



Sustainable Improvement of Soil Fertility and *Phaseolus vulgaris* Yield using Biochar, *Azolla pinnata* and Plant Growth-Promoting Rhizobacteria

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ABSTRACT

Background: Enhancing crop productivity while maintaining soil fertility remains a major challenge in arid and semi-arid regions, where sandy soils are low in organic matter and nutrient retention. Sustainable alternatives to chemical fertilizers are therefore essential. This study evaluated the effectiveness of integrating plant growth-promoting rhizobacteria (*Bacillus circulans*), *Azolla pinnata* and biochar as soil amendments to improve soil health and the productivity of *Phaseolus vulgaris* (common bean).

Methods: A field experiment was conducted over two consecutive growing seasons (2022-2023) at the Ismailia Agricultural Research Station, Egypt. Treatments included individual and combined applications of *B. circulans*, *Azolla* and biochar. Soil physicochemical properties, macronutrient availability (N, P and K), microbial activity, including dehydrogenase enzyme activity and plant growth and yield parameters were evaluated.

Result: The integrated treatment significantly improved soil properties, increasing organic matter (0.66-0.71%) while slightly reducing pH (7.61-7.59) and electrical conductivity (1.11-1.09 dS m⁻¹). Nutrient availability was enhanced, with nitrogen reaching 48.10-49.10 mg kg⁻¹, phosphorus 5.62-6.62 mg kg⁻¹ and potassium 185-187 mg kg⁻¹. Microbial biomass and enzymatic activity were markedly stimulated. Plant growth traits, including biomass, leaf number, plant height and photosynthetic pigments, were improved, resulting in higher seed yield. These findings demonstrate that the synergistic use of biochar, *Azolla* and *B. circulans* effectively enhances soil fertility, microbial activity, plant growth and yield, providing a practical and eco-friendly strategy for sustainable agriculture in nutrient-poor, semi-arid regions.

Key words: *Azolla pinnata*, *Bacillus circulans*, Biochar, *Phaseolus vulgaris*, Plant growth-promoting rhizobacteria, Soil fertility, Sustainable agriculture.

INTRODUCTION

The global land cover is diminishing due to the rapid growth of the human population. The use of chemical fertilizers in agriculture, the destruction of large ecological systems throughout the biosphere, such as plants that cannot absorb CO₂ and the production of energy fuels that both increase the demand for fossil fuels and offer low-energy fuels at high energy costs are examples of how human activity also affects the environment (Korsa *et al.*, 2024).

The continuous decline in fertile agricultural land has intensified the need for sustainable strategies to improve soil productivity, particularly in newly reclaimed areas. Land reclamation has become a common practice to expand agricultural production; however, reclaimed soils often suffer from low fertility, poor organic matter content and limited water-holding capacity, which restrict crop productivity and increase reliance on chemical fertilizers and irrigation (Hou *et al.*, 2024; Wang *et al.*, 2024). Sandy loam soils, frequently found in reclaimed lands, are characterized by high permeability but weak nutrient retention and low microbial activity, making them highly susceptible to nutrient loss and soil degradation (Musei *et al.*, 2024). Therefore, developing sustainable soil management strategies that enhance soil fertility and resource-use

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efficiency is essential for maintaining agricultural productivity in such environments.

Biological and organic soil amendments have emerged as promising approaches to improve soil health while reducing environmental impacts associated with intensive chemical fertilizer use. Among these, the aquatic fern *Azolla* has attracted considerable attention due to its

capacity to fix atmospheric nitrogen through its symbiotic association with the cyanobacterium *Anabaena azollae*. This natural biofertilization process enhances soil nutrient availability and reduces dependence on synthetic nitrogen fertilizers. In addition to its role as a biofertilizer, *Azolla* contributes to carbon sequestration, environmental remediation and sustainable biomass production and can be used as livestock and aquaculture feed (Korsa *et al.*, 2024; Al-Sayed *et al.*, 2022).

Several studies have demonstrated that the incorporation of *Azolla* into cropping systems improves nutrient availability and crop yield. For example, *Azolla* co-cultivation in rice systems significantly increased grain yield due to nutrient release during decomposition (Bazihizina *et al.*, 2025), while its application in wheat cultivation enhanced nitrogen uptake and productivity (Prabakaran *et al.*, 2022).

