

Article

Evaluating Biochar-Microbe Synergies for Improved Growth, Yield of Maize, and Post-Harvest Soil Characteristics in a Semi-Arid Climate

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Abstract: Arid and semi-arid regions are characterized by high temperature and low rainfall, leading to degraded agricultural soils of alkaline calcareous nature with low organic matter contents. Less availability of indigenous nutrients and efficacy of applied fertilizers are the major issues of crop production in these soils. Biochar application, in combination with plant growth promoting rhizobacteria with the ability to solubilize nutrients, can be an effective strategy for improving soil health and nutrient availability to crops under these conditions. Experiments were planned to evaluate the impact of biochar obtained from different sources in combination with acid-producing, nutrient-solubilizing Bacillus sp. ZM20 on soil biological properties and growth of maize (Zea mays L.) crops under natural conditions. Various biochar treatments, viz. wheat (Triticum aestivum L.) straw biochar, Egyptian acacia (Vachellia nilotica L.) biochar, and farm-yard manure biochar with and without *Bacillus* sp. ZM20, were used along with control. Soil used for pot and field trials was sandy loam in texture with poor water holding capacity and deficient in nutrients. Results of the pot trial showed that fresh and dry biomass, 1000 grain weight, and grain yield was significantly improved by application of biochar of different sources with and without Bacillus sp. ZM20. Application of biochar along with Bacillus sp. ZM20 also improved soil biological properties, i.e., soil organic matter, microbial biomass carbon, ammonium, and nitrate nitrogen. It was also observed that a combined application of biochar with Bacillus sp. ZM20 was more effective than a separate application of biochar. The results of wheat straw biochar along with Bacillus sp. ZM20 were better as compared to farm-yard manure biochar and Egyptian acacia biochar. Maximum increase (25.77%) in grain yield was observed in the treatment where wheat straw biochar (0.2%) was applied in combination with



Bacillus sp. ZM20. In conclusion, combined application of wheat straw biochar (0.2%) inoculated with *Bacillus* sp. ZM20 was the most effective treatment in improving the biological soil properties, plant growth, yield, and quality of maize crop as compared to all other treatments.

Keywords: aridity; Bacillus sp.; biochar; nutrient availability; organic matter; soil health

1. Introduction

The current world population is about 7.6 billion, which is increasing at an exponential rate and will be about 9.8 billion in 2050 and is further expected to rise to 11.2 billion in 2100, as reported by the United Nations [1]. About half of the added population will be concentrated in less developed countries. Due to this reason, there will be a marked decrease in agricultural lands, as most of the productive lands will be used for constructing new housing societies and infrastructure [2]. To feed the world population, utilization of less productive soils, and bringing such soils into the agricultural system by fighting desertification, salinization, and soil pollution is the major challenge for the scientific community [3]. Moreover, increasing per-hectare yield of the major crops along with exploring the unutilized arable lands can be helpful to meet the challenge of food requirements.

Maize, being the staple food of most of the world population, is an important cereal crop [4]. Its total production is even more than rice and wheat crops [5]. Maize has gained its popularity to meet the world food requirements due to higher yield per unit area as compared to other staple crops [6]. Although the per-acre yield of maize is adequate, it is an exhaustive crop that needs more nutrients and that is why it depletes more nutrients from the soil [7]. It has high demand for phosphatic-and zinc-containing fertilizers as compared to other major crops; therefore, nutrient deficiency is experienced more in the maize crop [8].

Biochar can be effective to rehabilitate degraded lands by improving the soil physical properties, nutrient-holding capacity, and soil carbon contents, leading to improvement in soil productivity [9]. It is a carbon-rich compound that is produced through a process known as pyrolysis and has beneficial implications as a potential soil amendment [10]. Use of biochar has gained popularity as a carbon negative material which resists environmental change as it draws carbon from the atmosphere into the soil and persists for hundreds to thousands of years [11]. Recent interest has been developed to use biochar as a soil amendment for improving soil quality through mitigation of soil salinization, soil acidity, and metal contamination, along with improvement in soil productivity [12–15]. Biochar application to soil positively affects the properties of soil, including soil structure, water retention capacity, fertility, and carbon sequestration of degraded soil [16,17]. It also improves soil microbial activity due to presence of micropores in biochar which allow the sorption of dissolved organic matter, thus, helping speed up the soil rehabilitation process [18]. However, the success highly depends upon the types and rates of biochar application, the nature of feedstock, and soil and climate variations. In this regard, utilizing biochar with other soil amendments such as plant growth-promoting rhizobacteria (PGPR) has proved to be a better approach to conserving the environment, resulting in increased efficacy and cost-effectiveness [3,9].

The use of microorganisms with the aim of improving nutrient availability for plants is an important practice and is considered necessary for agriculture these days [19]. The PGPR are the bacteria that inhabit either the rhizosphere, the soil in the immediate vicinity of plant roots, or inside the plant tissues, helping the plants with better growth through some direct and indirect mechanisms [20,21]. There are certain PGPR species which can solubilize insoluble mineral compounds in soil through the production of organic acids along with some other growth-promoting mechanisms [22,23]. Among these, phosphate solubilizing rhizobacteria [24], zinc solubilizing rhizobacteria [25], and potassium solubilizing rhizobacteria [26] are well documented. These nutrient-solubilizing bacterial species also have multiple plant growth-promoting traits such as siderophores production, chitin decomposition, hydrogen cyanide production, ammonia production, etc. [24]. They effectively colonize plant roots, thus helping the improvement of plant growth and nutrient acquisition [27,28]. These bacteria can also induce tolerance against different biotic and abiotic stresses in plants through several indirect mechanisms [27,29]. Moreover, bacterial inoculation improves soil health by fixing atmospheric nitrogen [30,31], production of plant hormones, siderophores and exopolysaccharides [32], and phytoremediation of heavy metals and other organic pollutants [33,34].

The integrated use of biochar and PGPR can reduce the use of chemical fertilizers for crop production in addition to improving soil health through increased soil organic matter contents, enhanced soil aggregation, better microbial activity, and increased soil fertility [35,36]. The improvement in soil health and maize growth has also been reported by the combined use of biochar and PGPR under water-stressed conditions [37]. Work on the use of biochar for increasing soil fertility and remediating the polluted soil has been carried out, but the use of biochar as soil amendment for improving the soil health, growth, and yield of maize in the degraded soils of arid and semi-arid regions has been least explored. It has been hypothesized that the use of biochar and PGPR can help improve barren desert soils to productive farmlands, and release the pressure off the ever-decreasing cultivated areas. Keeping with this view, current study was conducted to investigate the potential of biochar obtained from different sources along with acid-producing, nutrient-solubilizing *Bacillus* sp. for improving soil biological properties, growth, and yield of maize crop in desert regions.

