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Combined Use of Spent Mushroom Substrate Biochar and PGPR Improves Growth, Yield, and Biochemical Response of Cauliflower (*Brassica oleracea* var. *botrytis*): A Preliminary Study on Greenhouse Cultivation

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Abstract: This paper investigated the impact of the combined use of spent mushroom substrate (SMS) biochar and plant-growth-promoting rhizobia (PGPR) on the growth, yield, and biochemical response of cauliflower (*Brassica oleracea* var. *botrytis*). A preliminary study was conducted under greenhouse condition using six treatments (sextuplicate) as control (no addition), T1 (PGPR), T2 (5 g/Kg biochar), T3 (5 g/Kg biochar + PGPR), T4 (10 g/Kg biochar), and T5 (10 g/Kg biochar + PGPR) under greenhouse conditions. The Scanning Electron Microscopy (SEM-Zeiss), Energy Dispersive Spectroscopy (EDS), and Fourier's transform infrared spectroscopy (FTIR) analyses showed that biochar produced from slow pyrolysis of SMS had advantageous structural, functional, and morphological properties for agricultural use. Results showed that SMS biochar addition aids the acceleration of soil nutrient properties. SMS biochar and PGPR application also significantly ($p < 0.05$) improved the selected growth, yield, and biochemical parameters of cauliflower. In particular, the highest cauliflower yield (550.11 ± 10.05 g), fresh plant biomass (1.66 ± 0.04 Kg), dry plant biomass (149.40 ± 4.18 g), plant height (22.09 ± 0.14 cm), root length (11.20 ± 0.05 cm), plant spread (28.35 ± 0.18 cm), and the number of leaves (12.50 ± 0.50) were observed in T5 treatment. Similarly, the best values for biochemical parameters and enzyme activities such as total chlorophyll (TC: 3.13 ± 0.07 mg/g), superoxide dismutase (SOD: 79.12 ± 1.29 µg/g), catalase (CAT: 55.70 ± 2.52 µg/g), peroxidase (POD 30.18 ± 0.37 µg/g), total phenolics (TP: 19.50 ± 0.31 mg/g), ascorbic acid (AA: 14.18 ± 0.55 mg/g), and total carotenoids (TCT: 150.17 ± 8.20 µg/100 g) were also recorded in the T5 treatment. The application of SMS biochar and PGPR showed a positive correlation with growth, yield, and biochemical response of cauliflower, as indicated by the Pearson correlation analysis. The findings of

this study suggest efficient recycling of mushroom industry waste for biochar production and the use of PGPR to improve nutrient utilization in sustainable agriculture.

Keywords: agro-waste recycling; biochar; growth promotion; mushroom waste; sustainable horticulture

1. Introduction

Cauliflower (*Brassica oleracea* var *botrytis*) is an annual crop mostly consumed in Asian countries, including China and India, as well as in the USA, thus accounting for 78% of the world's cauliflower consumption. It is a good source of minerals, ascorbic acid, carotenoids, flavonoids, polyphenols, and vitamin C [1]. The production of cauliflower and broccoli was 26.9 million metric tons in 2019, with China and India contributing 70% of this value [2]. In 2021, the cauliflower and broccoli market hit 23.1 billion USD, and it is forecasted to grow at a compound annual growth rate of 4.19% between 2022 and 2027 [3]. As the global production of this valuable crop increases, scientific communities are in dire need of its sustainable production vis-à-vis improved crop productivity.

Spent mushroom substrate (SMS) is considered an important agro-industrial waste derived from mushroom production as it contains considerable amounts of organic and inorganic nutrients, which are retained after mushroom harvesting [4]. However, the disposal of SMS has become an issue of environmental pollution concern. Furtherance to resolving this issue, SMS is considered a supplement for ruminants' feed, subsequent mushroom growing media, and raw material for biogas production, as well as different implementations as a soil amendment and biofertilizer. Recently, the application of SMS in biogas production and its substrate for crop cultivation was reported. The spent Shiitake substrate in an admixture with cow dung was transformed anaerobically into biogas and methane, yielding a highly nutritional digestate. The obtained digestate was used as a biofertilizer for tomato (*Solanum lycopersicum* L.) crop production, improving the vegetative characteristics such as seed germination, seedling, and root lengths, crop height, and yield, while biochemical traits, including chlorophyll, flavonoids, phenolics, and tannins contents were also enhanced [5]. Moreover, several improvements in soil properties were noted, i.e., soil electrical conductivity (increased by 12.4–36.9%), organic carbon (increased by 114.6–344.7%), phosphorus, and potassium contents (increased by 6.2–18.8% and 23.5–76.5%, respectively) [5].

Biochar is a carbon-rich solid material produced from biomass conversion via pyrolysis [6]. Owing to its contribution in atmospheric carbon dioxide removal by helping in carbon sequestration, biochar plays a role in the reduction of greenhouse gas (GHG) emissions, an important sustainable development goal (SDG 13) of the United Nations [7]. Following the United Nations recommendations on waste minimization, Asian farmers, encouraged by institutional and governmental guidance, began the implementation of biochar in different agricultural activities. Some researchers have demonstrated the reliability of biochar as a soil amendment. For instance, the application of biochar to the soil revealed a relatively slow decomposition of organic carbon and an increase in soil organic matter content. It also improved the growth parameters of wheat (*Triticum monococcum* L.) crops, such as the germination rate, plant dry weight, and root fresh weight, including potassium and calcium contents [8]. The main benefits on the physical level in that study were the reduction of soil compaction and the increase in soil porosity and density. Meanwhile, positive results were observed at the chemical level in terms of an increases in pH resulting in acidity of the soils and in cation exchange capacity—a determining factor for crops nutrients uptake from soils and the resultant yield and nutritional composition of the produce [8]. Biochar is also famed for its ability to reduce heavy metals and pesticides that have detrimental impacts on soil, crop, and human health [9]. Additionally, a desirable result of biochar fertilization was boosting soil biota leading to a better suspension of un-

wanted fungi and nematodes acting as a natural plant-growth-promoting rhizobacterium (PGPR) [10].

