Scientific Reports
https://doi.org/10.1038/s41598-026-44003-8
Article in Press

Interactive effects of biochar and microbial biofertilizers on sandy soil fertility and cowpea yield in Egyptian agroecosystems

Received: 21 August 2025

Accepted: 9 March 2026

Published online: 11 March 2026

Cite this article as: Khalifa D.M., Hewait H.M., Stanciu A. *et al.* Interactive effects of biochar and microbial biofertilizers on sandy soil fertility and cowpea yield in Egyptian agroecosystems. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-44003-8

Doaa M. Khalifa, Heba M. Hewait, Alina-Stefania Stanciu, Ragheb M. Eladly, M. E. Shalaby & Aleksandra Głowacka

Interactive effects of biochar and microbial biofertilizers on sandy soil fertility and cowpea yield in Egyptian agroecosystems

Doaa M. Khalifa [1] *, Heba M. Hewait [2], Alina-Stefania Stanciu [3], Ragheb M. Eladly [4], M. E. Shalaby [5] and Aleksandra Głowacka [6,*]

[1] Soil Physics and Chemistry Department, Soils, Water and Environmental Research Institute (SWERI), Agriculture Research Center, (ARC) Giza, Egypt, dr.doaaamousa@gmail.com

[2] Microbiology Department, Soils, Water and Environmental Research Institute (SWERI), Agriculture Research Center, (ARC) Giza, Egypt, hebamhewait@arc.sci.eg

[3] Department of Agriculture-Horticulture, Faculty of Environmental Protection, University of Oradea, Oradea, Romania; astanciu@uoradea.ro, ORCID: https://orcid.org/0009-0008-3835-6981

[4] Department of Soil and Agricultural Chemistry, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt; raghebEladly3@alexu.edu.eg

[5] Department of Plant production, Faculty of Agriculture (Saba Basha), Alexandria University, 21531 Alexandria, Egypt; m-shalaby@alexu.edu.eg

[6] Department of Plant Cultivation Technology and Commodity Sciences, University of Life Sciences in Lublin, 13 Akademicka Street, 20-950 Lublin, Poland; aleksandra.glowacka@up.lublin.pl

* Correspondence: aleksandra.glowacka@up.lublin.pl; dr.doaaamousa@gmail.com

ABSTRACT

Cowpea (*Vigna unguiculata* ssp. *unguiculata*) is a promising legume crop for arid and semi-arid regions due to its short growth duration, nutritional value, and drought tolerance. However, sandy soils in Egypt suffer from low fertility, weak aggregation, and poor water-holding capacity, which limit crop productivity. This study evaluated the combined effects of biochar and microbial inoculants (*Bacillus amyloliquefaciens* and *Saccharomyces* *cerevisiae*) on sandy soil fertility and cowpea productivity in Egypt. A two-season (2020–2021) field experiment was conducted in the Ismailia Governorate, Egypt, using cowpea cv. Dokki 126 in a randomized complete block design with 12 treatments (biochar, microbial inoculants, nitrogen fertilizer, and their combinations) and three replicates. The integrated application of biochar and microbial inoculants significantly increased total microbial counts by up to 65% and dehydrogenase activity by 42% compared to the control. Soil bulk density decreased by 18%, total porosity increased by 22%, and available soil N, P, and K contents increased by 45%, 52%, and 39%, respectively, leading to higher chlorophyll content (+28%), plant length (+21%), and seed yield (+33%) relative to the control. These findings indicate that integrating biochar with microbial inoculants significantly enhances sandy soil fertility and cowpea productivity, offering a sustainable pathway for soil restoration in arid ecosystems.

Keywords: Cowpea; *Saccharomyces cerevisiae*; *Bacillus amyloliquefaciens*; biochar; sandy soil; soil fertility; crop productivity; sustainable agriculture.

Introduction

Agriculture in arid and semi-arid regions of Egypt faces critical challenges due to sandy soils characterized by low fertility, low organic matter content, weak structure, and poor water-holding capacity. These constraints severely limit the productivity of major crops and threaten agricultural sustainability[1,2]. Cowpea (*Vigna unguiculata* L. Walp.) is a short-duration legume valued for its high protein content, adaptability to drought, and potential to improve soil fertility through biological nitrogen fixation[3,4]. It is therefore considered a promising crop for sustainable agriculture in Egypt's desert reclamation areas[5].

Conventional strategies to overcome soil degradation typically rely on mineral fertilizers and organic amendments. While effective in the short term, excessive use of mineral fertilizers can degrade soil health, increase production costs, and cause environmental pollution. Organic amendments improve soil structure and nutrient retention, but their slow decomposition and limited availability restrict large-scale application[6-8]. Consequently, integrated and innovative soil management practices are required to enhance soil fertility and maintain crop productivity under arid conditions.

Biochar, a carbon-rich material produced by pyrolysis of organic biomass, has emerged as an effective soil amendment that improves soil physical, chemical, and biological properties, including bulk density, porosity, nutrient retention, and microbial activity[9,10]. Likewise, microbial biofertilizers such as *Saccharomyces cerevisiae* and *Bacillus amyloliquefaciens* can enhance soil biological fertility by decomposing organic matter, producing growth-promoting metabolites, and suppressing soil-borne pathogens[11]. However, the combined effects of biochar and microbial inoculants under Egyptian sandy soils remain poorly understood and may differ depending on soil type, crop species, and environmental conditions[12,13].

We hypothesized that biochar in combination with microbial inoculants would enhance soil properties and cowpea productivity more effectively than mineral fertilization alone. Therefore, the objectives of this study were to: (i) assess the effects of biochar and microbial inoculants on soil physical, chemical, and biological characteristics; (ii) evaluate their influence on cowpea growth and yield; and (iii) identify sustainable soil management strategies suitable for sandy soils in arid agroecosystems.

Results

Soil biological parameters

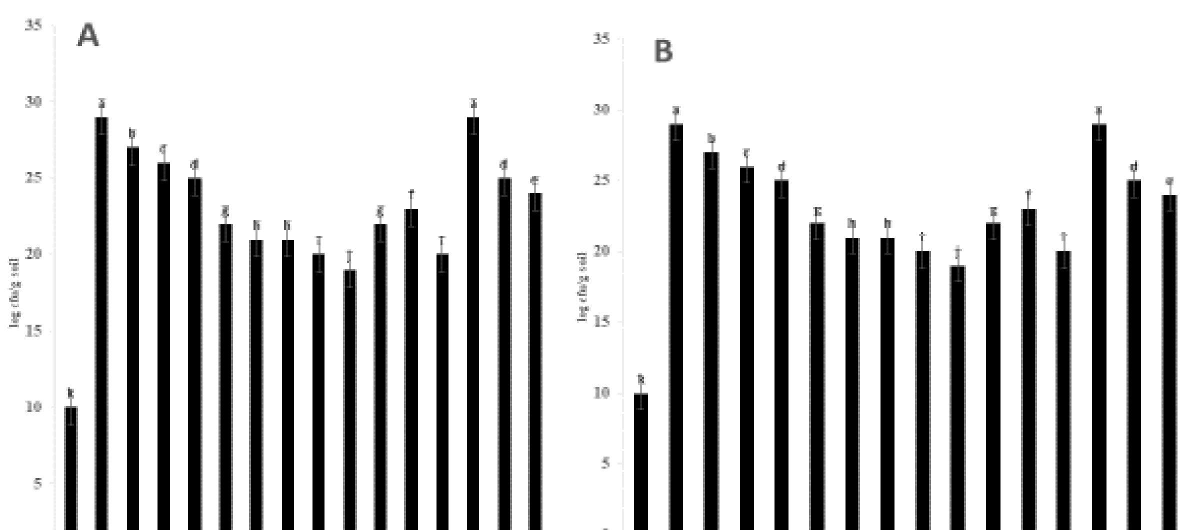
The results in the uploaded (Fig. 1) reveal significant findings on the impact of biochar combined with *S. cerevisiae* (yeast) and *B. amyloliquefaciens* on microbial count and dehydrogenase enzyme activity in soil across two seasons. These treatments were evaluated for their effects on bacterial and yeast counts, as well as soil dehydrogenase enzyme activity, to understand their roles in enhancing soil fertility and microbial ecosystem health.