2. Materials and Methods

2.1. Biochar Preparation and Characterization

Biochar was prepared from Egyptian acacia (*Vachellia nilotica* L.) stem, wheat straw, and dairy manure pyrolyzed at 450 °C following the method of Naeem et al. [38]. The dried branches of Egyptian acacia were chopped in small pieces of 2–3 inches and further dried at 105 °C for one hour. The oven-dried biomass was pyrolyzed at 450 °C. Finally, the prepared biochar was crushed into smaller particles for even distribution in soil. Before charring, the dairy manure was air-dried and sieved (≤ 2 mm), then pyrolyzed in a muffle furnace at 450 °C. Similarly, the air-dried, chopped wheat straw was also pyrolyzed at 450 °C. The weight of biomass used for each type of biochar was recorded prior and after pyrolysis. After cooling, the biochar was passed through a 250-µm sieve and stored in a refrigerator (at 4 °C) before use.

The biochar produced from different sources was analyzed for chemical characteristics (Table 1). Prepared biochar was analyzed for its turnover rate made from the pyrolysis of feedstock. The biochar production rate was calculated by using the total weight of raw material used to prepare that biochar. Biochar yield was estimated by following the method of Al-Wabel et al. [39]. The pH and electrical conductivity (EC) of the biochar was measured using a 1:20 solid/solution ratio after shaking for ninety minutes in deionized water in a mechanical shaker. The carbon contents of biochar were assessed using the loss-on-ignition approach [40,41]. Total nitrogen (N) contents were measured using Kjeldahl distillation equipment [42].

Table 1. Rate and	physicoch	nemical p	roperties of	biochar; val	lues are t	he mean of	f three replications \pm	SE.

Parameters	Egyptian Acacia Biochar	Farmyard Manure Biochar	Wheat Straw Biochar
Turnover rate (%)	28.45 ± 1.27	23.31 ± 0.94	38.76 ± 1.78
pН	9.3 ± 0.25	8.67 ± 0.12	8.43 ± 0.21
EC ($dS cm^{-1}$)	1.85 ± 0.03	2.1 ± 0.02	1.9 ± 0.03
Bulk density (g cm $^{-3}$)	0.36 ± 0.01	0.43 ± 0.01	0.46 ± 0.03
Nitrogen (%)	0.31 ± 0.02	0.28 ± 0.01	0.47 ± 0.02
Carbon (%)	68.45 ± 3.24	44.21 ± 2.16	52.67 ± 1.89

2.2. Collection of Rhizobacterial Strains

Pre-selected and characterized rhizobacterial strain *Bacillus* sp. *ZM20*, accession number KX086260, with strong ability to produce organic acids [23] under zinc deficient conditions was collected from the Soil Microbiology and Biotechnology Laboratory, Department of Soil Science, the Islamia University of Bahawalpur.

2.3. Soil Sampling and Analysis

A bulk soil sample (0–15 cm) was taken from the experimental field and the soil that was used for pot trial. The soil samples were air-dried and sieved through 2-mm sieve followed by analysis for basic soil characteristics (Table 2) as per standard protocols. The pH, EC, and organic matter were measured according to the method of Nelson and Sommers [43]. The available N was analyzed by the Kjeldhal method [42], while for available phosphorus (P), Olsen's method [44] was used. The extractable potassium (K) was measured using a flame photometer (Model; Model BWB-XP, BWB Technologies, UK). The saturation percentage referring to the field capacity of soil was estimated by oven-drying the soil sample at 105 °C to a constant weight, followed by calculations according to the method as described by Sarfraz et al. [45]. All the chemicals were of analytical grade (Sigma-Aldrich, Unichem, Merck) supplied by Wahid Scientific Store, Lahore, Pakistan.

Table 2. Characteristics of the soils; values are the mean of three replications \pm SE.

Parameter	Pot Trial	Field Trial
$EC_e (dS m^{-1})$	1.6 ± 0.01	1.8 ± 0.01
pH	8.1 ± 0.04	7.9 ± 0.02
Organic matter (%)	0.39 ± 0.02	0.47 ± 0.02
Available N (%)	0.024 ± 0.001	0.059 ± 0.003
Available P (mg kg ^{-1})	3.7 ± 0.01	4.5 ± 0.03
Extractable K (mg kg ^{-1})	53 ± 1.68	77 ± 3.21
Saturation percentage (%)	33 ± 0.76	36 ± 0.71
Water-holding capacity (Inches ft ⁻¹)	1.27 ± 0.05	1.29 ± 0.03
Textural class	Sandy loam	Sandy loam

2.4. Pot Trial

A pot trial was conducted in the wire house to evaluate the impact of biochar obtained from different sources in combination with acid-producing, nutrient-solubilizing Bacillus sp. ZM20 on soil biological properties, and the growth and yield of maize crops (Pioneer-30Y80) under natural environmental conditions in the February-March sowing season. The experiment was conducted in a wire house with natural growth conditions, protecting the experimental units from animals and birds with wire only. Various biochar treatments viz. Egyptian acacia biochar (0.1%), Egyptian acacia biochar (0.2%), farmyard manure (FYM) biochar (0.1%), FYM biochar (0.2%), wheat straw biochar (0.1%), and wheat straw biochar (0.2%) with and without Bacillus sp. ZM20 were used along with controls. Soil (8 kg per pot⁻¹) used to fill the pots (height 12", diameter 12") was sandy loam in texture with poor water-holding capacity (1.27 inches ft^{-1}) and deficient in nutrients (Table 2), as analyzed by following the standard protocols as defined by Ryan et al. [46]. The pots were arranged in the wire house following a completely randomized design (CRD) in factorial arrangement with three replications. Maize seeds were inoculated with a slurry of Bacillus sp. ZM20 prepared by mixing the inoculum, sugar solution, and peat in the ratio (04:01:05). The inoculated seeds were used to sow in one set of treatments while in the other set un-inoculated maize seeds were sown. The recommended doses of P and K at the rate of 90 kg ha⁻¹ and 60 kg ha⁻¹ while half of the recommended dose of N (120 kg ha^{-1}) were applied as basal doses in the form of diammonium phosphate, sulfate of potash, and urea. The remaining dose of N was applied in two splits. Good quality tap water meeting the irrigation quality criteria [47] was used to irrigate pots, and all other agronomic practices were carried

out according to requirements. Growth and yield parameters were recorded at the time of harvesting and grain samples were collected to analyze for N, P, and K.

