to the prepared microbial consortia suspension. The pH of the combined suspension was measured and adjusted to neutral (pH 7) using sterile pH-adjusting solutions (NaOH or HCl). A control spray was prepared by mixing the above reagents replacing the microbial consortia with sterilized distilled water to match the volume. This control mixture was used to treat the control pots.

Determination of the effect of the biofertilizer spray on plant growth

The growth medium for the pot experiment consisted of a blend of compost, coir dust, and soil in a 1:1:1 ratio. The physicochemical parameters of the potting media were determined. Each pot was prepared using 200 grams of the homogenized potting medium. The biofertilizers spray's effect on plant growth was assessed using lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*). The seeds were surface-sterilized by immersion in a 1% sodium hypochlorite (NaOCl) solution for 1 minute, followed by three rinses with sterilized distilled water. They were then dried using sterilized tissue paper before being incorporated into the potting mixture (**Osman *et al*., 2024**). Growth parameters measured for plants included shoot length, root length, wet weight, dry weight, leaf length, leaf width, leaf area, and no leaves after 30 days of seed germination. A 10 mL liquid bio-fertilizer spray was applied at five-day intervals onto the surface layer of the pot, and the soil was mixed using a sterilized spatula. A control pot was prepared, to which the biofertilizer spray was not applied. The pots were irrigated twice daily with 100 mL of water. The experiment followed a completely randomized block design with three replications per treatment.

Statistical Analysis

Data analysis and graphical representations were conducted using Microsoft Excel. Data from different treatments were calculated and statistically analyzed with a one-way analysis of variance (ANOVA) using Python 3.11 (VS Code).

RESULTS AND DISCUSSION

Soils are naturally rich in diverse microbial populations, making it feasible to utilize these native communities to enhance crop productivity to replace conventional chemical fertilizers (**Bertola *et al*., 2021; Gupta *et al*., 2022; Dincă *et al*., 2022**). In this study, open dump and compost sites soil were selected to isolate and analyze the presence of PSB, PSF, and NFB. Table 1 represents the physicochemical properties of the collected soil samples. The compost and open dump soil samples were found to be acidic, with the compost soil exhibiting the highest levels of moisture content (56.70±1.2%), total organic carbon (53.20±2.3%), and total organic nitrogen (0.46±0.01 ppm). The compost and open dump sites displayed moisture contents of 56.70±1.2% and 34.70±0.6%, respectively. Both compost and open dump soils were conducive to the growth of PSB, PSF, and NFB. Microbial growth and activity, especially for PSB, PSF, and NFB, are favored in slightly acidic to neutral pH ranges, with optimal organic carbon and moisture (**Rosita *et al*., 2023**). Acidic conditions in compost and open dump soils favour the growth of soil microorganisms. The acidic pH can also increase nutrient availability and microbial metabolic activity (**Naz *et al*., 2022**). Further, the higher moisture content and organic carbon in compost soils provide a resource-rich environment with abundant nutrients and energy sources for microbial proliferation and metabolic activities of microorganisms in soils (**Muscolo *et al*., 2018; Dijkstra and Keitel, 2024**).

Table 1 Physiochemical properties of collected soil samples

Parameter	Compost Soil	Open dumpsite
Soil pH	3.13±0.02	6.73±0.02
Soil moisture (%)	56.70±1.2	34.70±0.6
Soil total organic carbon (%)	53.20±2.3	45.22±2.3
Soil total organic nitrogen (ppm)	0.46±0.01	0.39±0.01

The genotypic identification results revealed that all isolated PSB and NFB strains belonged to the genus *Bacillus* spp. Moreover, the isolated PSF strains were identified as two strains of *Aspergillus* spp. Table 2 illustrates the genotypically confirmed isolated PSB, PSF, and NFB strains.

Table 2 Genotypically confirmed isolated PSB, PSF, and NFB strains.

Biofertilizer	Species
PSB	*Bacillus cereus* ATCC 14579
	Bacillus siamensis KCTC 13613
PSF	*Aspergillus oryzae*
	Aspergillus niger
NFB	*Bacillus subtilis* NCIB 3610
	Bacillus cereus CCM 2010

The phosphate-solubilizing efficiency of isolated PSB and PSF strains was evaluated qualitatively and quantitatively (**Janati *et al*., 2022**). The qualitative assessment measured the PSI on PVK solid media. Figure 1 illustrates the clear zone formation for TCP solubilization on PVK agar after 7 days of incubation by *Bacillus siamensis* KCTC 13613 and *Aspergillus niger*.