Another promising soil amendment is biochar, a carbon-rich material produced through the pyrolysis of organic biomass. Biochar has been widely recognized for its ability to improve soil structure, increase water-holding capacity and enhance nutrient retention, particularly in sandy soils with low fertility (Sharma, 2024). Its highly porous structure facilitates the adsorption and gradual release of nutrients, thereby reducing nutrient leaching and improving plant nutrient uptake (Das and Ghosh, 2023). Furthermore, biochar amendments have been shown to stimulate soil microbial activity and enhance biological processes involved in nutrient cycling (Wang *et al.*, 2022). These improvements in soil physicochemical and biological properties can enhance chlorophyll synthesis, photosynthetic activity and ultimately crop productivity (Zhang *et al.*, 2024).

Plant growth-promoting rhizobacteria (PGPR) represent another key component of sustainable soil management. These beneficial microorganisms stimulate plant growth through multiple mechanisms, including phytohormone production, nitrogen fixation and phosphate solubilization (Kunal *et al.*, 2023; Singh and Srivastava, 2025). Their application as biofertilizers has gained increasing attention due to their ability to improve nutrient availability, enhance root development and increase crop productivity (Ling *et al.*, 2022; Singh and Singh, 2018). For instance, inoculation with *Azospirillum brasilense* has been reported to increase wheat yield while reducing nitrogen fertilizer requirements (Galindo *et al.*, 2022). In addition, phosphate-solubilizing bacteria such as *Pseudomonas* and *Bacillus* improve phosphorus availability and may also suppress soil-borne pathogens, thereby enhancing plant health and productivity (Sun *et al.*, 2023).

The common bean (*Phaseolus vulgaris*) is an important leguminous crop widely cultivated in temperate, subtropical and tropical regions. The crop is valued for its high nutritional content, providing significant amounts of protein, dietary fiber, vitamins and essential minerals (Lisciani *et al.*, 2024). In Egypt, common bean cultivation plays an important role in both human nutrition and agricultural economies,

particularly for smallholder farmers (Karavidas *et al.*, 2022; Draz *et al.*, 2022). As a leguminous species capable of biological nitrogen fixation, *P. vulgaris* also contributes to improving soil fertility and supports sustainable cropping systems (Amer *et al.*, 2025; Singh *et al.*, 2016). Despite its importance, the productivity of common bean in reclaimed sandy soils remains limited due to poor soil fertility and unfavorable physicochemical properties.

Although previous studies have separately demonstrated the beneficial effects of *Azolla*, biochar and PGPR on soil fertility and crop performance, research investigating their combined and synergistic effects in reclaimed sandy soils remains limited. Integrated soil amendment strategies may provide a more effective approach to improving soil health, enhancing nutrient availability and increasing crop productivity under nutrient-poor conditions.

Therefore, the objective of this study was to evaluate the integrated effects of biochar, *Azolla* and plant growth-promoting rhizobacteria inoculation (*Bacillus circulans*) on the physicochemical properties and fertility of sandy soil, as well as their combined influence on the growth and productivity of *Phaseolus vulgaris* cultivated in reclaimed agricultural land.

MATERIALS AND METHODS

Site description and soil characterization

The field experiments were conducted during the two successive summer seasons of 2022 and 2023 at the Ismailia Agriculture Research Station farm, Ismailia Governorate, Egypt (30°35'41.9"N, 32°16'45.8"E). To determine the initial physical and chemical properties, representative soil samples were collected from the surface layer (0-30 cm depth) before planting in each season. The analyses were performed following the standard methodologies described by Page *et al.* (1982) and Bottomley *et al.* (2020) and the results are presented in Table 1. Preliminary analysis confirmed the soil was sandy in texture and exhibited low fertility status with respect to available macronutrients.

Experimental design and treatments

The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each experimental plot measured 12 m² (4 m length × 3 m width). Eight treatments were evaluated to assess the individual and combined effects of three soil amendments: *Azolla pinnata*, biochar and the plant growth-promoting rhizobacterium (PGPR) *Bacillus circulans*. All treatments, including the control, received the full recommended dose of NPK chemical fertilizers as described in Section 2.3. The eight treatments were as follows:

- 1. Control:** No organic or bio-fertilizer amendments applied.
- 2. *Bacillus circulans* (B.c.):** Soil inoculated with *B. circulans*.
- 3. *Azolla pinnata* (A):** Soil amended with *Azolla pinnata*.
- 4. Biochar (B):** Soil amended with biochar.