2.5. Field Trial

A field trial was conducted in the February–March (Spring 2019) sowing season to verify the results of the pot trial and further recommendation to farming community. The same treatment plan was followed as observed in the pot trial. The field trial was conducted in the field area of the Department of Soil Science, The Islamia University of Bahawalpur, Pakistan. The soil of the experimental field was sandy loam in texture with poor water-holding capacity and deficient in nutrients (Table 2). The randomized complete block design was used for the field trial with a factorial arrangement and three replications. The size of the plots was $22' \times 16'$ with a row-to-row distance of 2.5'. Maize seeds were inoculated before sowing by following the same procedure as described above in the pot trial. The recommended doses of P and K at the rate of 90 and 60 kg ha⁻¹ while half of the recommended dose of N (120 kg ha⁻¹) were applied as basal doses in the form of diammonium phosphate, sulfate of potash, and urea. The remaining dose of N was applied in two splits. Canal water was used for irrigation purposes and all other agronomic practices were carried out according to requirements. Growth and yield parameters were recorded at the time of harvesting and grain samples were collected to analyze for N, P, and K.

2.6. Nutrient Analyses in Grains

Grains were digested according to the protocol as described by Wolf [48]. The P in grain samples was analyzed using a UV-visible spectrophotometer (Agilent Carry 60, USA), while K in grains was determined on a flame photometer (Model; Model BWB-XP, BWB Technologies, Newbury, UK) by following the standard methods [46]. For the analysis of N in grains, an automatic digestion unit (DK 6), semi-automatic distillation unit (UDK 126) of Kjeldahl apparatus (VELP Sci., Italy) was used, followed by standard titration as described in the Kjeldahl method [49]. All the chemicals were of analytical grade (Sigma-Aldrich, Unichem, Merck) supplied by Wahid Scientific Store, Lahore, Pakistan.

2.7. Post-Harvest Soil Sample Collection and Analysis

The post-harvest soils samples were collected from the pot (harvested in July) and field trials (harvested in July), and analyzed for organic matter, microbial biomass carbon (MBC), ammonium N, and nitrate N under pot and field conditions. The composite soil sampling method was used, and the samples were air-dried and sieved through a 2-mm sieve before analysis. The prepared soil samples were stored in a refrigerator at 4 °C and analyzed within seven days. The organic matter contents were measured according to the method of Nelson and Sommers [43]. For the analysis of microbial biomass carbon (MBC), chloroform fumigation and extraction methods were used [50,51]. For the analyses of ammoniacal N and nitrate N in soil, the methods of Kamphake et al. [52] and Sims and Jackson [53], respectively, were used. All the chemicals were of analytical grade (Sigma-Aldrich, Unichem, Merck) supplied by Wahid Scientific Store, Lahore, Pakistan. Replicated measurements were always performed to ensure the accuracy of the data.

2.8. Statistical Analysis

All data reported here are means of three replicates which were analyzed using one-way analysis of variance (ANOVA) in Statistix 8.1. The mean values were compared through a least significant difference (LSD) test as described by Steel et al. [54].

3. Results

3.1. Pot Trial

Integrated use of biochar and *Bacillus* sp. ZM20 improved soil properties in the pot trial. Results (Figure 1A) showed that FYM (Farm yard manure) biochar treatments increased the organic matter in the pot trial. The application of biochar without inoculation increased the soil organic matter contents, but the results of Egyptian acacia biochar at both levels were non-significant when compared with the control under un-inoculated and inoculated sets of treatments. Under inoculated treatments, the maximum organic matter (0.449%) was observed in the treatment where wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20; this treatment was, however, non-significant with FYM biochar application (0.2%), and wheat straw biochar application (0.1%) under inoculated treatments. Combined inoculation of biochar and *Bacillus* sp. ZM20 showed better results than separate application of biochar in all treatments.

The application of biochar from different sources significantly improved the MBC in the soil (Figure 1B). Maximum improvement in MBC under an un-inoculated set of treatments was observed by the application of wheat straw biochar (0.2%), which was statistically at par with the application of wheat straw biochar (0.1%) and FYM biochar (0.2%). These treatments, however, were significantly better than all other treatments under un-inoculated conditions. The application of biochar from all sources in the presence of *Bacillus* sp. ZM20 was significantly better than separate use, except for Egyptian acacia biochar (0.1%), where the increase was non-significant with that of the respective un-inoculated treatment. Maximum MBC (342 mg kg⁻¹) was observed in the treatment where wheat straw biochar was applied (0.2%), and it was statistically similar with that of the wheat straw biochar (0.1%) treatment.

The sole and combined application of biochar and inoculated with *Bacillus* sp. ZM20 to improve the ammonium N and nitrate N in the pot trial was observed (Figure 1C,D). The application of biochar separately, and in combination with *Bacillus* sp. ZM20, significantly enhanced the ammonium N and nitrate N, except for Egyptian acacia biochar treatments, which gave non-significant improvement in both cases as compared to the control. A maximum increase in ammonium N and nitrate N was recorded due to the combined application of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 as compared to the inoculated control. Overall, inoculated treatments showed better results regarding ammonium N and nitrate N than un-inoculated treatments.