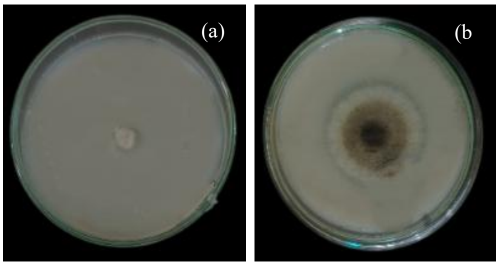

Figure 1 Clear zones formation on PVK agar after 7 days (a) *Bacillus siamensis* KCTC 13613, (b) *Aspergillus niger*

Table 3 illustrates the PSI for isolated PSB and PSF strains, qualitatively assessing their phosphate-solubilizing capabilities. The PSI of *B. siamensis* KCTC 13613 and *Aspergillus niger* was 2.05 ± 0.02 and 2.16 ± 0.01, respectively. However, the PSI of *Bacillus cereus* ATCC 14579 and *Aspergillus oryzae* was 1. This low PSI value indicates that the visible zone of solubilization around microbial colonies was limited (**Jokkaew *et al*., 2022**). However, this does not necessarily mean low phosphate-solubilizing potential. It suggests that the solid media environment may restrict the diffusion of organic acids and other solubilizing agents, thereby limiting zone formation.

Table 3 PSI of isolated PSB and PSF

Biofertilizer	Species	PSI
PSB	*Bacillus cereus* ATCC 14579	1
	Bacillus siamensis KCTC 13613	2.05±0.02
PSF	*Aspergillus oryzae*	1
	Aspergillus niger	2.16±0.01

Quantitative analysis in PVK broth supplemented with 0.5% Tricalcium Phosphate (TCP) provided a more comprehensive view, and it showed that all isolated PSB and PSF strains produced bioavailable phosphorus over time (**Abawari *et al*., 2021**). This increase in available phosphorus in liquid media indicates that these isolates can solubilize phosphate effectively. The decrease in pH over the incubation period further confirmed the production of organic acids, which play a critical role in solubilizing phosphate (**Ahmad *et al*., 2023**). The quantitative results demonstrate that while PSI values on solid media provide a preliminary indication of solubilization potential, liquid media assessments more accurately reflect the strains' efficiency in converting insoluble phosphorus into forms accessible to plants (**Shaffique *et al*., 2023**). Figures 2 and 3 represent the pH variation and available phosphorus levels for the PSB and PSF strains over the incubation period, illustrating the gradual decrease in pH and the corresponding increase in available phosphorus.

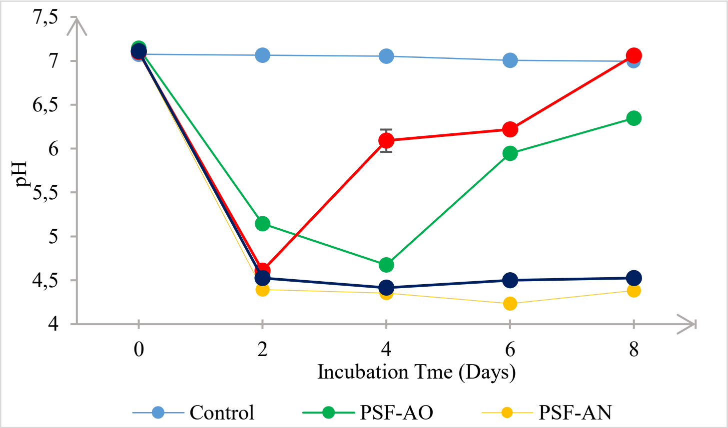

Figure 2 pH values of TCP containing PVK broth inoculated with PSB and PSF isolates after 0th, 2nd, 4th, 6th and 8th of incubation.