5. A + B.c.: Soil amended with *Azolla pinnata* and inoculated with *B. circulans*.

6. A + B: Soil amended with both *Azolla pinnata* and biochar.

7. B.c. + B: Soil inoculated with *B. circulans* and amended with biochar.

8. B.c. + B + A: Soil inoculated with *B. circulans* and amended with both biochar and *Azolla pinnata*.

Crop management and fertilizer application

The common bean (*Phaseolus vulgaris* L.) cultivar 'Dokki 126' was used as the test crop. A uniform basal dose of phosphorus fertilizer as calcium superphosphate (15% P₂O₅) was applied to all plots at a rate of 200 kg fed⁻¹ just before sowing. Potassium fertilizer, in the form of potassium sulfate (48% K₂O), was applied at a rate of 50 kg fed⁻¹ in two equal splits: at sowing and 30 days after sowing (DAS). Nitrogen fertilizer, as ammonium nitrate (33.5% N), was applied at a rate of 350 kg N fed⁻¹ in four equal splits at 15, 30, 45 and 60 DAS.

Amendments and their characterization

- **Biochar (B):** Biochar was produced from sugarcane bagasse *via* slow pyrolysis at 450°C. The produced biochar was ground and sieved to a particle size of <0.5 mm. Its physicochemical properties, including pH, EC and nutrient content (N, P, K), were determined according to Page *et al.* (1982) and are presented in Table 2. Biochar was applied to the soil at a rate of 17.5 m³ fed⁻¹ before sowing.
- **Azolla (A):** Fresh *Azolla pinnata* was obtained from the Agricultural Microbiology Research Department, SWERI, ARC, Giza, Egypt. The primary chemical composition (*e.g.*, N, P, K, organic matter) was analyzed following the procedures of (El-Shahat, 1988) and is presented in Table 3. *Azolla* was applied into the soil as a dose of 430 kg ha⁻¹ (180 kg fed⁻¹) at the time of sowing.
- **Bacillus circulans (B.c.):** The PGPR strain *Bacillus circulans* was used in this study. The bacterial culture was maintained on nutrient broth with 15% glycerol at -80°C (Salem *et al.*, 2024). For the field application, the bacterium was cultivated aerobically in nutrient broth on a rotary shaker (150 rpm) at 28°C for 48 hours. The final concentration of the inoculum was adjusted to 10⁹ CFU mL⁻¹. This bacterial suspension was diluted with irrigation water and applied to the soil at a rate of 10 L fed⁻¹ immediately after sowing and again 45 days later.

Soil sampling and post-harvest analysis

At the end of the growing season (harvest), soil samples were collected from a depth of 0-30 cm from each experimental plot. The samples were air-dried, crushed, homogenized and passed through a 2 mm sieve before analysis for the following parameters according to standard methods (Cottenie *et al.*, 1982).

Table 1: Chemical and physical characteristics of the investigated surface layer of soil (0-30 cm).

Soil properties		Values
Physical properties		
Particle size distribution	Fine sand (%)	40.4
	Silt (%)	3.2
	Clay (%)	6.0
	Coarse sand (%)	50.4
	Soil texture	Sandy
Available water (%)	7.20	
Wilting point (%)	1.90	
Field capacity (%)	9.13	
Organic matter (%)	0.41	
Bulk density (g cm ⁻³)	1.62	
Total porosity %	47.5	
Saturation percentage (%)	21.23	
Calcium carbonate (%)	5.95	
*pH (Soil paste)	7.59	
**EC (dS m ⁻¹)	1.31	
Chemical properties		
Soluble cations (meq L ⁻¹)	Ca ⁺⁺	5.51
	Mg ⁺⁺	2.75
	Na ⁺	10.69
	K ⁺	1.03
Soluble anions (meq L ⁻¹)	CO ₃ ⁻	7.70
	HCO ₃	3.20
	Cl ⁻	14.80
	SO ₄ ²⁻	2.91
Available nutrients (mg kg ⁻¹)	Nitrogen	44.2
	Phosphorus	4.99
	Potassium	171.0

*Determined in soil paste; **Determined in soil paste extract.

Table 2: Chemical and physical characteristics and available nutrients of biochar.

Biochar properties		Values
Physical properties		
Moisture content (%)		25.72
Organic matter (%)		46.39
Bulk density (g cm ⁻³)		0.16
Chemical properties		
C, %		82.48
N, %		0.70
H, %		1.35
*pH (Sus.1:10 d Sm ⁻¹ :2.5: water suspension)		6.87
*EC (d Sm ⁻¹) (1:10 bio char water suspension)		0.85
Available nutrients		
Macronutrients (%)	Total N	2.55%
	Total P	0.65%
	Total K	1.18%

- Soil pH was measured in a 1:2.5 (soil: water) suspension.
- Electrical conductivity (EC) was measured in a 1:5 (soil: water) extract.
- EC of biochar was determined in a 1:10 biochar-water suspension.
- Organic matter (OM) content was determined.
- Available nitrogen (N), phosphorus (P) and potassium (K) were extracted and quantified.

