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Enhancement of Plant Growth with Plant-Based Compost and the Heterotrophic *Azotobacter* and *Streptomyces* Inoculation under Greenhouse Conditions

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Abstract

Compost is a natural and sustainable way to improve soil fertility and enhance plant growth. Moringa leaves have high mineral, cytokinin, and vitamin content which are useful for growth so that they can be used as organic fertilizer. *Azotobacter* and *Streptomyces* are from soil and have many biological activities. This study aimed to detect the importance of bioagents formula with Moringa Compost (MC) to enhance plant growth in poor sterile soil and plants were irrigated with half strength of Hoagland nutrient solution. Moringa leaves were collected and cleaned, and organic compost was prepared and analyzed for microbial and chemical composition. The prepared MC was rich in nitrogen and minerals and had high content of bacteria and fungi. The two bioagents used were isolate MB5 and MB11 which were characterized and molecular identified as *Azotobacter chroococcum* MB5 and *Streptomyces griseus* MB11. The free-living *A. chroococcum* can fix atmospheric nitrogen while Streptomyces is a filamentous bacterium with a high ability to produce secondary metabolites. The addition of 20% MC to soil increased soil EC and microbial counts compared to MC-free soil. Moreover, inoculation of soil with either AZ or ST increased the microbial counts and soil EC and the clearest increase was in the case of inoculation of soil with MC+AZ+ST. It also found that MC extract alone with the bacterial filtrates increases seed germination of *Phaseolus vulgaris* L. (common bean), which is a herbaceous annual worldwide plant, grown for its edible dry seeds or green unripe pods. In this regard, inoculation of soil with inoculum of both *A. chroococcum* MB5, and *S. griseus* MB11, in the presence of MC has the most pronounced effect and enhances both the growth, fresh and dry weights, leaf number, plant height, and root length of *P. vulgaris* grown under greenhouse conditions for one month and chemical content of the plant protein carbohydrates, P, N, Ca++ and K+. In conclusion, the combined application *A. chroococcum* MB5 and *S. griseus* MB11, as a biofertilizers with Moringa compost is recommended to enhance *P. vulgaris* growth. The use of these biofertilizers can reduce the use of chemical fertilizers, which can have detrimental effects on soil and the environment. Therefore, further research on the inoculation and application of these microorganisms with MC is essential for sustainable agriculture.

Keywords: Moringa, Compost, Inoculum, *Phaseolus vulgaris, Azotobacter chroococcum, Streptomyces griseus*

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INTRODUCTION

To deliver the nutrients required to feed the world's population, which is expected to increase from 7.3 to 9.8 billion by 2050, new environmentally friendly uses must be developed.[1] Enhancing growth could be performed in one of three ways: directly by adding plant growth-promoting bacteria, which can directly boost growth, decrease pathogen activity, and adjust soil microbial balance.[2] Several microorganisms have been introduced into the soil to boost plant tolerance to various environmental factors. These bacteria can be employed in conjunction with other microbial agents to boost growth due to plant growth-stimulating substances and the production of potential antagonistic agents.[3]

The production of crops for food, fuel, and other industrial purposes is essential for human survival. However, the quality of the soil is a vital factor in crop production. Soil fertility is a complex process that depends on several factors, including the presence of organic matter, beneficial microorganisms, and nutrients. Chemically, using fertilizers is expensive and severely harms the environment and the beneficial soil microorganisms that live there in addition, it poses a risk to human health and can create situations that help in the rapid emergence of chemical-resistant pathogens. To increase plant growth, the application of bioagents with a high degree of safety and minimal environmental impact is recommended.[4] The application of organic fertilizers is a common practice to improve soil fertility and increase crop yield.

Moringa (*Moringa oleifera*) is a drought-tolerant tree native to some parts of Asia and arid regions and has been used traditionally as a source of food and medicine. It produces huge quantities of leaves and composting their wastes can be an excellent way to create nutrient-rich organic matter for soil improvement and sustainable agriculture. Many researchers documented the preparation of compost and organic fertilizer from Moringa leaves which can be used as organic fertilizer and this compost has gained popularity due to its high nutrient content like nitrogen, phosphorus, potassium, and other essential nutrients which improve soil fertility and increase crop yield.[5] Kartika[6] reported that *Brassica rapa* growth and development were increased by Moringa compost which was rich in protein, vitamins A, B, C, D, E, and K, folic acid, biotin, and cytokinin in addition to Ca^{+2}, Fe^{+3}, K^{+1}, Zn^{+2} ions.

Primarily, bacteria are the smallest and the most numerous living organisms in the compost, which account for 80-90% of cells found in a gram of compost. Bacteria are responsible for compost degradation and heat generation by a variety of enzymes that chemically degrade a wide range e of organic materials. In the beginning mesophilic bacteria (0-40°C) predominate but when the compost temperature rises above 40°C, thermophilic bacteria like *Bacillus* dominate at this phase, followed by mesophilic bacteria and actinomycetes at the end of the process when the temperature decreased. Also, Actinomycetes, contribute significantly to composting by degrading complex organics such as cellulose, lignin, chitin, and proteins. Because of their enzymes, they can chemically decompose resistant debris. Some Actinomycete species emerge during the thermophilic phase, whereas others emerge during the cooler curing phase.[7]

The microaerophilic Gram-negative plant growth-promoting *A. chroococcum* is a mesophile, dark-brown water-soluble pigment producer (melanin) and fixes nitrogen under aerobic conditions in the presence of P^{-3}, K^{+}, S^{-2}, Mg^{+2}, and Ca^{+2}.[8,9] *Azotobacter* is commonly found in soil and is used as a bio-fertilizer to enhance plant growth and yield due to making nitrogen available to plants. The application of *Azotobacter* with Moringa compost has been shown to increase the nitrogen content of the soil and improve crop yield.[10]