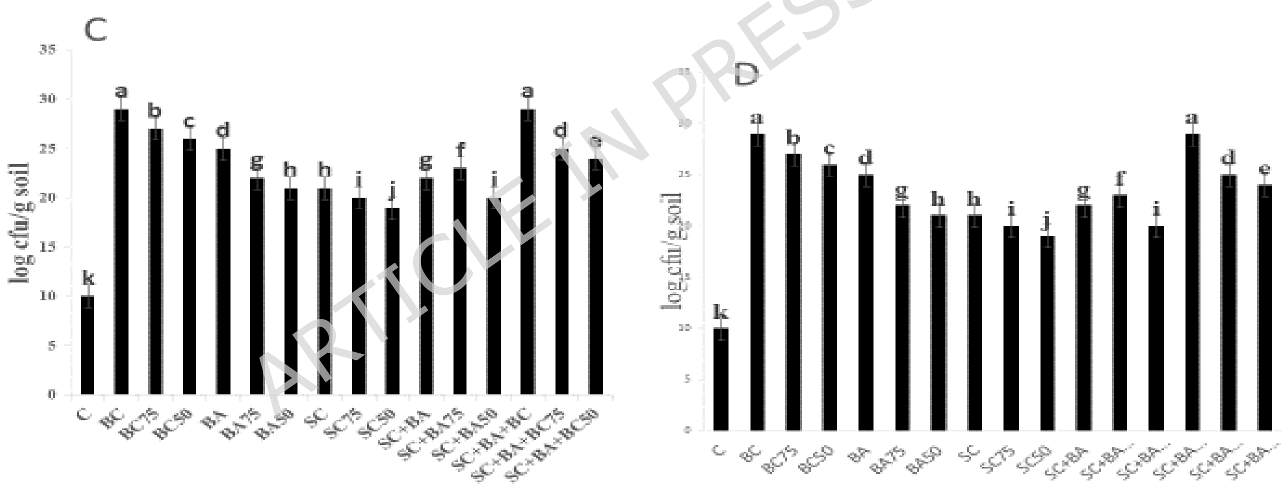
Microbial count

The microbial count increased significantly under treatments involving biochar combined with microbial inoculants compared to the control. In the first season, the combination of *S. cerevisiae*, *B. amyloliquefaciens*, and biochar achieved the highest bacterial count (27 log cfu/g soil), representing a 170% increase compared with the control (10 log cfu/g soil). A similar trend was observed in the second season, with slightly higher microbial counts across all treatments, indicating sustained enhancement of microbial growth and soil microbial fertility (Fig. 1A–B). This trend was mirrored in the second season, albeit with slightly higher microbial counts across all treatments, signifying a persistent enhancement of microbial growth due to the applied treatments.

Soil dehydrogenase activity

Dehydrogenase enzyme activity also increased significantly under biochar + microbial inoculant treatments. In the first season, the combined treatment (*S. cerevisiae* + *B. amyloliquefaciens* + biochar) recorded 31 µg TPF/g soil/day, more than 106% higher than the control (15 µg TPF/g soil/day). In the second season, activity rose to 33 µg TPF/g soil/day, confirming persistent improvement of soil metabolic activity and nutrient cycling (Fig. 1C). The second season reinforced these findings, as dehydrogenase activity generally increased, with the highest recorded activity of 33 µg TPF/g for the same treatment. This result indicates that biochar and microbial inoculants not only improve microbial populations but also boost metabolic processes crucial for nutrient cycling and soil respiration.




C
log cfu/g soil
35
30
25
20
15
10
5
0
C
BC
BC75
BC50
BA
BA75
BA50
SC
SC75
SC50
SC+BA
SC+BA75
SC+BA50
SC+BA+BC
SC+BA+BC75
SC+BA+BC50
k
a
b
c
d
g
h
h
i
j
g
f
i
a
d
e
D
log cfu/g soil
C
BC
BC75
BC50
BA
BA75
BA50
SC
SC75
SC50
SC+BA
SC+BA...
SC+BA...
SC+BA...
SC+BA...
SC+BA...

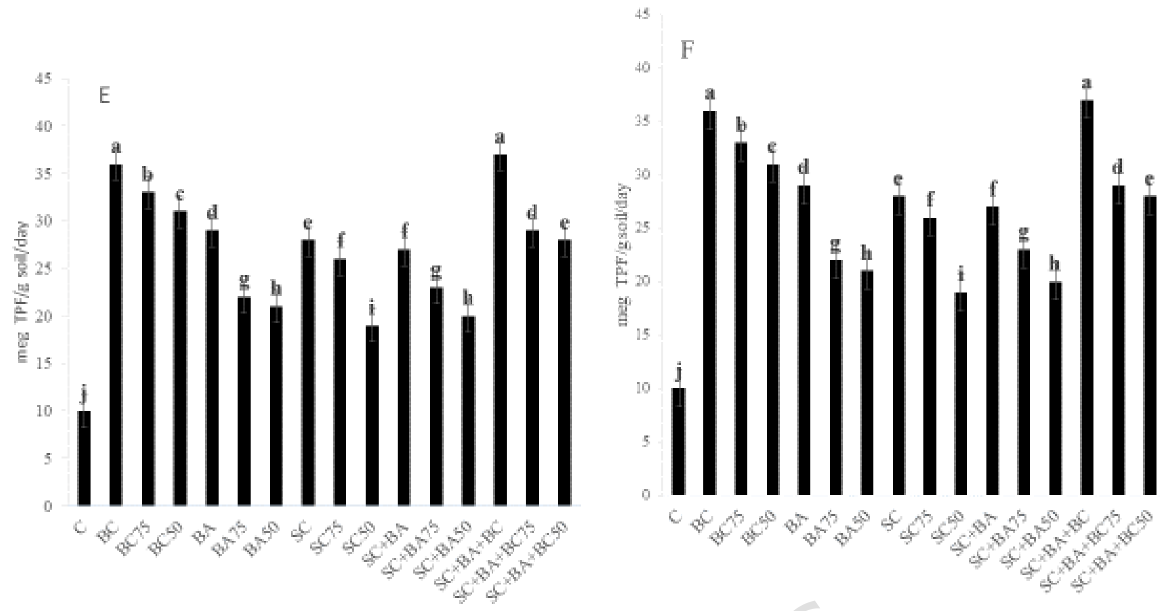


Figure 1. Effects of biochar (BC), *Saccharomyces cerevisiae* (SC), and *Bacillus amyloliquefaciens* (BA), applied alone or in combination, on soil microbial properties during two growing seasons. (A, B) Bacterial count in the first and second seasons, respectively; (C, D) yeast count in the first and second seasons, respectively; and (E, F) dehydrogenase activity in the first and second seasons, respectively. Bars represent mean ± standard error (SE) (n = 3). Different letters above bars indicate significant differences among treatments according to Duncan's multiple range test at $p \leq 0.05$.

Treatments were as follows: C (Control); BC (Biochar); BC75 (Biochar + 75% N); BC50 (Biochar + 50% N); BA (*B. amyloliquefaciens*); BA75 (*B. amyloliquefaciens* + 75% N); BA50 (*B. amyloliquefaciens* + 50% N); SC (*S. cerevisiae*); SC75 (*S. cerevisiae* + 75% N); SC50 (*S. cerevisiae* + 50% N); SC+BA75 (*S. cerevisiae* + *B. amyloliquefaciens* + 75% N); SC+BA50 (*S. cerevisiae* + *B. amyloliquefaciens* + 50% N); SC+BA+BC (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar); SC+BA+BC75 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75% N); and SC+BA+BC50 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50% N).

Soil physical parameters

Bulk Density (BD) and total porosity (TP)

Effects of biochar, *S. cerevisiae* and *B. amyloliquefaciens* on soil bulk density (BD) and total porosity (TP), over two growing seasons. The results in Table 1 show significant variations between different treatments. In terms of bulk density, the combined treatment of yeast, *B. amyloliquefaciens*, and biochar produced the lowest BD (1.33 g/cm³ in the first season and 1.28 g/cm³ in the second season), indicating a reduction in soil compaction. This contrasts with the control group, which had the highest BD (1.62 g/cm³ in the first season and 1.65 g/cm³ in the second season). Lower BD suggests improved soil structure and potentially better root penetration and water movement. Total porosity (TP) followed a similar trend. The highest TP values were observed in the combined yeast, *B. amyloliquefaciens*, and biochar treatments, reaching 40.3% in the first season and 39.3% in the second. The control group had significantly higher TP values (47.5% and 47.8%), but this higher TP doesn't necessarily translate into better soil health if it results from poor soil structure. In summary, combining biochar with *S. cerevisiae* and *B. amyloliquefaciens* positively influenced soil structure by reducing bulk density and enhancing total porosity across both seasons.