PGPRs are intracellular endophytes inhabiting around/on the root surface of plants and promote beneficial effects on soil biota and plants by improving water and nutrient uptake through direct and indirect mechanisms. They act by increasing the surface area of plant roots to aid nitrogen fixation and phosphorus solubilization in soils via their secretions [11]. PGPRs were also reported to produce several types of plant hormones such as auxins (i.e., indole-3-acetic acid, known as IAA), cytokinin, and gibberellins and suppress ethylene production by plants. PGPRs also produce HCN, which suppresses pathogens and induces plant growth [12]. Potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) are increased in PGPR-amended soils, whereas some heavy metals such as copper (Cu) and zinc (Zn) were suppressed [8]. PGPR also acts as a bio-pesticide by increasing the systemic resistance of plants through the synthesis of proteins, lipopolysaccharides, and siderophores, which improves the structure of the cell wall and strengthens the defensive mechanisms of plants [13].

Biochar derived from SMS is capable to remove several heavy metals from aqueous solutions [9]. It has also been demonstrated that SMS conversion to biochar prevents its leaching (expressed by the liberation of unwanted phosphates, nitrates, and chemical oxygen demand), thus a reduction of soil and underground water pollution [14]. The addition of SMS-biochar in an admixture with pig manure and rice straw reduced the compost's organic matter loss, while improving phosphorus, potassium, calcium, and nitrogen contents [15]. However, the production of SMS biochar and its implication in plant fertilization is scantly investigated in the literature. Recent reports showed that biochar produced from spent Shiitake mushroom and spent black fungi substrates had a high porosity and an improved surface area [16]. The incorporation of PGPR in *Brassicaceae* cultivation has also been previously acknowledged. PGPR has been shown to play a crucial role in the alleviation of silver-nanoparticles (AgNPs) stress –the main elements responsible for growth inhibition of brown mustard (*Brassica juncea* L.) seedlings; thus, reducing its toxicity [17]. Similarly, PGPR showed promising improvements in seed yield and fat content of winter oilseed (*Brassica napus* L.) (7.7% and 9.2%, respectively, compared to control) [18]. A combination of biochar and PGPR resulted in minor but positive effects on soil physico-chemical properties and plant growth parameters [8]. Cauliflower cultivation on soils amended with PGPR has received less attention from researchers despite being one of the most nutritious human diets. An admixture of PGPR, NPK fertilizer, and farmyard manure (FYM) increased yield (by 42.8%), ascorbic acid (by 5.0%), total carotenoids (by 7.1%), total soluble sugars (by 20.3%) and proteins (by 5.1%) contents in cauliflower compared to control (100% NPK + FYM) [19].

Considering the aforementioned, investigating the combined use of SMS biochar and PGPR is required. To the best of our knowledge, this is the first preliminary study that evaluates the combined effect of SMS biochar and PGPR on soil nutrient profile, production, and quality of cauliflower (*Brassica oleracea* var. *botrytis*) under greenhouse conditions.

2. Materials and Methods

2.1. Collection of Experimental Materials

For the current study, spent mushroom substrate (SMS) was obtained from the macro-fungi cultivation facility of Agro-ecology and Pollution Research Laboratory, Gurukula Kangri (Deemed to be University), Haridwar, India. The wheat straw-based SMS was collected from *Agaricus bisporus* (white button) mushroom. The white button mushroom (commercial stain: A15) was previously grown on wheat straw (70%) supplemented with wheat bran (15%), urea (1%), calcium ammonium nitrate (9%), gypsum (3%), and potash-based fertilizers (2%). SMS was obtained after harvesting the third flush, i.e., after the termination of the mushroom cultivation crop. The casing soil was carefully removed from the top of cultivation bags and SMS was sun dried before final application in biochar production. Besides this, the healthy seeds of cauliflower (*Brassica oleracea* var. *botrytis*;

PSBK-1 variety—a high-yielding variety) were procured from Beej Bhawan, Pusa Complex, New Delhi, India. This variety of cauliflower is very popular, high yielding, and seeds are also easily available within the study area Moreover, consortia of plant growth-promoting rhizobia (PGPR II) with proportionate counts (8×10^7 cfu/g) of *Bacillus subtilis* (MTCC 441) and *Pseudomonas fluorescence* (MTCC 103^T) were procured from the National Centre of Organic Farming (NCOF) located in Ghaziabad, India. For the cauliflower cultivation experiments, arable soil of loam texture was also collected from the agriculture field located in the Kulheri village, Saharanpur, India i.e., near the experimental site.

2.2. Biochar Production and Characterization

Prior to the biochar production, the SMS was oven-dried at 60 °C until a constant weight was achieved. Then, a total of 50 g of air-dried SMS was placed in a crucible disc and placed inside a muffle furnace (NSW-101, Narang Scientific, New Delhi, India). The heating rate of the muffle furnace was adjusted at 10 °C/min. The biomass was slowly provided for 1 h until the temperature reached up to 600 °C. Finally, the muffle furnace was turned off and cooled to room temperature. The biochar yield was calculated using Equation (1).

$$\text{Biochar yield } (\%) = \frac{\text{Weight of Biochar Obtained } (g)}{\text{Weight of SMS Used } (g)} \times 100 \quad (1)$$

The pyrolyzed biomass was characterized using Scanning Electron Microscopy (Zeiss Gemini SEM, Carl Zeiss, Oberkochen, Germany) attached with Energy Dispersive Spectroscopy (EDS) detector (Octane Eliter Plus, New Jersey, USA). In this, SEM-EDS was used to study the surface morphology and ultimate elemental composition of produced biochar. The SMS and biochar were also subjected to Fourier's transform infra-red (FTIR-8400S, Shimadzu, USA) analysis to understand the comparative changes in the biomass before and after pyrolysis. For this, laboratory-grade KBr pellets were used as reference material and a 3% smoothening was applied to the obtained spectra to reduce the measurement noise. Besides this, the selected properties of biochar such as pore size (nm) and Brunauer-Emmett-Teller (BET) surface area (m^2/g) follow the standard protocols [20–22].

