Middle East Journal of Agriculture Research
Volume: 13 | Issue: 04| Oct. – Dec.| 2024

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2024.13.4.62
Journal homepage: www.curresweb.com
Pages: 1042-1057

Biochar and Biofertilizer: A Green approach for Improving Wheat Yields in Egypt

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Received: 10 Oct. 2024 **Accepted:** 11 Dec 2024 **Published:** 20 Dec. 2024

ABSTRACT
Wheat is a key cereal crop; it is crucial for ensuring worldwide food security and is the world's second most produced crop. The current national priority is to improve wheat productivity in Egypt. Excessive mineral fertilizers boost wheat yields but are costly and harm ecosystems. Using organo or / and bio fertilizers containing growth promoting or nitrogen fixing microbes offers a sustainable, cost-effective alternative. A field experiment evaluated the impact of integrating biochar, inoculating *Tildeniella torsiva* NA3 (*T. torsiva* NA3), *Anabaena fertilissima* (*A. fertilissima*), and *Azolla pinnata* extract (biofertilizer), along with recommended fertilizers, on the yield of two wheat genotypes (*Triticum aestivum* cvs. Sids 14 and Sakha 95) during season (2022 / 2023). Our findings reveal that the addition of biochar and biofertilizer notably improved soil health, increased chlorophyll a and b contents, enhanced grain quality, and boosted wheat yield components in Sids 14 and Sakha 95. For instance, integrating 50% N and biofertilizer (N6) with biochar significantly increased ($P \leq 0.05$) nitrogenase (N-ase) enzyme activity and CO_2 evolution in Sids-14 by 2808.39% and 25.27%, respectively, compared to the full-recommended dose 100%N, (N1). Additionally, in Sids-14, chlorophyll a and b levels rose by 17.18% and 20%, while in Sakha 95, chlorophyll a and b increased by 20.68% and 21.12% under the N2 treatment (100% N, biofertilizer) in presence of biochar, comparing to standard dose of mineral fertilizer to each cultivar. These findings suggest that biochar, when combined with biofertilizers, can be an effective strategy for improving soil health, wheat growth, nutrient uptake, and overall productivity, offering a sustainable approach for enhancing agricultural performance in wheat cultivation.

Keywords: biochar, biofertilizer, cyanobacteria, azolla, wheat.

1. Introduction
Wheat (*Triticum aestivum* L.) is the most important cereal crop of the Poaceae family and has served as Egypt's primary strategic food crop for over 7,000 years. It is primarily used for bread-making, various industrial applications, and as a key source of straw fodder for animal feed. It is essential for ensuring food security on a global scale. Grown globally, wheat ranks as the second most widely produced crop. (Kumar *et al*., 2023). The current national focus is on enhancing wheat efficiency to close the gap between Egypt's wheat production and consumption by expanding cultivated areas and increasing yield per unit area. (Zaki *et al*., 2021). Wheat production per unit area can be increased by cultivating high-yielding varieties and applying certain agronomic practices, especially the addition of nitrogen and phosphorus fertilizer. (Tabak *et al*., 2020 and Mussarat *et al*., 2021)

The regular application of chemical fertilizers, while enhancement crop yields, comes with significant environmental and health challenges. These fertilizers can cause soil degradation, reducing its fertility over time, and disrupt ecological balance by contaminating water sources through leaching and runoff. Moreover, plants absorb only about half of the chemical fertilizers applied, resulting in

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nutrient loss and environmental pollution, such as eutrophication in water bodies. Chemical fertilizer usage contributes to climate change by emitting greenhouse gases, and its excessive use causes health risks to animals and humans by exposure to harmful substances in food and water sources. (Savci 2012; Elhanafi *et al*., 2019; Kumar *et al*., 2019 and Bisht and Chauhan, 2020). As a result, it has become an essential imperative to identify safe and environmentally sustainable Thus, it has become an urgent necessity to find a safe and eco-friendly alternatives as biofertilizers (Choudhury and Kennedy, 2005; Babu *et al*., 2015).

The World Health Organization (WHO) intends to exploit the "Green Revolution" to increase global food production by around 50% by 2029. It aims to increase agricultural output while minimizing environmental and health dangers associated with chemical fertilizers. Therefore, the application of bio or/and organic fertilizers has become one of the most important alternatives for enhancement sustainable agriculture (Gao *et al*., 2020).

A biofertilizer consists of live microorganisms that, when applied to soil, seeds, or plant surfaces, enhance plant growth and increase crop yield. It is commonly acknowledged, that microorganisms have the ability to fix atmospheric nitrogen and solubilize phosphorus in the soil, which in turn improves the availability of essential nutrients, such as nitrogen, phosphorus, and potassium. (Kumar *et al*., 2023).

Among the most common microorganisms on the earth are Cyanobacteria, a class of photosynthetic gram-negative bacteria (Hall *et al*., 1995; Deepali *et al*., 2020). Without a host, Soil microalgae are able to grow, develop, and produce vital compounds. They fix, nitrogen produce phyto-growth hormones, amino acids, and vitamins. They also improve the structure of the soil by producing sticky compounds, keeping water in the soil, lowering its saltiness, producing organic acids that make phosphorus more available and absorbing heavy metals on their surface (Malik *et al*., 2001 Song *et al*., 2005 and Deepali *et al*., 2020). Because of their affordability, accessibility, and environmental friendliness, soil microalgae-based biofertilizers are now a promising alternative.

Rice, wheat, soybeans, cotton, and maize are among the row crops that were fertilized by using cyanobacteria (Karthikeyan, *et al*., 2007; Prasanna *et al*., 2012; Kholssi *et al*., 2022; Kumar *et al*., 2023 and Chanda *et al*., 2024). Studies have identified Anabaena species as effective biofertilizers in many paddy fields for rice cultivation (Subash & Arka, 2020; Jaiswal *et al*., 2021) as well as, in fields used for wheat fertilization. (Boghdady and Ali, 2013 and Kholssi *et al*., 2022). Additionally, *Spirulina sp*. and *Oscillatoria sp*. are suggested for use as a biofertilizers in organic crop production (Jamal Uddin *et al*., 2019).

Azolla sp. is one of the most significant biofertilizers known today. It is a small, free-floating aquatic fern which thrives on the surface of still or slow-flowing freshwater bodies, including ponds, lakes, and rice paddies. Azolla species are economically significant because of their fast growth and symbiosis with Anabaena azollae, enabling nitrogen fixation. (Kollah *et al*., 2016). Azolla naturally supplies nitrogen for agriculture, fixing 30–60 kg/ha and showing promise as a crop nitrogen source (Kollah *et al*., 2016). It enhances rice nutrition in paddies, reducing urea needs (Malyan *et al*., 2019), and provides essential vitamins, stimulants, amino acids, intermediates, and minerals like Ca, Mg, K, P, Fe, and Cu (Maswada *et al*., 2021). Furthermore, Azolla extract, known as the "green gold mine," has recently demonstrated its effectiveness as an organic fertilize to wheat plants (Yadav *et al*, 2014).

Biochar has recently gained recognition as a potential organo-fertilizer. Biochar, a stable form of bio- carbon, is produced by heating organic materials like plant residues, wood, or agricultural waste in an oxygen deficient environment through pyrolysis. It is characterized by a finely grained texture carbonate containing high level of organic carbon content with poor degradability (Sanchez *et al*., 2009 and Malińska *et al*., 2015).

In addition to maintaining ecological balance, healthy soil encourages robust plant development and resistance, which raises crop yields and overall production. Nutrient-rich, well-structured soil is conducive to a wide variety of plant life. It is home to microorganisms that improve soil performance and fertility, including fungi, bacteria, and earthworms. According to research, using biochar as a soil conditioner has all of the previously mentioned advantages, enhancing the general quality and improving soil health. (Ró˙zyło *et al*., 2017; Gou *et al*., 2018; Medy´nska-Juraszek *et al*., 2020 Bahuguna *et al*., 2021; Dai *et al*., 2021; Razzaghi *et al*., 2020; Nkoh *et al*., 2022 and Wyzi´nska *et al*., 2024).