Table 1. Effect of biochar and microbial inoculants on soil bulk density (BD) and total porosity (TP) during two growing seasons.

Treatment	BD (g cm⁻³) Season 1	BD (g cm⁻³) Season 2	TP (%) Season 1	TP (%) Season 2
C	1.62b ± 0.87	1.65b ± 0.88	47.5d ± 6.01	47.8b ± 6.01
BC	1.38a ± 0.79	1.31a ± 0.80	41.1a ± 5.71	40.1a ± 5.61
BC75	1.37a ± 0.88	1.32a ± 0.81	41.3a ± 5.66	40.6a ± 5.56
BC50	1.40a ± 0.91	1.33a ± 0.87	41.4a ± 5.57	40.5a ± 5.41
BA	1.43a ± 0.95	1.41a ± 0.91	43.7b ± 5.72	42.1a ± 5.61

BA75	1.46a ± 0.95	1.42a ± 0.92	41.5a ± 5.21	41.3a ± 5.17
BA50	1.44a ± 0.85	1.41a ± 0.82	45.3c ± 5.22	41.8a ± 5.02
SC	1.46a ± 0.86	1.43a ± 0.83	41.7a ± 4.98	41.6a ± 4.79
SC75	1.44a ± 0.89	1.39a ± 0.87	42.3a ± 4.79	43.1a ± 4.81
SC50	1.41a ± 0.91	1.41a ± 0.89	44.1b ± 5.33	43.1a ± 5.13
SC+BA75	1.43a ± 0.82	1.42a ± 0.81	42.7a ± 5.43	42.0a ± 5.41
SC+BA50	1.42a ± 0.91	1.43a ± 0.92	42.5a ± 5.44	41.7a ± 5.14
SC+BA+BC	1.33a ± 0.83	1.28a ± 0.81	40.3a ± 4.71	39.3a ± 4.66
SC+BA+BC75	1.37a ± 0.89	1.30a ± 0.84	40.0a ± 4.76	40.1a ± 4.71
SC+BA+BC50	1.38a ± 0.86	1.31a ± 0.83	40.1a ± 4.71	40.2a ± 4.53
$LSD_{(0.05)}$	0.14	0.32	1.01	1.09

Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (LSD, p ≤ 0.05). Legend: C - Control, BC - Biochar, BC75 - Biochar + 75%N, BC50 – Biochar + 50%N, BA - *B. amyloliquefaciens*, BA75 - *B.* amyloliquefaciens + 75%N, BA50 - *B. amyloliquefaciens* + 50%N, SC - *S. cerevisiae*, SC75 - *S. cerevisiae* + 75%N, SC50 - *S. cerevisiae* + 50%N, SC+BA75 - *S. cerevisiae* + *B. amyloliquefaciens* + 75%N, SC+BA50 - *S. cerevisiae* + *B. amyloliquefaciens* + 50%N, SC+BA+BC - *S. cerevisiae* + B. amyloliquefaciens + Biochar, SC+BA+BC75 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75%N, SC+BA+BC50 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50%N. Values represent means ± standard deviations (SD).

Soil chemical parameters

Soil pH, electrical conductivity (EC) and organic matter (OM)

Value Table 2 data shows that the soil pH value is slightly lower after adding different soil substitute biochar, *S. cerevisiae* and *B. amyloliquefaciens* compared with the control group. However, on the other hand, the effect of soil change on soil pH is temporally.

The data in (Table 2) also shows the electrical conductivity (EC) value is affected by the addition of biochar, *S. cerevisiae* and *B. amyloliquefaciens* but there is no significant difference with treatments compared with control, the EC value in the second season was lower to 1.091 (dS/m).

Data represented in Table 2 shows that after the addition of the mixture of biochar, *S. cerevisiae* and *B. amyloliquefaciens*, the percentage of organic matter content of the soil increased significantly in both seasons. The percentage increase in organic matter in the *S. cerevisiae* + *B. amyloliquefaciens* + biochar mixtures during the first and second seasons was 32.5% and 37.2%, respectively.

Table 2. Effect of biochar and microbial inoculants on soil pH, EC, and OM during two seasons.

Treatment	pH (Season 1)	pH (Season 2)	EC (dS m-1) Season 1	EC (dS m-1) Season 2	OM (%) Season 1	OM (%) Season 2
C	7.59 a ± 0.01	7.81 d ± 0.03	1.31 e ± 0.09	1.31 d ± 0.08	0.50 a ± 0.08	0.53 a ± 0.07
BC	7.59 a ± 0.01	7.81 d ± 0.03	1.32 e ± 0.08	1.31 d ± 0.08	0.51 a ± 0.09	0.53 a ± 0.07
BC75	7.66 ae ± 0.02	7.65 abc ± 0.03	1.17 abc ± 0.09	1.18 abc ± 0.07	0.61 bcde ± 0.05	0.67 cd ± 0.05
BC50	7.63 abcd ± 0.04	7.67 abc ± 0.06	1.21 bc ± 0.09	1.21 bc ± 0.09	0.63 cde ± 0.07	0.65 bcd ± 0.07
BA	7.72 bcdef ± 0.01	7.71 bcd ± 0.02	1.25 de ± 0.10	1.24 cd ± 0.07	0.54 abc ± 0.04	0.59 abc ± 0.08
BA75	7.71 bcdef ± 0.00	7.72 bcd ± 0.97	1.24 cd ± 0.10	1.27 cd ± 0.06	0.53 ab ± 0.05	0.52 a ± 0.07
BA50	7.70 abcdef ± 0.03	7.74 cd ± 0.02	1.27 de ± 0.16	1.28 cd ± 0.06	0.54 abc ± 0.04	0.57 ab ± 0.05
SC	7.74 cdef ± 0.08	7.77 cd ± 0.04	1.25 de ± 0.17	1.26 cd ± 0.08	0.57 abe ± 0.05	0.58 ac ± 0.07
SC75	7.78 fg ± 0.05	7.74 cd ± 0.03	1.28 de ± 0.19	1.29 d ± 0.09	0.58 abe ± 0.04	0.61 abcd ± 0.08

SC50	7.77 deg ± 0.93	7.75 cd ± 0.08	1.26 de ± 0.11	1.24 cd ± 0.07	0.56 abcd ± 0.06	0.58 abc ± 0.06
SC+BA75	7.78 fg ± 0.95	7.77 cd ± 0.04	1.29 e ± 0.09	1.27 cd ± 0.05	0.55 abc ± 0.05	0.54 ab ± 0.09
SC+BA50	7.79 g ± 1.06	7.76 cd ± 0.03	1.28 de ± 0.01	1.29 d ± 0.06	0.54 abc ± 0.06	0.57 ab ± 0.08
SC+BA+BC	7.61 ac ± 0.05	7.59 a ± 0.95	1.11 a ± 0.02	1.09 a ± 0.05	0.66 e ± 0.03	0.71 d ± 0.07
SC+BA+BC75	7.63 ad ± 0.02	7.60 a ± 0.91	1.14 ab ± 0.03	1.14 ab ± 0.04	0.65 de ± 0.05	0.69 c ± 0.06
SC+BA+BC50	7.64 abcd ± 0.01	7.62 ab ± 0.94	1.15 ab ± 0.01	1.13 ab ± 0.01	0.64 cde ± 0.02	0.68 cd ± 0.07
LSD (0.05)	0.12	0.11	0.08	0.09	0.10	0.11

Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (LSD, p ≤ 0.05). Legend: C - Control, BC - Biochar, BC75 - Biochar + 75%N, BC50 – Biochar + 50%N, BA - *B. amyloliquefaciens*, BA75 - *B. amyloliquefaciens* + 75%N, BA50 - *B. amyloliquefaciens* + 50%N, SC - *S. cerevisiae*, SC75 - *S. cerevisiae* + 75%N, SC50 - *S. cerevisiae* + 50%N, SC+BA75 - *S. cerevisiae* + *B. amyloliquefaciens* + 75%N, SC+BA50 - *S. cerevisiae* + *B. amyloliquefaciens* + 50%N, SC+BA+BC - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar, SC+BA+BC75 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75%N, SC+BA+BC50 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50%N. Values represent means ± standard deviations (SD).