2.3. Experimental Design

The cauliflower cultivation experiments were performed in a greenhouse (10 × 5 × 3 m; length × width × height) located in Kulheri village, Saharanpur, India (29°52′50.7″ N and 77°16′17.6″ E) from August 2021 to November 2021 (100 days). The greenhouse was constructed using thermostable high-density polyethylene (HDPE) plastic sheets with 50% ultraviolet (UV) stabilization capacity. The cauliflower cultivation was done in round-shaped plastic bags of 40 Kg capacity. For this, the bags were pre-sterilized using a 10% formalin solution and sun-dried. Specifically, a total of six treatments (sextuplicate) consisting of six replicates each such as control (no addition), T1 (PGPR), T2 (5 g/Kg biochar), T3 (5 g/Kg biochar + PGPR), T4 (10 g/Kg biochar), and T5 (10 g/Kg biochar + PGPR) were given as shown in Figure 1. The biochar was mixed proportionally with arable soil (30 Kg) and then filled in the bags. The seeds were pre-treated for a period of 2 h in 1 L of hot water (60 °C) to facilitate germination. The seeds were placed on filter paper (Whatman no. 42) to remove excess moisture and one healthy seed was planted at a depth of 2 cm in each bag. Each bag had 1 cauliflower plant, therefore, each treatment had 6 plants. On the other hand, 20 g of PGPR consortia were mixed with 180 mL of distilled water to prepare the stock biofertilizer inoculum. Then, a 5 mL of biofertilizer inoculum was added to the top surface of the soil (mixed with biochar or not), aseptically. The mean values of environmental conditions such as temperature, humidity, and light intensity of greenhouses during the cultivation period were noted as 28 °C, 65%, and 5400 lx, respectively. The bags were equally irrigated using a potable water supply from a borewell at an interval of each 7–10 days. The gas exchange and climatic conditions of the greenhouse were maintained using a digital control facility.

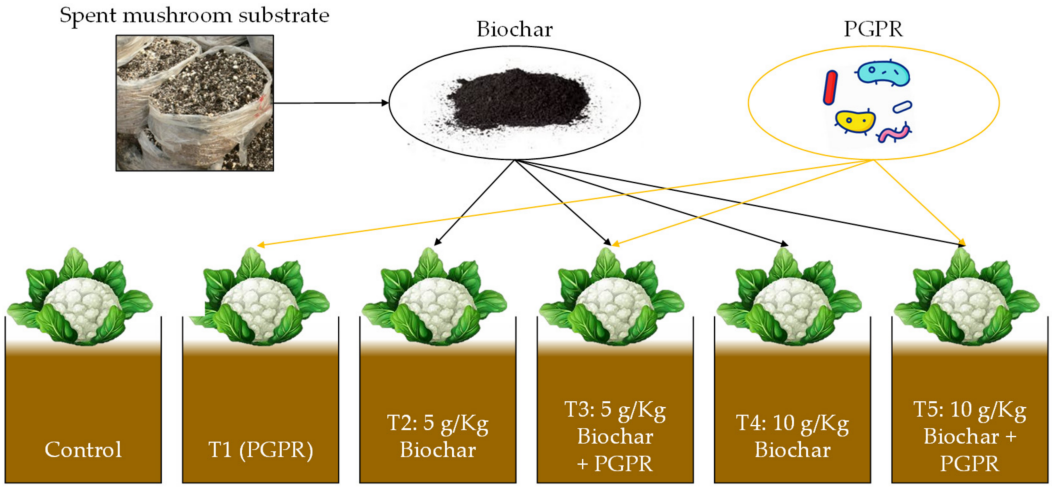

Figure 1. Experimental design for cauliflower cultivation using different treatments of the spent mushroom substrate (SMS) biochar and PGPR biofertilizer.

2.4. Chemical Analyses

In this study, the arable soil, SMS, and biochar obtained after pyrolysis were analyzed for selected physicochemical and nutrient properties. For this, standard laboratory protocols were adopted as recommended by APHA [23], AOAC [24], and Kumar et al. [5]. Specifically, the pH and electrical conductivity (EC) were measured using a digital meter (1615, ESICO, Parwanoo, India). Moisture content was determined using oven-drying (KI-181, Khera Instruments, Delhi, India) and weighting (Samson HI-600K, Edapally, India) methods. Organic carbon (OC) and total nitrogen (N) were estimated using Walkley and Black [25] and Kjeldahl's [26] acid digestion and distillation methods, respectively. Total phosphorus was determined using spectroscopy (60 Cary, Agilent, Santa Clara, CA, USA) after extraction using ammonium molybdate solution while the contents of potassium were analyzed using a calibrated flame photometer (1382, ESICO, Parwanoo, India). The contents of selected micronutrient elements such as manganese (Mn), iron (Fe), and zinc (Zn) in arable soil and SMS were estimated using atomic absorption spectroscopy (Analyst 800 AAS, PerkinElmer, Waltham, Massachusetts, USA) [5].

2.5. Growth and Biochemical Analyses

The growth parameters of cauliflower such as cauliflower yield (g), fresh plant biomass (Kg), dry plant biomass (g), plant height (cm), root length (cm), plant spread (cm), and the number of leaves were estimated as per the previous study of Kumar et al. [27]. Moreover, the biochemical parameters and enzyme activities such as total chlorophyll (TC: mg/g), superoxide dismutase (SOD: µg/g), catalase (CAT: µg/g), peroxidase (POD), total phenolics (TP: mg/g), ascorbic acid (AA: mg/g), and total carotenoids (TCT: µg/100 g) were also examined as per the standard protocols previously adopted by dos Reis et al. [28] and Jabeen et al. [29]. In particular, the contents of TC were determined using 80% acetone extraction followed by spectroscopic determination at wavelengths of 645 and 663 nm [30]. The SOD activity was analyzed using an extraction agent prepared by mixing proportionate amounts of nitro-blue tetrazolium chloride, L-methionine, potassium phosphate buffer Triton-X, riboflavin, and enzyme extract followed by spectrophotometric determination at 560 nm wavelength. The contents of CAT were analyzed using H_2O_2 and potassium phosphate buffer as extraction reagents followed by measuring the absorbance at 240 nm. Similarly, POD activity was measured at 470 nm wavelength using potassium phosphate buffer, H_2O_2, and a guiacol-based reagent. Moreover, TP contents were determined using the procedure of acetone extraction and Folin-Ciocalteau's phenol reagent measurement (750 nm). The ascorbic acid content was extracted with tri-chloroacetic acid, neutralized with dinitrophenyl hydrazine, and absorbance was measured at 530 nm wavelength. Finally,

TCT was assessed using acetone and saponification in a potassium hydroxide solution, followed by spectroscopic measurements at 450 nm wavelength.