In order to gradually reduce reliance on chemical fertilizers for long-term use, modern agriculture seeks to incorporate mineral, organic, and biofertilizers. This integrated fertilization approach optimizes

EISSN: 2706-7955 ISSN: 2077-4605 *DOI: 10.36632/mejar/2024.13.4.62*

nutrient availability, increases metabolite production, enhances chlorophyll synthesis and photosynthesis, and improves yield, quality, and crop components. It also reduces agricultural pollution and lowers costs, according to numerous studies on the use of Azolla or biochar, either alone or in combination with other organic materials (Sharifi *et al.,* 2019; Kimani *et al*., 2021 and Al Sayed *et al*., 2022).

To the best of our knowledge, no research has assessed the combined impact of cyanobacteria, Azolla extract, and biochar on crop growth and productivity. The integration of biochar, Azolla extract, and cyanobacteria provides an effective strategy for sustainable agricultural management.

The goal of this study is to assess the positive impact of incorporating biochar into the soil, in combination with inoculating two cyanobacteria species (*Tildeniella torsiva* NA3 and *Anabaena fertilissima*) and applying an *Azolla pinnata* extract, along with the recommended mineral fertilizer, on the productivity of two wheat genotypes (*Triticum aestivum* cv. Sids 14 and Sakha 95). Additionally, examine the combined impact of biofertilizer and biochar on soil health by evaluating CO_2 evolution as a marker of microbial activity in the soil and nitrogenase enzyme activity as a marker of nitrogen fixation and microbial activity.

2. Material and Method

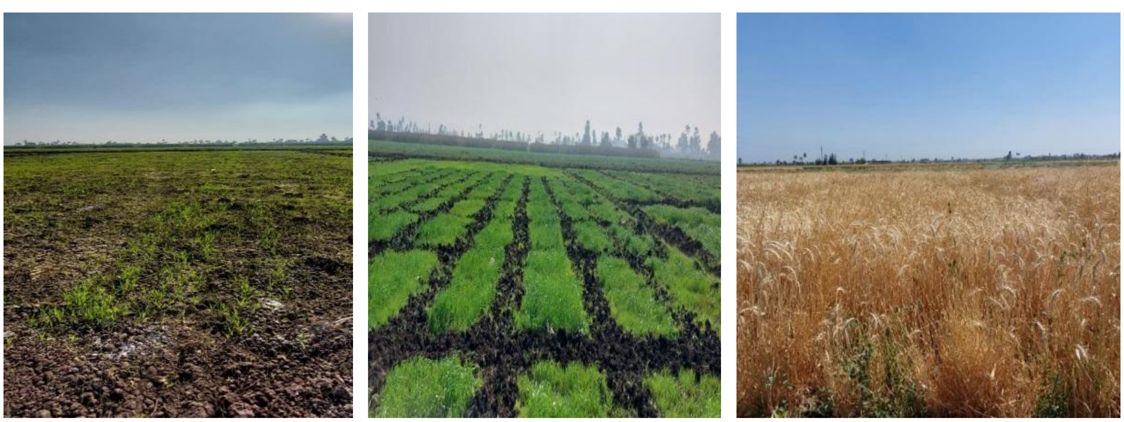
2.1 Research area

The research was carried out at Sids Agricultural Research Station (SARS), Agricultural Research Center, Beni-Suef Governorate, Egypt (Latitude: 29º 04 N, Longitude: 31º05 E) during season (2022 / 2023). The initial soil analysis, conducted in accordance with A.O.A.C. (1986) guidelines, classified the experimental soil as clay with a slightly alkaline pH of 7.7, with low salinity (1.3 $dS.m^{-1}$), low organic matter (1.9 %) and available nitrogen, phosphorus & potassium (NPK) were 20.0, 15.0 and 170 ppm respectively.

2.2. Experimental design

A field study was carried out to assess the positive impacts of incorporating biochar into the soil, combined with inoculation of two cyanobacteria species (*Tildeniella torsiva* NA3 and *Anabaena fertilissima*) and the extract of *Azolla pinnata*, (biofertilizer) alongside the recommended mineral fertilizer, on the production of two wheat genotypes (*Triticum aestivum* cv. Sids 14 and Sakha 95).

After preparing the experimental field through plowing and puddling. The experiments were laid out in a split split plot design with three replicates. Each plot measured 4.2 m² (6 lines × 0.2 m width × 3.5 m length), while the harvest area was 2.8 m² (4 lines × 0.2 m width × 3.5 m length). All agronomic practices carried out following the guidelines of the Crop Field Research Institute, Agricultural Research Center (Fig. 1).



A B C

Fig. 1: Field experiments design on effect of incorporation biochar into soil with addition of AZ bio inoculant in presence of recommended mineral fertilizers on the yield of wheat plant **A:** after 3 weeks of germination, **B:** branching stage and **C:** heading and flowering stage.

Urea (46% N), a mineral nitrogen fertilizer, was applied based on the specific needs of each treatment. The full recommended nitrogen dose (100% N) was 180 kg/fed, with reduced levels of 75% N (135 kg/fed) and 50% N (90 kg/fed). Additionally, all plots supplied with 30 kg/fed of superphosphate (15.5% P_2O_5) and 50 kg/fed of potassium sulphate (48% K_2SO_4) as sources of phosphorus and potassium, respectively.

The experimental area was divided into three main groups, and the interaction among them was as follows:

I. Main plots (Soil conditioner)
Soil with biochar
Soil without biochar

II. Sub plots (Cultivars)
Sids 14
Sakha 95

III. Sub Sub Plots (Fertilizer),
N1: 100 % N of the recommended
N2: 100% N + biofertilizer
N3: 75 % N of the recommended dose
N4: 75 % N + biofertilizer
N5: 50% N of the recommended dose
N6: 50% N + biofertilizer

2.2.1. Biochar incorporation.

Biochar mix well with the investigated soil before sowing the wheat at the rate of one ton/faddan. Biochar was obtained from private company and its chemical constituents analyzed and recorded at Table (1).

Table 1: Chemical constituents of the biochar

Type of analysis	Value
pH	8.6
EC dS/m	0.12
Total – Nitrogen %	2.83
Potassium (K) %	0.51
Magnesium (Mg) %	0.16
Calcium (Ca) %	2.35
Silicon mg/ kg	22.3
Organic matter %	3.7
Organic carbon %	2.51

2.2.2 Preparation of the biological inoculant.

The biological inoculants comprised two cyanobacterial cultures, *Tildeniella torsiva* NA3 (*T. torsiva* NA3) and *Anabaena fertilissima* (*A. fertilissima*) along with an extract of *Azolla pinnata* (*A. pinnata*) in a ratio of 0.25:0.25:0.5, respectively. These inoculants were applied twice to the soil as a drench at a rate of 119 L/ha (50 L/fed), 30 and 55 days after planting the wheat seeds.

2.2.2.1. *Azolla pinnata*

Fresh Azolla cultivated in clean water obtained from Sids Agricultural Research Staion (SARS). Rinse the Azolla thoroughly to remove dirt, debris, and contaminants. Place the Azolla in a blender with a small amount of water. The electric mixer should break down the plant cells, allowing the release of intracellular contents like proteins, lipids, pigments, and other bioactive compounds (Rahim and Ali

2023), Then filter it through a cheesecloth, or centrifuge to separate the solid plant material from the liquid extract. The filtrate contains the desired compounds.

2.2.2.2. Cyanobacterial culture

Tildeniella torsiva NA3 was isolated from Sids Agricultural Research Station' farm, while *Anabaena fertilissima* sourced from Microbiology department, Soils, Water and Environment Research Institute. Both of them maintained and propagated in liquid BG_{11} medium Allen and Stanier (1968), until the stationary phase.

The hormonal contents of the bio inoculant was analyzed chromatographically according to (Kannangara *et al.,* 1983) and recorded in, table (2):

Table 2: Phyto hormonal contents of the bio inoculant, Abscisic acid (Abc.), Gibberellic (Gib.), Indole -3-acetic acid (IAA) and Cytokinin (Cyt.) in mg/l.