Soil biological activity

- **Dehydrogenase activity (DHA):** Soil dehydrogenase activity was assessed at 30 and 60 DAS following the method described by Thalmann, (1967).
- **Total bacterial count:** The total bacterial population in the soil was enumerated at 30 and 60 DAS using the method of Holm and Jensen, (1972).

Plant analysis

At harvest, plants from each plot were collected to determine the yield and its components. Plant samples (straw and seeds) were oven-dried at 70°C to constant weight, then ground. The dried plant material was digested using a H₂SO₄-H₂O mixture (Page *et al.*, 1982). The digests were analyzed for total N, P and K content following the methodologies described by Cottenie *et al.* (1982).

Photosynthetic pigment determination

Fresh leaf samples were collected at the flowering stage to determine photosynthetic pigments. Chlorophyll *a*, chlorophyll *b* and carotenoids were extracted and their concentrations were measured spectrophotometrically at

wavelengths of 663, 647 and 470 nm, respectively. The pigment concentrations were calculated using the formulas provided by (Moran, 1982) and expressed as mg 100 g⁻¹ fresh weight (F.W.).

Statistical analysis

All data collected were subjected to a two-way analysis of variance (ANOVA) appropriate for a Randomized Complete Block Design (RCBD) using SPSS software (Version 20). When a significant F-test was observed ($P \leq 0.05$), treatment means were compared using Duncan's multiple range test. All figures were generated using Origin Pro software (Version 2024).

RESULTS AND DISCUSSION

The integration of biochar, *Azolla pinnata* and *Bacillus circulans* markedly enhanced soil properties, nutrient availability, microbial activity and *Phaseolus vulgaris* growth and yield over two seasons.

Soil physicochemical properties

The integrated application of biochar, *Azolla pinnata* and *Bacillus circulans* significantly improved soil physicochemical properties over two seasons. Organic matter increased to 0.66-0.71%, pH decreased slightly to 7.61-7.59 and electrical conductivity (EC) was reduced to 1.11-1.09 dS m⁻¹ (Table 4). Biochar and *Azolla* applied individually also improved these parameters moderately, whereas the *Azolla* + *B. circulans* combination showed less pronounced effects. These results align with Kabir *et al.* (2023), Fakhar *et al.* (2025) and Kozioł *et al.* (2024), who reported biochar amendments enhance soil aggregation, organic carbon

Table 3: Some characteristics of *Azolla pinnata*.

Total nitrogen	3.00	C/N: Carbon/nitrogen	15/1
Organic matter	78.20	TP: Total phosphorus,	0.7
Organic carbon	45.35	TK: Total potassium	3.5
Ash	21.8		

Table 4: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* (B.c.) on soil pH, electrical conductivity (EC) and organic matter (OM) during two growing seasons.

Treatments	First season			Second season		
	pH	EC (dS m ⁻¹)	OM (%)	pH	EC (dS m ⁻¹)	OM (%)
Control	7.80±0.01h	1.32±0.16i	0.50±0.01h	7.81±0.02h	1.31±0.08i	0.53±0.01j
Biochar	7.62±0.09b	1.15±0.11a	0.65±0.08b	7.60±0.18b	1.14±0.14b	0.69±0.19b
B.c.	7.67±0.10b	1.17±0.13b	0.61±0.03c	7.65±0.11c	1.18±0.13c	0.67±0.17c
<i>Azolla</i>	7.64±0.18b	1.13±0.14a	0.65±0.06b	7.60±0.10b	1.14±0.16b	0.69±0.13b
<i>Azolla</i> + B.c.	7.73±0.10d	1.24±0.10e	0.54±0.04g	7.71±0.10d	1.27±0.16e	0.59±0.09f
<i>Azolla</i> + Biochar	7.67±0.10b	1.17±0.19b	0.61±0.09c	7.65±0.10c	1.18±0.11c	0.67±0.17c
B.c. + Biochar	7.64±0.19b	1.12±0.14a	0.65±0.09b	7.60±0.19b	1.14±0.15b	0.69±0.01b
<i>Azolla</i> + B.c + Biochar	7.63±0.10a	1.11±0.12a	0.66±0.07a	7.59±0.11a	1.09±0.19a	0.71±0.12a
LSD ($P \leq 0.05$)	0.39	0.02	0.02	0.32	0.02	0.03