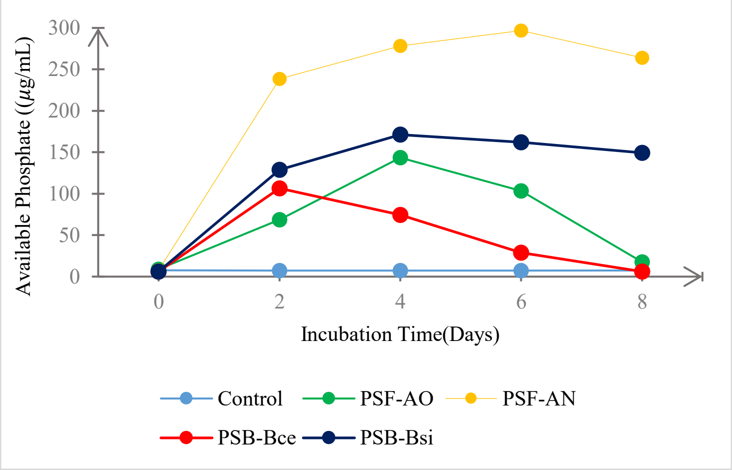

Figure 3 Solubilized P concentrations after 0th, 2nd, 4th, 6th and 8th day of incubation in TCP containing PKV broth inoculated with PSB and PSF isolates (PSF-AO- Aspergillus oryzae, PSF-AN- Aspergillus niger, PSB-Bce *Bacillus cereus* ATCC 14579, PSB-Bsi – *Bacillus siamensis* strain KCTC 13613)

Among the PSB strains, *B. siamensis* showed the highest phosphate solubilization efficiency in PVK broth media, equal to 171.19 ± 0.13 µg/mL. In contrast, *Bacillus cereus* (106.5 ± 0.1 µg/mL) exhibited higher phosphate solubilization on the second day of incubation. A previous study by **Janati *et al.* (2023)** has demonstrated that the morphological and biochemical characteristics of the strain allowed us to identify around nine different bacterial genera, including *Bacillus* spp., *Pseudomonas* spp., and *Rhizobium* spp. This study suggested that the phosphorus solubilization average of rock phosphorus and TCP of all strains that were isolated from each of the four regions ranged from 18.69 mgL^{-1} to 40.43 mgL^{-1} and from 71.71 mgL^{-1} to 94.54 mgL^{-1}, respectively. Among isolated PSF strains, *Aspergillus niger* showed the highest phosphorus availability, with 296.79 ± 0.21 µg/mL. In contrast, *Aspergillus oryzae* peaked on the fourth day at 143.7 ± 0.3 µg/mL. These results align with previous studies demonstrating that *Aspergillus* species convert insoluble phosphate into plant-available forms through organic acid secretion. **Doilom *et al.* (2020)** showed that fungal strains were tested for their ability to solubilize TCP on both solid qualitatively and liquid Pikovskaya (PVK) media quantitatively. This study suggested that isolated *Aspergillus* spp showed the most significant phosphate solubilizing activity on a solid PVK medium with the solubilization index (SI) (2.58 ± 0.04 cm) and the highest solubilized phosphates (1523.33 ± 47.87 µg/mL) on a liquid PVK medium.

The nitrogen-fixing potential of isolated strains was measured by measuring ammonia, nitrite, and nitrate production after incubation in the Ashby broth medium. Figure 4 illustrates the results of determining ammonia, nitrite, and nitrate concentrations in ash using broth media inoculated with NFB strains.

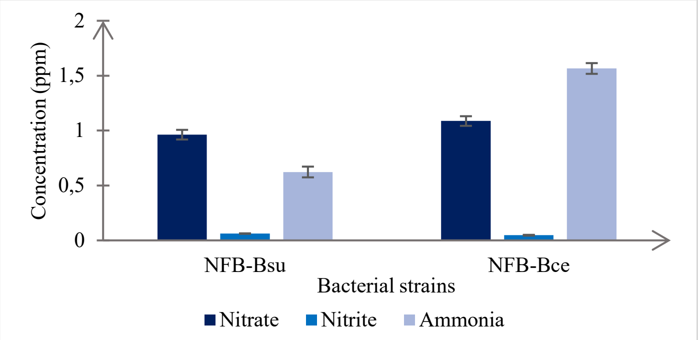

Figure 4 Results of Ammonia, nitrite and nitrate production of isolated NFB strains (NFB- Bsu-*Bacillus subtilis* strain NCIB 3610, NFB-Bce-*Bacillus cereus* strain CCM 2010)