The results of the impact of the integrated use of biochar and *Bacillus* sp. ZM20 on plant height (Figure 2A) revealed that the separate as well as combined use of biochar with *Bacillus* sp. ZM20 significantly improved plant height in the pot trial, except for Egyptian acacia biochar, at both levels, which was statistically non-significant compared to the control. In the inoculated treatment, the combined use of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 was carried out and showed the maximum plant height. In the case of root length, the results of separate applications of wheat straw biochar at both levels and FYM biochar (0.2%) were significantly better than those of the control; however, other treatments gave non-significant improvement in root length when compared with the control. The combined use of biochar and *Bacillus* sp. ZM20 was better than the separate use of biochar in improving the root length, but the results were non-significant with un-inoculated treatments in all cases. Maximum improvement in root length as compared to control was observed with the combined use of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20; however, it was statistically non-significant with the treatments of wheat straw biochar (0.1%) and FYM biochar (0.2%) in combination with Bacillus sp. ZM20 (Figure 2B). Application of biochar significantly improved the shoot fresh and dry biomass separately and in combination with Bacillus sp. ZM20 as compared to respective controls. Maximum improvement in shoot fresh biomass and shoot dry biomass were observed with the application of wheat straw biochar in combination with *Bacillus* sp. ZM20 (Figure 2C,D). The improvement due to the inoculation of *Bacillus* sp. ZM20 in both the parameters over the respective un-inoculated treatment was non-significant in all the cases.

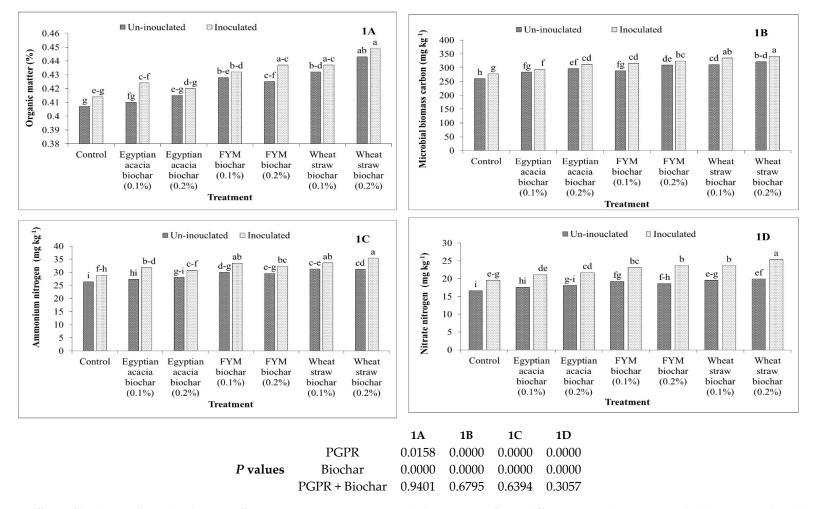


Figure 1. Effects of biochar with and without *Bacillus* sp. ZM20 on organic matter (**A**): Least significant difference (LSD) 0.0156, microbial biomass carbon (**B**): LSD 14.290, ammonium N (**C**): LSD 2.096, and nitrate N (**D**): LSD 1.6386 under pot trial; (n = 3); bars sharing same letters are statistically not different from each other at $p \le 0.05$.

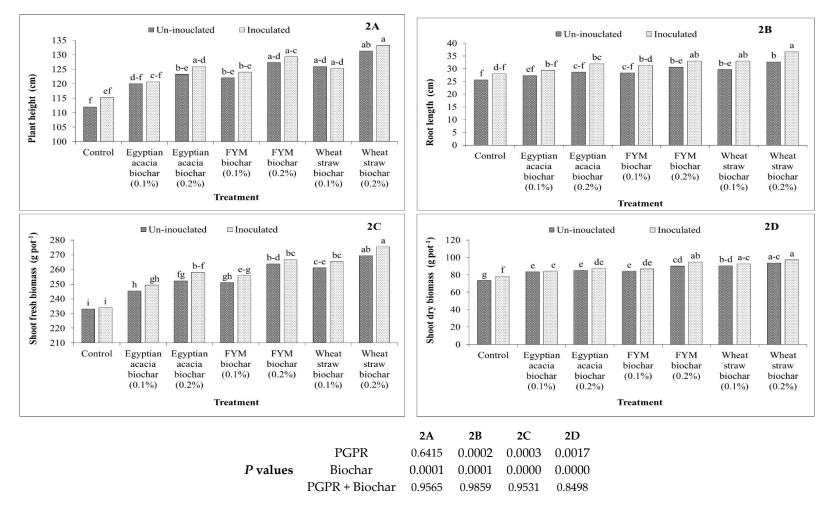


Figure 2. Effects of biochar with and without *Bacillus* sp. ZM20 on plant height (**A**): LSD 8.7945, root length (**B**): LSD 3.6996, shoot fresh biomass (**C**): LSD 6.8333 and shoot dry biomass (**D**): 4.6543 of maize under pot trial; (n = 3); bars sharing same letters are statistically not different from each other at $p \le 0.05$.

Results of the effects of separate as well as combined applications of biochar inoculated with *Bacillus* sp. ZM20 significantly improved the root fresh biomass in the pot trial (Table 3). A maximum increase (29.63%) in root fresh biomass was observed due to the combined use of wheat straw biochar (0.2%) with *Bacillus* sp. ZM20. The results of the separate use of biochar (all treatments) without inoculum were, however, non-significant with the control in most of the cases, except wheat straw biochar (0.2%), which gave significantly better results than the control. The results of the improvement in root dry biomass were non-significant with the control due to the biochar application with and without in most of the cases, except for FYM biochar (0.2%) and wheat straw biochar (0.2%) in both sets of treatments, i.e., inoculated and un-inoculated. A maximum increase (23.36%) in root dry biomass was observed due to the combined use of wheat straw biochar (0.2%) with *Bacillus* sp. ZM20 (Table 3).