Values represent mean±standard error. Different letters within the same column indicate significant differences among treatments according to the LSD test at $P \leq 0.05$.

and pH stabilization (Kabir *et al.*, 2023; Fakhar *et al.*, 2025; Koziol *et al.*, 2024). Similarly, Marzouk *et al.* (2024) observed *Azolla* incorporation reduces pH in calcareous soils (Marzouk *et al.*, 2024). Contrastingly, some studies report excessive *Azolla* can over-acidify soils (Thapa and Poudel, 2021), highlighting the need for balanced application. Biochar improved soil structure, moisture retention and carbon stabilization; *Azolla* decomposition released organic acids enhancing phosphorus solubility (Marzouk *et al.*, 2024; Kumar *et al.*, 2024) and *B. circulans* contributed to phosphate solubilization (Setiawati *et al.*, 2022). Individually, biochar or *Azolla* moderately improved soil properties, while *Azolla* + *B. circulans* was less effective. Biochar and microbial inoculants also stabilized EC by adsorbing Na⁺ and Cl⁻ ions (Wang *et al.*, 2024).

Soil nutrient availability

Soil NPK levels were highest in the integrated treatment (N: 48.10-49.10 mg/kg, P: 5.62-6.62 mg/kg, K: 185-187 mg/kg; Table 5, whereas individual treatments showed moderate improvements. These findings are consistent with Al Tawaha *et al.* (2025) and Marzouk *et al.* (2025), who reported combined organic amendments enhance nutrient cycling (Al Tawaha *et al.*, 2025; Marzouk *et al.*, 2025). Biochar's high cation exchange capacity (CEC) and *Azolla*'s rapid N release explain the observed effects (Ngambia *et al.*, 2024; Ebrahim *et al.*, 2024). *Bacillus circulans* further mobilizes phosphorus through enzyme activity, agreeing with (Mehta *et al.*, 2010). Some studies, however, suggest biochar alone may not significantly improve P availability in very alkaline soils (Dai *et al.*, 2017), contrasting with the strong synergistic effect seen here (Dai *et al.*, 2017). Biochar enhances nutrient retention by increasing CEC and reducing nutrient leaching, particularly in sandy soils (Ngambia *et al.*, 2024; Wang *et al.*, 2024; Bekchanova *et al.*, 2024).

Soil microbial activity

Microbial counts and dehydrogenase activity increased markedly under integrated amendments (Fig 1), reflecting improved biological soil health. This agrees with (Abd-Elhameed *et al.*, 2022) and (Chhetri *et al.*, 2022), demonstrating biochar provides microbial habitats, while *Azolla* and *B. circulans* supply nutrients and promote enzymatic activity. Seasonal variation influenced microbial dynamics, consistent with environmental effects reported by Wu *et al.* (2024).

Plant growth, photosynthetic pigments and yield

Plant height, branch and leaf numbers increased (Fig 3) indicating enhanced nutrient uptake, root development and overall plant vigor. Fresh and dry biomass improved illustrated in Fig 4, higher fresh weight indicates improved water retention and nutrient absorption, while increased dry weight reflects enhanced carbon assimilation.

Chlorophyll and carotenoid content rose as shown in Fig 2, indicating enhanced photosynthesis (Al-Sayed *et al.*, 2022). Seed yield reached 3.18 t ha⁻¹ (~75% higher than control), with 31.8 pods per plant, 100-seed weight 41.2 g, biological yield 7.23 t ha⁻¹ and harvest index 44%. Seed and biological yields, as well as pods per plant and 100-seed weight, were significantly enhanced by all soil amendments compared to the control, with the combined treatment of *Azolla* + *Bacillus circulans* + Biochar showing the highest improvement in both seasons, reflecting synergistic effects on plant growth and productivity (Table 6) reflecting more efficient nutrient allocation and higher productivity. These results align with Mayly and Hidayat, (2024), Nadeem *et al.* (2017) and Zhang *et al.* (2024), confirming synergistic biochar, *Azolla* and PGPR application improves growth and yield (Mayly and Hidayat, 2024; Nadeem *et al.*, 2017; Zhang *et al.*, 2024). Contrastingly, some single amendments show less

Table 5: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* on available soil nitrogen (N), phosphorus (P) and potassium (K) during two growing seasons.