Soil N, P, and K Availability

The availability of soil nitrogen, phosphorus, and potassium increased significantly in all treatments compared to the control (Table 3). The combined *S. cerevisiae* + *B. amyloliquefaciens* + biochar treatment exhibited the highest nutrient levels — 48.1 mg/kg N, 5.62 mg/kg P, and 185.0 mg/kg K in the first season — representing increases of 22.7%, 12.6%, and 22.5% over the control, respectively. A similar pattern was observed in the second season, confirming the positive role of biochar and microbial inoculants in nutrient availability and retention.

Table 3. Effect of biochar and microbial inoculants on soil N, P, and K availability in two seasons.

Treatment	N mg kg⁻¹ (Season 1)	N mg kg⁻¹ (Season 2)	P mg kg⁻¹ (Season 1)	P mg kg⁻¹ (Season 2)	K mg kg⁻¹ (Season 1)	K mg kg⁻¹ (Season 2)
C	39.20 a ± 4.76	45.10 a ± 4.65	4.99 a ± 1.91	5.09 a ± 1.92	151 a ± 15.81	166 a ± 18.45
BC	45.97 g ± 5.12	48.61 c ± 5.16	5.56 fg ± 2.31	6.51 fg ± 2.33	182 ef ± 18.01	185 hi ± 18.11
BC75	45.77 fg ± 5.61	48.53 c ± 5.77	5.41 ef ± 2.11	6.45 efg ± 2.32	183 fg ± 18.04	184 gh ± 18.02
BC50	45.21 ef ± 5.09	48.51 c ± 5.71	5.39 de ± 2.12	6.43 efg ± 2.41	180 e ± 18.01	184 gh ± 18.04
BA	44.32 ef ± 5.01	47.09 b ± 5.61	5.33 cde ± 2.05	6.11 bc ± 2.44	175 bcd ± 17.91	175 de ± 17.79
BA75	44.41 ef ± 5.02	47.00 b ± 5.53	5.24 bc ± 2.01	6.14 bcd ± 2.51	176 cd ± 17.78	172 bc ± 17.89
BA50	42.90 cd ± 4.78	46.87 b ± 5.44	5.22 bc ± 2.05	6.08 b ± 2.63	174 bc ± 17.78	170 b ± 17.77
SC	43.90 de ± 4.66	47.08 b ± 5.41	5.35 ce ± 2.10	6.25 be ± 2.71	175 bd ± 17.68	177 e ± 18.14
SC75	42.01 a ± 4.45	46.89 b ± 5.44	5.36 ce ± 2.04	6.22 bcd ± 2.11	173 b ± 17.05	175 de ± 18.21
SC50	41.98 a ± 4.34	46.77 b ± 5.44	5.30 cde ± 2.05	6.15 bcd ± 2.23	173 b ± 17.11	173 cd ± 18.22
SC+BA75	42.54 a ± 4.33	47.05 b ± 5.51	5.11 ab ± 2.01	6.33 def ± 2.33	177 d ± 17.87	180 f ± 18.01
SC+BA50	41.51 a ± 4.44	46.44 ab ± 5.33	5.09 ab ± 2.00	6.29 cde ± 2.36	174 bc ± 17.87	175 de ± 18.66
SC+BA+BC	48.10 c ± 5.03	49.10 c ± 2.11	5.62 g ± 2.19	6.62 g ± 2.67	185 g ± 17.98	187 i ± 18.67
SC+BA+BC75	46.09 b ± 5.11	48.80 c ± 5.71	5.59 g ± 2.33	6.54 g ± 2.55	183 fg ± 18.12	186 hi ± 18.71
SC+BA+BC50	46.05 b ± 5.23	48.77 c ± 5.51	5.56 fg ± 2.13	6.51 fg ± 2.35	181 ef ± 17.92	182 fg ± 18.61
LSD (0.05)	1.50	1.40	0.15	0.20	2.50	2.30

Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (LSD, $p \leq 0.05$). Legend: C -

Control, BC - Biochar, BC75 - Biochar + 75%N, BC50 – Biochar + 50%N, BA - *B. amyloliquefaciens*, BA75 - *B. amyloliquefaciens* + 75%N, BA50 - *B. amyloliquefaciens* + 50%N, SC - *S. cerevisiae*, SC75 - *S. cerevisiae* + 75%N, SC50 - *S. cerevisiae* + 50%N, SC+BA75 - *S. cerevisiae* + *B. amyloliquefaciens* + 75%N, SC+BA50 - *S. cerevisiae* + *B. amyloliquefaciens* + 50%N, SC+BA+BC - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar, SC+BA+BC75 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75%N, SC+BA+BC50 - *S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50%N. Values represent means ± standard deviations (SD).

Plant growth and yield parameters

Photosynthetic Pigments

Treatments involving biochar and microbial inoculants significantly enhanced chlorophyll and carotenoid contents in cowpea leaves (Fig. 2). In both seasons, the combination of yeast, *B. amyloliquefaciens*, and biochar yielded the highest chlorophyll and carotenoid contents (48.10 and 5.62 mg/100 g FW in the first season, 49.10 and 6.62 mg /100 g FW in the second), showing significant improvement in pigment accumulation compared to the control.

The combined treatment increased chlorophyll content from 30.2 to 48.1 mg/100 g FW (59% increase) and carotenoids from 2.11 to 5.62 mg/100 g FW (66% increase) in the first season. Similar enhancements were observed in the second season, confirming improved photosynthetic efficiency and plant vigor.

The results suggest that the integration of biochar and microbial inoculant applications enhances the photosynthetic pigments in cowpea leaves, thereby improving plant health and productivity.