2.6. Data Analysis and Software

Data were analyzed using one-way analysis of variance (ANOVA) and Duncan tests to compare the significant differences between treatment groups. Pearson Correlation was performed to understand the positive or negative relationship between spent mushroom (SMS) biochar and PGPR applications on growth, yield, biochemical, and enzyme activity characteristics of cauliflower (*B. oleracea*).

3. Results and Discussion

3.1. Characteristics of Spent Mushroom Substrate and Biochar

The comparison between SMS and its biochar properties revealed that there was a change in pH level from an acid to a basic level. (Table 1). This is mostly attributed to the increase in ash content due to the high pyrolysis temperature [31]. Although a higher SMS- based biochar's pH (by 34.6%) was noticed by other researchers [15]. In addition, the transformation of SMS into biochar reduced the electrical conductivity by 52.6%. The obtained value (2.09 dS/m) was similar to the average EC of monolithic biochar [32] but lower by 1.8-fold than formerly reported on SMS-based biochar. SMS was much wetter than its biochar, which is obvious as the latter was processed at a high temperature which makes it dry. Chang et al. [15] also reported a higher moisture content (by 4.1-folds) of SMS-based biochar compared to the obtained one in the current study. Our findings outlined that SMS-based biochar had a low organic carbon content.

Table 1. Physicochemical, nutrient, and morphological properties of the spent mushroom substrate (SMS) and its biochar.

Properties	Spent Mushroom Substrate (SMS)	Biochar @ 600 °C (10 °C/min for 1 h)
pH (H_2O)	5.02 ± 0.02	8.10 ± 0.01
Electrical Conductivity (EC: dS/m)	4.83 ± 0.20	2.09 ± 0.02
Moisture Content (MC: %)	56.66 ± 3.70	0.40 ± 0.03
Organic Carbon (OC: %)	23.40 ± 1.53	*Na*
Total Nitrogen (N: %)	0.92 ± 0.06	7.15 ± 0.02
C:N ratio	25.43 ± 0.10	8.67 ± 0.02
Total Phosphorus (P: %)	0.21 ± 0.02	*Na*
Total Potassium (K: %)	0.70 ± 0.04	*Na*
Biochar Yield (%)	*Na*	19.80 ± 0.15
Pore Size (nm)	*Na*	0.92 ± 0.20
BET Surface Area (m^2/g)	*Na*	5.10 ± 0.08
Carbon (C: %)	*Na*	62.06 ± 0.52
Oxygen (O: %)	*Na*	25.23 ± 0.18
Manganese (Mn: %)	0.16 ± 0.02	0.12 ± 0.01
Iron (Fe: %)	0.53 ± 0.04	0.35 ± 0.01
Zinc (Zn: %)	0.22 ± 0.02	0.10 ± 0.01

Values are mean ± SD of six analyses; *Na*: not analyzed or applicable.

It was acknowledged that spent substrate derived from mushroom production has most of its organic carbon sources depleted as fungi tend to use them to "fuel" and promote their growth [33,34]. Moreover, the high pyrolysis temperature led to the loss of organic matter in general and carbon sources (i.e., cellulose) in specific [35]. Biochar was richer in total nitrogen than SMS. As a result, the C:N ratio of biochar was reduced and far below earlier acknowledgments [11,15]. This could as a result of different types of agricultural or industrial wastes previously used or the nature of fungi grown. Biochar has intra-pore diameters which play an important role in its nutrient holding capacity [11,36]. Similarly, BET surface area was 48.2-folds lower than reported by the same authors, outlining a relatively robust nature of herein obtained SMS-based biochar, which reduces the adsorp-

tion performance. This point is observed in Figure 2a. Therefore, further investigations should take the lead in order to improve BET and pore size of SMS-based biochar, thus the improvement of adsorption performance. The increase in pyrolysis temperature would be extremely beneficial as it raises the total carbon and oxygen contents of biochar resulting in higher surface area and larger pore size [37]. The transformation of SMS into biochar leads to promising reductions in total manganese (Mn), iron (Fe), and zinc (Zn) contents (reduction by 1.33-fold, 1.51-fold, and 2.2-folds, respectively). These results pointed out the beneficial transformation of SMS into biochar in reducing the non-desirable heavy metals amounts. Our findings showed that total Mn, Fe, and Zn contents in SMS were 3.2-folds, 6.2-folds, and 27.5-folds, respectively, higher than formerly reported [35]. Whereas the resultant biochar held 2.3-folds lower total Mn, 1.3-fold lower total Fe, and 2.2-folds higher total Zn compared to the same study. Noteworthy is the increase of heavy metals in SMS-based biochar obtained in the aforementioned study controverting our findings.