— · · ·		Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
1	reatment	Root Fresh Biomass (g pot ⁻¹)		Root Dry Biomass (g pot ⁻¹	
	Control	28.00 ^f	36.00 ^с -е	14.33 ^d	15.67 ^{c,d}
Egyptian acacia biochar (0.1%)		29.33 ^f	38.33 ^{b–d}	15.33 ^{c,d}	17.33 ^{a-d}
Egyptian ac	cacia biochar (0.2%)	31.67 ^{e,f}	42.00 ^{a,b}	16.33 ^{a-d}	17.67 ^{a-c}
FYM b	piochar (0.1%)	30.67 ^{e,f}	41.33 ^{a-c}	16.00 ^{b-d}	17.67 ^{a-c}
FYM ł	piochar (0.2%)	33.00 ^{d-f}	43.67 ^{a,b}	17.67 ^{a-c}	19.00 ^{a,b}
Wheat stra	aw biochar (0.1%)	31.00 ^{e,f}	43.00 ^{a,b}	17.33 ^a -d	18.33 ^{a-c}
	aw biochar (0.2%)	35.67 ^{d,e}	46.67 ^a	18.00 ^{a-c}	19.33 ^a
LSI	$D \ (p \le 0.05)$	5.56	636	3.1110	
	PGPR	0.0000 0.0020		0.0192 0.0314	
p value	Biochar				
	PGPR + Biochar	0.9586		0.9996	
		100 Grain Weight (g)		Grain Yield (g pot⁻¹)	
	Control	18.67 ^c	19.67 ^{b,c}	103.3 ^f	106.3 e,f
Egyptian ac	cacia biochar (0.1%)	20.33 ^{b,c}	21.33 ^{a-c}	114.3 ^{d,e}	116.0 ^d
	cacia biochar (0.2%)	21.00 ^{a-c}	21.67 ^{a-c}	116.3 ^d	121.0 ^{b-d}
	piochar (0.1%)	21.00 ^{a-c}	21.67 ^{a-c}	116.0 ^d	121.7 ^{b-d}
	piochar (0.2%)	21.67 ^{a-c}	23.67 ^{a,b}	122.0 ^{b-d}	127.0 ^{a-c}
	aw biochar (0.1%)	21.33 ^{a-c}	23.00 ^{a,b}	120.0 ^{c,d}	126.0 ^{a-c}
Wheat straw biochar (0.2%)		22.33 ^{a-c}	24.67 ^a	129.0 ^{a,b}	133.7 ^a
LSD ($p \le 0.05$)		4.3030		8.9777	
	PGPR	0.10)39	0.0134	
p value	Biochar	0.14	421	0.0000	
	PGPR + Biochar	0.99	956	0.97	55
					. = 0 (1 1 (

Table 3. Effects of biochar with and without *Bacillus* sp. ZM20 on root fresh biomass, root dry biomass, 100 grain weight, and grain yield of maize in pot trial.

Values sharing same letter(s) within a parameter are statistically non-significant with each other at 5% level of probability; values are the mean of three replications \pm SE. Lower case words show difference in treatment means.

Results regarding the effects of separate and combined applications of biochar with *Bacillus* sp. ZM20 on 100-grain weight are presented in (Table 3). In most of the cases, regarding the use of all types of biochar, individually as well as in combination with *Bacillus* sp. ZM20, the results were nonsignificant with the control, except for the wheat straw biochar (0.2%) application in combination with *Bacillus* sp. ZM20. Statistical analyses showed that all treatments of the separate and combined uses of biochar showed significantly better results than the control in improving the grain yield of maize in the pot trial. A maximum increase (25.77%) in grain yield was observed in the treatment where wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20 (Table 3). Data (Table 4) showed that the use of all types of biochar, individually as well as in combination with *Bacillus* sp. ZM20, gave non-significant results in improving the stover yield in all the cases when compared to the control.

		Un-Inoculated	Inoculated	Un-Inoculated	Inoculate	
Treatment		Stover Yield	Stover Yield (g pot ⁻¹)		Nitrogen Conc. in Grains (%	
	Control	20.33 ^c	23.00 ^{a-c}	2.17 ^g	2.19 ^{f,g}	
Egyptian acacia biochar (0.1%)		22.00 ^{b,c}	24.33 ^{a-c}	2.21 ^{f,g}	2.21 ^{e–g}	
Egyptian ac	acia biochar (0.2%)	23.00 ^{a-c}	26.33 ^{a,b}	2.24 ^{c–g}	2.25 ^{c-f}	
FYM b	piochar (0.1%)	26.00 ^{a-c}	25.67 ^{a-c}	2.22 ^{d–g}	2.25 ^{c-f}	
FYM b	biochar (0.2%)	25.00 ^{a-c}	27.00 ^{a,b}	2.28 ^{a–e}	2.30 ^{a-c}	
Wheat stra	aw biochar (0.1%)	24.33 ^{a-c}	26.33 ^{a,b}	2.28 ^{b-е}	2.29 ^a -d	
Wheat stra	aw biochar (0.2%)	25.67 ^{a-c}	28.00 ^a	2.32 ^{a,b}	2.35 ^a	
LSI	$D \ (p \le 0.05)$	5.97	63	0.0705		
PGPR		0.07	37	0.1857 0.0000		
<i>p</i> value	Biochar	0.2071				
	PGPR + Biochar	0.98	62	0.9971		
		Phosphorus Conc. in Grains (%)		Potassium Conc. in Grains (
	Control	0.373 ^e	0.390 ^с -е	2.57 ^h	2.60 g,h	
Egyptian ac	acia biochar (0.1%)	0.380 ^{d,e}	0.403 ^{b-d}	2.61 ^{g,h}	2.63 ^{f,g}	
Egyptian ac	acia biochar (0.2%)	0.390 ^{с-е}	0.410 ^{a-c}	2.64 ^{e-g}	2.69 ^{с-е}	
FYM b	piochar (0.1%)	0.387 ^{с–е}	0.407 ^{a-c}	2.62 ^{g,h}	2.67 ^{d-f}	
FYM b	piochar (0.2%)	0.397 ^{b-е}	0.417 ^{a,b}	2.70 ^{b-d}	2.74 ^{a,b}	
Wheat stra	aw biochar (0.1%)	0.400 ^{b-d}	0.410 ^{a–c}	2.67 ^{d-f}	2.72 ^{b,c}	
Wheat straw biochar (0.2%)		0.407 ^{a–c}	0.430 ^a	2.74 ^{a,b}	2.77 ^a	
LSI	$D \ (p \le 0.05)$	0.02	0.0250		0.0499	
	PGPR	0.00	00	0.0003		
<i>p</i> value	Biochar	0.00	00	0.0000		
	PGPR + Biochar	0.0000		0.9546		

Table 4. Effects of biochar with and without *Bacillus* sp. ZM20 on stover yield, and nitrogen, phosphorus and potassium concentration in grains of maize in pot trial.

Values sharing same letter(s) within a parameter are statistically non-significant with each other at 5% level of probability; values are the mean of three replications \pm SE. Lower case words show difference in treatment means.