Streptomyces is a genus of soil bacteria that are known for their ability to produce a wide range of bioactive compounds and lytic enzymes. The use of *Streptomyces* as a bio-fertilizer has been shown to enhance plant growth and yield. Applying *Streptomyces* with Moringa compost has been shown to improve soil fertility and increase crop yield.[11]

Using Moringa compost with *Azotobacter* or *Streptomyces* to enhance plant growth and yield is a sustainable and eco-friendly approach to improving soil fertility[5] due to their ability to fix atmospheric nitrogen and produce bioactive compounds. Sagar *et al*.[12] reported that

Azotobacter and Moringa leaf extract significantly improved the growth and yield of tomato plants. The treated plants had higher plant height, number of branches, number of fruits, and fruit yield compared to the control group. Similarly, Kumar *et al*.[11] detected an increase in the growth and yield of wheat plants after the application of *Streptomyces* and Moringa compost. The increase was clear in plant height, number of grains/spike, and grain yield compared to the control group. The use of this combination enhances plant growth and yield due to the potential of these microorganisms on soil fertility.

Phaseolus vulgaris is one of the most widely cultivated and economically important legume crops, that face drought, salinity, and nutrient deficiencies that adversely affect their growth and production. To address these challenges and promote sustainable agriculture; this study aimed to explore a promising innovative strategy that detects the effect of the combined use of plant-based compost and the inoculation of rhizosphere soil with the heterotrophic diazotroph *Azotobacter* alone or in combination with *Streptomyces* under greenhouse conditions.

MATERIALS AND METHODS

Moringa peregrine compost preparation

The abundant and nutrient-rich Moringa healthy and fresh leaves were collected, dried under sunlight for 5 days, and powdered because smaller particles decompose faster which increases the composting efficiency. The powder was mixed with soil (3:1 w/w) to facilitate the decomposition process and the pile was watered and mixed well every two days to maintain moisture contents around 55% to maintain microbial activities.[13] Regularly, turn the compost pile to aerate it, providing oxygen to the microorganisms responsible for decomposition and speeding up the composting process.[13,14] The compost pile is left to mature for 3 months, depending on environmental conditions and the size of the pile, and the produced heat during this process kills the pathogens and weed seeds. Finally, the organic matter breaks down into rich, dark, and crumbly compost.

The effect of compost extract and the bacterial filtrates on seed germination

Phaseolus vulgaris (common *bean)* seeds were obtained from Al-Tawfeeq Agricultural Supplies, Jeddah, and surface sterilized using 10% NaOCl (3 min), washed with sterile distilled water, and air dried. Compost extract was prepared by shaking 50 g of compost in 50 ml sterile water and the mixture was filtered through filter paper. Surface sterile seeds were soaked in sterile compost extract, filter sterilized (Millipore filter, 0.45 mm) culture filtrate of *Azotobacter, Streptomyces,* their mixture (1:1, V/V) or distilled water for 24 hrs and 5 seeds were transferred to glass Petri dishes plates with sterile filter paper at the bottom, filled with water and all plants were incubated in the dark for 6 days until complete germination.[15] Three replications of each treatment were carried out and germination percentage (%) and index were calculated.[16]

Germination Index= Sum of germinated seed for a certain period / Total days × Total seeds

Seedlings were transplanted in filled sterile pots in the outdoor greenhouse under optimum conditions of temperature, humidity, light, and day/night rhythm from April to May 2022.

Determination of moisture content, pH and EC, and the organic matter of compost

Compost sample (100 g) were oven-dried for 24 hrs at 105°C, weighted and moisture content was calculated by the variance in weight. Moreover, the pH, EC, and organic matter were measured according to Motsara and Roy.[17] After acid digestion, nitrogen content (N), organic matter, P, K, and Fe ions was detected as described in Allen *et al.*[18] and the C/N ratio in compost samples was estimated.[19]

Microbial analyses of compost and soil

Bacteria, actinomycetes, and fungi had a role in compost preparation and their types and counts changed every time. Compost or soil samples were collected, and serially diluted, and microbial counts were estimated on media of Nutrient agar and nitrogen-free agar for bacteria, Starch nitrate agar for actinomycetes, and PDA for fungi using plate count agar.[20] All plates were incubated at 25°C and 45°C for 1-2 days for bacteria and 25°C for actinomycetes and fungi. Also, total counts of phosphate-solubilizing bacteria were detected using Pikovskaya's medium. Compost suspension was spread on the previous agar medium and the plates were incubated at 30°C for 2 days. The colonies with clear zones around bacterial growth were counted and recorded as positive results for phosphate solubilization.[21]

Bacterial identification

The most dominant free-living bacterial isolate MB5 which grows well in nitrogen-free medium and actinomycete isolate MB11 which showed leathery and powdery colonies on Starch nitrate agar were selected, characterized, and identified using morphological and physiological methods. The selected isolates were gram staining and examined under light microscopes and biochemically characterized by starch hydrolysis, oxidase test, carbohydrate fermentation, and color of diffusible pigment[22,23] in addition to molecular methods using partial sequencing of 16S rDNA. The universal eubacterial primers 16S rDNA 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) were used.[24] The PCR reactions were performed and the amplification process was confirmed by ethidium bromide fluorescence in 1% agarose gel.

Plant-promoting bacterial growth and activities

The growth of the two isolated bacteria was measured by determining the optical density at 500 nm. The bacterial cells were collected by centrifugation at 5000 rpm for 10 min and cells were prepared in a sterile saline solution to inoculate soil while filtrate was filtered sterilized and used for seed soaking experiment and determining the percentage of seed germination. The two selected bacterial isolates were screened in vitro for their phosphate solubilizing activity using Pikovskaya's medium,[25] indole Acetic Acid (IAA) was detected after growth in a medium containing 2 g/L tryptophan as described by Ndeddy Aka and Babalola[26] while HCN production was detected as described by Bakker and Schippers.[27]

Soil preparation and plant experiment

Air-dried sand soil (sieved acid-washed sand) was sterilized 2 times in two successive days, air dried, and mixed well with 20% Moringa compost. Sterile pots were filled with either 2 kg of sterile sand soil only (G1) or filled with 2 Kg of the previous soil mixture (G2-G5) and were inoculated with AZ (G3), ST (G4), and AZ+ST (G5). Each group contained 10 replicates and five seedlings per pot which were grown at 25°C and soil water potential stayed near field capacity.