Treatments	First season			Second season		
	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
Control	39.20h±6.09	4.99g±0.11	171h±33.1	45.10f±6.01	5.09h±0.12	166g±31.12
Biochar	46.09b±7.98	5.59b±0.17	183b±41.1	48.80b±7.91	6.54b±0.17	186.10d±44.56
<i>Bacillus circulans</i>	42.90g±9.01	5.22f±0.16	174g±35.03	46.87e±8.11	6.08g±0.17	170f±38.21
<i>Azolla</i>	44.41e±10.1	5.24f±0.13	176e±37.1	47.00d±9.64	6.14f±0.22	172f±35.01
<i>Azolla</i> + <i>Bacillus circulans</i>	43.90f±6.65	5.35e±0.16	175f±39.1	47.08d±8.77	6.25e±0.19	177e±37.31
<i>Azolla</i> + Biochar	45.97c±8.91	5.56c±0.12	182c±44.19	48.61c±7.89	6.51c±0.20	185.90b±44.56
<i>Bacillus circulans</i> + Biochar	45.21d±20.72	5.39d±0.13	180d±39.1	48.51c±9.13	6.43d±0.16	184.50c±45.01
<i>Azolla</i> + <i>Bacillus circulans</i> + Biochar	48.10a±12.09	5.62a±0.10	185a±41.09	49.10a±10.10	6.62a±0.21	187a±47.11
LSD (P≤0.05)	0.39	0.21	1.57	0.32	0.21	0.31

Values represent mean±standard error. Means followed by different letters within the same column are significantly different according to the LSD test at P≤0.05.

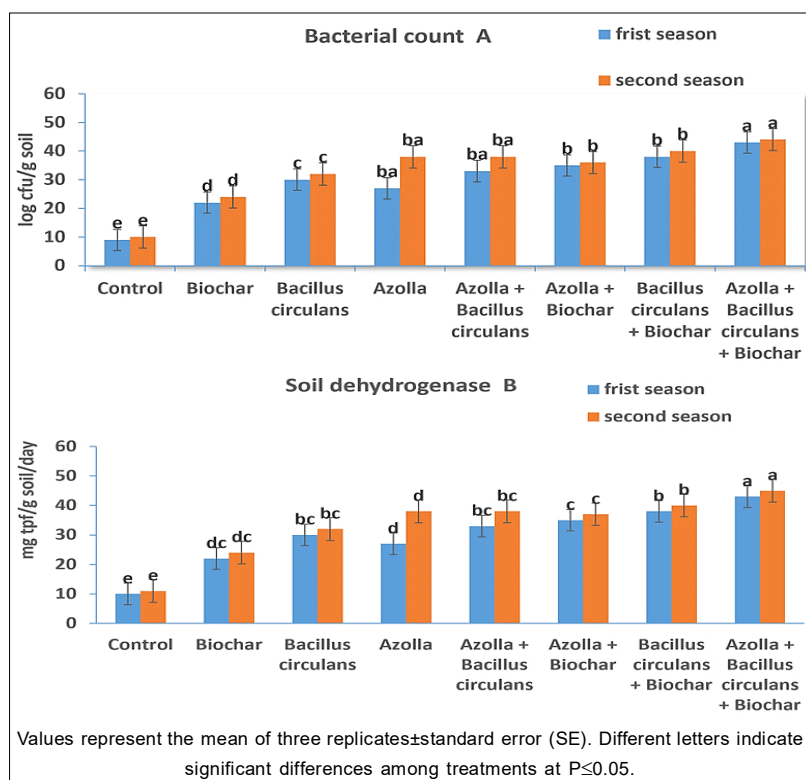


Fig 1: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* on soil microbial count (A) and dehydrogenase activity (B) during two growing seasons.