Results (Table 4) showed that N concentration in grains of maize was significantly improved due to the separate as well as combined application of all types of biochar and *Bacillus* sp. ZM20. The results of Egyptian acacia biochar (both levels) and FYM biochar (0.1%) gave non-significant results in both sets of treatments. A maximum increase (7.30%) in N concentration in maize grains was observed due to the combined use of wheat straw biochar (0.2%) with *Bacillus* sp. ZM20. The results of the impact of biochar (all treatments), with and without inoculum, on P concentration in maize grains were non-significant with the control in most of the cases, except for the wheat straw biochar (at both levels) application in un-inoculated set of treatments, and the FYM biochar (0.1%) and wheat straw biochar (0.2%) application in combination with *Bacillus* sp. ZM20. Similar results were observed in case of K concentration in maize grains, where the maximum improvement (6.5%) over control was observed due to combined use of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 (Table 4).

3.2. Field Trial

Results (Figure 3) showed that all treatments significantly increased the organic matter and MBC under field conditions, except the application of Egyptian acacia biochar (0.1%), which gave non-significant improvement in organic matter when compared with the control under the un-inoculated treatment (Figure 3A). Under inoculated treatments, maximum improvement (5.78%) in organic matter contents over the control was observed in treatment where wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20; this treatment was, however, non-significant with the use of wheat straw biochar (0.1%) under inoculated treatments. The combined inoculation of biochar and *Bacillus* sp. ZM20 showed better results than the separate application of biochar in all treatments.

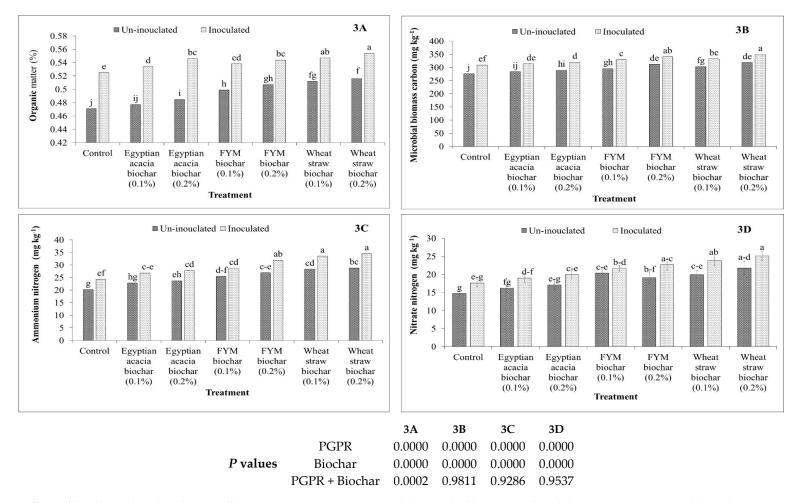


Figure 3. Effects of biochar with and without *Bacillus* sp. ZM20 on organic matter (**A**), microbial biomass carbon (**B**), ammonium nitrogen (**C**), and nitrate nitrogen (**D**) under field trial; (n = 3); bars sharing same letters are statistically not different from each other at $p \le 0.05$.

The application of biochar from different sources also significantly improved the MBC under field conditions in a semi-arid climate (Figure 3B). A maximum improvement (23.39%) in MBC under an un-inoculated set of treatments was observed by the application of wheat straw biochar (0.2%), which was statistically at par with application of FYM biochar (0.2%). These treatments, however, were significantly better than all other treatments under un-inoculated conditions. The application of biochar from all sources in the presence of *Bacillus* sp. ZM20 was significantly better than the separate use, except for Egyptian acacia biochar (0.1%), where the increase was non-significant with that of respective un-inoculated treatment. A maximum MBC (22.89%) was observed in the treatment where the wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20, and it was statistically similar with that of the FYM biochar (0.2%) treatment.

The application of biochar separately and in combination with *Bacillus* sp. ZM20 significantly enhanced the ammonium N and nitrate N, except for the Egyptian acacia biochar treatments (both levels), which gave non-significant improvement in both cases as compared to the control (Figure 3C,D). A maximum increase in ammonium N (22.61%) and nitrate N (29.59%) as compared to the inoculated control was recorded due to the combined application of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20. Overall, inoculated treatments showed better results regarding ammonium N and nitrate N than un-inoculated treatments.

The results of the impact of integrated use of biochar and *Bacillus* sp. ZM20 on plant height (Table 5) under field conditions revealed that the separate as well as combined use of biochar with *Bacillus* sp. ZM20 improved plant height, but that this improvement was statistically non-significant with the control in most of the cases. In the inoculated treatment, the combined use of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 was carried out and showed the maximum improvement (12.7%) in plant height. The application of biochar separately and in combination with *Bacillus* sp. ZM20 significantly improved the shoot fresh and dry biomass as compared to respective controls. Maximum improvement in shoot fresh biomass (16.6%) and shoot dry biomass (20.75%) was observed with the application of wheat straw biochar (0.2%) in combination with *Bacillus* sp. ZM20 (Table 5). Results regarding the effects of separate and combined applications of biochar with *Bacillus* sp. ZM20 on 1000-grain weight and grain yield showed a significant improvement in most of the cases, except for Egyptian acacia biochar (0.1%), which gave non-significant improvement when compared with the control. A maximum increase (21.9%) in grain yield was observed in the treatment where wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20 (Table 6).

Treatment –		Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
		Plant Height (cm)		Shoot Fresh Biomass (g pot ⁻¹)	
Control		136.3 ^f	139.3 ^{e,f}	243.6 ^g	245.3 ^g
Egyptian acacia	a biochar (0.1%)	141.0 ^{d-f}	143.3 ^{c-f}	255.0 ^f	260.3 ^{e,f}
Egyptian acacia biochar (0.2%)		144.0 ^{e,f}	150.3 ^{a-c}	262.3 ^e	266.0 ^{d,e}
FYM biochar (0.1%)		143.7 ^{c–f}	146.7 ^{b-е}	261.7 ^{e,f}	265.7 ^{d,e}
FYM biochar (0.2%)		147.0 ^b -e	154.3 ^{a,b}	273.3 ^{b,c}	278.0 ^{b,c}
Wheat straw b	piochar (0.1%)	146.3 ^{b-е}	149.7 ^{a–d}	271.0 ^{c,d}	276.3 ^{b,c}
Wheat straw biochar (0.2%)		153.0 ^{a,b}	157.0 ^a	279.0 ^{a,b}	286.0 ^a
LSD ($p \le 0.05$)		8.9702		7.2668	
PGPR		0.0174		0.0022	
p value	Biochar	0.000)3	0.0000	
	PGPR + Biochar	0.977	74	0.9678	

Table 5. Effects of biochar with and without *Bacillus* sp. ZM20 on plant height, shoot fresh biomass, shoot dry biomass, and 1000-grain weight of maize in field trial.