Inoculation of soil by bacteria and plant growth studies

Azotobacter cells were grown on nitrogen-free agar (Agar 1.5%, Sucrose 0.5%, $CaCO_3$ 0.5%, $MgSO_4$ 0.02%, NaCl 0.02%, KH_2PO_4 0.02%, $FeSO_4$ 0.0005%) and isolate MB11 was grown on starch nitrate agar for 4 days at 100 rpm and 25°C. and cells were collected, re-suspended in saline solution (A_{500nm} =0.5) and 2 ml of the suspension (2x10^6 CFU/ml) was used to inoculate nitrogen-free broth or starch nitrate broth in 250 ml Erlenmeyer flasks containing 50 ml of the medium. After 4 days of inoculation, cells were collected, and, re-suspended in saline solution (A_{500nm} 0.45-0.59) and used to inoculate soil with Azotobacter (AZ) or Streptomyces (ST). About 20 ml of the bacterial suspension (2x10^6 CFU/ml) was used to inoculate each pot with 2 kg of soil. The filtrate was used for soaking the *P. vulgaris* (common *bean)* seeds.

Greenhouse experiment was carried out 5 seedlings were transferred to each plastic pot (25x25 cm^2), filled with 2 kg of steam-sterilized sand soil mixed with 20% Moringa compost. The pots were divided into 5 groups (G), G1: control plants (normal soil without compost), G2: plants grown in normal soil, G3: the plants grown in soil and inoculated with *Azotobacter* (20 ml of cell suspension of 8x10^5 CFU/ml), G4: plants grown in soil with compost and inoculated with *Streptomyces* (20 ml of cell suspension of 8x10^5 CFU/ml), and G5: plants grown in soil with compost and inoculated with both bacteria (40 ml of a mixture of cell suspensions of *Azotobacter* and *Streptomyces,* 2x10^6 CFU/ml, V/V). After a week, irrigation was applied with 200 ml two times/week of half strength of Hoagland nutrient solution, composed of these materials in mM: KH_2PO_4, 1.0; KNO_3, 5; $Ca(NO_3)_2$, 5, $(NH_4)Mo_7O_{24}$, 0.0002, $MgSO_4$, 2, Fe/ EDTA, 0.1, H_3BO_3, 0.005, $MnCl_2$, 0.010, $ZnSO_4$, 0.008, $CuSO_4$,0.004.[28]

Plant growth and chemical analysis

After one month, plant growth was measured by detected root depth, shoot length, shoot and root fresh, and dry weights,[29] dry weight was obtained after oven drying by at 70°C for 2 days. In addition, the fresh leaf content of chlorophylls and carotenoids were measured using UV-VIS Spectroscopy after extraction with 95% ethyl alcohol.[30] Chlorophyll's concentrations (µg/ml) were calculated as follows:

Chlorophyll a (Chl_a)= (13.36 A664) - (5.19 A649).
Chlorophyll b (Chl_b)= (27.43 A649) - (8.12 A664),
Carotenoid (Car) = 1000 A470 - (2.13 Chl_a - 97.63 Chl_b) /209.

Moreover, plant shoots of each treatment were collected, dried, and analyzed for protein, and sugar according to protocols methods described in Allen *et al.*[18] The soluble carbohydrate of the shoot was estimated by the Anthrone method while the total content of soluble proteins was estimated according to the Lowry method.[31] After acid digestion, shoot or phosphorus and nitrogen were detected using the methods of Wilde *et al*.[32] while mineral contents of K^+, and Ca^{++} were determined using Shimadzu Atomic Absorption Spectrophotometer (AA -7800 Series).

Statistical analysis

Data of all parameters were collected and analyzed by using the statistical software SPSS. The data were subjected to a one-way analysis of variance (ANOVA) to evaluate the difference in mean values ($P < 0.05$) of treatments.

RESULTS AND DISCUSSION

Composting refers to the dynamic process of converting macromolecular organic substances in organic solid waste into carbon dioxide, water, NH_3, and humus substances through microbial metabolism in a high-temperature.[33] One of the main challenges for crop production in sandy soils is the limitations of available nutrients. These soils suffer from continuous and significant losses in nutrients, and this might negatively affect plant growth. To improve the physical and chemical characteristics of these soils, organic compost is recommended.[34]

Moringa leaves were collected mixed with soil and fermented for about 3 months with watering and shaking until a black color and good smell were obtained. Compost's chemical and microbial properties, including pH, electrical conductivity (EC), temperature, moisture, organic matter, and mineral content were summarized in Table 1. The color was brown and the compost temperature was 30-35°C, pH 7.5, EC was 4.16 dS m^{-1} with 45% moisture content while mineral contents were summarized in Table 1. Compost contained the highest count of true bacteria (2.201×10^7 CFU/g) followed by thermophilic bacteria (0.113×10^7 CFU/g), then actinomycetes (0.009×10^7 CFU/g) and fungi (0.006×10^7 spore/g). The lowest counts were recorded for P-solubilizing bacteria (2.111×10^4 CFU/g) and Free-living N-fixing bacteria (0.003×10^4 CFU/g).

The pH level is an essential parameter during composting.[35] Microbial activities during the composting process are influenced by time.