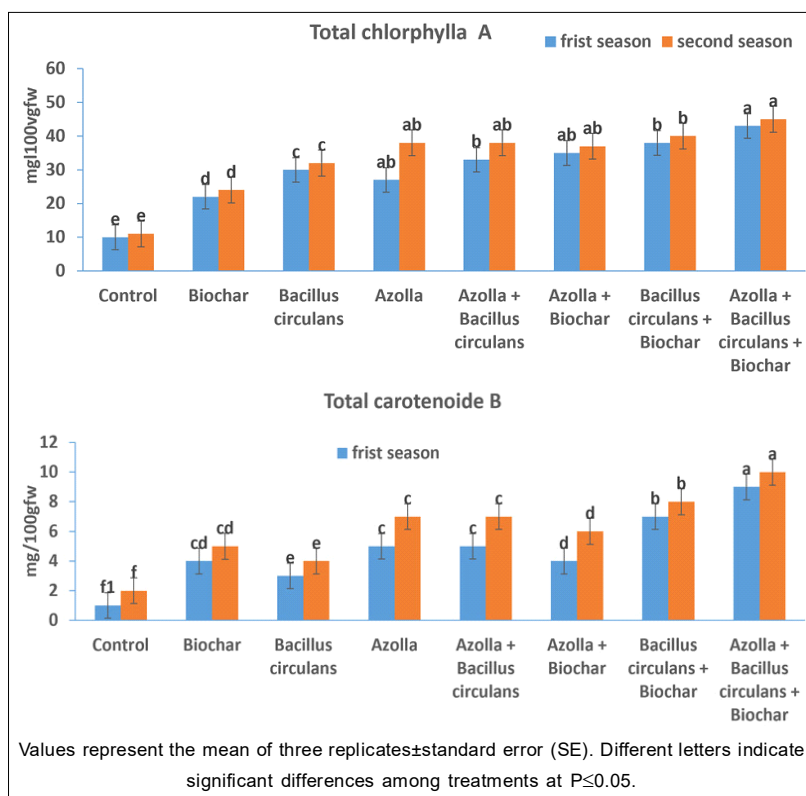


Fig 2: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* on carotenoid pigment content (A) and chlorophyll pigment content (B) of *Phaseolus vulgaris* during two growing seasons.

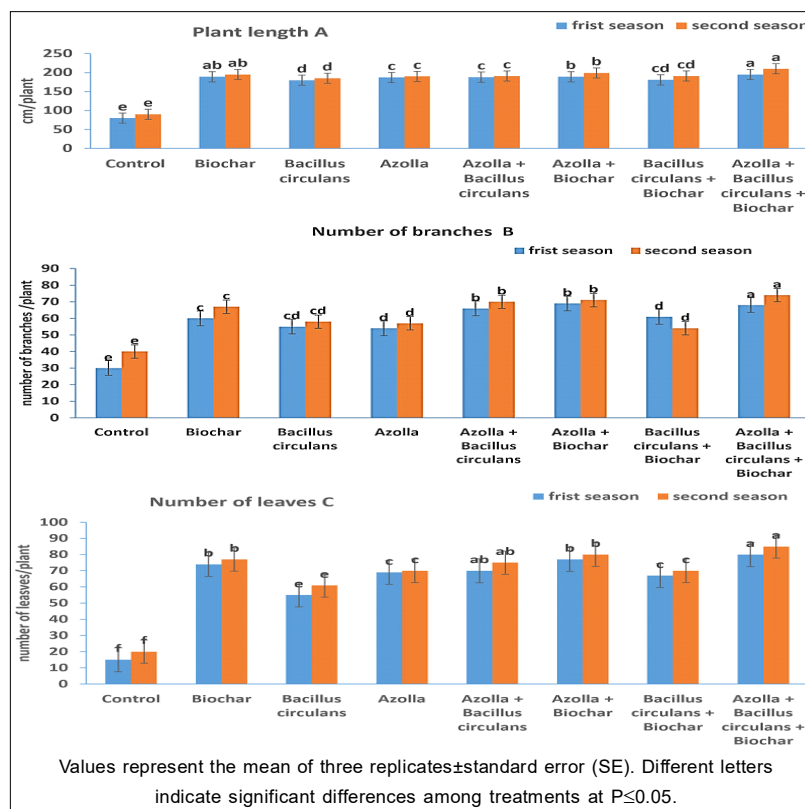


Fig 3: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* on plant length (A), number of branches (B) and number of leaves (C) of *Phaseolus vulgaris* during two growing seasons.