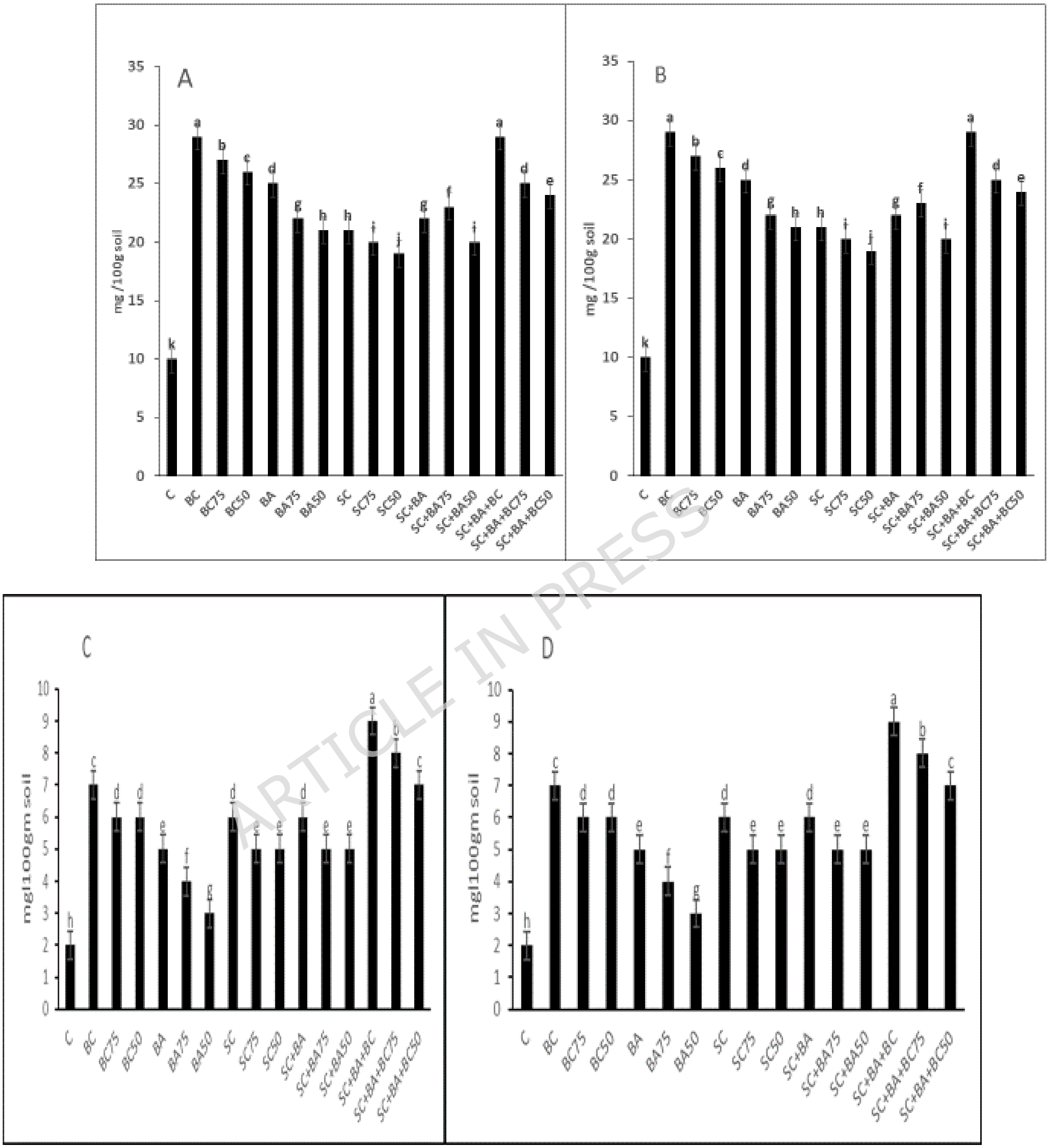


Figure 2. Effects of biochar (BC), *Saccharomyces cerevisiae* (SC), and *Bacillus amyloliquefaciens* (BA), applied alone or in combination, on photosynthetic pigments in cowpea leaves during two growing seasons. (A, B) Chlorophyll content in the first and second seasons, respectively;

(C, D) carotenoid content in the first and second seasons, respectively. Bars represent mean ± standard error (SE) (n = 3). Different letters above bars indicate significant differences among treatments according to Duncan's multiple range test at $p \leq 0.05$.
Treatments were as follows: C (Control); BC (Biochar); BC75 (Biochar + 75% N); BC50 (Biochar + 50% N); BA (*B. amyloliquefaciens*); BA75 (*B. amyloliquefaciens* + 75% N); BA50 (*B. amyloliquefaciens* + 50% N); SC (*S. cerevisiae*); SC75 (*S. cerevisiae* + 75% N); SC50 (*S. cerevisiae* + 50% N); SC+BA75 (*S. cerevisiae* + *B. amyloliquefaciens* + 75% N); SC+BA50 (*S. cerevisiae* + *B. amyloliquefaciens* + 50% N); SC+BA+BC (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar); SC+BA+BC75 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75% N); and SC+BA+BC50 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50% N).

Plant Morphological Traits

Number of Branches

The number of branches increased with biochar and microbial treatments compared to the control group, both in the first and second seasons Fig. 3 (B). Specifically, biochar with *B. amyloliquefaciens*, and *S. cerevisiae*, had similar effects to biochar with 75% nitrogen, with marginal differences. The combined treatment of biochar, *B. amyloliquefaciens, S. cerevisiae*, resulted in the highest increase in branch numbers, with the first season recording 50 branches and the second season 52 branches. This increase reflects the positive interaction between biochar, microorganisms, and nitrogen on plant branching.

Fresh and Dry Weight

In terms of fresh and dry weight (Fig. 3 D-E) of plant, the control treatment resulted in significantly lower values (80 g in the first season and 70 g in the second one). However, treatments involving biochar, microorganisms, and nitrogen consistently showed higher dry weight values. The highest dry weight was observed in the treatment with *S. cerevisiae*, *B. amyloliquefaciens*, biochar, and recording 160 g in the first season and 167 g in the second. Dry weight improved from 80 g to 160 g (100% increase) in the first season. These results demonstrate strong synergistic effects of biochar and beneficial microbes on growth and productivity.

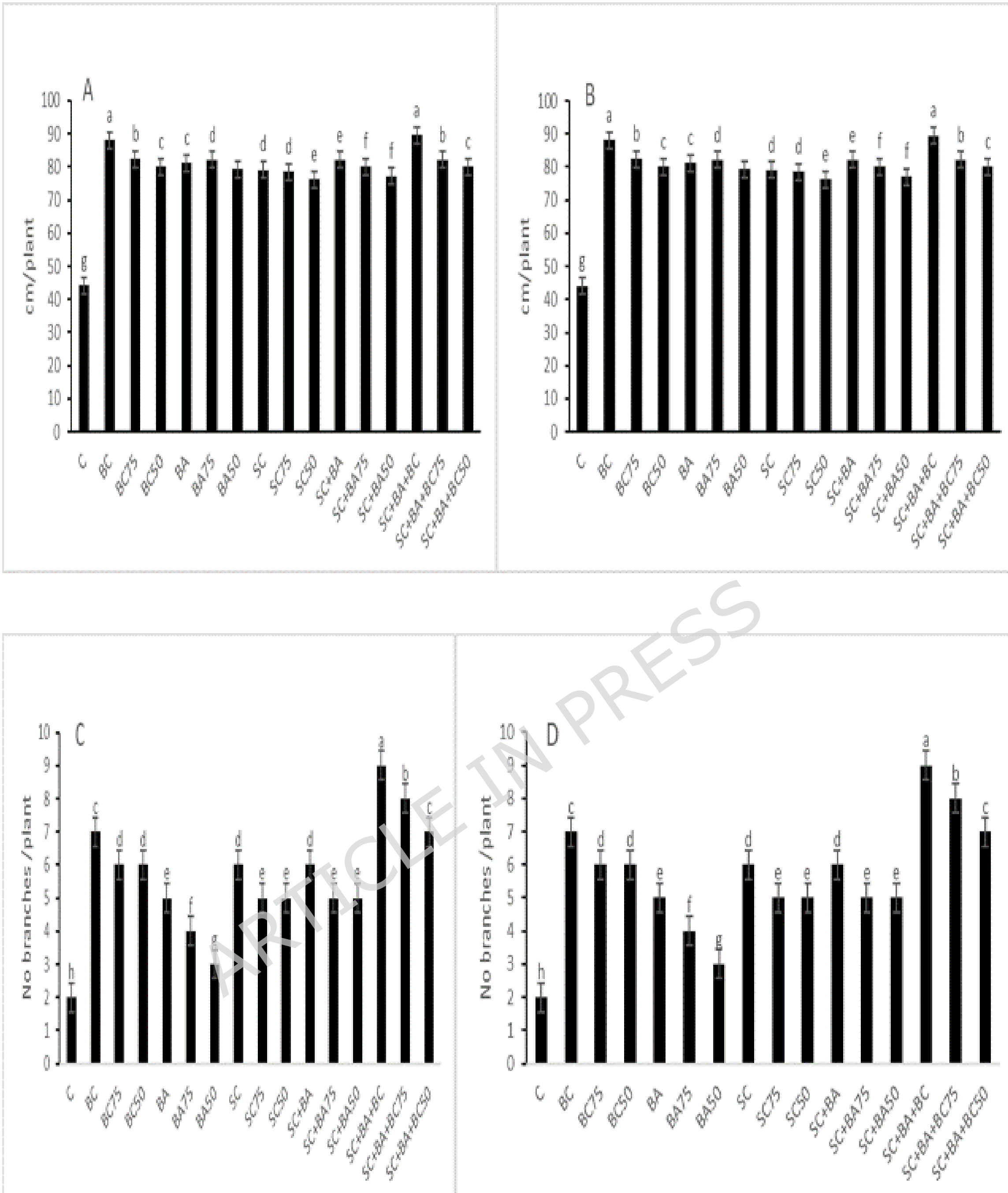
This demonstrates that these microbial inoculants, when used with biochar and nitrogen, significantly enhance the biomass of cowpea plants.