Table 1. Compost chemical and microbial properties, including pH, electrical conductivity (EC), temperature, moisture, organic matter, mineral content and microbial counts

Parameter	Results	Parameter	Results	Microbe	Counts (CFUorspore/g)
Color	Brown	Total N %	2.760 ± 0.80	Total bacterial counts	2.201×10^7
EC (dS m^{-1})	4.163 ± 0.34	Total P %	0.467.30 ± 0.06	Thermophilic bacterial counts	0.113×10^7
Temperature °C	30-35	Total Ca %	0.379 ± 0.51	Actinomycetes	0.009×10^7
pH	7.5 ± 0.14	Total Fe %	0.121 ± 1.09	Fungi	0.006×10^7
Moisture %	45.1	Total K %	0.271± 1.23	P solubilizing bacteria	1.111×10^4
Organic matter %	27.661 ± 1.5	C/N	17.961 ± 0.30	Free-living N fixing bacteria	0.003×10^4

Data are means of three replicates ± standard deviation

Table 2. The tested morphological, physiological and biochemical characters of the isolate MB5 and MB11

Tested character	Isolate MB5	Isolate MB11	Tested character	Isolate MB5	Isolate MB11
Shape	Cocci	filamentous	Temperature (°C)	25-32	ND
Gram stain	Negative	Positive	Growth in nitrogen-free medium	+	-
Color	Black	Dark Gray	pH range	5-8	5-11
Soluble pigment	Found	Found	Cyst formation	Found	Not Found
Mycelia and spore chain	ND	Found	Phosphate solubilization	+	+
HCN production	+	+	ACC	+	+
H2S production	+	+	Siderophore production	+	+
IAA production	+	+	Nitrate reduction	+	+
Utilization of phenylalanine	+	+	Gelatinase, catalase and oxidase	+	+
Decomposition of xanthine, casein, chitin, pectin, urea	-	+	Resistance to Kanamycin, Rifampin and Tetracycline	+	+
Tolerance to NaCl (10%)	+	+	Tolerance to NaCl (15%)	-	+

+: positive results, -: negative results

Typically, compost has a pH ranging from 6 to 9.5.[36] The EC of compost is a crucial salinity indicator and indicates its suitability for use. EC typically rises during composting as a result of organic matter degradation, which produces inorganic compounds. The EC values of the mature composts fell within the previously documented range of 2.8-10.1 dS m^{-1} for composts derived from solid fraction wastes. One of the most important elements for monitoring the composting process is temperature. Composting is an exothermic process that is affected by the starting substrate, temperature and biodegradability of the microbe present.[37] Under suitable moisture content, Moringa leaves give a good bioavailability to composting by soil microorganisms with high metabolic activities.[35] It is reported that under high moisture content, bacteria and fungi convert wastes into humus.[13] The ideal moisture content was 45-60% is the most suitable and 53% gives the maximum results and the longest duration of the thermophilic period (15 days) which enhances nitrogen content.[38] Moringa wastes were degraded by microbial activity to compost with a high decomposition rate which was noticed by the reduction in the pile volume and change in color. The detected organic matter was 27%, while the high-quality compost is in the range of 25-80%.[39] Aeration and the produced high temperature are required to enhance compost formation.[40] Carbon, nitrogen, and carbon/nitrogen ratio were important measured for the compost and the C/N ratio in compost may vary depending on the bioavailability of carbon and nitrogen.[41] The high carbon content may be due to the high presence of lignin, cellulose, and hemicelluloses complex structures which are difficult to degrade.[42] Moreover, the detected total nitrogen content (2.7%) is lower than that estimated which was 3.2%.[43] It was documented that compost with a C/N ratio of 15-20 is satisfactory as a good nitrogen source while low C/N ratio are due to carbon release as CO_2.[44] Microorganisms need a C/N ratio of approximately 10, which is optimal for their metabolism. Microorganisms require carbon, nitrogen, phosphorus, phosphate, and potassium as primary nutrients for optimal microbial activities. Moringa wastes stayed only for three months to be composted but in contrast to our study, it may stay for a year or even more without complete decay and extending composting time is necessary to produce compost with high nitrogen content.

Compost is a rich source of P and other nutrients e.g., Ca, Fe, and N (Table 1) which were essential elements for numerous physiological functions of the plants.[45] In this work, the most abundant bacteria that were noticed during compost preparation were selected and counted using different agar media. The counts of total mesophilic and thermophilic bacteria, Nitrogen fixing and phosphate solubilizing bacteria in addition to actinomycetes and fungi were recorded in the compost. Most microbes maintained their growth ability when the temperature was 25°C. Total counts of mesophilic ≥ phosphate solubilizing ≥ thermophilic bacteria ≥ actinomycetes ≥ fungi ≥Nitrogen-fixing bacteria. Various bacteria like the genera of *Thermus, Bacillus,* and *Streptomyces* were detected.[46] The type of raw materials in the compost leads to different microbiota compositions during composting. Simultaneously, bacteria might have different responses to nutrients in the raw material, and the bacteria that can degrade these materials rapidly increase in abundance and counts.[42]

The most abundant isolates MB5 and MB11 were selected and identified using morphological and physiological methods (Table 2). The isolate MB5 belongs to free-living bacteria that grow well in the nitrogen-free medium as gelatinous colonies with dark black color and the cells were cocci, motile Gram-negative, cyst forming (Figure 1A and B), oxidase, and catalase positive. They grew well at 25-32°C and generated indole, citrate, catalase, and oxidases according to,[47] and using several approaches, it was recognized as a species belonging to the genus *Azotobacter*.[40,48] Using the previous morphological and biochemical characters in addition to molecular methods using partial sequencing of 16S rDNA, the isolate MB5 was identified as *A. chroococcum* MB5 (Figure 2). The cells are abundant in soil, increasing soil nitrogen concentration and agricultural sustainability.