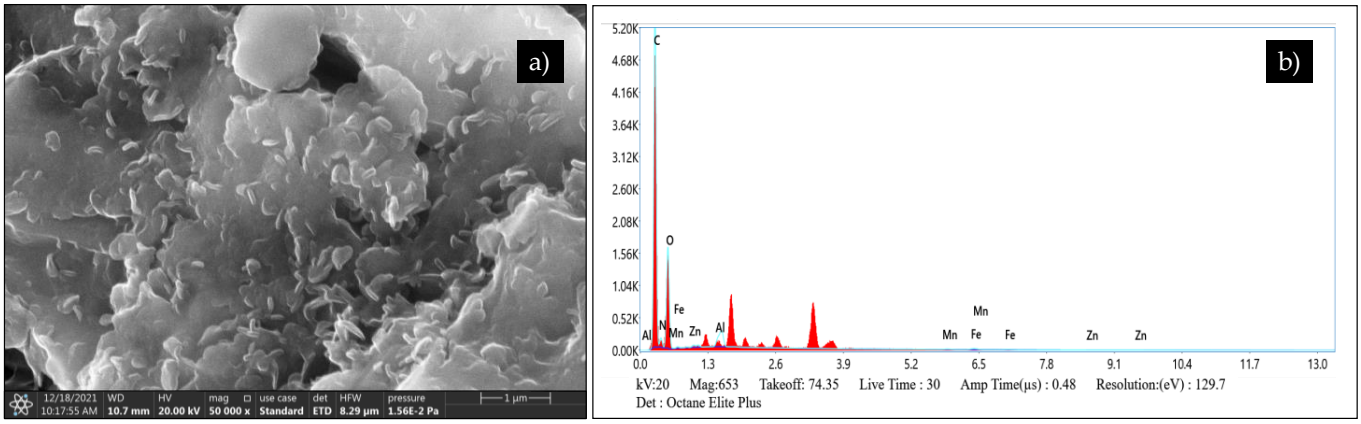

Figure 2. (**a**) Scanning electron microscopy (SEM-Zeiss) image and (**b**) energy-dispersive X-ray spectroscopy (EDS) spectrum of biochar derived from pyrolysis of the spent mushroom substrate (SMS).

The energy-dispersive X-ray spectroscopy (EDS) spectrum shows the ionization energy (abscissa) and the counts (ordinate). The higher the counts the higher element's presence would be. Generally, the observation of Figure 2b showed the presence of minor amounts of impurities being in line with the findings presented in Table 1. The highest ionization energy peaks were detected at low counts for C and O (which is obvious). Although insignificant, peaks of Al observed in the EDS spectrum should be further investigated within SMS-based biochar. Therefore, EDS showed to be a fast and accurate tool for impurities detection within SMS-based biochar. In order to perform a comparative evaluation between SMS and its biochar, both recorded spectra were averaged (Figure 3). A high similarity was generally observed among both spectra through the transmittance was higher by 12% (on average) in SMS-based biochar compared to SMS. Negative peaks of IR transmittance refer to positive wavelength (1/cm) peaks of absorption. A general decreasing pattern of SMS spectrum was noticeable at 850–1250 IR transmittance regions, which simulate strong C=C bending between mono- and di-substituted (alkane) compounds and strong C-O stretching between alkyl aryl ether compounds mostly found in carbohydrates [38]. A similar negative peak among both spectra at 550–600 IR transmittance regions assumes strong C-I stretching between halo compounds. Whereas, a similar positive peak among both spectra at the 1745 IR transmittance region refers to strong C=O stretching between esters (mainly originating from triglycerides). A similar negative peak among both spectra at 2750–2810 IR transmittance regions outlines a medium C-H stretching between aldehydes (possibly originating from lipids). Moreover, SMS-based biochar showed three peaks (two positive and one negative) in its IR transmittance. Positive peaks were noticed at the 1550 IR transmittance region outlining a strong N-O stretching between nitro compounds found in proteins [38] as well as at the 3550 IR transmittance region denoting a strong O-H stretching

alcohols (intermolecularly bonded). A negative peak was observed at around 3400 IR transmittance region; this simulates an N-H stretching of aliphatic primary amines found in SMS-based biochar.

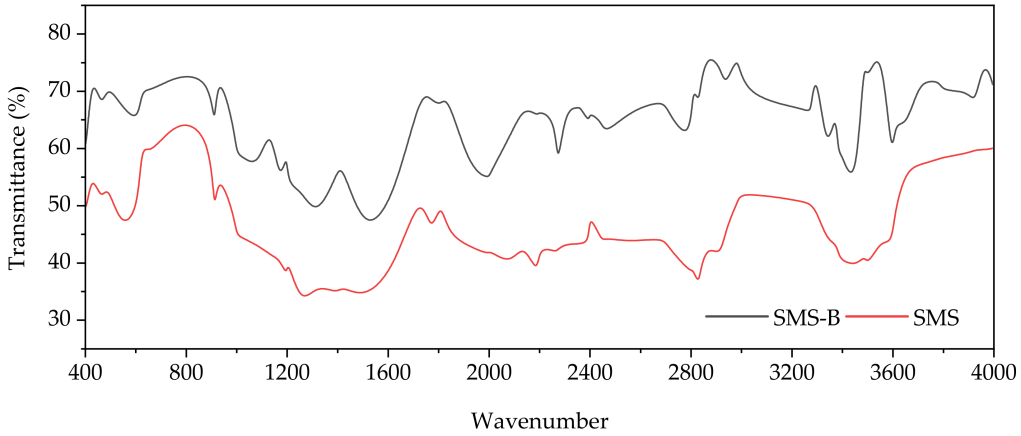

Figure 3. FTIR spectra of the spent mushroom substrate (SMS)-based and its biochar (B) derived through pyrolysis (unit of wavenumber: 1/cm).

3.2. Effect of Biochar Addition on Soil Nutrients

The implementation of SMS-based biochar in low and high doses of 5 g/kg and 10 g/kg, respectively, via arable soil supplementation significantly ($p < 0.05$) improved several traits such as pH (1.1–11.3%), EC (5.3–11.2%) and total nitrogen (90–180%) (Table 2). A higher pH increases the solubility of soil nutrients for subsequent crop production [39]; whereas a higher EC results in higher nutrient uptake by plants [40]. The high improvement in total nitrogen content of arable soil makes SMS-based biochar an excellent candidate for soil supplementation prior to vegetable cultivation, especially those belonging to the Brassicaceae family. SMS-based biochar could partially substitute chemical fertilizers, including some organics containing considerable amounts of potentially toxic elements. C:N ratio of arable was significantly reduced ($p < 0.05$) by 89.0% and 177.4% following the implementation of 5 g/kg and 10 g/kg, respectively, of SMS-based biochar. Total iron significantly ($p < 0.05$) increased by 19.0% following implementation of 10 g/kg SMS-based biochar into arable soil compared to control. Statistically insignificant ($p > 0.05$) changes were detected between treatments in terms of organic carbon, total P, total Mn, and total Zn, indicating the beneficial supplementation of arable lands with SMS-based biochar without enhancing the detrimental amounts of heavy metals. An earlier study reported improved soil properties after single and/or joint application of biochar and vermicompost for cucumber (*Cucumis sativus* L.) cultivation [41]. These authors noted that soil pH increased by 1.2–2.5% after biochar application, while a co-application of vermicompost and biochar resulted in decreased EC and increased dissolved organic carbon (DOC).