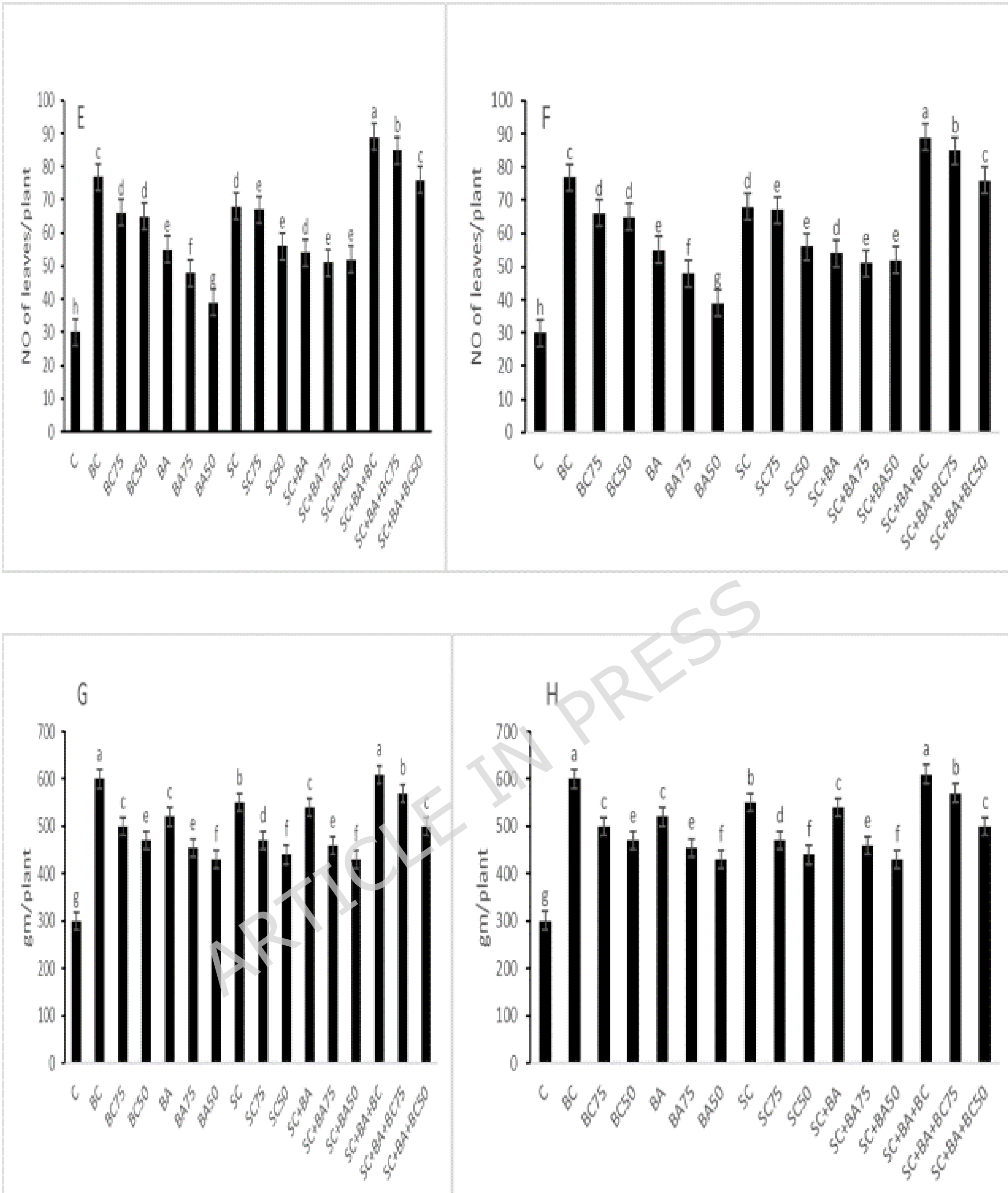
Plant Length

The study on the effect of biochar, *S. cerevisiae*, and *B. amyloliquefaciens* on cowpea plant criteria across two seasons provides valuable insights into sustainable agricultural practices. The results likely highlight the synergistic benefits of these amendments in enhancing plant growth, yield, and resilience. Plant height increased from 80 cm (control) to 160 cm (100% increase) in the first season (Fig. 3 A). In the control group, plant length was 80 cm in the first season and 90 cm in the second. The combination of *S. cerevisiae*, *B. amyloliquefaciens*, biochar, produced the highest plant length, with 160 cm in the first season and 170 cm in the second season. This indicates that biochar and microbial amendments may enhance plant growth by improving nutrient availability and uptake in the plants.

Number of Leaves

The number of leaves per plant followed a similar trend to the number of branches, with biochar and microbial treatments producing more leaves compared to the control (Fig. 3 C). For instance, the combination of *S. cerevisiae, B. amyloliquefaciens*, biochar, resulted in 80 leaves in the first season and 90 in the second, the highest among all treatments. Number of leaves rose from 40 to 80 (100% increase) and number of branches from 25 to 50 (100% increase). This increase in leaf number suggests improved plant vigor and photosynthetic capacity under biochar and microbial treatments.


A
B
C
D
cm/plant
No branches /plant
C
BC
BC75
BC50
BA
BA75
BA50
SC
SC75
SC50
SC+BA
SC+BA75
SC+BA50
SC+BA+BC
SC+BA+BC75
SC+BA+BC50


E
F
NO of leaves/plant
G
H
gm/plant
C
BC
BC75
BC50
BA
BA75
BA50
SC
SC75
SC50
SC+BA
SC+BA75
SC+BA50
SC+BA+BC
SC+BA+BC75
SC+BA+BC50

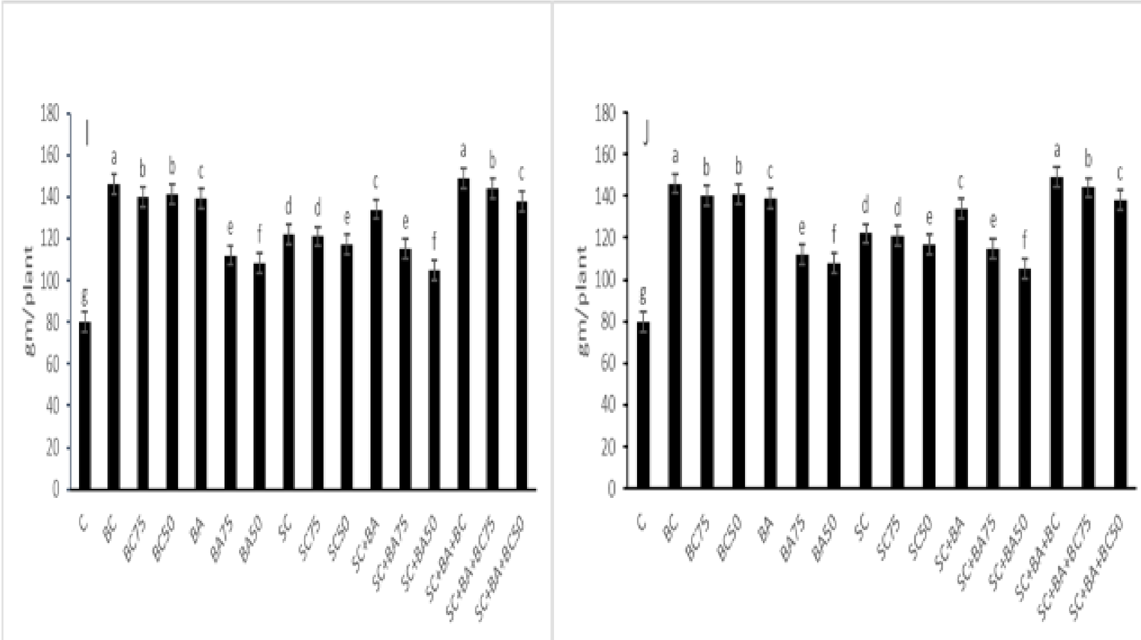


Figure 3. Effects of biochar, *Saccharomyces cerevisiae*, and *Bacillus amyloliquefaciens* on growth traits of cowpea plants during two growing seasons. (A, B) Plant length in the first and second seasons, respectively; (C, D) number of branches; (E, F) number of leaves; (G, H) fresh weight; and (I, J) dry weight. Bars represent mean ± standard error (SE) (n = 3). Different letters above bars indicate significant differences among treatments according to Duncan's multiple range test at $p \leq 0.05$.
Treatments were as follows: C (Control); BC (Biochar); BC75 (Biochar + 75% N); BC50 (Biochar + 50% N); BA (*B. amyloliquefaciens*); BA75 (*B. amyloliquefaciens* + 75% N); BA50 (*B. amyloliquefaciens* + 50% N); SC (*S. cerevisiae*); SC75 (*S. cerevisiae* + 75% N); SC50 (*S. cerevisiae* + 50% N); SC+BA75 (*S. cerevisiae* + *B. amyloliquefaciens* + 75% N); SC+BA50 (*S. cerevisiae* + *B. amyloliquefaciens* + 50% N); SC+BA+BC (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar); SC+BA+BC75 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 75% N); and SC+BA+BC50 (*S. cerevisiae* + *B. amyloliquefaciens* + Biochar + 50% N).