Abc. mg / l	Gib. mg / l	IAA. mg / l	Cyt. mg / l
3.3	79.2	2207.5	3868.0

2.3. Soil microbial activity.

2.3.1. CO_2 evolution

Soil microbial activity, indicated by CO_2 evolution, was assessed in the wheat rhizosphere after 65 days of plant growth using the method Pramer and Schmidt (1965). To collect soil samples, ten grams of soil were placed in a 500 ml serum bottle with rubber stoppers. A cylindrical bag made of polyethylene was then suspended over a mixture of 100 ml 0.05 N NaOH and 3 ml 50% $BaCl_2$, which was incubated at 30°C for three days. CO_2 concentration in mg/100 g of soil was determined by titrating the remaining NaOH with 0.05 N HCl (1 ml HCl = 1 mg CO_2). A control bottle without soil was used as a blank.

2.3.2. Nitrogenase enzyme activity

Nitrogenase enzyme activity (nmole C_2H_4/g dry soil/h) was measured in wheat soil's rhizosphere to assess free-living N_2-fixation capability, as described by Dilworth (1966). To activate soil microorganisms, homogenize 15 gm of each soil sample with 2 ml of 10% glucose Okafor and Macrea (1973). Soil samples were put in 100 ml serum vials with tight rubber silicon closures and were incubated at 30°C for 24 hours. Sharp needle syringes were used to replace 10% (v/v) of headspace gas with an equivalent amount of acetylene gas (C_2H_2). The injected bottles were re-incubated for an additional 4 hours. One millilitre of headspace gas was examined to determine the amount of produced ethylene gas.

2.4. Plant analysis

2.4.1. Pigments contents

After 65 days of vegetative development, three randomly selected plants were collected from each plot. The pigments content (chlorophyll a, b, and carotenoids) were assessed using Lichtenthaler's method (Lichtenthaler, 1987). After soaking 50 mg of middle leaf tissue in 10 ml of 80% acetone, the samples were frozen for 48 hours in darkness. The pigment extract was centrifuged at 3000 rpm for 10 minutes. The concentrations of chlorophyll (Chl) a, b, and carotenoids (mg/g fresh wt) were estimated by measuring absorbance at 663, 647, and 470 nm using Spectronic 21D spectrophotometer (Milton Roy, USA), and calculated using the formula below.

$$\text{Chl.a (mg/g)} = 12.25(A_{663}) - 2.79(A_{647})$$
$$\text{Chl.b (mg/g)} = 21.50(A_{647}) - 5.10(A_{663})$$
$$\text{Carotenoids (mg/g)} = (1000(A_{470}) - 1.82\,[\text{Chla}] - 85.02[\text{Chlb}])198$$

2.4.2 Estimation of total nitrogen, phosphorus and potassium (NPK)

Half gram of grounded seeds was digested using sulphuric -perchloric – acids mixture. ($HClO_4$ + H_2SO_4) acids according to the procedure of Chapman and Pratt (1961).

1- Total nitrogen in plant samples was measured using the Kjeldahl method Jackson (1973).
2- Total potassium in plant samples was measured using a flame photometer, as outlined by Jackson (1967).
3- The total phosphorus content in plant samples was measured using Inductively Coupled Plasma Spectrometry (ICP) with an Ultima 2JY Plasma instrument.

2.5 Wheat yield components

Plant samples of 1.0 m^2 were randomly selected from each plot at the harvest stage for assessment. The number of spikes/m^2, grains/spike, weight of 1000 grains wt (gram), and grain yield (tone/fed) determined.

2.6. Statistical analyses

The collected data were statistically analysed using the analysis of variance method as described by Gomez and Gomez (1993). Mean values were compared using Gen-Stat software at a 5% significance level. The traits analysed as a split -split plot design with three replications separately as described by (Snedecor and Cochran 1967)

3. Results

3.1. Integrated effects of biofertilizers and biochar on soil microbial activity represented by N2-fixation efficiency and CO_2 evolution.

Tables (3,4,5) demonstrates the integrated effects of biofertilizer, biochar and different levels of mineral nitrogen fertilizer on soil microbial activity indicators. Nitrogenase enzyme activity and CO_2 emissions. This suggests an inverse relationship between the percentage of nitrogen fertilization and soil microbial activity, regardless of whether the soil amended with biochar or not. For example, Sids 14 treated with 100% nitrogen fertilization (N1) with biochar application showed the lowest values for N-ase enzyme activity and CO_2 evolution, measuring 1.31 nmol C_2H_4/g dry soil/hour and 180 mg/100 g soil, respectively, compared to 50% nitrogen fertilization (N5), which recorded 29.4 nmol C_2H_4/g dry soil/hour and 220 mg/100 g soil.

The addition of biochar significantly enhanced N-ase enzyme activity and CO_2 evolution in both wheat genotypes compared to treatments without biochar. Among all treatments, soil amended with both biochar and biofertilizer demonstrated the highest levels of N-ase enzyme activity and CO_2 evolution. For instance, the N6 treatment led to a remarkable increase ($P \leq 0.05$) in N-ase enzyme activity and CO_2 evolution in the Sids-14 genotype by 2808.39% and 25.27%, respectively, compared to the full recommended dose (FRD) of nitrogen (100% N). Similarly, the N4 treatment showed the greatest effectiveness in the Sakha 95 genotype, significantly boosting ($P \leq 0.05$) N-ase enzyme activity and CO_2 evolution by 359.31% and 78.57%, respectively, compared to the FRD (100% N) treatment.

3.2. Integrated effects of biofertilizers and biochar on wheat pigmentation

The results showed a direct relationship between pigment content and mineral nitrogen application in both genotypes, irrespective of whether the soil was treated with biochar or not, as shown in Table (3,4,5). However, wheat plants grown in biochar-amended soil and treated with biofertilizer showed a significantly higher content of chlorophyll a and b ($P \leq 0.05$) compared to those in the other treatments. The N2 treatment, when applied to soil with biochar, was the most effective, significantly increasing chlorophyll a and b compared to the full-recommended dose (FRD) in both cultivars. In Sids 14, chlorophyll a increased by 17.18% and chlorophyll b by 20%, while in Sakha 95, chlorophyll a rose by 20.68% and chlorophyll b by 21.12%.

Table 3: Effect of soil conditioner, cultivars and the interaction between soil conditioner x Cultivars on Nitrogenase enzyme activity, CO_2 evolution, Chlorophyll a, b and Carotenoid .

Parameters / Treatments	Cultivars	Nitrogenase enzyme activity (nmole C_2H_4/g dry soil/h)	CO_2 evolution mg/100 g soil	Chlorophyll a (mg/g fresh wt)	Chlorophyll b (mg/g fresh wt)	Carotenoid (mg/g fresh wt)
Soil conditioner						
With Biochar		20.76	169.51	1.86	0.7075	0.36
Without Biochar		11.99	110.69	1.55	0.5569	0.31
LSD 0.05		**0.584**	**0.987**	**1.885**	**0.05**	**0.07**
Cultivars						
Sids 14		19.18	163.42	1.749	0.6497	0.33
Sakha 95		13.57	116.78	1.675	0.6147	0.34
LSD 0.05		**0.450**	**0.379**	**0.08**	**0.02**	**0.02**
Fertilizer						
N1		3.88	105.78	1.71	0.62	0.36
N2		11.51	147.30	2.01	0.78	0.42
N3		11.11	129.20	1.48	0.54	0.27
N4		33.15	148.88	1.94	0.73	0.38
N5		17.26	147.47	1.29	0.46	0.26
N6		21.33	161.97	1.81	0.66	0.33
LSD 0.05		**0.766**	**1.12**	**0.08**	**0.045**	**0.04**
With Biochar	Sids 14	20.43	202.67	1.908	0.7206	0.37
	Sakha 95	21.08	136.35	1.824	0.6944	0.36
Without Biochar	Sids 14	17.92	124.17	1.589	0.5789	0.30
	Sakha 95	6.06	97.22	1.526	0.5350	0.32
LSD 0.05		**0.52**	**0.791**	**0.14**	**0.04**	**0.06**

Table 4: Effect of interactions between soil conditioner x fertilizer and cultivars x fertilizer on Nitrogenase enzyme activity, CO_2 evolution, Chlorophyll a, b and Carotenoid.