The most dominant actinomycete isolate MB11 was obtained from compost and showed leathery and powdery grey color colonies on Starch nitrate agar. After examination under light and electron microscopes, the cells were Gram-positive with well-developed filamentous aerial and substrate mycelia carrying straight long spore chains with smooth surfaces and colonies produced pale yellow soluble pigments on agar medium (Figure 1C and 1D). Cells were gram-positive and molecular analysis showed that these isolates belong to genus *Streptomyces* and identified as *S. griseus* MB11 with a 94% similarity level (Figure 3). Similar results was recorded by the previous species.[49]

In this work, IAA was found in the culture filtrate of *A. chroococcum* MB5 and *S. griseus* MB11. It was confirmed that bacteria and actinomycetes produce IAA in growth media.[50,51] *Streptomyces rochei, S. livaceoviridis,* and *S. rimosus* were a producer of IAA, which increase plant growth, and other bacteria were found

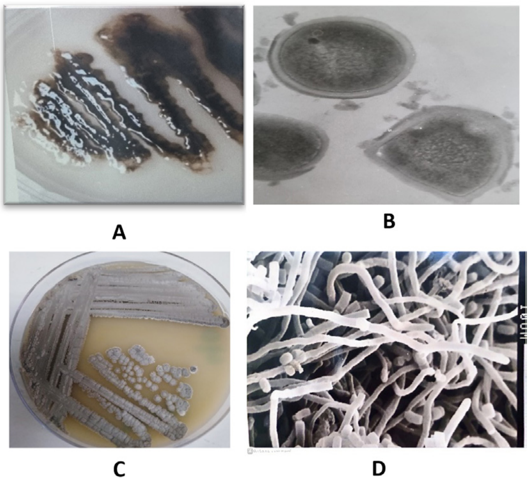

Figure 1. The isolate MB5 on nitrogen-free medium after 5 days of growth at 25°C (A), Under transmission electron microscope (B), isolate MB11 on starch nitrate agar after 7 days of growth at 25°C (C), under scanning electron microscope (D)

Figure 2. The phylogenic tree of the isolate MB5 and the most related isolates

as efficient producers of IAA, siderophore, and phosphate-dissolving organisms.[52-54] Soil bacteria synthesize phytohormones that promote plant growth and alter the morphology and structure of roots.[55] These bacteria are considered eco-friendly biofertilizers since they are inexpensive and provide a sustainable source of nutrients to plants, reducing reliance on chemical fertilizers. They also play an important role in boosting nutrient availability, which promotes plant growth.[56] In this investigation, the filtrates of *A. chroococcum* MB5 or *S. griseus* MB11 or their mixture increased seed germination, which could be related to the presence of IAA and other secondary metabolites.

Soil pH, EC, and microbial counts of the detected bacteria and fungi in inoculated and inoculated soil after one month of plant growth were summarized in Table 3. After plant growth, soil pH was not affected by the inoculated microorganism except, in the case of using cells of AZ+ST, a significant increase was noticed (Table 3). Also, the addition of compost to the soil increases soil pH from 6.5 to 6.9. Moreover, soil EC was increased with soil inoculation compared to control (soil with 20% Moringa compost only). Total bacterial counts at 25°C were increased while the counts at 45°C were under detection limits but the counts of AZ or ST were increased in their inoculated soil. In contrast, there is non-significant increase in fungi counts compared to control.

Plant growth enhancement by soil bacteria and compost

The percentage of seed germination and Germination Index were increased by soaking in MC or AZ or ST extracts and the significant increase was clear in the case of using MC+AZ or MC+AZ+ST extracts (Table 4). The effect of Zn and

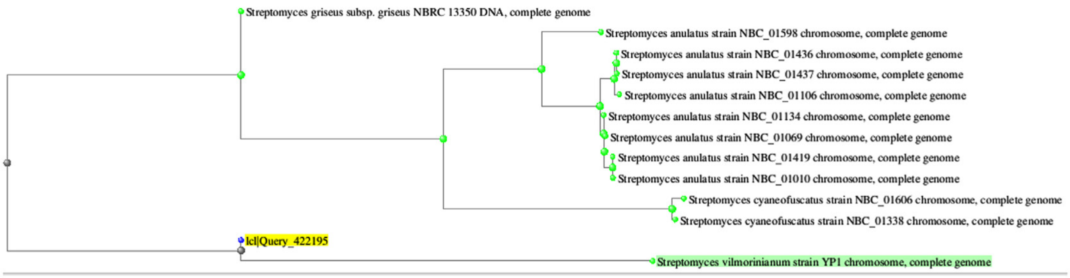

Figure 3. The phylogenic tree of the isolate MB11 and the most related isolates

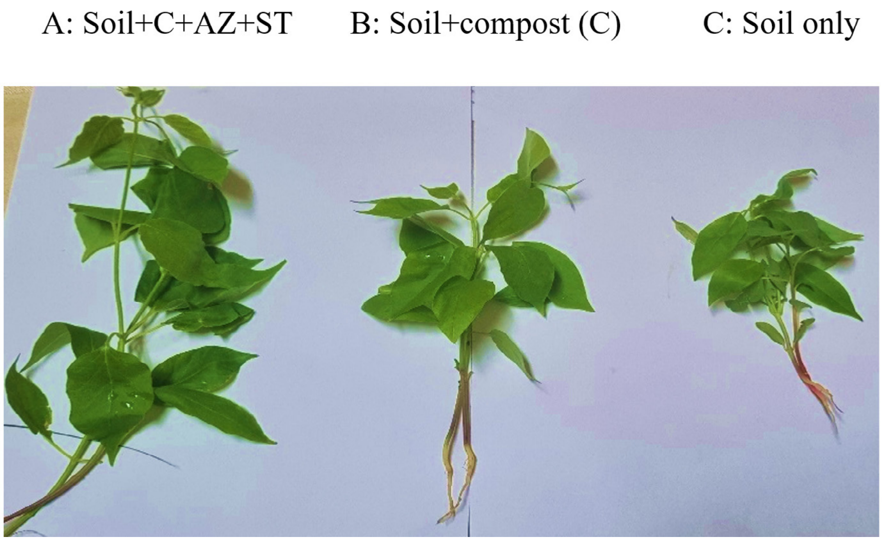

Figure 4. The growth of plants for 30 days after seedling in soil with 20% Moringa compost and inoculated with both *Azotobacter* (AZ) and *Streptomyces* (AZ) or uninoculated and compared to control plants grown in soil only