Discussion

The present study aimed to test the hypothesis that the combined application of biochar and microbial inoculants (*S. cerevisiae* and *B. amyloliquefaciens*) would improve soil fertility, biological activity, and cowpea productivity under sandy soil conditions. The findings strongly support this hypothesis, demonstrating that the integration of biochar with beneficial microbes significantly enhanced soil biological, physical, and chemical properties, as well as plant growth and yield. These results highlight the synergistic potential of biochar–microbe interactions in promoting sustainable agricultural productivity in arid regions such as Egypt[2,4].

Biochar as a Soil Amendment

Biochar is well known for improving soil structure, water retention, and nutrient-holding capacity due to its high porosity and surface area. In the present study, biochar application notably reduced soil bulk density, increased total porosity, and enhanced electrical conductivity, thereby facilitating root penetration and aeration[13]. Such improvements are consistent with previous reports that biochar increases cation exchange capacity (CEC) and water retention, enhancing nutrient availability and reducing leaching losses[5]. These structural changes also stimulated microbial proliferation and enzymatic activity, contributing to higher organic matter accumulation and overall soil fertility[14].

Microbial Inoculants and Their Role in Plant Growth

The application of microbial inoculants such as *B. amyloliquefaciens* and *S. cerevisiae* played a pivotal role in improving soil nutrient dynamics and plant growth. *B. amyloliquefaciens* is known for its ability to solubilize phosphorus, fix atmospheric nitrogen, and secrete phytohormones that promote plant root growth and nutrient uptake[14,15]. In this study[16], *Bacillus* inoculation significantly enhanced cowpea growth parameters, including plant height, branch number, and pod length, which can be attributed to improved N and P availability.

Similarly, *S. cerevisiae* contributed to plant growth through multiple mechanisms, including the production of growth-promoting substances such as indole acetic acid (IAA) and vitamins, and the enhancement of photosynthetic pigments. Previous studies[16,17] reported that yeast inoculation increases chlorophyll content and photosynthetic efficiency, consistent with the present findings where chlorophyll and carotenoid levels markedly increased in yeast-treated plants. These improvements indicate that *S. cerevisiae* enhances physiological activity and stress tolerance in plants[14].

Synergistic Effects of Biochar and Microbial Inoculants

The combined use of biochar and microbial inoculants produced a pronounced synergistic effect on soil and plant parameters. Biochar serves as a habitat for beneficial microbes, providing micro-niches and protection from environmental stress, while microbial inoculants actively improve nutrient solubilization and cycling[17]. This complementary relationship results in higher microbial activity and diversity, reflected in the observed increase in soil dehydrogenase activity—a key indicator of microbial metabolism. as reported by[18] who found that biochar enhances the colonization and activity of beneficial microbes, thereby improving soil nutrient availability and crop performance[19].

Impact on Soil Health and Nutrient Availability

The co-application of biochar, *S. cerevisiae*, and *B. amyloliquefaciens* substantially improved soil organic matter, enzymatic activity, and nutrient availability, confirming the positive impact on soil fertility[20]. Enhanced organic matter contributes to better soil aggregation, nutrient retention, and microbial proliferation, which in turn improve plant nutrient uptake. The observed[21] increase in soil N, P, and K availability corroborates previous findings that biochar and microbial inoculants jointly promote nutrient cycling and reduce nutrient losses. *B. amyloliquefaciens* also enhances phosphorus solubilization, while yeast inoculation improves microbial diversity and organic carbon turnover, leading to a more resilient soil ecosystem.

Overall Implications

Collectively, these results demonstrate that the integrated use of biochar and microbial biofertilizers represents an effective, eco-friendly approach to improving soil fertility and crop productivity in sandy soils[22]. The synergistic interaction between biochar and beneficial microbes supports the study hypothesis and aligns with global trends toward sustainable soil management. These findings provide a scientific basis for recommending the combined use of biochar and microbial inoculants as a practical strategy for enhancing soil health and crop performance under resource-limited condition[20].

Limitations and future research

Although the present study demonstrates significant improvements in soil fertility and cowpea productivity through the synergistic use of biochar and microbial inoculants, several limitations should be acknowledged. First, the experiment was conducted over only two growing seasons in a single location (Ismailia Governorate, Egypt), which may limit the generalizability of results to other soil types or climatic regions. Second, only one cowpea cultivar (cv. Dokki 126) was tested, and responses might differ among other genotypes. Third, while biochar and microbial inoculants were applied at fixed rates, variations in application rates or combinations could further influence outcomes, requiring optimization for broader agricultural systems. Finally, this study focused on short-term soil and crop responses, and long-term effects such as carbon sequestration, microbial community dynamics, and sustainability of yield improvements remain to be investigated. Future research should address these limitations by testing across multiple environments, evaluating long-term impacts, and exploring economic feasibility to facilitate practical adoption by farmers.

Materials and Methods

Study site

The field experiment was conducted during two consecutive summer seasons (2021 and 2022) at the Ismailia Agricultural Research Station, Ismailia Governorate, Egypt (30°35′41.9″ N, 32°16′45.8″ E).

Soil characteristics

Surface soil samples (0–30 cm) were collected before sowing for physical and chemical analyses (Table 4). The soil was sandy in texture, low in organic matter, and relatively poor in available macronutrients according to the classification of [22].

Cowpea cultivar

Cowpea (*Vigna unguiculata* ssp. *unguiculata*), cultivar Dokki 126, was obtained from the Legume Research Department, Field Crops Research Institute, ARC, Giza, Egypt.

Table 4. Some physical and chemical properties of the experimental 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	151.0

Biochar

Biochar was produced from agricultural residues at 500°C for 2 h, ground, and sieved (<0.5 mm). It was applied to the soil at a rate of 7.35 m^3 $feddan^{-1}$. The physical and chemical characteristics are shown in Table 5[22].

Table 5. Some physical and chemical properties of the tested 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 dS m^{-1})1:2.5 : water suspension		6.87
*EC (dS m^{-1}) (1:10 biochar water suspension)		0.85
Available nutrients		
Macronutrients (mg kg^{-1})	N	470
	P	38
	K	137

Microbial strains

Two microbial inoculants were used: *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*, provided by the Microbiology Department, Soil, Water and Environment Research Institute (SWERI), ARC, Giza, Egypt.

- ***B. amyloliquefaciens*** was cultured on nutrient agar and grown aerobically in nutrient broth at 28°C for 48 h (150 rpm). The inoculum (10^9 CFU/mL) was diluted in irrigation water and applied at 10 L/feddan immediately after sowing and again after 45 days.
- ***S. cerevisiae*** was grown on YPD agar (supplemented with 10 mg/mL ampicillin) and incubated at 25°C for 3–5 days. Colonies were transferred to YPD broth, and the inoculum was applied at 20 L/feddan in irrigation water at sowing and 45 days later.

Field management and experimental design

Before sowing, the soil was prepared with agricultural gypsum ($CaSO_4 \cdot 2H_2O$, 1190 kg/feddan). Potassium sulfate (41.5% K_2O) was added in two equal doses

(119 kg/feddan), at sowing and 30 days later. Superphosphate was applied during soil preparation. Cowpea seeds were coated with rhizobium (*Actinorhizobium* spp.) using gum Arabic prior to sowing. The experiment included 15 treatments combining biochar, microbial inoculants, and different nitrogen fertilizer levels (100%, 75%, and 50% of the recommended dose). Treatments were arranged in a completely randomized design (CRD) with three replicates. Each plot measured 18 m² (6 × 3 m). Total bacterial counts were determined 30 and 60 days after sowing [22].

Methods

- **Bacterial counts:** Determined at 30 and 60 days after sowing according to[23].
- **Dehydrogenase activity:** Measured according to[24].
- **Soil physical and chemical properties:** Bulk density and total porosity were determined following[22]; soil pH, EC, soluble ions, and available N, P, and K were analyzed as described in[22, 25].