Parameters / Treatments	Fertilizer	Nitrogenase enzyme activity (nmole C_2H_4/g dry soil/h)	CO_2 evolution mg/100 g soil	Chlorophyll a (mg/g fresh wt)	Chlorophyll b (mg/g fresh wt)	Carotenoid (mg/g fresh wt)
With Biochar	**N1**	6.51	136.2	1.830	0.7	0.37
	N2	14.0	157.7	2.13	0.75	0.47
	N3	15.23	159.95	1.61	0.64	0.28
	N4	38.21	184.25	2.12	0.80	0.42
	N5	22.15	178.2	1.5	0.54	0.27
	N6	28.44	200.75	2.0	0.80	0.37
Without Biochar	**N1**	1.25	75.35	1.592	0.53	0.35
	N2	9.03	136.9	1.79	0.65	0.37
	N3	6.99	98.45	1.35	0.45	0.26
	N4	28.1	113.5	1.77	0.66	0.35
	N5	12.36	116.75	1.08	0.38	0.25
	N6	14.21	123.2	1.75	0.65	0.29
LSD 0.05		**1.025**	**1.521**	**0.155**	**0.06**	**0.07**
Sids 14	**N1**	0.81	137.85	1.77	0.6	0.34
	N2	11.89	187.4	2.0	0.74	0.45
	N3	13.1	148.0	1.54	0.60	0.27
	N4	37.44	162.25	2.0	0.79	0.40
	N5	23.89	169.0	1.3	0.45	0.27
	N6	27.92	176.0	1.8	0.69	0.28
Sakha 95	**N1**	6.95	73.70	1.64	0.64	0.37
	N2	11.14	107.2	1.9	0.66	0.38
	N3	9.11	110.4	1.42	0.48	0.28
	N4	28.87	135.5	1.84	0.67	0.37
	N5	10.62	125.95	1.28	0.46	0.25
	N6	14.73	147.95	1.95	0.75	0.38
LSD 0.05		**1.039**	**1.47**	**0.13**	**0.06**	**0.06**

Table 5: Effect of interactions between soil conditioner x cultivars x fertilizer on Nitrogenase enzyme activity, CO_2 evolution, Chlorophyll a, b and Carotenoid.

Parameters / Treatments	Cultivars	Fertilizer	Nitrogenase enzyme activity (nmole C_2H_4/g dry soil/h)	CO_2 evolution mg/100 g soil	Chlorophyll a (mg/g fresh wt)	Chlorophyll b (mg/g fresh wt)	Carotenoid (mg/g fresh wt)
With Biochar	Sids 14	N1	1.31	180.0	1.92	0.7	0.37
		N2	15.35	201.0	2.25	0.84	0.59
		N3	15.73	186.0	1.68	0.69	0.3
		N4	22.68	203.0	2.2	0.84	0.4
		N5	29.40	220.0	1.5	0.51	0.24
		N6	38.1	225.5	1.9	0.74	0.3
	Sakha 95	N1	11.7	92.4	1.74	0.71	0.37
		N2	12.65	114.4	2.1	0.86	0.35
		N3	14.73	133.9	1.54	0.59	0.27
		N4	53.74	165.0	2.0	0.77	0.45
		N5	14.9	136.4	1.5	0.57	0.3
		N6	18.79	176.0	2.0	0.66	0.44
Without Biochar	Sids 14	N1	0.31	95.7	1.63	0.5	0.32
		N2	8.43	173.8	1.9	0.75	0.32
		N3	10.47	110.0	1.4	0.52	0.24
		N4	52.2	121.0	1.79	0.64	0.4
		N5	18.38	118.0	1.1	0.4	0.3
		N6	17.75	126.5	1.7	0.65	0.26
	Sakha 95	N1	2.2	55.0	1.55	0.56	0.38
		N2	9.63	100.0	1.8	0.67	0.42
		N3	3.5	86.9	1.3	0.37	0.29
		N4	4.0	106.0	1.8	0.65	0.3
		N5	6.35	115.5	1.067	0.36	0.2
		N6	10.68	119.9	1.64	0.58	0.32
LSD 0.05			**1.457**	**2.11**	**0.19**	**0.089**	**0.09**

The data indicate that the application of biochar and biofertilizer effectively enhances pigment content, Thus N2 and N4 showing the highest levels in both cultivars. The Sids 14 cultivar responds more positively than Sakha 95 to these treatments, especially under higher nitrogen availability. These observations emphasize the beneficial role of biochar and biofertilizer in boosting chlorophyll and carotenoid levels, which has the potential to improve plant health and productivity.

3.3. Integrated effects of biofertilizers and biochar on wheat grain quality

The integrated effect of Biochar and biofertilizer also, treatments on nitrogen, phosphorus, potassium, and protein content in Sids 14 and Sakha 95 Cultivars were recorded in the Tables (6,7,8).

Treatments involving biochar and biofertilizer generally exhibit higher levels of nitrogen (N), phosphorus (P), potassium (K), and protein percentages than those without biochar with notably strong effects observed in the N2 and N4 treatments, Sids 14 under N2 with biochar, nitrogen content reaches 1.45%, potassium 0.88%, and protein content 8.33%, all surpassing the levels observed in treatments without biochar. Sakha 95 cultivar demonstrates slightly higher phosphorus percentage than Sids 14 in some treatments. For instance, under the biofertilizers treatments (N2, N4 and N6) with biochar, Sakha shows P % values of 0.36, 0.36, and 0.31, respectively, compared to 0.32, 0.3, and 0.24% in Sids 14. On the other side, Sids 14 cultivar generally shows higher nitrogen, potassium and protein levels than Sakha 95.

The N5 treatment, which has lower nitrogen levels, consistently exhibits the lowest nutrient and protein content across both cultivars. Wheat grains harvested from plots treated with N5 in absence of biochar, exhibited lower nitrogen, phosphorus, potassium and protein content (0.94; 0.03; 0.08 and 5.4 %), respectively, in Sids 14, wherease, (0.8, 0.24, 0.32 and 4.6), respectively in Sakha 95. This suggests that lower nitrogen availability limits nutrient uptake and protein synthesis.

3.4. Integrated effects of biofertilizers and biochar on wheat yield components.

At the harvest stage, the wheat yield index reflected in the number of grains per spike, number of spikes to each m^2, the weight of 1000 grains, and the total grain yield per feddan were determined and recorded at Tables (6,7,8).

The application of biochar seems to significantly enhance all parameters (number of kernels/ spike (NK/S), number of spikes/m^2 (Ns/m²), 1000 grains weight, and grain yield (Gy/fed) in both cultivars. Additionally, biologically treatments paired with biochar resulted in even more pronounced improvements, making N2 the most effective treatment for both cultivars.

Genotype of Sids 14 treated both N2 and biochar achieves 69 kernels per spike, 423 spikes per m², a 1000-grains weight of 52.92 g, and a grain yield of 3.7 tons per fed. Without biochar, these values dropped to 67 kernels per spike, 372 spikes per m², 49.43 g for 1000-grains weight, and 3.19 tons per fed, indicating a clear improvement. While Sakha 95 displays similar trends; under N2 with biochar, the number of kernels per spike and spikes per m² reach 63 and 355, compared to 60 and 338 without biochar. Additionally, the 1000-grains weight and grain yield per feddan increased from 45.02 g and 2.73 tons to 46.6 g and 3.04 tons, respectively.