Among the isolates, *Bacillus cereus* demonstrated the highest levels, producing 1.56 ± 0.04 ppm of ammonia and 1.09 ± 0.04 ppm of nitrate, demonstrating the efficiency in nitrogen transformation into ammonia and nitrate. *Bacillus subtilis* strain NCIB 3610 also showed prominent nitrate production, with concentrations of 0.96 ± 0.04 ppm. Additionally, *Bacillus subtilis* strain NCIB 3610 exhibited relatively high ammonia production, 0.62 ± 0.05 ppm. The study by **Fretes *et al* (2018)** further supported the nitrogen-fixing ability of *Bacillus* spp. It emphasized these strains' potential as effective nitrogen fixers for agricultural applications. These findings align with **Satapute *et al* (2012)**, who demonstrated that the soil isolate *Bacillus subtilis* strain AS-4 has nitrogen-fixing ability. Further, it suggests that *Bacillus subtilis* strain AS-4 could be exploited as soil inoculants and used for nitrogen fixation in soils with high salt concentrations, which are eco-friendly and cost-effective in the long run.

Table 4 illustrates the physicochemical properties of soil, compost, coir dust, and prepared potting media. The potting media were created by mixing compost, soil, and coir dust in a 1:1:1 ratio to grow mung bean plants. This ratio aligns with the findings of **Herath *et al* (2013),** who used the same coir dust: compost: sand (1:1:1) mixture in their pot experiments.

Table 4 The physicochemical properties of soil, compost, coir dust, and prepared potting media.

Parameter	Soil	Compost	Coir dust	Mix (1:1:1)
pH	5.22±0.01	6.51±0.01	5.43±0.01	6.07±0.02
EC (mS)	0.16±0.001	3.74±0.006	1.26±0.001	1.35±0.001
Total organic carbon (%)	33.70±0.28	56.75±0.07	60.70±0.28	43.35±0.35
Total nitrogen (mg/Kg)	90±28.3	1050±99.0	1010±113.1	790±84.9
Available phosphate (mg/Kg)	50±14.1	170±28.3	1035±91.9	195±35.4

The pH of the mixture was measured at 6.07 ± 0.02, which is optimal for plant growth as it promotes nutrient availability. Also, a previous study has demonstrated that the acidic pH value is more favourable for the proliferation and growth of soil microorganisms (**Musarrat and Khan, 2014; Khan *et al*., 2024**). Electrical conductivity (EC) measurements are moderate at 1.35 ± 0.001 mS/cm. It indicates that the ionic concentration is appropriate for healthy root development in the soil environment. Furthermore, the total organic carbon content of 43.35 ± 0.35% suggests a nutrient-rich profile that fosters microbial activity, essential for promoting plant growth. This microbial activity includes the roles of PSB, PSF, and NFB. The total nitrogen content of 790 ± 84.9 mg/kg provides a sufficient nitrogen source, while the available phosphate level of 195 ± 35.4 mg/kg points to enhanced phosphorus availability. These findings indicate that the 1:1:1 blend of soil, compost, and coir dust creates a supportive environment conducive to the effective growth of plants. **Tariq *et al* (2012)** demonstrated the benefits of coir as a potting medium, noting a significant increase in plant height, with the highest average height of 23.51 cm observed in coconut coir alone (T1). This was followed by a height of 21.78 cm in a silt and coconut coir mix (T5), and 21.59 cm in silt alone (T0) showed comparable growth. This supports the role of coir in potting mixtures, as it contributes to improved growth outcomes, as seen in the soil-compost-coir 1:1:1 blend, by maximizing nutrient uptake and plant resilience.

The antagonistic effect was determined before preparing the microbial consortium from the selected bacterial and fungal strains. Assessing the antagonistic effect is crucial before preparing a microbial consortium to ensure compatibility among the strains. This helps prevent any negative interactions that could hinder the growth or activity of the microbes, thereby optimizing their collective performance in promoting plant growth. Figure 5 illustrates the antagonistic interactions between the bacterial and fungal strains. No antagonistic effects were observed between the selected bacterial strains and fungal strains. The lack of antagonistic interactions indicates that the introduced microbial strains have a high chance of co-colonizing and remaining in the rhizosphere, which is necessary for reliable functional expression and effectiveness in field settings.