Plant analyses

Growth and yield attributes: when harvested, plant growth patterns were collected 85 days after planting to define yield parameters as follows: Plant length (cm), Number of leaves/plants, Number of branches/plants, Plant fresh weight (g), Plant dry weight (determined by drying 100 g in an oven at 70ºC until constant weight)

- **Photosynthetic pigments:** chlorophyll, and carotenoids were quantified spectro photometrically at 663, and 470 nm according to[26].

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using CoStat software (version 6.45). Means were compared using Duncan's multiple range test at the 5% probability level. Duncan's multiple range test was used to compare treatment means. The analyses of variance approach described b[27,28] was used to tabulate and analyze the data at a 5% significance level (one-way analysis) using the CoStat 6.45 program analysis

Conclusions

In conclusion, the application of biochar, *S. cerevisiae*, and *B. amyloliquefaciens* has a significant positive impact on cowpea productivity and soil health in Egyptian agriculture. The synergistic effects of biochar and microbial inoculants lead to improved nutrient availability, enhanced plant growth, and better soil health indicators. These findings support the use of biochar and microbial inoculants as sustainable agricultural practices that can improve crop productivity while minimizing environmental impacts. Future research should focus on long-term field studies to further evaluate the benefits of these treatments under different soil and environmental conditions.

Supplementary data

Not applicable.

CRediT authorship contribution statement

D.M.K. and H.M.H: Conceptualization, data curation. **D.M.K.** and **H.M.H**: Formal analysis. **A.G.**: Funding acquisition. **A.G. and D.M.K**: Investigation. **A-S.S.** and **M.E.S., D.M.K.** and **R.M.E.**: Methodology. **D.M.K**: Resources. **D.M.K.** and **R.M.E.**: Software. **D.M.K**: Writing – original draft. **A-S.S., A.G.** and **R.M.E:** Writing – review and editing.

Funding

The author(s) reported that there is no funding associated with the work featured in this article.

Conflicts of Interest

All the authors declared that they have no competing interests

Consent to participate

All the authors have consented to participate in this submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

References

1- Elsobky, E. E., & Hassan, H. H. (2021). Optimizing cowpea productivity by sowing date and plant density to mitigate climatic changes. Egyptian Journal of Agronomy, 43, 317–331.

2- Lateef, E. M. A. E., Eata, A. E. M., Wali, A. M., & El-Salam, M. S. A. (2020). Evaluation of mungbean (Vigna radiata L. Wilczek) as green pod and seed crop under different cropping systems in Egypt. Asian Journal of Crop Science, 12(3), 115–123.

3- Hernández-Fernández, M., Ruiz-Muñoz, M., & Cantoral, J. M. (2021). Culturable yeasts as biofertilizers and biopesticides for a sustainable agriculture: A comprehensive review. Plants, 10(5), 822. https://doi.org/10.3390/plants10050822

4- Hamed, S. M., El-Gaml, N. M., & Eissa, S. T. (2022). Integrated biofertilization using yeast with cyanobacteria on growth and productivity of wheat. Beni-Suef University Journal of Basic and Applied Sciences, 11, 112.

5- Hosseinzadeh-Bandbafha, H., Shariat, H. H. K., Panahi, M., Dehhaghi, Y., Orooji, H., Shahbeik, O., ... & Tabatabaei, M. (2023). Applications of nanotechnology in biodiesel combustion and post-combustion stages. Renewable and Sustainable Energy Reviews, 182, 113414. https://doi.org/10.1016/j.rser.2023.113414

6- Ng, F. L., Lin, T.-C., Wang, E., Lee, T. Y., Chen, G. T., Su, J.-F., & Chen, W. L. (2025). Bacillus-based biofertilizer influences. Sustainability, 17(14), 6293. https://doi.org/10.3390/su17146293

7- Luo, L., Zhao, C., Raza, A., & Yin, C. (2022). *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. Current Research in Environmental Sustainability, 3, 100063.

8- Barros-Rodríguez, A., García-Gálvez, C., Pacheco, P., Kalyuzhnaya, M. G., & Manzanera, M. (2023). Isolation of methane-enriched bacterial communities and application as wheat biofertilizer under drought conditions. Plants, 12(19), 2487.

9- Garrido, A., Atencio, L. A., Bethancourt, R., Bethancourt, A., Guzmán, H., Gutiérrez, M., & Durant-Archibold, A. A. (2020). Antibacterial activity of volatile organic compounds produced by the octocoral-associated bacteria Bacillus sp. BO53 and Pseudoalteromonas sp. GA327. Antibiotics, 9(12), 923.

10- Kumar, S., Kaushik, G., Dar, M. A., Nanda, M., & Ahmed, M. (2022). Biochar in agriculture: The role of biochar in enhancing nutrient use efficiency and crop productivity. Journal of Plant Nutrition, 45(3), 387–401.

11- Neogi, S., Sharma, V., Khan, N., Chaurasia, D., Ahmad, A., Chauhan, S., ... & Bhargava, P. C. (2022). Sustainable biochar: A facile strategy for soil and environmental restoration, energy generation, mitigation of global climate change and circular bioeconomy. Chemosphere, 293, 133474.

12- Lehmann, J., Cowie, A., Masiello, C. A., Kammann, C., Woolf, D., Amonette, J. E., ... & Whitman, T. (2021). Biochar in climate change mitigation. Nature Geoscience, 14, 883–892. https://doi.org/10.1038/s41561-021-00852-8

13- James, A., Sánchez, A., Prens, J., & Yuan, W. (2022). Biochar from agricultural residues for soil conditioning: Technological status and life cycle assessment. Current Opinion in Environmental Science & Health, 25, 100314. https://doi.org/10.1016/j.coesh.2021.100314

14- Xin, Z., Tong, J., Wang, J., Ruan, C., Lyu, J., & Shi, J. (2024). Research progress on activated persulfate by biochar: Soil and water environment remediation, mechanism exploration and simulation calculation. Journal of Hazardous Materials, 152718.

15- Kang, K., Hu, Y., Khan, I., He, S., & Fatehi, P. (2023). Recent advances in the synthesis and application of magnetic biochar for wastewater treatment. Journal of Environmental Chemical Engineering, 129786.

16- Singh, V., & Kumar, B. (2024). A review of agricultural microbial inoculants and their carriers in bioformulation. Journal of Cleaner Production, 100843.

17- Lu, P., Wang, H., Wang, Z., Liu, J., Li, Y., Xia, L., & Song, S. (2024). Critical steps in the restoration of coal mine soils: Microbial-accelerated soil reconstruction. Journal of Environmental Management, 368, 122200.

18- Kim, K.-H., & Kwon, E. E. (2023). Biochar as a tool for the improvement of soil and environment. Frontiers in Environmental Science, 11, 100018.

19- Ali, A., Khalifa, A., & Al-Malki, M. (2021). Plant growth-promoting rhizobacteria from Ocimum basilicum improve growth of Phaseolus vulgaris and Abelmoschus esculentus. South African Journal of Botany, 139, 200–209.

20- Choudhary, R. S., & Yadav, N. K. (2021). Biofertilizers (microbial inoculants). Just Agriculture, 1, 1–10.

21- Shyam, S., Ahmed, S., Joshi, S. S., & Sarma, H. J. (2025). Biochar as a soil amendment: Implications for soil health, carbon sequestration, and climate resilience. Sustainable Agriculture, 2, 18.

22- Page, A. L., Miller, R. H., & Keeney, D. R. (1982). Methods of soil analysis: Part 2. Chemical and microbiological properties. American Society of Agronomy.

23- Thalman, A. (1967). Über die microbiello aktivität und ihr beziehung zu frucht barkeits merkmalen einger a charboden unter besonderer berücksichtigung der dehydrogenase aktivität (TTC reduction). Ph.D. Thesis, University of Gießen, Germany.

24- Herbert, D., Phipps, P. J., & Strang, R. E. (1971). Chemical analysis of microbial cells. In J. R. Norris & D. Ribbons (Eds.), Methods in microbiology (Vol. 5B, pp. 210–336). Academic Press.

25- Soltanpour, P. N. (1985). Use of AB-DTPA soil test to evaluate element availability and toxicity. Communications in Soil Science and Plant Analysis, 16(3), 323–338.

26- Moran, R. (1982). Formula for determination of chlorophyllous pigments extracted with N.N dimethyl formamide. Plant Physiology, 69, 1376–1381.

27- Snedecor, G. W., & Cochran, W. G. (1980). Statistical methods (7th ed.). Iowa State University Press.

28- Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11(1), 1–42.