Table 3. Soil pH, EC and microbial counts of the detected bacteria and fungi in inoculated and inoculated soil after 2 months of plant growth

Treatment	Soil pH	Soil EC	Counts in soil after plant growth x10^4 CFU/g				
			Bacterial Counts		Nitrogen fixing	Actino-mycetes	Fungi
			25°C	45°C			
Soil alone#	6.5 ± 0.45*	0.16 ± 0.01*	ND	ND	ND	ND	ND
Soil+MC (control)	6.9 ± 0.24	0.29 ± 0.05	81	0.009	0.005	0.23	0.11
Soil+MC+AZ	6.9 ± 0.56	0.33 ± 0.06	198*	ND	0.196*	0.28	0.17
Soil+MC+ST	6.9 ± 0.33	0.63 ±0.06*	106*	ND	0.003	0.88*	0.14
Soil+MC+AZ+ST	7.0 ± 0.19*	0.61 ± 0.11*	191*	ND	0.194*	0.97*	0.19

ND: under detection limits, *: significant differences at p > 0.05, #: acid washed sterile washed soil, MC: Moringa compost, AZ: *Azotobacter*, ST: *Streptomyces*

Mn on seed quality and germination in common beans (*P. vulgaris* L.) was studied.[57] The bacterial biofertilizer *Azotobacter*, and of gibberellic acid affect the germination of guava seeds and the single effect of each agent was superior in all studied characteristics and the control treatment recorded the lowest rate.[58]

The bacterial inoculum can be applied to the soil containing organic fertilizers, such as compost or manure, or by spraying it onto the soil surface during the seedling stage and the efficacy of *Azotobacter* or *Streptomyces* as a biofertilizer depends on several factors, including the soil type, crop species, and environmental conditions.[12,59,60] In this experiment, soil containing compost was inoculated with the bacterial inoculum before seedlings were transferred to the pots. After two months of growth, inoculation of soil with AZ, ST and a mixture of AZ+ST had a beneficial effect on plant growth and chemical analysis. Microbial inoculation or the presence of compost enhances the availability of nitrogen and other nutrients to plants, promotes nutrient cycling, suppresses plant pathogens, and enhances plant growth-promoting activities.

In the present study, plant height, root depth, and leaf number, as well as fresh and dry weight, were significantly affected after applying compost treatments or bioagents to the soil (Table 4). It was clearly shown that *P. vulgaris* shoot height, root depth, fresh weight, and dry weight were significantly increased in all treatments compared to the control (compost only). Moringa compost increased all tested plant parameters compared to plants grown in soil without compost (Figure 4). In agreement with the present study, previous reported that compost addition to sandy soil led to higher plants,[61] while the application of different levels of termite mound compost material to sandy soil significantly improved plant height and leaf number.[62] Adding compost and dry *Azolla* to the soil improved both the quality and growth of squash plants.[63] Thus, enhancing soil quality facilitates nutrient absorption by the plant and promotes optimal root development and growth, increasing the total number of roots.[34,64] The increase may be related to N fixation and production of IAA.

Many researchers demonstrated that the enhanced mineralization of nutrients and decomposition of organic matter by bacteria could be ascribed to improved plant growth and productivity.[65,66] Furthermore, it is hypothesized that the compost with growth-promoting bacteria exerts a direct influence on plants through hormonal mechanisms (cytokinins and auxin-like activity) and an indirect influence via metabolic soil microorganisms in response to changes in soil nutrient uptake and physical properties.[67]

Chlorophyll and carotenoids are pigments widely distributed in the chloroplasts in plant leaves and play an important role in plant photosynthesis and there is a close correlation between the leaf chlorophyll content and nitrogen availability. As shown in Table 5, there were significant differences in chlorophyll contents of the leaves in all treatments. However, C+AZ+ST showed significant improvement in chlorophyll a and b, with 1.86 ± 0.02 and 9.69 ± 0.04 mg/g, respectively. Meanwhile, carotenoid content slightly increased in plants inoculated with C+AZ+ST. It was reported that chlorophyll contents

Table 4. Effect of Moringa compost and inoculation with bioagents on Fresh and dry weights, Leaf number, plant height, and root length of Phaseolus vulgaris grown under greenhouse conditions for one month

Property	Soil	Soil+ MC (Control)	MC+AZ	MC+ST	MC+AZ+ST
% of seed germination	66	69	79*	74	79.3*
Germination Index	0.12	0.13	0.15	0.14	0.15
Leaf number	7.9*	8.9 ± 1.22[a]	8.3 ± 1.58[a]*	8.3 ± 1.50[a]*	10.3 ± 2.11[b]*
Fresh weight (g)	9.0*	10.1 ± 0.90[a]	12.12 ± 0.85[a]*	16.62 ± 0.93[b]*	19.10 ± 0.92[c]*
Dry weight(g)	2.3	2.9 ± 0.18[a]	2.99 ± 1.18[a]	3.40 ± 0.06[b]	4.9 ± 0.15[c]
Plant height (cm)	23.8	31.0 ± 4.4[a]	33.30 ± 2.50[c]	37.00 ± 0.99[b]	39.11 ± 0.50[d]
Root depth (cm)	14.0	16.33 ± 1.7[d]	21.5 ± 2.01[b]	28.66 ± 1.07[a]	18.33 ± 1.97[c]

Data are means values of 10 replicates ± standard deviations. *: statistically significant results compared to control at $p < 0.05$. The results with different letters in the same row were statistically significant ($p < 0.05$), MC: moringa compost, AZ: *Azotobacter*, ST: *Streptomyces*

were positively and significantly correlated to organic fertilizers. There is a correlation between nutrient uptake by plant tissues and leaf content of chlorophyll. Therefore, the continuous nutrient supply from the soil by compost addition or inoculation with plant growth-promoting bacteria increases the adsorption of essential elements needed for effective plant photosynthesis.