Treatment –		Un-Inoculated	Inoculated	Un-Inoculated	Inoculated
		Plant Height (cm)		Shoot Fresh Biomass (g pot ⁻¹)	
		Shoot Dry Bion	nass (g pot ⁻¹)	1000-Grain	Weight (g)
Con	itrol	79.67 ^g	80.33 ^{f,g}	222.33 ^g	232.67 ^{e,f}
Egyptian acacia	u biochar (0.1%)	84.33 ^{d-f}	83.67 ^{e–g}	229.00 ^{f,g}	237.67 ^{d,e}
Egyptian acacia		85.00 ^{d,e}	87.67 ^{с–е}	236.00 ^{b-f}	243.33 ^{b-d}
FYM biochar (0.1%)		84.33 ^{d-f}	87.00 ^{с–е}	234.00 ^{e,f}	242.67 ^{b-d}
FYM biocl	har (0.2%)	88.67 ^{c,d}	95.00 ^{a,b}	243.00 ^{b-d}	248.33 ^{a,b}
Wheat straw b	piochar (0.1%)	88.67 ^{c,d}	93.67 ^{a,b}	239.33 ^{с-е}	246.33 ^{a-c}
Wheat straw b	biochar (0.2%)	91.33 ^{b,c}	97.00 ^a	247.67 ^{a,b}	251.33 ^a
LSD (p	LSD ($p \le 0.05$)		4.5478		190
PGPR		0.0008		0.0000	
p value	Biochar	0.00	00	0.0000	
PGPR + Biochar		0.2527		0.8961	

Table 5. Cont.

Values sharing same letter(s) with in a parameter are statistically non-significant with each other at 5% level of probability; values are the mean of three replications \pm SE.

Table 6. Effects of biochar with and without *Bacillus* sp. ZM20 on grain yield, and nitrogen, phosphorus, and potassium concentrations in grain of maize in field trial.

Treatment		Un-Inoculated	Inoculated	Un-Inoculated	Inoculated	
iicut			Grain Yield (t ha ⁻¹)		Nitrogen Conc. in Grains (%)	
Control		7.40 ^f	7.90 ^{d,e}	2.20 ⁱ	2.24 ^{g–i}	
Egyptian acacia biochar (0.1%)		7.57 ^{e,f}	8.23 ^{c,d}	2.23 ^{h,i}	2.31 ^{e,f}	
Egyptian acacia	a biochar (0.2%)	8.07 ^d	8.60 ^c	2.28 ^{f,g}	2.34 ^{b–e}	
FYM bioc	har (0.1%)	7.93 ^{d,e}	8.57 ^c	2.27 ^{f-h}	2.34 ^{b–e}	
FYM bioc	har (0.2%)	8.33 ^{c,d}	9.10 ^b	2.33 ^{с–е}	2.38 ^{a,b}	
Wheat straw l	biochar (0.1%)	8.27 ^{c,d}	9.10 ^b	2.31 ^{d-f}	2.35 ^{b-d}	
Wheat straw	biochar (0.2%)	8.63 ^c	9.63 ^a	2.37 ^{a–c}	2.41 ^a	
LSD (p	≤ 0.05)	0.448	35	0.04	34	
	PGPR	0.0000		0.0000		
p value	Biochar	0.0000		0.0000		
	PGPR + Biochar	0.6905		0.7951		
		Phosphorus Conc. in Grain (%)		Potassium Conc. in Grain (%)		
Cor	ntrol	0.393 g	0.403 ^{e-g}	2.64 ⁱ	2.69 ^{g–i}	
Egyptian acacia	a biochar (0.1%)	0.400 ^{fg}	0.417 ^{c-f}	2.68 ^{h,i}	2.74 ^{d–g}	
	a biochar (0.2%)	0.410 ^d -g	0.423 ^{b-d}	2.71 ^{f-h}	2.80 ^{b-d}	
071	har (0.1%)	0.407 ^d -g	0.420 ^{b-e}	2.71 ^{f-h}	2.78 ^{с–е}	
FYM bioc	har (0.2%)	0.420 ^{b-e}	0.437 ^{a,b}	2.76 ^{c-f}	2.85 ^{a,b}	
Wheat straw l	biochar (0.1%)	0.413 ^{c–f}	0.430 ^{a-c}	2.74 ^{e–h}	2.82 ^{b,c}	
Wheat straw l	biochar (0.2%)	0.430 ^{a-c}	0.447 ^a	2.82 ^{b,c}	2.89 ^a	
LSD ($p \le 0.05$)		0.0197		0.0622		
	PGPR	0.000	0.0004		00	
p value	Biochar	0.000)1	0.0000		
PGPR + Biochar		0.9981		0.93	0.9359	

Values sharing same letter(s) with in a parameter are statistically non-significant with each other at 5% level of probability; values are the mean of three replications \pm SE. Lower case words show difference in treatment means.

The results in Table 6 show that the N concentration in grains of maize was significantly improved due to the separate as well as combined application of all types of biochar and *Bacillus* sp. ZM20, except for Egyptian acacia biochar (0.1%), which gave non-significant improvement in the

N concentration in grains under an un-inoculated set of treatments. A maximum increase (7.6%) in the N concentration in maize grains was observed due to the combined use of wheat straw biochar (0.2%) with *Bacillus* sp. ZM20. The results of the impact of biochar (all treatments), with and without inoculum, on P and K concentrations in maize grains were significantly better than the control in most of the cases. A maximum improvement in both the parameters over the control was observed due to combined use of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 (Table 6).

4. Discussion

The present study was conducted in arid and semi-arid regions on sandy loam soil characterized by low rainfall and high temperature, associated with low organic matter content. Due to low organic matter, biochar in combination with bacterial inoculation can have the ability to improve the soil health and crop yield under such a scenario. The application of biochar can be effective at rehabilitating degraded lands by improving the soil structure, nutrient- and water-holding capacity, and soil carbon contents, leading to improvement in soil productivity [9,55]. A carbon-rich compound called charcoal is produced through a process known as pyrolysis and has beneficial implications such as soil amendment for improving soil health and crop yield [10,56]. The physicochemical properties of biochar are crucial in determining its functionality and impact on plant growth and soil health [57]. It was observed that biochar contains a high carbon-to-nitrogen ratio (Table 1), which makes it stable against decomposition. The carbon contents of Egyptian acacia biochar were higher compared to the other two sources, but wheat straw biochar had a higher turnover rate as compared to the other sources. In previous studies, scientists have also reported that biochar is rich in carbon contents along with other nutrients like C, N, and S [58,59] which have shown promising results in improving crop growth and yield characteristics similar to the findings of the current study.