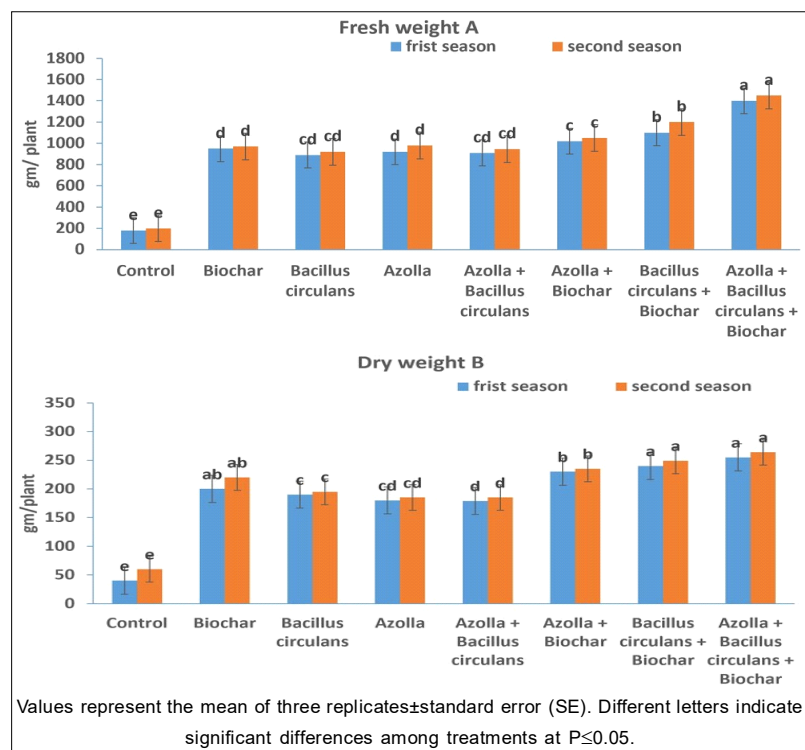


Fig 4: Effect of biochar, *Azolla pinnata* and *Bacillus circulans* on fresh weight (A) and dry weight (B) of *Phaseolus vulgaris* during two growing seasons.

Table 6: Yield parameters of *Phaseolus vulgaris* as influenced by biochar, *Azolla pinnata* and *Bacillus circulans* during two growing seasons.

Treatments	First season					Second season				
	Pods plant ⁻¹	100-seed weight (g)	Seed yield (t ha ⁻¹)	Biological yield (t ha ⁻¹)	Harvest index (%)	Pods plant ⁻¹	100-seed weight (g)	Seed yield (t ha ⁻¹)	Biological yield (t ha ⁻¹)	Harvest index (%)
Control	18.4	34.6	1.82	5.06	36.0	19.0	35.1	1.90	5.20	36.5
Biochar	23.2	36.8	2.29	6.00	38.2	24.0	37.3	2.35	6.12	38.4
<i>Bacillus circulans</i>	21.7	36.0	2.15	5.78	37.2	22.4	36.6	2.21	5.90	37.5
<i>Azolla</i>	22.8	36.5	2.22	5.92	37.5	23.5	37.0	2.28	6.05	37.7
<i>Azolla</i> + <i>Bacillus circulans</i>	24.9	37.4	2.40	6.18	38.8	25.7	38.0	2.47	6.32	39.1
<i>Azolla</i> + Biochar	26.1	38.3	2.55	6.45	39.5	27.0	38.8	2.62	6.60	39.7
<i>Bacillus circulans</i> + Biochar	25.6	37.9	2.49	6.33	39.3	26.4	38.5	2.56	6.47	39.6
<i>Azolla</i> + <i>Bacillus circulans</i> + Biochar	31.8	41.2	3.18	7.23	44.0	32.6	41.8	3.29	7.40	44.5
LSD (P≤0.05)	2.15	1.47	0.26	0.54	2.80	2.18	1.50	0.28	0.57	2.90

Means followed by different letters within the same column are significantly different according to the LSD test at P≤0.05.

pronounced effects, highlighting the importance of combined application (Sadegh *et al.*, 2019).

Implications and future perspectives

This study highlights the potential of combining biochar, *Azolla* and *Bacillus circulans* to improve soil health, nutrient availability and crop productivity in nutrient-poor soils. Their synergistic interaction provides a sustainable strategy for enhancing agricultural productivity.

Future research should evaluate the long-term effectiveness of this integrated approach across different agro-climatic regions and cropping systems. Advanced molecular techniques could also be used to investigate changes in soil microbial communities and functional diversity. In addition, economic analyses and optimization of application rates and timing are needed to ensure practical scalability and maximize the benefits of these amendments under field conditions (Zhang *et al.*, 2024).

CONCLUSION

This study highlights the benefits of integrating plant growth-promoting rhizobacteria (PGPR), *Azolla pinnata* and biochar to improve soil fertility and the productivity of *Phaseolus vulgaris*. The combined application of *Bacillus circulans*, *Azolla* and biochar showed a synergistic effect, improving soil properties by increasing organic matter while reducing soil pH and electrical conductivity, creating more favorable conditions for plant growth.

These amendments also enhanced nutrient availability, resulting in better plant performance. The integrated treatment significantly improved growth parameters, including plant height, number of branches and leaves and fresh and dry biomass. In addition, higher levels of photosynthetic pigments such as chlorophyll and carotenoids indicated improved photosynthetic efficiency and yield potential.

Overall, the results demonstrate that combining PGPR, *Azolla* and biochar is an effective and sustainable strategy for improving soil health and crop productivity. This integrated approach can help reduce dependence on chemical fertilizers while supporting resilient agricultural systems in Egypt and similar agro-ecological regions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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