Biofertilizer treatments tend to increase yield compared to their non-treated counterparts. For instance, Sids 14 treated with N4 and biochar reaches a 3.4 tons grain yield/fed, compared to 3.07 tons Gy/fed for non-treated one (N3). Similarly, Sakha 95 with biochar under N4 achieves a yield of 2.83 tons Gy/fed compared to 2.47 tons in N3.

Wheat yield index in Sids14 was generally higher than in Sakha 95 under most treatment conditions, suggesting that Sids 14 may be more responsive to both biochar addition and nitrogen treatments than Sakha 95.

Table 6: Effect of soil conditioner, Cultivars and the interaction between soil conditioner x Cultivars on N,P,K, protein, NK/S, NS/m^2, 1000 grains weight (g) and GY/fed (ton).

Parameters / Treatments	Cultivars	N %	P %	K %	Protein %	NK/S	NS/m^2	1000 grains wt (g)	GY/fed ton
Soil conditioner									
With Biochar		1.22	0.25	0.36	7.01	62.39	362.1	45.11	2.76
Without Biochar		1.06	0.19	0.3	6.13	59.61	339.5	42.08	2.39
LSD 0.05		**0.039**	**0.06**	**0.017**	**0.22**	**5.01**	**11.71**	**2.27**	**0.43**
Cultivars									
Sids 14		1.14	0.17	0.33	6.56	63.03	365.8	45.64	2.853
Sakha 95		1.14	0.27	0.33	6.59	58.97	335.8	41.55	2.31
LSD 0.05		**0.036**	**0.03**	**0.012**	**0.2**	**2.9**	**10.32**	**1.465**	**0.32**
Fertilizer									
N1		1.1	0.22	0.34	4.32	60.83	384.4	48.02	2.97
N2		1.3	0.3	0.5	5.16	64.58	372.2	46.43	3.17
N3		1.1	0.17	0.31	4.0	61.58	347.4	43.14	2.58
N4		1.2	0.28	0.34	4.67	66.08	380.2	47.70	2.94
N5		0.96	0.14	0.21	3.66	56.83	299.5	36.46	1.72
N6		1.16	0.23	0.3	4.46	56.08	320.8	39.81	2.08
LSD 0.05		**0.06**	**0.02**	**0.03**	**0.38**	**3.04**	**14.17**	**1.887**	**0.06**
With Biochar	Sids 14	1.22	0.24	0.41	7.01	63.94	378.3	47.28	3.08
	Sakha 95	1.22	0.26	0.3	7.02	60.83	345.8	42.39	2.44
Without Biochar	Sids 14	1.06	0.1	0.25	6.12	62.11	353.2	42.99	2.61
	Sakha 95	1.07	0.29	0.35	6.14	57.11	325.8	40.16	2.17
LSD 0.05		**0.039**	**0.05**	**0.015**	**0.22**	**4.04**	**11.28**	**1.882**	**0.37**

Middle East J. Agric. Res., 13(4): 1042-1057, 2024
EISSN: 2706-7955 ISSN: 2077-4605 *DOI: 10.36632/mejar/2024.13.4.62*

Table 7: Effect of interactions between soil conditioner x fertilizer and cultivars x fertilizer on N,P,K, protein, NK/S, NS/m2, 1000 grains weight (g) and GY/fed (ton).

Parameters / Treatments	Fertilizer	N %	P %	K %	Protein %	NK/S	NS/m^2	1000 grains wt (g)	GY/fed tons
With Biochar	N1	1.27	0.22	0.35	7.33	63.83	388.2	48.81	3.11
	N2	1.3	0.34	0.6	7.53	66.50	389.3	48.43	3.39
	N3	1.15	0.27	0.3	6.64	64.83	358.2	44.4	2.77
	N4	1.18	0.2	0.28	6.7	69.33	403.5	50.66	3.13
	N5	1.13	0.24	0.27	6.49	55.00	302.2	36.97	1.91
	N6	1.28	0.23	0.35	7.36	54.83	331.0	41.37	2.27
Without Biochar	N1	0.90	0.22	0.32	5.17	57.83	380.7	47.22	2.83
	N2	1.1	0.25	0.3	6.32	62.67	355.2	44.42	2.96
	N3	1.07	0.2	0.2	6.15	58.33	336.7	41.88	2.39
	N4	1.23	0.17	0.29	7.1	62.83	356.8	44.74	2.75
	N5	1.05	0.13	0.32	6.03	58.67	296.8	35.95	1.53
	N6	1.05	0.21	0.39	6.03	57.33	310.7	38.25	1.89
LSD 0.05		**0.088**	**0.05**	**0.04**	**0.51**	**4.717**	**19.09**	**2.678**	**0.36**
Sids 14	N1	1.13	0.19	0.31	6.49	64.00	400.3	50.22	3.27
	N2	1.35	0.25	0.57	7.76	68.33	397.7	49.58	3.46
	N3	1.0	0.11	0.17	5.75	64.0	373.5	46.52	2.84
	N4	1.2	0.2	0.25	6.9	67.67	395	49.82	3.2
	N5	0.99	0.1	0.33	5.7	57.33	301.0	36.8	1.98
	N6	1.18	0.16	0.37	6.7	56.83	327.0	40.39	2.34
Sakha 95	N1	1.04	0.25	0.36	6.0	57.67	368.5	45.81	2.67
	N2	1.06	0.33	0.33	6.09	60.83	346.8	42.37	2.89
	N3	1.22	0.36	0.34	7.04	59.17	321.3	39.77	2.32
	N4	1.21	0.17	0.32	6.98	64.50	365.3	45.59	2.67
	N5	1.18	0.27	0.26	6.81	56.33	298.0	36.12	1.46
	N6	1.15	0.28	0.38	6.61	55.33	314.7	38.13	1.82
LSD 0.05		**0.09**	**0.04**	**0.04**	**0.52**	**4.476**	**19.75**	**2.657**	**0.31**

Table 8: Effect of interactions between soil conditioner x cultivars x fertilizer on N,P,K, protein, NK/S, NS/m^2, 1000 grains weight (g) and GY/fed (ton).

Parameters / Treatments	Cultivars	Fertilizer	N %	P %	K %	Protein %	NK/S	NS/m^2	1000 grains wt (g)	GY/fed ton
With Biochar	Sids 14	N1	1.26	0.22	0.35	7.24	68.0	402.0	51.01	3.5
		N2	1.45	0.32	0.88	8.33	69.33	423.3	52.92	3.7
		N3	1.05	0.19	0.36	6.03	66.67	399.3	49.56	3.07
		N4	1.3	0.3	0.38	7.47	70.0	408.0	51.40	3.4
		N5	1.03	0.17	0.26	5.92	55.33	297.3	36.32	2.2
		N6	1.23	0.24	0.27	7.07	54.33	340.0	42.50	2.57
	Sakha 95	N1	1.29	0.22	0.32	7.41	59.67	374.3	43.95	2.73
		N2	1.33	0.36	0.36	7.6	63.67	355.3	46.60	3.04
		N3	1.21	0.22	0.3	6.95	63.0	317.0	39.25	2.47
		N4	1.28	0.36	0.36	7.36	68.67	399.0	49.93	2.83
		N5	1.06	0.1	0.18	6.09	54.67	307.0	37.62	1.61
		N6	1.17	0.31	0.33	6.72	55.33	322.0	40.25	1.97
Without Biochar	Sids 14	N1	1.0	0.16	0.31	5.75	61.3	398.7	46.25	3.04
		N2	1.25	0.19	0.36	7.18	67.0	372.0	49.43	3.19
		N3	0.97	0.04	0.27	5.57	60	347.7	43.47	2.61
		N4	1.13	0.1	0.28	6.49	65.33	382.0	48.24	2.97
		N5	0.94	0.03	0.08	5.4	59.33	304.7	37.28	1.76
		N6	1.1	0.08	0.24	6.32	59.33	314.0	39.28	2.11
	Sakha 95	N1	0.97	0.28	0.37	5.57	55.67	362.7	42.60	2.61
		N2	1.37	0.36	0.43	7.87	60.33	338.3	45.02	2.73
		N3	0.95	0.24	0.32	5.4	55.33	325.7	40.28	2.16
		N4	1.17	0.35	0.34	6.72	58.0	331.7	41.25	2.52
		N5	0.8	0.24	0.32	4.6	55.33	289.0	34.62	1.3
		N6	1.16	0.31	0.34	6.67	38	307.3	37.22	1.66
LSD 0.05			**0.126**	**0.06**	**0.06**	**0.72**	**6.4**	**27.39**	**3.750**	**0.38**

4. Discussion

Soil amended with both biochar and biofertilizer, comprising of *Anabaena fertilissima*, *Tildeniella torsiva* NA3, and azolla extract, achieving the highest levels of nitrogenase enzyme activity and CO_2 evolution indicates a synergistic effect of these two amendments on soil microbial processes. This could attributed to; Biochar's high porosity and surface area create a favorable environment for microbial communities, including nitrogen-fixing bacteria. Combined with biofertilizers that provide beneficial microbes (nitrogen fixing microbes) or nutrients, these results in a robust microbial population, increasing nitrogenase activity and enhancing nitrogen availability for plants. (Ghazal *et al*. 2010; Renuka, 2018; Dai *et al*., 2021 and Hamed *et al*., 2022).