Following inoculation with AZ, ST, or a combination of the two, soil microbiota boosted root and shoot growth, mineral, and protein content which may be due to nitrogen fixation, auxins, and the synthesis of unidentified chemicals. Similarly, plants inoculated with *A. chroococcum, Azospirillum brasilense,* and *S. mutabilis* showed a significant increase in growth, mineral contents like P, Mg, and N, and total soluble sugars due to IAA release and/or nitrogen fixation by soil microbiota.[68,69]

Due to the presence of nutrient elements, particularly potassium, which aids in photosynthesis, carbohydrate transport, regulation of stomatal opening, and respiration, the physiological properties of plants treated with final compost are enhanced.[70] These results are consistent with prior research that demonstrated the effectiveness of chlorophyll as well as confirmed the final compost's maturity and high quality.[71] Furthermore, compost increased the levels of chlorophyll and carotenoids in comparison to control, as demonstrated.[72]

In soil, plant residues or compost are changed to value-added products by microorganisms and this increases plant growth and nutrient contents, namely protein, carbohydrates, and lipids. In addition, compost is a good bio-fertilizer that contains nutrients that have the potential to be utilized in agriculture.[73] However, during composting, microorganisms degraded the nutrients to simple forms at the same time, N contents were increased in plants grown in inoculated soil with AZ compared to Control, ST, and AZ+ST, respectively. In this study, addition of compost to soil increased plant protein and carbohydrate and P, N, Ca, and K contents (Table 4) compared to control (soil only) and inoculation of soil with AZ, ST, or AZ+ST improved all the previous parameters compared to plants grown in soil with compost only, therefore, addition of Moringa compost and inoculation with benefit microorganisms can be utilized to enhance plant growth and productivity and may lead to distinct functional evolutions of microorganisms. In the present study, compost slightly increased the protein and carbohydrate content of the plant compared to control. This result is in close agreement with other results[74] that reported carbohydrates and protein mainly were increased due to increased microbial activity during composting and plant growth. The inoculum of three strains of nitrogen-fixing bacteria, two concentrations of Moringa leaf extract, and their combinations significantly increased fennel plant growth, yield, and oil components in addition to plant height, branch number per plant, herb fresh weight, fruit weight, umbel number per plant, and fruit yield. Photosynthetic pigments, total phenols, and oil components were also improved.[75] It was

Table 5. Chlorophyll a, b, and carotenoid and macro-nutrient and mineral contents of common bean grown in the presence of Moringa compost and bioagents

treatments	Chlorophyll (mg/g FW)			Essential compounds (mg/g)		Mineral (mg/g)			
	Chl a	Chl b	Car	Carbo-hydrates	Protein	P	N	Ca	K
Soil alone	0.66 ± 0.01^{b}	4.56 ± 0.17^{b}	2.36 ± 0.05^{a}	0.97 ±0.07^{c}	1.55 ± 0.02^{a}		5.26^{b}	5.13^{d}	15.99^{a}
Soil+MC	0.89 ± 0.01^{b}	6.27 ± 0.17^{a}	2.31 ± 0.05^{a}	1.36 ± 0.03^{b}	2.44 ± 0.006^{b}	5.92^{a}	6.29^{d}	6.24^{a}	25.23^{d}
Soil+MC+AZ	0.89 ± 0.04^{b}	8.69 ± 0.04^{c}	2.99 ± 0.08^{b}	1.65 ± 0.02^{a}	3.89 ± 0.07^{c}	7.3^{c}	8.57^{a}	8.23^{b}	26.44^{d}
Soil+MC+ST	1.08 ± 0.01^{c}	8.45 ± 0.07^{c}	2.23 ± 0.03^{a}	1.79 ± 0.02^{a}	4.38 ± 0.004^{d}	785^{b}	6.51^{d}	8.00^{b}	25.23^{d}
Soil+MC+ AZ+ST	1.86 ± 0.02^{a}	9.69 ± 0.04^{c}	2. 99 ± 0.10^{b}	1.91 0.02^{a}	5,.99 ± 0.02^{d}	8.05^{d}	8.16^{a}	8.11^{b}	26.23^{d}

The results with different letters in the same column were statistically significant ($p < 0.05$), MC: Moringa compost, AZ: *Azotobacter*, ST: *Streptomyces*

reported that bacteria might have been capable of absorbing nutrients to endure the composting process. Nitrogen-fixing bacteria *Azotobacter* and plant growth-promoting Streptomyces have many biological activities like nitrogen fixation and production of IAA, phosphate solubilization, and other eco-friendly activities that increase plant growth. When N was easily accessible, plants would predominantly produce compounds with high N content (e.g., proteins for growth). When N availability was scarce, metabolism shifted toward carbon-containing compounds like starch and cellulose, phenolics, and terpenoids. A balanced proportion of nitrogen in soil enhances protein production, hence increasing microbial activity resulting in a faster degradation rate of unsoluble compounds and good compost which increases plant growth. Moreover, K^+ uptake is extremely selective and is intimately linked to plant metabolic activity. Potassium acts as an activator or cofactor for numerous enzymes involved in metabolizing carbohydrates and proteins. Meanwhile, potassium ions are necessary for the activity of over fifty enzymes and plants prefer it in soluble form.[75] Soil N content affects K^+ uptake consequently influencing the levels of carbohydrates and proteins. The nitrogen concentration in the compost was relatively low, thus, the presence of bioagents increased the nitrogen content of the soil, which impacted the carbohydrate and protein content of the plant.