Table 2. Effect of the spent mushroom substrate (SMS)-based biochar supplementation on physico-chemical and nutrient properties of arable soil.

Soil Properties	Treatments		
	Control-T1	T2-T3 (5 g/Kg)	T4-T5 (10 g/Kg)
pH	7.20 ± 0.02 a	7.28 ± 0.03 b	8.01 ± 0.02 c
Electrical Conductivity (EC: dS/m)	1.87 ± 0.03 a	1.97 ± 0.02 b	2.08 ± 0.03 c
Organic Carbon (OC: %)	3.44 ± 0.04 a	3.45 ± 0.02 a	3.46 ± 0.01 a
Total Nitrogen (N: %)	0.40 ± 0.02 a	0.76 ±0.02 b	1.12 ± 0.04 c
C:N ratio	8.60 ± 0.10 a	4.55 ± 0.05 b	3.10 ± 0.03 c

Table 2. *Cont.*

Soil Properties	Treatments		
	Control-T1	T2-T3 (5 g/Kg)	T4-T5 (10 g/Kg)
Total Phosphorus (P: %)	0.35 ± 0.03 a	0.36 ± 0.02 a	0.37 ± 0.01 a
Total Potassium (K: %)	0.33 ± 0.04 a	0.33 ± 0.01 a	0.31 ± 0.01 a
Manganese (Mn: %)	0.17 ± 0.01 a	0.18 ± 0.01 a	0.18 ± 0.01 a
Iron (Fe: %)	0.21 ± 0.02 a	0.23 ± 0.01 a	0.25 ± 0.01 b
Zinc (Zn: %)	0.09 ± 0.01 a	0.10 ± 0.01 a	0.10 ± 0.01 a

Values are mean ± SD of six analyses; the same letters (a–c) show no significant difference among treatment group values at $p < 0.05$; Control: no PGPR or biochar; T1: PGPR; T2: 5 g/Kg biochar; T3: 5 g/Kg biochar + PGPR; T4: 10 g/Kg biochar; T5: 10 g/Kg biochar + PGPR.

3.3. Effect of SMS Biochar and PGPR on Growth and Yield of Cauliflower

Table 3 shows the impact of SMS biochar and PGPR treatments on the growth and yield traits of the cauliflower. The results showed that SMS biochar with PGPR treatments significantly ($p < 0.05$) affected the growth and yield performance of the cauliflower. However, the response values were highest in combined SMS biochar and PGPR treatments (T3 and T5) and lesser in individual treatments (T1, T2, T4) compared to the control treatment. Specifically, the highest cauliflower yield (550.11 ± 10.05 g), fresh and dry plant biomass (1.66 ± 0.04 and 149.40 ± 4.18 g), plant height (22.09 ± 0.14 cm), root length (11.20 ± 0.05 cm), plant spread (28.35 ± 0.18 cm) and the number of leaves (12.50 ± 0.50) were observed using T5 treatment as compared to the control treatment. Overall, the increasing order of cauliflower yield ranged as control < T1 < T2 < T4 < T3 < T5. The improvement in growth and yield attributes of cauliflower using SMS biochar might be because it is known as a promising supplement that can enhance soil fertility, thereby increasing nutrient availability to the plant [14]. The Pearson correlation plots in Figure 4a show that the growth and yield parameters of cauliflower were positively correlated ($p < 0.05$). Moreover, the PGPR inoculation has shown significant improvement in the plant's ability to absorb the nutrients available within their rhizosphere region [42]. In the current investigation, the cauliflower plant dually benefitted from SMS biochar and PGPR treatments, which resulted in accelerated growth and yield. However, a 10 g/Kg dose of biochar addition yielded better cauliflowers compared to those in 5 g/Kg, which might be associated with a lesser supplemented biochar dose. Moreover, microbes present in the PGPR biofertilizer may attach to the biochar particle either physically (on pores) or biochemically (on free functional sites). Biochar particle acts as host particle that provides carbon niche to the microbes, thereby accelerating their growth rates [43]. A recent co-application of biochar and vermicompost resulted in increased cucumber yields by 29.2–56.0% owing to the increased fungi and bacteria communities in soil [41]. Since PGPRs are well known to assist the plant in efficient nutrient utilization, the cauliflower yields also increased in this study.

Table 3. Effect of different treatments of the spent mushroom substrate (SMS)-based biochar and PGPR on growth and yield characteristics of cauliflower (*B. oleracea*).

Properties (per Plant)	Treatments					
	Control	T1 (PGPR)	T2 (5 g/Kg B)	T3 (5 g/Kg B + PGPR)	T4 (10 g/Kg B)	T5 (10 g/Kg B + PGPR)
Cauliflower Yield (g)	360.50 ± 3.76 a	380.75 ± 3.20 b	410.92 ± 6.15 c	520.45 ± 8.82 c	510.02 ± 5.30 c	550.11 ± 10.05 d
Fresh Plant Biomass (Kg)	1.10 ± 0.07 a	1.16 ± 0.02 a	1.20 ± 0.06 a	1.47 ± 0.05 b	1.38 ± 0.03 b	1.66 ± 0.04 c
Dry Plant Biomass (g)	98.10 ± 1.10 a	105.10 ± 2.05 b	108.56 ± 2.09 b	132.30 ± 1.12 d	124.21 ± 3.10 c	149.40 ± 4.18 e
Plant Height (cm)	14.65 ± 0.17 a	16.44 ± 0.06 b	17.84 ± 0.22 c	21.25 ± 0.10 d	18.46 ± 0.22 c	22.09 ± 0.14 d
Root Length (cm)	9.57 ± 0.05 a	9.78 ± 0.09 b	10.10 ± 0.07 c	10.76 ± 0.13 d	9.80 ± 0.08 b	11.20 ± 0.05 d
Plant Spread (cm)	20.09 ± 0.28 a	22.38 ± 0.15 b	23.37 ± 0.26 c	26.55 ± 0.42 d	24.91 ± 0.25 c	28.35 ± 0.18 e
Number of Leaves	8.50 ± 0.50 a	8.50 ± 0.50 a	9.50 ± 0.50 ab	11.00 ± 1.00 bc	10.50 ± 0.50 b	12.50 ± 0.50 c

Values are mean ± SD of six replicates; B: spent mushroom substrate biochar; the same letters (a–e) show no significant difference among treatment group values at $p < 0.05$; Control: no PGPR or biochar; T1: PGPR; T2: 5 g/Kg biochar; T3: 5 g/Kg biochar + PGPR; T4: 10 g/Kg biochar; T5: 10 g/Kg biochar + PGPR.