Furthermore, Biochar improves soil characteristics such as aeration, water retention, and cation exchange capacity, while cyanobacteria based biofertilizers enhance nutrient availability. Combined, they support microbial activity, resulting in higher CO_2 evolution as an indicator of microbial respiration and organic matter breakdown. (Wyzi´nska *et al*., 2024)

It is well established that Azolla extracts are abundant in bioactive compounds, such as growth hormones, organic acids, enzymes, and cofactors, (Maswada, *et al*., 2021) which could be provide energy to soil microbes. This results in increased respiration rates and CO_2 production, as well as enhanced nitrogenase enzyme activity in the microorganisms involved.

In this study, we found that raising nitrogen levels to 100% N notably reduced nitrogenase enzyme activity and CO_2 evolution, a change we linked to a decline in the number of free-living nitrogen-fixing microorganisms in the soil. A similar results reported by Hamed *et al*. (2022).

Results highlight the combined advantages of utilizing biofertilizer, the full-recommended nitrogen dose and biochar, particularly in enhancing chlorophyll a and b levels and increasing photosynthetic activity. These findings are consistent with the observations made by de Bever *et al.* (2013), Maswada *et al*. (2021), Eman *et al*. (2023), and Ghulam *et al.* (2024). This observation is logical, as the effectiveness of biofertilizer, combined with biochar, significantly boosts nitrogenase enzyme activity which, in turn, increases nitrogen availability, a crucial element of the chlorophyll molecule (Hamed *et al*., 2022). Consequently, higher nitrogenase enzyme activity leads to enhanced chlorophyll content, improving photosynthesis and promoting plant growth.

On the other side, Sids 14 appears to have higher pigment concentrations overall compared to Sakha 95, suggesting that it may have a better genetic predisposition for photosynthetic efficiency.

The results revealed that the combined use of biofertilizer and biochar greatly increases the levels of total nitrogen, phosphorus, potassium, and grain protein production. Notably, protein content exhibited a pattern consistent with nitrogen levels, as nitrogen serves as a key component of amino acids and proteins. These findings are in agreement with those of Kimani *et al.* (2021), who reported that the combination of biochar and Azolla enhances rice yield and nitrogen use efficiency; Hamed *et al.* (2022), who discovered that combining cyanobacteria with yeast and partial nitrogen fertilization increased NPK uptake (kg/fed) and the percentage of protein in wheat grains. Similarly, Marta Wyzińska *et al*. (2024) reported that biochar with different types had a remarkable impact on the characters of wheat grain.

In most treatments, the Sids 14 cultivar shows somewhat higher percentages of nitrogen, potassium, and protein than Sakha 95, indicating a more significant response to nitrogen availability. In certain conditions, the Sakha 95 cultivar has a slightly higher P percentage than Sids, perhaps as a result of cultivar-specific nutrient uptake efficiency.

This integrated impact is attributable to a number of factors affecting soil health, nutrient availability, and plant physiology. The nitrogen-fixing strain *Anabaena fertilissima*, which is present in the biofertilizer, fixes atmospheric nitrogen into ammonia, a type of nitrogen that plants can easily absorb. This makes more nitrogen available, which is essential for the production of proteins and chlorophyll. (Kholssi *et al*., 2022). Furthermore, biofertilizer, which is abundant in growth hormones as indicated by its analysis, enhances the root system's nutrient absorption capacity by promoting root growth and strengthening interactions with soil microbes. This results in increased absorption of essential nutrients, particularly nitrogen, phosphorus, and potassium, which are important for plant growth and metabolic functions (Maswada *et al*., 2021). Biochar has a high cation exchange capacity, allowing it to retain and exchange essential nutrients such as nitrogen, phosphorus, and potassium. This improves nutrient availability for plant uptake, reduces nutrient leaching, and promotes more efficient use of fertilizers (Dai *et al*., 2021).

Similarly, our data highlight the superiority of the biofertilizer treatment combined with biochar in improving wheat yield indices. This 1000-grains weight, the number of spikes per square meter, the number of grains per spike, and the overall grain yield per feddan all show this improvement. This finding makes sense and was expected as the combination increases microbial activity, boosts photosynthesis, and enhances grain quality by raising the percentages of protein and NPK. Al Sayed *et al*. (2022), who reported that incorporating biochar as a soil organic amendment in combination with Azolla represents an effective agricultural management practice, further support our findings. This approach plays a significant contribution to improving nutrient availability, metabolite production, and chlorophyll biosynthesis, thereby improving the photosynthesis process. These improvements are ultimately reflected in enhanced yield, yield components, and grain quality.

The results support our hypothesis that the combined treatments greatly enhanced the nutritional quality of wheat grains as well as their growth, nutrient absorption, photosynthetic pigment levels, yield, and its constituent parts.

5. Conclusion

In conclusion, the two wheat genotypes (*Triticum aestivum* cvs. Sids 14 and Sakha 95) were more productive when biochar, *Tildeniella torsiva* NA3 and *Anabaena fertilissima* inoculants, and *Azolla pinnata* extract were applied in conjunction with the suggested mineral fertilizers. When it came to improved growth and yield under the combination treatment, Sids 14 responded more than Sakha 95. This combination strategy showed promise for improving wheat output and soil health, indicating that it is a viable sustainable agricultural method for increasing crop productivity.

Acknowledgements

The authors wish to thank Soils, Water and Environment Research Institute (SWERI), and Sids Agricultural Research Station, Agriculture Research Center, Giza, Egypt, for facilities provided to conduct this research work.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or Analyzed during the current study.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding received from any agency.

Author contributions

NA & SEA designed the experimental approach, contributed to full plant and soil analyses, interpreted the results and drafted the manuscript. NA and STE carried out the field crop study, data analysis and drafting of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

References

Allen M.M. and R.Y. Stanier, 1968. Growth and division of some unicellular blue-green algae. J. Gen. Microbiol. 51:199–202. https:// doi. org/ 10. 1099/ 00221 287- 51-2- 199.

Al-Sayed H.M., A.M. Ali, M.A. Mohamed and M.F. Ibrahim, 2022. Combined Effect of Prickly Pear Waste Biochar and Azolla on Soil Fertility, Growth, and Yield of Roselle (*Hibiscus sabdariffa* L.) Plants. J. of Soil Sci. and Plant Nutrition, 22:3541–3552. https://doi.org/10.1007/s42729-022-00908-7

AOAC 1986. Official Methods of Analysis. 14th Edition, Association of Official Analytical Chemists, Washington DC.

Babu, S., R. Prasanna, N. Bidyarani and R. Singh, 2015. Analysing the colonisation of inoculated cyanobacteria in wheat plants using biochemical and molecular tools. J. Appl. Phycol., 27:327–338. https://doi.org/10.1007/ s10811- 014- 0322-6

Bahuguna, A., S. Sharma, and J. Yadav, 2021. Effect of different organic sources on physical, chemical and biological properties of soil in inceptisols of Varanasi. Int. J. Plant Soil Sc., 33: 41-52.

