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Biochar as a novel carrier affects *Brassica napus* L. growth, soil phosphorus fractions, and its uptake

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Contribution Ibrahim, M. designed the experiment and wrote the manuscript. M. Rubab, A. Hassan & T. Nasr analyzed, interprets and verified the data. S. Ayaz validates the data and improved the figures

Biochar increases soil fertility by enhancing nutrients retention in amended soil. In addition, biochar increases phosphorus availability in soil by reducing leaching process. The performance of biochar serving as an inoculum carrier depends on physicochemical properties (pH, porosity and, surface area) that impact the establishment of plant growth-promoting bacteria in amended soil. Biochar is largely used as a soil amendment for soil remediation and plants growth. However, little work has been conducted considering biochar suitability when applied as a carrier, which has resulted in inconsistent outcomes. In the present study, six biochar carriers including rice straw biochar (RSB), rice husk biochar (RHB), soybean straw biochar (SSB), peanut shells biochar (PNB), corn cobs biochar (CCB), and wood biochar (WB) were prepared and inoculated with liquid suspension of a phosphate solubilizing bacteria (PSB) identified as *Bacillus megaterium* KU647234 in a pot experiment. The data revealed that rape (*Brassica napus* L.) growth and biomass were significantly ($P \leq 0.05$) improved by up to 304% with amendments of carriers, particularly soybean straw (SSB). P bioaccumulation in roots, stems, and leaves increased significantly ($P \leq 0.05$) by up to 74%, 111%, and 75% respectively. In addition, RSB, SSB, RHB, PNB, WB, and CCB amendments Plate count assay data revealed that six carriers sustained the *Bacillus megaterium* abundance to varying degrees. The SSB carrier was identified as the best carrier that sustained higher rape biomass, and P bioaccumulation compared with other carriers.

Keywords: Carriers, *Brassica napus L*, P fractions, P uptake.

INTRODUCTION: Biochar application increases plant growth and soil quality. The combine application of biochar and plant growth promoting bacteria (PGPR) increased the production capacity of pepper (*Capsicum annuum*) in southwest China (Zhang *et al.*, 2023). Nutrients play a key role in plants growth and development. For optimum plant growth and development Phosphorus (P) is a crucial macronutrient in the agricultural sector (Wahid *et al.*, 2020). However, suitable P availability in soil, enhances plant growth, is a severe issue throughout the globe, especially in developing countries such as China, Pakistan, and India where phosphate fertilizers are scarce (Metson *et al.*, 2016). The non-available P forms such as inorganic P (Pi) as well as organic P (Po) in agricultural soil are immobilized by mineral compounds of Iron (Fe), Aluminum (Al), and Calcium (Ca) and are the main cause of P deficiency in soil (Muhmood *et al.*, 2019). The inoculation of plant growth-promoting bacteria (PGPR), which is used as alternative biological inoculants, was frequently studied previously to increase agricultural yield based on phytohormones secretion, P solubilization mechanism, organic P mineralization, N_2 fixation, and vitamin synthesis (Saxena *et al.*, 2013; Glick and Gamalero, 2021). The majority of studies stressed the PGPR use efficiency which has the ability linked with the P solubilization mechanism that enhances P availability to the host plant in specific environments (Basu *et al.*, 2021; de Andrade *et al.*, 2023). In the last few decades, a large number of PGPR particularly species of *Pseudomonas, Enterobacter, Bacillus*, and *Serratia* isolated and studied associated with phosphorus mobilization mechanisms such as by decreasing pH of the medium coupled with dissolving Ca-P minerals, mineralization of organic P by the activity of enzyme phosphatase and desorbing P mineral compound trapped by Al and Fe molecules by exudation of organic acids (Zheng *et al.*, 2018). Based on these mechanisms were referred to as potentially successful inoculants for increasing plant productivity and crop yield. PGPR inoculation into the agricultural soil did not increase the plant biomass or P uptake efficiency leading to abnormal changes in soil P and enzyme activities (Martínez *et al.*, 2015). Direct inoculation of PGPR into the soil revealed low survival due to competition with indigenous native microbes, nematodes, protozoan, and fungal populations as well as low nutrient availability (Hale *et al.*, 2014). Researchers are in search of a viable material that retains the maximum number of PGPR population densities for a long time when introduced into the soil. In this regard, biochar seems a suitable candidate. Biochar is a carbon-rich black porous material produced from thermal pyrolysis (300-700 ^{0}C) of different raw biomasses derived from organic wastes (Hale *et al.*, 2015; Ibrahim *et al.*, 2019). Biochar has a large surface area and internal porosity can offer positive opportunities for inoculation of PGPR thus providing safe habitats for survival. In amended soil, biochar might also provide essential nutrients to PGPR (Zimmerman *et al.*, 2011). The excessive and intensive applications of inorganic P fertilizers particularly in the Taihu Lake Regions (TLR) were reported (Wang *et al.*, 2015). This massive application of phosphate fertilizers (120 kg P2O5 ha-1) in agricultural fields by local farmers has resulted in dramatic P imbalance and accumulation in the TLR region (Wang *et al.*, 2012; Jiang *et al.*, 2021). Therefore, it is necessary to achieve ecological and economic goals, to increase P availability, and to decrease the phosphate fertilizers input through the feasible application of novel materials in modern agriculture systems. Most studies reported specific biochar and PGPR effects on plant growth (Cantrell *et al.*, 2012). However, there is limited information about the ability of various biochars as an inoculum carrier. Therefore, PGPR called *Bacillus megaterium* having the inorganic P solubilization ability was loaded with six low-cost biochar called corn cob biochar (CCB), soybean straw biochar (SSB), rice husk biochar (RHB), rice straw biochar (RSB), wood biochar (WB) and peanut shells biochar (PNB) and amended in a pot experiment to determine their effects on 1) rape growth and biomass production, 2) soil P fractions, 3) P uptake and bioaccumulation, d) inoculum abundance.