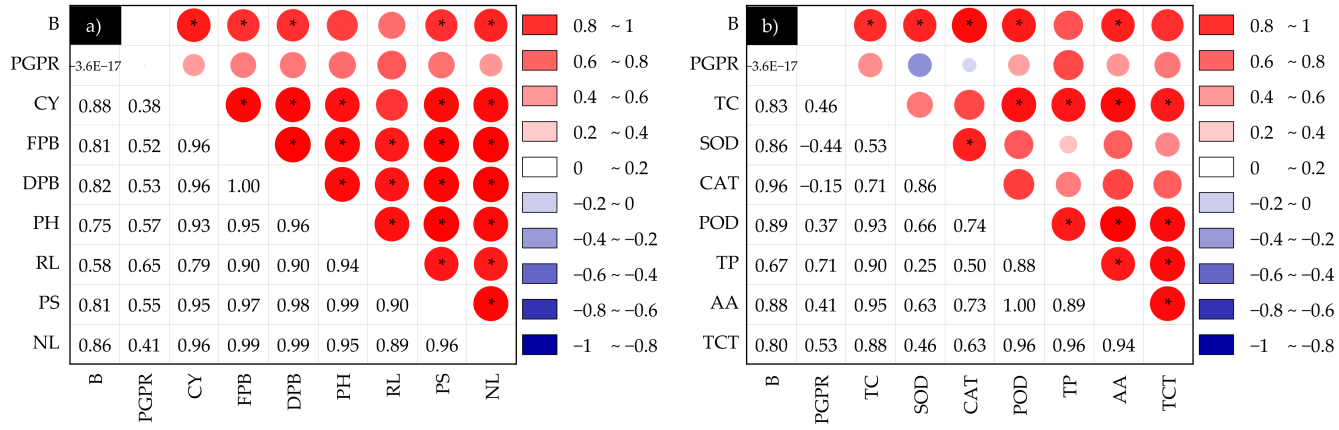

Figure 4. Correlation between spent mushroom (SMS)-based biochar and PGPR treatments on (**a**) growth, yield (**b**) biochemical, and enzyme activity characteristics of cauliflower (*B. oleracea*) (B: spent mushroom substrate biochar dose; PGPR: plant growth-promoting rhizobia; CY: crop yield; FPB: fresh plant biomass; DPB: dry plant biomass; PH: plant height; RL: root length; PS: plant spread; NL: number of leaves; TC: total chlorophyll; SOD: superoxide dismutase; CAT: catalase; POD: peroxidase; TP: total phenolics; AA: ascorbic acid; TCT: total carotenoids: *: correlation is significant at $p < 0.05$).

The findings of this work agree with the results from the previous studies [44–46]. However, the studies on the collective use of SMS biochar and PGPR are limited. Among the few available related studies, Kumar et al. [44] investigated the effect of biochar and fly ash in combination with two PGPRs (*Bacillus* sp. strain A30 and *Burkholderia* sp. strain L2) on the growth of tomato (*Lycopersicon esculentum*) crop. They reported that a significant ($p < 0.05$) increase was seen in plant parameters (dry and fresh biomass, length, number of flowers) using the combined use of PGPR and biochar. Similarly, Nadeem et al. [45] studied the synergistic impact of biochar, compost, and PGPR (*Pseudomonas fluorescens*) on the cucumber (*Cucumis sativus*) grown on water-deficient soil. They found that while cucumber production was affected by the water shortage, the use of biochar, compost, and PGPR under 50% field capacity gave positive results in terms of cucumber yield with an increase in shoot length by 88%, shoot biomass by 77%, root length by 89%, and root biomass by 74%, respectively. Further, Ullah et al. [46] explored the integrated effect of algal biochar and PGPR on maize (*Zea maize*) crops cultivated in deficit irrigation soils. They observed that maize crops grown with integrated use of algal biochar and PGPR increased the yield and growth traits as compared to the control treatment. Therefore, it was concluded that the cauliflower growth and yield parameters in the current study can be improved by the integrated use of SMS biochar and PGPR.

3.4. Effect of SMS Biochar and PGPR on Biochemical Response of Cauliflower

As shown in Table 4, SMS biochar and PGPR treatments indicated significant ($p < 0.05$) variations in biochemical constituents and enzyme activities of cauliflower. In particular, maximum values of biochemical parameters and enzyme activities such as total chlorophyll (TC: 3.13 ± 0.07 mg/g), peroxidase (POD: 30.18 ± 0.37 µg/g), total phenolics (TP: 19.50 ± 0.31 mg/g), ascorbic acid (AA: 14.18 ± 0.55 mg/g), and total carotenoids (TCT: 150.17 ± 8.20 µg/100 g) were also recorded in the T5 treatment followed by T3, T4, T2, T1, and control, respectively. However, the enzyme activities of superoxide dismutase (SOD: 79.12 ± 1.29 µg/g) and catalase (CAT: 55.70 ± 2.52 µg/g) were lesser in T5 and T3 as compared to other treatments. This might be because SOD and CAT are stress tolerating enzymes secreted by plants under high nutrient stress. SOD and CAT are important antioxidant enzymes that prevent the degeneration of plant cells under stress conditions. Herein, SMS biochar application on the soil significantly increased the soil nutrient availability. However, PGPRs are known to ameliorate high nutrient stress and slow down the SOD and CAT production, thereby efficient plant survival [47]. Thus, high values of SOD and CAT

activities in cauliflower might have been suppressed by the PGPR application. Besides this, the other biochemical parameters such as chlorophyll content, phenolics, ascorbic acid, and carotenoids have their distinct role in plant growth and metabolism, which also justifies the nutritional properties of cauliflower [48]. These parameters were positively correlated with SMS biochar and PGPR addition as shown in Figure 4b. Similarly, CAT and POD help in the conversion of H_2O_2 into H_2O and O_2, thereby protecting the plant from highly reactive oxygen species incorporated from stressed environments. On the other hand, phenolics are the most crucial byproducts of plant cell metabolism, which play an important role as antioxidants against oxidative stresses [49]. Moreover, AA (or vitamin C) and TC are major biochemical constituents that work as a redox buffer during hormone and photosynthetic regulation [42]. In the current study, the optimum levels of these biochemical constituents in T3 and T5 treatments suggested the advantageous application of SMS biochar and PGPR for cauliflower cultivation.