Bisht, N., and P.S. Chauhan, 2020. Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. Soil contamination-threats and sustainable solutions, 1-10.

Boghdady, M.S., and S.A. Ali, 2013. Comparison between effect of *Azospirillum brasilense* and *Anabaena oryzae* on growth, yield and anatomical characters of wheat plants. J. Appl. Sci. Res., 9:627–37

Chanda, S., S. Dattamudi, K. Jayachandran, L.J. Scinto, and M. Bhat, 2024. The Application of Cyanobacteria as a Biofertilizer for Okra (*Abelmoschus esculentus*) Production with a Focus on Environmental and Ecological Sustainability. Environments, 11(3): 45. https://doi.org/10.3390/environments11030045

Chapman, H.D. P.F. Pratt, 1961. Methods of analysis for soil, plant and water.

Choudhury, A.T.M.A., and I.R. Kennedy, 2005. Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. Commun Soil Sci. Plant Anal. 36(11–12):1625–1639. https://doi.org/10.1081/CSS- 200059104.

Combinations of hoagland's solution and Azolla filiculoides on photosynthesis and chlorophyll content in Beta vulgaris subsp. Cycla "fordhook giant" grown in hydroponic cultures. African J Biotechnol., 12 :2006–2012.

Dai, Z., X. Xiong, H. Zhu, H. Xu, P. Leng, J. Li, C. Tang, and J. Xu, 2021. Association of biochar properties with changes in soil bacterial, fungal and fauna communities and nutrient cycling processes. Biochar, 3: 239–254.

de Bever, A., P.A. Ndakidemi, and C.P. Laubscher, 2013. Effects of different combinations of Hoagland's solution and *Azolla filiculoides* on photosynthesis and chlorophyll content in Beta vulgaris subsp. Cycla'fordhook giant' grown in hydroponic cultures. African Journal of Biotechnology, 12(16): 2006-2012. DOI: 10.5897/AJB12.1582.

Deepali, C., M. Mukesh, B. Tansukh, S. Prashant, and S. Kanika, 2020. Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochem. Biophys. Rep. 22: 100737. https://doi.org/10.1016/j.bbrep.2020.100737

Dilworth, M.J., 1966. Acetylene reduction by nitrogen-fixing preparations from Clostridium pasteurianum. Biochimicaet Biophysica Acta 127:285–294. https:// doi. org/ 10. 1016/ 0304- 4165(66) 90383-7

EBC. European Biochar Certificate Guidelines for a Sustainable Production of Biochar; European Biochar Foundation EBC: Arbaz, Switzerland, 2012–2022; Available online: http://european-biochar.org (accessed on 20 November 2023).

Elhanafi, L., M. Houhou, C. Rais, I. Mansouri, L. Elghadraoui, and H. Greche, 2019. Impact of excessive nitrogen fertilization on the biochemical quality, phenolic compounds, and antioxidant power of *Sesamum indicum* L seeds. J. Food Qual 2019:1–6. https://doi.org/10.1155/2019/9428092

Eman H. Ashour, Aida H. Afify, Ahlam A. Mehesen, Randa M. Zaki and M. Ahmed, 2023. El-Sawah Response of wheat to cyanobacteria and compost tea applications as a tool to achieve bio-organic farming concept World Journal of Advanced Research and Reviews, 17(01): 1046–1058.

Gao, C., A.M. El-Sawah, D.F.I. Ali, Y. Alhaj Hamoud, H. Shaghaleh, and M.S. Sheteiwy, 2020. The Integration of Bio and Organic Fertilizers Improve Plant Growth, Grain Yield, Quality and Metabolism of Hybrid Maize (*Zea mays* L.). Agronomy 10: 319. https://doi.org/10.3390/agronomy10030319

Ghazal, F.M., L.A.A. Moussa, and N.A.H. Fetyan, 2010. Cyanobacteria and Rhizobium radiobacter as possible biofertilizers in wheat production. J. Agric. Chemi. Biotechnol., 1(7):383–399. https:// doi. org/ 10. 21608/ jacb. 2010. 90050

Ghulam, M., R. Muhammad , U. Muhammad , H. Sajjad , I.A. Muhammad, Maha Deeb Jawaher Alkahtani , M.A. Bandar, A.S. Hendy, R.A. Mohamed, I. Rashid , H. Wiwiek , H.R. Muhammed and R. Muhammad, 2024. Biochar enhances the growth and physiological characteristics of Medicago sativa, Amaranthus caudatus and *Zea mays* in saline soils. Murtaza et al. BMC Plant Biology 24:304 https://doi.org/10.1186/s12870-024-04957-1

Gomez, M.A., and A.A. Gomez, 1993. Statistical procedure for agricultural research 2nded. John Wile & Sons, New York, p 680.Chapman HD Pratt PF (1961) Methods of analysis for soil, plant and water. Univ. of California Div. of Agric Sci.

Gou, M.M., Z.Y. Qu, F. Wang, X.Y. Gao, M. Hu, 2018. Progress in research on biochar affecting soil-water environment and carbon sequestration-mitigating emissions in agricultural fields. Transactions of the Chinese Society for Agricultural Machinery, 49: 1–12.

Hall, D.O., S.A. Markov, Y. Watanabe, and K.K. Rao, 1995. The potential applications of cyanobacterial photosynthesis for clean technologies, Photosynth. Res., 46: 159–167. https://doi.org/10.1007/BF00020426

Hamed, S.M., N.M. El-Gaml and S.T. Eissa, 2022. Integrated biofertilization using yeast with cyanobacteria on growth and productivity of wheat. Beni-Suef Univ. J. Basic. Appl. Sci. 11:112. https://doi.org/10.1186/s43088-022-00288-y

Jackson, M.L., 1973. "Soil Chemical Analysis" Prentice-Hall of India private limited – New Delhi, India

Jackson, M.L., 1967. Soil Chemical Analysis. Prentice-Hall of India Pvt. Ltd., New Delhi, 498.

Jaiswal, P., D.W. Dhar, N. Sharma, S. Jain, P. Nehra, B. Singh, Y.V. Singh, and S. Saxena, 2021. Evaluating the role of endophytic cyanobacterial isolates on growth promotion and N/P status of rice crop. Vegetos, 35:244–250.

Jamal Uddin, A.F.M., M. Rakibuzzaman, E.W. Wasin, M.A. Husna, and A.K. Mahato, 2019. Foliar application of *Spirulina* and *Oscillatoria* on growth and yield of okra as bio-fertilizer. Journal of Bioscience and Agriculture Research, 22(02): 1840-1844. https://doi.org/10.18801/jbar.220219.227

Kannangara, T., R.C. Durley, G.M. Simpson, and N. Seetharama, 1983. Drought resistance of sorghum bicolor. 6. Changes in endogenous growth regulators of plants grown across an irrigation gradient. Canadian Journal of Plant Science. 63(1): 147-155. https://doi.org/10.4141/cjps83-014.

Karthikeyan, N., R. Prasanna, L. Nain, and B.D. Kaushik, 2007. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. Eur. J. Soil Biol. 43(1): 23–30. https://doi.org/10.1016/j.ejsobi.2006.11.001

Kholssi, R., E.A.N. Marks, J. Miñón, O. Montero, J.F. Lorentz, and D.A.C. Rad, 2022. Biofertilizing effects of *Anabaena cylindrica* biomass on the growth and nitrogen uptake of wheat. Commun Soil Sci. Plant Anal. 53(10):1216–1225. https:// doi. org/ 10. 1080/ 00103 624. 2022. 20433 50.