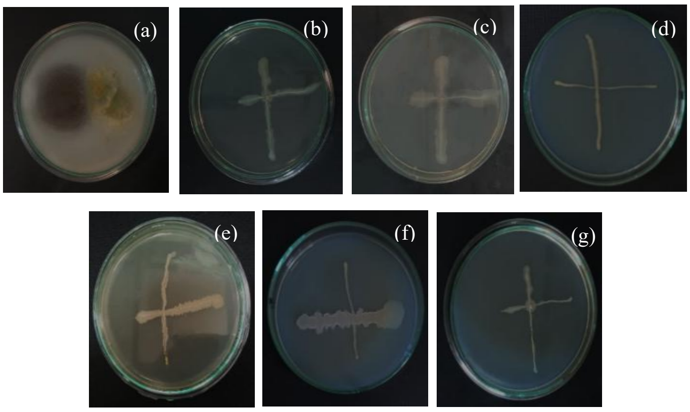

Figure 5 The antagonistic interactions between the bacterial and fungal strains. (a) PSF-AN-PSF-AO (b) PSB-Bce- PSB-Bsi (c)NFB-Bce-NFB-Bsu (d) PSB-Bce - NFB-Bsu (e) PSB-Bce - NFB-Bce (f) PSB-Bsi - NFB-Bce (g) PSB-Bsi - NFB-Bsu (PSB-Bce *Bacillus cereus* ATCC 14579, PSB-Bsi - *Bacillus siamensis* strain KCTC 13613, NFB- Bsu1-*Bacillus subtilis* strain NCIB 3610, NFB-Bce-*Bacillus cereus* strain CCM 2010, PSF-AO- Aspergillus oryzae, PSF-AN- Aspergillus niger)

Evaluating the effectiveness of the developed biofertilizer spray involved two different plant species, including non-leguminous plants (lettuce - *Lactuca sativa*) and fast-growing plants (radish -*Raphanus sativus*). This indicates that applying this biofertilizer spray is versatile and can promote growth for different plant categories. Figure 6 illustrates the highest growth parameters observed in plants treated with biofertilizer spray, compared to the control group, which did not receive microbial inoculation. The data were derived from three replicates for both treatment and control groups in a pot experiment. The observed enhancement in growth parameters for the treated plants suggests the positive influence of the microbial consortia on nutrient availability and uptake, resulting in improved plant development.

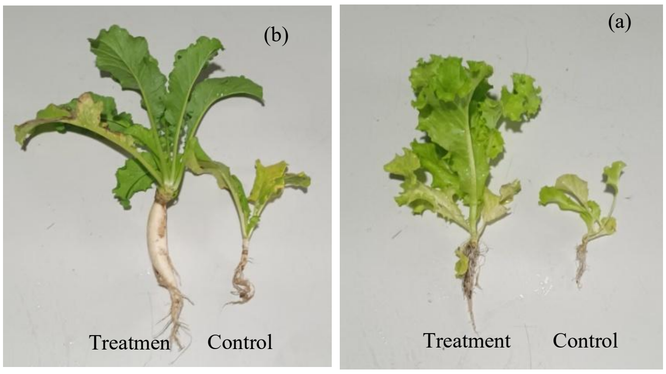

Figure 6 Comparison of plant growth parameters between treatment and control groups for plant species (a) lettuce (b) radish

Figure 7 illustrates the effect of the prepared biofertilizer spray on the growth of lettuce and radish. Growth parameters comprising shoot length, root length, wet weight, dry weight, seed germination time, leaf area, leaf length, leaf width, and the number of leaves were significantly affected by applying biofertilizer spray compared to control pots ($p<0.05$).