Table 4. Effect of different treatments of the spent mushroom substrate (SMS)-based biochar and PGPR on biochemical and enzyme activity characteristics of cauliflower (*B. oleracea*).

Properties	Treatments					
	Control	T1 (PGPR)	T2 (5 g/Kg B)	T3 (5 g/Kg B + PGPR)	T4 (10 g/Kg B)	T5 (10 g/Kg B + PGPR)
Total Chlorophyll (TC: mg/g)	1.57 ± 0.02 a	1.90 ± 0.05 b	1.86 ± 0.03 b	3.02 ± 0.06 cd	2.89 ± 0.04 c	3.13 ± 0.07 d
Superoxide Dismutase (SOD: µg/g)	62.49 ± 3.50 b	52.09 ± 2.71 a	84.98 ± 4.10 cd	73.28 ± 0.90 bc	92.66 ± 5.15 d	79.12 ± 1.29 c
Catalase (CAT: µg/g)	32.08 ± 0.74 a	34.22 ± 1.03 a	45.02 ± 1.65 bc	40.93 ± 0.82 b	64.24 ± 3.60 d	55.70 ± 2.52 c
Peroxidase (POD: µg/g)	12.36 ± 0.04 a	14.23 ± 0.16 b	20.14 ± 0.54 c	26.05 ± 1.10 d	24.08 ± 0.85 d	30.18 ± 0.37 e
Total Phenolics (TP: mg/g)	11.20 ± 0.13 a	13.56 ± 0.08 bc	12.09 ± 0.06 b	16.88 ± 0.24 d	14.58 ± 0.16 c	19.50 ± 0.31 e
Ascorbic Acid (AA: mg/g)	6.82 ± 0.02 a	8.21 ± 0.05 b	10.18 ± 0.08 c	13.04 ± 0.12 e	12.05 ± 0.20 d	14.18 ± 0.55 e
Total Carotenoids (TCT: µg/100 g)	80.50 ± 2.53 a	91.58 ± 4.32 a	101.25 ± 7.21 b	121.87 ± 5.92 c	110.39 ± 6.07 b	150.17 ± 8.20 d

Values are mean ± SD of six replicates; B: spent mushroom substrate biochar; the same letters (a–e) show no significant difference among treatment group values at $p < 0.05$; Control: no PGPR or biochar; T1: PGPR; T2: 5 g/Kg biochar; T3: 5 g/Kg biochar + PGPR; T4: 10 g/Kg biochar; T5: 10 g/Kg biochar + PGPR.

Previously, numerous researchers have explored the effect of biochar in combination with PGPR on the biochemical response of different crops. Among them, Lalay et al. [50] found that integrated application of biochar and PGPR (*Pseudomonas* sp. and *Staphylococcus* sp.) induced the catalase (CAT) activity and also increased the chlorophyll pigments, carotenoids, and anthocyanin content of Rapeseed (*Brassica napus* L.). In another study, Abbas et al. [51] cultivated wheat (*Triticum aestivum* L.) using rice straw biochar (0%, 1.5%, 3.0%, and 5% w/w) in an aged contaminated soil. They found that biochar application increased the growth parameters of the wheat plant while decreasing the Cd and Ni bioavailability. Also, a significant ($p \leq 0.05$) effect of biochar was observed on the activities of antioxidant enzymes (SOD, POD, and CAT) in the shoot and root parts. Moreover, Mirzaei et al. [52] studied the effect of PGPR (*Pseudomonas* and *Azotobacter* spp.) inoculation on lemon grass (*Cymbopogon citratus*) grown under water stress conditions. They reported that CAT and SOD activity altered by 77% and 71%, respectively, in the stress condition, while total phenolic and flavonoid, and essential oil content were significantly enhanced. Similarly, Thakur et al. [19] investigated the effect of PGPR and nutrient sources on cauliflower. They observed that the combined application of PGPR and nutrients (75% NPK + 50% farm yard manure and 50% vermicompost on an N-equivalence basis) significantly increased the growth and yield performance of the cauliflower. They found that the cost-efficient and highest crop yield was 392.45 q/ha, with a fruit weight of 981.05 g, and AA content of 74.87 mg/100 g, respectively. Therefore, these results are in good agreement with the findings of the present study on variation in biochemical and enzyme acidity response of cauliflower.

4. Conclusions

This study concluded that the combined use of spent mushroom substrate (SMS) biochar and plant-growth-promoting rhizobia (PGPR) improved the growth, yield, and biochemical parameters of cauliflower (*Brassica oleracea* var. *botrytis*) at pot-scale cultivation. SMS biochar has favorable characteristics, including sufficient pore size, surface area, and functional groups for the better attachment and growth of beneficial microbes that support plant growth. Thus, a 10 g/Kg dose of SMS biochar with PGPR application gave the highest crop yield and optimum biochemical response. It was found that growth, yield, and biochemical parameters of cauliflower were positively correlated with SMS biochar and PGPR application except for superoxide dismutase and catalase. Therefore, this study presented an efficient approach for SMS management through biochar production and synergistic crop cultivation as a combined application with PGPR biofertilizer. Further studies on understanding the mechanisms and pathways by which PGPR assists in efficient nutrient assimilation by crops from SMS biochar are highly recommended. Also, the efficacy of the combined use of PGPR and SMS biochar should be tested in open field conditions.

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