Kimani, S.M., P.O. Bimantara, V. Kautsar, K. Tawaraya, and W. Cheng, 2021. Poultry litter biochar application in combination with chemical fertilizer and Azolla green manure improves rice grain yield and nitrogen use efficiency in paddy soil. Biochar. 3:591–602. https:// doi.org/10.1007/s42773-021-00116-z

Kimani, S.M., P.O. Bimantara, V. Kautsar, K. Tawaraya, and W. Cheng, 2021. Poultry litter biochar application in combination with chemical fertilizer and Azolla green manure improves rice grain yield and nitrogen use efficiency in paddy soil. Biochar., 3:591–602. https:// doi.org/10.1007/s42773-021-00116-z

Kollah, B., A.K. Patra and S.R. Mohanty, 2016. Aquatic microphylla Azolla: a perspective paradigm for sustainable agriculture, environment and global climate change. Environ. Sci. Pollut. Res., 23: 4358-4369.

Kumar, D., R. Anand and P. Kumari, 2023. Field Test of Cyanobacteria as Biofertilizer for the Cultivation of *Triticum Aestivum* (Wheat) Plant. International Journal for Multidisciplinary Research, 5(6): November-December.

Kumar, R., R. Kumar, and O. Prakash, 2019. Chapter 5. The Impact of Chemical Fertilizers on Our Environment and Ecosystem. In: Sharma, P., Ed., Research Trends in Environmental Sciences, AkiNik Publications, New Delhi, 69-86.

Lichtenthaler, H.K., 1987 Chlorophylls and carotenoids: Pigments of phtosynthetic biomembranes. Meth. Enzymol., 148:350–382. https:// doi. org/ 10. 1016/ 0076- 6879(87) 48036-1.

Mali ´nska, K., 2015. Prawne i jako´sciowe aspekty dotycz ˛ace wymaga ´n dla biow˛egla. In˙zynieria I. Ochr. Sr. 18: 359–371.

Malik, F.R., S. Ahmed, and Y.M. Rizki, 2001. Utilization of lignocellulosic waste for the preparation of nitrogenous biofertilizer. Pakistan J. Biol. Sci. 4(10):1217–1220. https://doi.org/10.3923/pjbs.2001.1217.1220

Malyan, S.K., 2019. Mitigation of greenhouse gas intensity by supplementing with *azolla* and moderating the dose of nitrogen fertilizer. Biocatal. Agric. Biotechnol. 20, e101266.

Marta, W., K.B. Adam and G. Jerzy, 2024. Impact of Biochar Dose and Origin on Winter Wheat Grain Quality and Quantity Agriculture, 14: 39. https://doi.org/ 10.3390/agriculture14010039 .

Maswada, H.F., U.A. Abd El-Razek, A.N.A. El-Sheshtawy, and Y.S.A. Mazrou, 2021. Effect of *Azolla filiculoides* on growth, physiological and yield attributes of maize grown under water and nitrogen deficiencies. J. Plant Growth Regul. 40: 558–573.

Medy´nska-Juraszek, A., P.A. Rivier, D. Rasse, and E.J. Joner, 2020. Biochar affects heavy metal uptake in plants through interactions in the rhizosphere. Appl. Sci. 10: 5105.

Mussarat, M., M. Shair, D. Muhammad, I.A. Mian, S. Khan, M. Adnan, S. Fahad, E.S. Dessoky, and E.L. Sabagh, 2021. Accentuating the role of nitrogen to phosphorus ratio on the growth and yield of Wheat crop. Sustainability, 13:2253. https://doi.org/10.3390/su13042253.

Nkoh, J.N., F.O. Ajibade, E.O. Atakpa, M. Abdulaha-Al Baquy, S. Mia, E.C. Odii, and R. Xu, 2022. Reduction of heavy metal uptake from polluted soils and associated health risks through biochar amendment: A critical synthesis. J. Hazard. Mater. Adv. 6, 100086.

Okafor, N., and I.C. Macrea, 1973. The influence of moisture level, light aeration and glucose upon acetylene reduction by black Earth soil. Soil Biol. Biochem., 5:181–186. https:// doi. org/ 10. 1016/ 0038- 0717(73) 90108-9.

Pramer, D. and E.L. Schmidt, 1965. Experimental Soil Microbiology. Burgess Publishing Co., Minneapolis.

Prasanna, R., M. Joshi, A. Rana, Y. Shivay, and L. Nain, 2012. Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C-N sequestration in soil under rice crop. World J. Microbiol. Biotechnol. 28:1223–1235.https:// doi. org/ 10. 1007/ s11274- 011- 0926-9

Rahim, B.A., and R.A.A. Ali, 2023. Effect of Azolla Extract on Yield, Its Components and Protein Content in three Cultivars of Barley (*Hordeum vulgare* L.) J. For Agric Sci. 10: 02. http://doi.org/10.52113/mjas04/10.2/31

Razzaghi, F., P.B. Obour, and E. Arthur, 2020. Does biochar improve soil water retention? A systematic review and meta-analysis. Geoderma. 361:114055.

Renuka, N., A. Guldhe, R. Prasanna, P. Singh, and F. Bux, 2018. Microalgae as multi-functional options in modern agriculture: Current trends, prospects and challenges. Biotechnol. Adv., 36:1255–73.

Ró˙zyło, K., M. ´ Swieca, U. Gawlik-Dziki, M. Stefaniuk, and P. Oleszczuk, 2017. The potential of biochar for reducing the negative effects of soil contamination on the phytochemical properties and heavy metal accumulation in wheat grain. Agric. Food Sci. 26: 34–46.

Sanchez, M.E., E. Lindao, D. Margaleff, O. Martinez, and A. Moran, 2009. Pyrolysis of agricultural residues from rape and sunflower: Production and characterization of bio-fuels and biochar soil management. J. Anal. Appl. Pyrolysis 85: 142–144. [CrossRef]

Savci, S., 2012. An Agricultural Pollutant: Chemical Fertilizer. Int. J. Environ. Sci. Develop. 3: 77–80. http://www.ijesd.org/papers/191-X30004.pdf

Seham, M.H., M.E. Naayem and T.E. Sherif, 2022. Integrated biofertilization using yeast with cyanobacteria on growth and productivity of wheat. Beni-Suef Univ. J. Basic. Appl. Sci., 11:112.

Sharifi, P., M. Shorafa, and M.H. Mohammadi, 2019. Comparison of the effect of cow manure, vermicompost, and *azolla* on safflower growth in a saline-sodic soil. Commun Soil Sci. Plant Anal., 50:1417–1424. https://doi.org/10.1080/00103624.2019.1621331

Snedecor, G.W. and W.G. Cochran, 1967. Statistical Methods. Oxford and IBH, New Delhi, India. 381

Song, T., L. Mårtensson, T. Eriksson, W. Zheng, and U. Rasmussen, 2005. Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. FEMS Microbiol. Ecol. 54(1): 131–140. https://doi.org/10.1016/j.femsec.2005.03.008

Subash, K.G., and P.C. Arka, 2020. Cyanobacterial Biofertilizer for sustainable agriculture and environment. Journal of Creative Research Thoughts, 8:2320–882.

Tabak, M., A. Lepiarczyk, B. Filipek-Mazur, and A. Lisowska, 2020. Efficiency of Nitrogen Fertilization of Winter Wheat Depending on Sulfur Fertilization. Agronomy, 10: 1304. https://doi.org/10.3390/agronomy10091304
Univ. of California, Los Angeles, Div. of Agric Sci, 60-61, 150-179.

Wyzi´nska, M., A.K. Berbe´ c and J. Grabi´nski, 2024. Impact of Biochar Dose and Origin on Winter Wheat Grain Quality and Quantity. Agriculture, 14(39): 1-15. https://doi.org/ 10.3390/agriculture14010039.

Yadav, R.K., G. Abraham, Y.V. Singh and P.K. Singh, 2014. Advancements in the utilization of *Azolla Anabaena* system in relation to sustainable agricultural practices. Proc. of the Indian Nat. Sci. Academy, 80(2): 301-316.

Zaki, M.R., A.A.M. Mehesen, E.H. Ashour, and A.H. Afify, 2021. Characterization of soil-indigenous cyanobacterial strains and bioactivity assessment. J. of Agric. Chem. and Biotechnol., Mansoura Univ., 12(11):195-199.