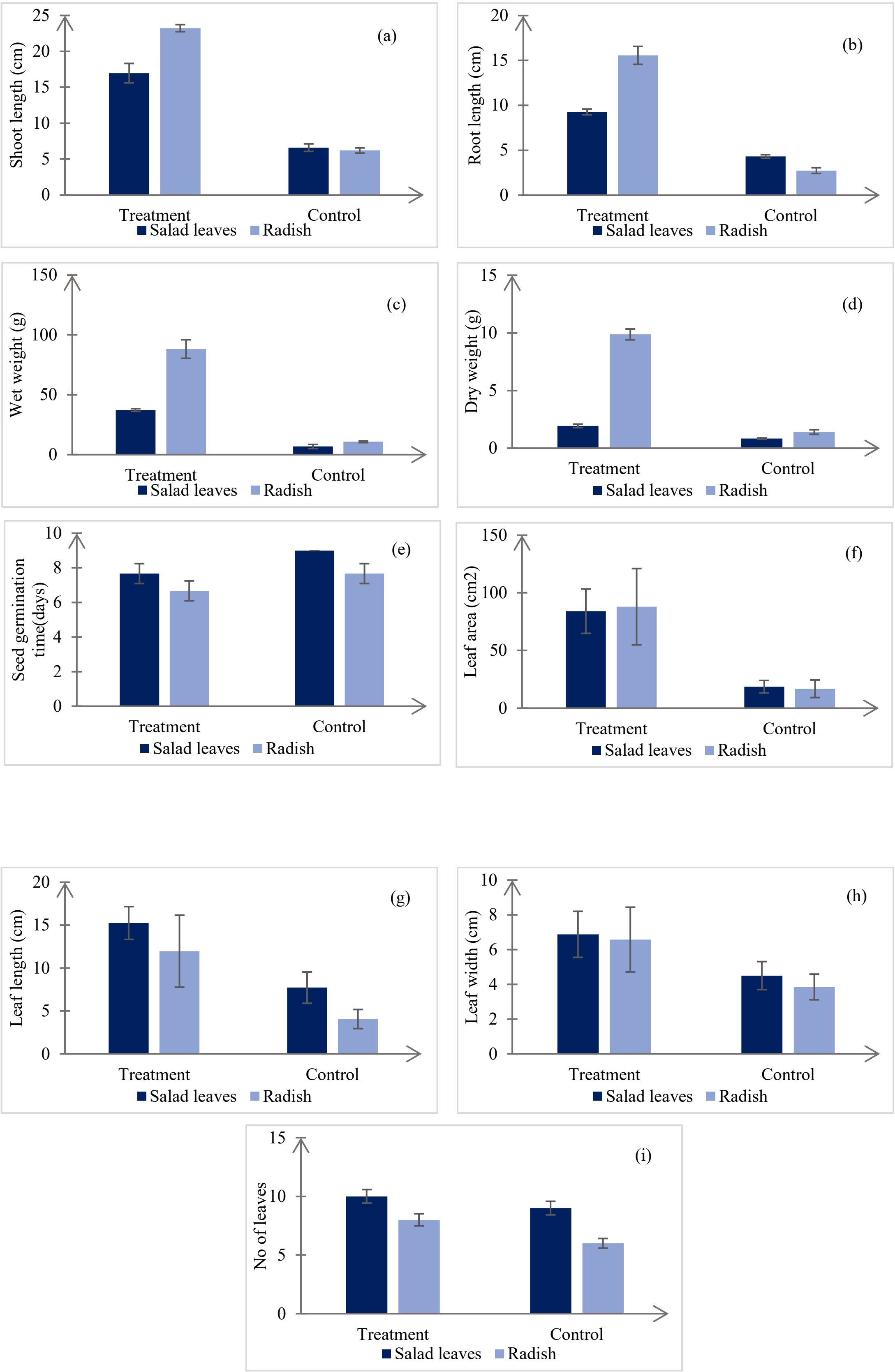

Figure 7 The effect of the prepared potting pellets on the growth of lettuce and radish (a) Shoot length (b) Root length (c) Wet weight (d) Dry weight (e) Seed germination time (f) Leaf area (g) Leaf length (h) Leaf width (i) Number of leaves

Shoot length in treated pots was significantly higher at 16.97±1.35 cm compared to the control pots at 6.6±0.53 cm. The biofertilizer application also increased the root length from 4.3 ± 0.20 cm in the controls to 9.27 ± 0.32 cm in the treated pots. Wet weight and dry weight also increased in the treated pots, with values recorded as 37.27 ± 1.15 g and 1.93 ± 0.15 g, respectively, compared to 6.8 ± 1.73 g and 0.83 ± 0.06 g in controls. The seed germination time was fast in the treated pots, 7.67 ± 0.58 days, compared to the control pots, 9.0 ± 0.0 days. Similarly, leaf area was significantly more significant in the treated group (84.10 ± 19.24 cm²) compared with the controls (18.61 ± 5.43 cm²). Moreover, the length and width of leaves were improved by the application of biofertilizers to 15.24 ± 1.91 cm and 6.87 ± 1.32 cm for treated pots, as compared to 7.71 ± 1.83 cm and 4.5 ± 0.81 cm for the control, respectively. Furthermore, leaves were significantly higher in treated plants (10.0 ± 0.58) than in the controls (9.0 ± 0.58). The shoot length, wet weight, and leaf area of salad leaves grown under treatment conditions increased by 157.07%, 448.04%, and 351.96%, respectively compared to the control. These results confirm the effectiveness of the biofertilizer spray in improving all measured parameters compared to the untreated control. These findings align with research conducted by **Abdel-Ilah *et al* (2022)**, which demonstrated the beneficial effects of plant growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), and compost on the growth of lettuce (*Lactuca sativa*) under field conditions. Their field experiments demonstrated that organic and biological fertilizers significantly enhanced the growth parameters of lettuce plants compared to control groups, achieving results comparable to those obtained using conventional NPK fertilizers.