The nutrient content in the soil significantly increased after adding compost which is rich in nutrients, P, N, K, and Ca contents. For plant development, phosphorus is always an essential nutrient and microbial P solubilization seems to be an effective process to release the precipitated P in soil. In the present work, the two selected isolates belong to P-solubilizing bacteria which has beneficial effects on plant yields. Similarly, P solubilizers *Pseudomonas, Bacillus,* and *Paenibacillus* were isolated from Jujube rhizosphere.[9]

In the results of this work, the total P content was shown to be higher in all treatments compared to compost as the control. The gradual increase in P content during the composting process occurred due to the increase in the P water solubility by bacteria during the decomposition of plant wastes. The Ca, P, K and N content in plant shoots increased in microbial-treated plants compared to the control. Organic fertilizers improve soil's organic matter content and nutrient availability which balance plant nutrition and improve soil structure, organic matter, and microbial activity.

The importance of bioagents formula with compost of Moringa to enhance plant growth was noticed (Figure 4), whereas the free-living *A. chroococcum* and Streptomyces were used as bioagents, which are known to have many biological activities and the presence of plant-based compost like Moringa compost plays a vital role in improving soil structure and fertility and enhance plant growth. The results also emphasize the importance of sustainable agriculture and the use of eco-friendly dry *Azolla* increased the nutrient efficiency in soil compared to other fertilization methods.[63] Microorganisms from the genera *Streptomyces, Bacillus, Trichoderma, Pseudomonas*, and nitrogen-fixing bacteria in addition to their enzymes have a role in biological process and plant infection resistance.[76] The use of compost and some plant growth-promoting (PGR) bacteria for enhancing plant growth is more practical, easy to handle, less expensive, and low-cost due to their presence in the soil as agricultural wastes or soil microflora.[77] The use of Moringa compost increases plant growth helps in recycling organic matter, contributes to sustainable agriculture, and enhances soil fertility.[78] The incorporation of compost into the soil enhances water retention, aeration, and nutrient availability, thereby mitigating the adverse effects of stress conditions and promoting beneficial microbial activity, which aids in nutrient cycling and the suppression of soil-borne pathogens.[79] Nitrogen-fixing *Azotobacter* introduces an additional source of nitrogen, reducing the plants' reliance on external nitrogen inputs and potentially mitigating nutrient deficiencies,[59] thus enhancing plant growth and development, especially when nitrogen availability is limited. There are synergistic effects of compost and *A. vinelandii* inoculation when used in combination and increase tomato growth due to many benefits, the first through enhanced stress tolerance to drought and salinity, improved soil structure, and increased nutrient availability resulting from compost application and the additional nitrogen supply from *A. vinelandii*.[80] There is increased nutrient uptake and utilization by plants due to compost solubilization and nutrient release by the action of the soil microbes[81] and there is disease suppression by promoting the healthy soil microbiome, which can help suppress soil-borne pathogens.[80] Plant-based compost reduces environmental impacts and makes it a highly desirable option for sustainable and productive plants. With the evidence from various scientific studies supporting its effectiveness, the use of plant-based compost should be encouraged as a natural and eco-friendly approach to maximizing plant yield, improved soil structure also reduces the risk of soil erosion and compaction, which are critical factors for successful plant growth.[81,82] Compost is a valuable source of essential nutrients for plant growth and contained a variety of macronutrients (nitrogen, phosphorus, and potassium) and micronutrients (such as calcium, magnesium, and iron) that are slowly released into the soil as the compost decomposes.[83] This gradual nutrient release provides a consistent supply of essential elements to plants, promoting steady growth and healthy fruit production.

The Saudi Arabia Standard Organization has released guidelines for organic fertilizers and compost to promote sustainability, environmental protection, and public health. According to the guidelines, compost should be produced from organic materials that are free from harmful substances, weed seeds, and contaminants. Additionally, the compost must have a balanced nutrient composition of nitrogen, phosphorus, and potassium to qualify as an effective fertilizer.[84,85] A quality control system should also be in place to ensure that the compost meets the required standards. Common metals that are tested for in compost include P, N, Ca, and P. A compost sample analysis results were compared to regulatory limits or guidelines to determine if the compost is safe for use in agriculture or other applications. Furthermore, Actinobacteria has shown a biosynthetic potential to produce large amounts of bioactive secondary metabolites with novel structures and remarkable biological activity in agriculture.[86]

Plant-based composts also contribute to developing a diverse and thriving soil microbial community. A healthy soil microbiome is particularly crucial for plant growth. Research has shown that the incorporation of composts and bioagents in the soil can lead to a reduction in the population of harmful pathogens, creating a more disease-resistant environment for plant growth.[87] Utilizing compost for plants reduces the environmental footprint of agriculture and supports eco-friendly farming which aligns with sustainable agricultural practices, reduces the need for chemical fertilizers, helps divert organic waste from landfills, and reduces greenhouse gas emissions, which can have detrimental effects on the environment.[88]

CONCLUSION

The characterization and identification of two bacterial isolates, *A. chroococcum* MB5 and *S. griseus* MB11, and studying their ability to promote plant growth through the production of phytohormones, and nutrient solubilization is one of agriculture's promising and sustainable approaches. Both isolates were found to produce indole acetic acid, which facilitates plant growth because they are considered eco-friendly biofertilizers that enhance nutrient availability and reduce dependence on chemical fertilizers. Inoculum of beneficial microorganisms with eco-friendly organic bio-fertilizer Moringa compost is an effective way to enhance plant growth, improve soil fertility, increase crop yield, and reduce the dependence on chemical fertilizers. This strategy improves soil structure, enriches the soil with essential nutrients, and promotes the growth of beneficial microorganisms. Moreover, the nitrogen-fixing ability of bacteria offers a sustainable and eco-friendly solution for addressing nutrient deficiencies, particularly in environments where stress factors are prevalent. Further research and field trials are necessary to optimize the application of this approach for various stress conditions, ensuring the continued advancement of sustainable plant cultivation.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was apporved by the Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia, on 20/May/2022.

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