In this study, the application of biochar improved the soil biological properties (soil organic matter contents, MBC), along with improvement in ammonium and nitrate N contents in soil (Figures 1 and 3). The increase in the levels of biochar increased the content of organic matter and MBC in studied soil. The presence of high carbon and other nutrients might have helped in the improvement of soil fertility as reported by Oni et al. [17], suggesting that biochar application positively affects the soil structure, water retention capacity, fertility, and soil carbon sequestration, leading to improvement in crop growth and productivity. Similarly, biochar application increased the ratio of below-ground biomass to above-ground biomass due to an increase in water-holding capacity, as reported previously [60], and a reduction in soil strength [61]. The integration of biochar and PGPR is a win-win strategy as biochar provides a niche for microbes due to its microporous structure, which in turn increases microbial activity and hence the sorption of dissolved organic matter [18]. The increase in carbon and organic matter contents in the present study due to the addition of different biochar types is in good agreement with Shenbagavalli and Mahimairaja [62]. The integrated use of biochar and PGPR can improve soil health through increasing soil organic matter contents, enhancing soil aggregation, promoting better microbial activity, and increasing soil fertility [35,36]. In our study, the integrated use of biochar inoculated with *Bacillus* sp. ZM20 was significantly better in improving soil organic matter and MBC, which might have supported crop growth. Our results are in good agreement with previous reports by Ullah et al. [37], in which they reported the increased growth, physiology, and production of crops under the combined application of biochar and PGPR. This increase in growth and yield of wheat in present study might be attributed to enhanced supply of nutrients that are scarcely available in the soil including nitrogen, phosphorus, zinc, and iron. This may also be due to the positive effects of applied PGPR which are well recognized candidates equipped with plenty of mechanisms, i.e., the production of siderophores that helps in iron acquisition, synthesis of plant growth regulators, and exopolysaccharides [15,32,36].

Biochar application as a soil amendment increases the growth parameters of plants (plant root and shoot growth), and their nutrient uptake by improving the water status of plants and water-use efficiency [63,64], thus leading to improved yield of crop plants. In the present study, the application

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of biochar from different sources improved the maize root and shoot growth and nutrient uptake, along with the yield and yield contributing factors (Tables 3 and 5). A maximum increase (25.77%) in grain yield was observed in the treatment where wheat straw biochar (0.2%) was applied in combination with *Bacillus* sp. ZM20. This might be due to the enhanced water-holding capacity of the soil [65] that resulted in enhanced nutrient availability [65], thus improving the growth of crop plants under the applied biochar [38] and PGPR [28]. As stated by Hussain et al. [31], the combined use of PGPR and biochar at the rate of 0.5 tons/ha have shown enhanced water-holding capacity of the soil, and hence the growth and yield of maize (*Zea mays* L.). Recently, Shen et al. [66] reported that biochar application improved plant growth; however, willow woodchip biochar was significantly better than pine-based biochar in improving plant growth and nutrient uptake of *Lotus pedunculatus*. The improved growth and yield characteristics of maize under the applied biochar are in good agreement with previous studies [14,67,68]. The enhanced soil characteristics and crop growth responses in the present study under the application of biochar and PGPR might be attributed to the differences in soil characteristics and the alkaline nature of biochar in the soil studied here.

The PGPR inhabits either the rhizosphere, the soil in the immediate vicinity of plant roots, or inside the plant tissues, and helps the plants exhibit better growth through some direct and indirect mechanisms [20,21]. The phosphorus-solubilizing bacteria (PSB), zinc-solubilizing bacteria (ZSB), and potassium-solubilizing bacteria (KSB) can increase plant nutrient availability along multiple plant growth promoting traits, such as siderophores production, chitin decomposition, hydrogen cyanide production, and ammonia production [25,26,69]. In the current study, the combined use of biochar and Bacillus sp. ZM20 improved maize growth, the uptake of N, P, and K, and the yield, which might be due to solubilization of nutrients through acid production, along with other growth-promoting characteristics such as siderophore production, exopolysaccharides production, and HCN production exhibited by this strain, as reported in previous studies [14,25]. The application of biochar improves the quality of soil and makes it conducive for better microbial activity [70]. Previous studies have reported that the integrated use of biochar and *Pseudomonas fluorescens* enhanced the growth of cucumber by improving plant–water relations under water deficit conditions. It has been reported that PGPR effectively colonize plant rhizosphere, thus helping in improving the growth, yield, and nutrient acquisition [29]. One possible reason behind increased uptake of N, P, and K in the present study (Tables 4 and 6) might be due to the promoting effects of PGPR and the applied biochar, which resulted in enhanced nutrient use efficiency, as has been reported previously [19,28,36]. Moreover, the presence of biochar in addition to PGPR might have helped to increase the sorption capacity of soil, resulting in higher mineral (NPK) concentration in wheat grains (Tables 4 and 6). These results are substantiated with those reported previously [37].

5. Conclusions

Low organic matter and depleted nutrients are the major issues of agricultural soils in arid and semi-arid regions. In the present study, the application of biochar from different sources significantly improved soil biological properties, growth, yield, and quality of maize grains. The integrated use of biochar and *Bacillus* sp. ZM20 was more effective as compared to the separate application. Biochar application along with *Bacillus* sp. ZM20 also improved soil biological properties, i.e., soil organic matter, MBC. Moreover, the biochar source and rate also influenced the soil properties and plant growth with different degrees of efficacy. The use of wheat straw biochar along with inoculated with the *Bacillus* sp. ZM20 bacterial strain was better as compared to farm-yard manure biochar and Egyptian acacia biochar. It is concluded that the combined application of wheat straw biochar (0.2%) and *Bacillus* sp. ZM20 was the most effective treatment in improving the soil properties, plant growth, yield, and quality of maize crops as compared to all other treatments in the pot and field trials.

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