The growth parameter of radish plants showed significant enhancement ($p < 0.05$) from biofertilizer spray-treated pots compared to the control pots. Shoot length in treated pots measured 23.23 ± 0.49 cm and was considerably higher when compared with control pots that measured 6.2 ± 0.36 cm. Similarly, the root length was highly significantly enhanced to reach 15.57 ± 1.00 cm in the treated plants against 2.73 ± 0.32 cm in the controls. Wet weight and dry weight were also considerably increased in the treated plants to values of 88.23 ± 7.77 g and 9.87 ± 0.47 g, respectively, compared to 10.77 ± 0.72 g and 1.4 ± 0.20 g in the control group. The seed germination time of treated pots was slightly faster (6.67 ± 0.58 days) than that of the control pots (7.67 ± 0.58 days). The leaf area of the treated plants was significantly higher, at 87.98 ± 33.11 cm², compared to the control group with 16.8 ± 7.62 cm². Biofertilizer treatment also significantly enhanced the leaf length and width to 11.96 ± 4.19 cm and 6.57 ± 1.86 cm, respectively, against 4.05 ± 1.11 cm and 3.85 ± 0.74 cm in control plants. The number of leaves in treated pots was higher (8.0 ± 0.52) than in the control pots (6.0 ± 0.41). The treated plants showed significant biomass accumulation and better vegetative development, as evidenced by increases in shoot length, wet weight, and leaf area of about 274.7%, 719.5%, and 423.7%, respectively, in comparison to the controls. These outcomes demonstrate the biofertilizer spray's significant beneficial effects on radish plants' overall growth performance.

Similarly, Biswas and **Shivaprakash (2021)** investigated the effects of co-inoculation with selected biofertilizers, including PSB, on radish growth, yield, and nutrient uptake (*Raphanus sativus*). Their findings revealed that microbial consortia significantly improved growth parameters, such as tuber diameter, length, and weight, representing the capacity of these rhizospheric microorganisms to solubilize and mobilize essential nutrients, making them readily available for plant uptake. Furthermore, **Maheswari and Elakkiya (2014**) conducted a similar study that demonstrated the effect of liquid biofertilizers on the growth and yield of *Vigna mungo*. The findings revealed that among all treatments, the combined inoculation of liquid biofertilizers (T7) exhibited the most favourable response across all tested parameters on the 60th day of observation. This treatment consistently outperformed others, highlighting its potential to optimize plant growth and productivity.

The application of a biofertilizer spray consisting of *Bacillus* spp. and *Aspergillus* spp. significantly enhanced growth parameters of lettuce (*Lactuca sativa*) and Radishes (*Raphanus sativus*). The present study highlights the potential of prepared liquid biofertilizer spray as an eco-friendly alternative to chemical fertilizers. It provides a promising solution for enhancing soil fertility and agricultural productivity. Moreover, this approach provides sustainable agriculture by lowering input costs, improving crop yield, and minimizing environmental impact.

CONCLUSION

The study has focused on the vital role of a biofertilizer consortium comprising *Bacillus* spp. and *Aspergillus* spp. for encouraging plant growth and maintaining soil fertility. The consortium effectively improved critical growth parameters of lettuce and radishes, including shoot and root lengths, leaf area, and biomass accumulation. The pot treated with biofertilizer spray had significant increases compared to the control. These findings emphasize the potential of *Bacillus* spp. and *Aspergillus* spp. as influential nitrogen fixers and phosphate solubilizers, contributing to the sustainability of agriculture by substituting synthetic fertilizers. This will provide valued information on microbial biofertilizers for their use as eco-friendly, efficient alternatives for crop management and will fall in line with global sustainability goals. Further long-term field applications and optimization of consortia composition in future studies shall help strengthen the understanding and scalability of such biological interventions in agriculture.

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