OBJECTIVES: The main objectives of the present study were such that whether biochar loaded with inorganic phosphate solubilizing bacteria (iPSB) has the ability to increase soil P concentration, rape plant growth and inoculum survival in amended soil.

MATERIALS AND METHODS: Study area, strain isolation, biochar production, and inoculation: Ten soil samples were randomly collected from the field in Hailun City, Heilongjiang Province, China. Five strains called A1, Y9-5, Y9-9, Y9-24, and Y14-12 having the ability of P solubilization were isolated and classified (table 1). Briefly, Pikovskaya medium (PVK) was used as a culture medium with some little modification without yeast extract and was supplied to 96-well plates in advance Nautiyal (1999). Bromocresol was used as an indicator and was added (10 µM) to the respective PVK medium. In 100 mL of sterilized water, soil samples (1 g) were homogenized well and the respective suspensions were serially diluted ranging from 10 to 10^5 and were supplied (1 µL) to each microplate carefully and incubated for 72 h at 30 ^{0}C. Control treatment without inoculation was included in each batch. Wells without obvious strain growth were recognized as negative, while wells without prominent colour change but with bacterial growth were considered uncertain strains. However, wells with a yellow color and clear bacterial growth were considered positive. Each dilution having three replicates was tested further. Finally, on solid modified PVK plates, the usefulness of the present method was further verified for positive strains carefully.

Five strains that had the ability of phosphate solubilization of the tri-calcium phosphate were selected. Quantitative estimation of the release of ortho-phosphate divided by total P that was determined by the addition of 0.1M HCl was measured for every 24 h. Strain Y9-9 was selected as the best due to the highest phosphate solubilization efficiency after 72 h incubation. Furthermore, genomic DNA was extracted and deposited to the National Centre for Biotechnology Information (NCBI) centre with GenBank accession number KU647234.

Strain	Code	Accession number	pH of medium	Soluble P concentration	Closest reference strain	Accession number	Similarity %
Bacillus megaterium	A1	KU647215	5.00	100.51	*Bacillus* sp. B2(2010b)	HM104462	99
B. megaterium	Y9-5	KU647233	4.44	134.49	*Bacillus* sp. S10	HE662645	99
B. megaterium	Y9-9	KU647234	4.41	159.48	*B. megaterium* BS17	KR063197	99
B. megaterium	Y9-24	KU647222	4.55	136.83	*Bacillus* sp. BDH4	KF933626	99
B. megaterium	Y14-12	KU647242	4.60	138.68	*B. megaterium* HNS88	KF933685	99

Table 1: Classification, and P solubilization of the isolated strains.

Carrier preparation: Corn cobs, wood, peanut shells, rice husk, rice straw and soybean straw waste residues were used as feedstock for the production of biochar. Corn cobs, rice straw, soybean straw, and rice husk residues are produced in large amount during crop harvesting. In-addition peanut shell is produced in large amount during cooking oil production on daily basis. To manage these wastes biochar RHB, SSB, WB, RSB, PNB, and CCB were prepared through thermal pyrolysis under a continuous flow of N_2 in the absence of O_2 at a temperature of 450 ^{0}C for 5 h in an automatically controlled furnace. Biochars images are shown in figure 1.

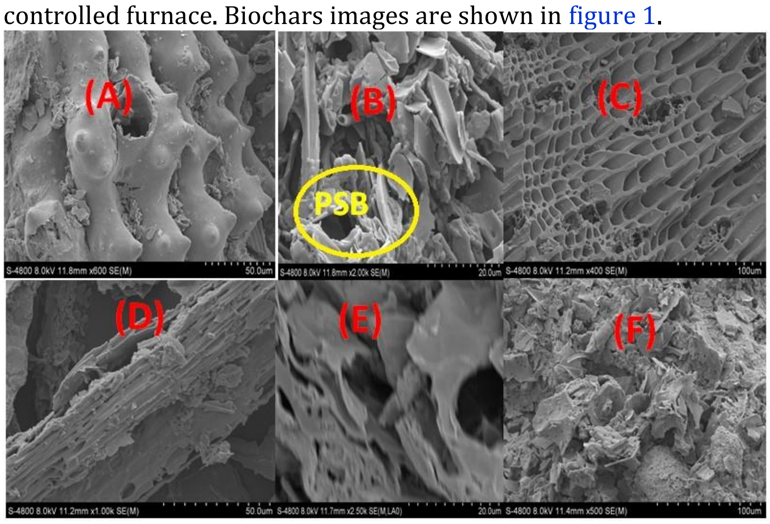

Figure 1: Scanning Electron Microscope (SEM) pictures of rice husk biochar (RHB), soyabean straw biochar (SSB), wood biochar (WB), rice straw biochar (RSB), peanut shell biochar (PNB), and corn cobs biochar (CCB).

Afterward, the basic physiochemical properties of biochars were measured. The soaking method was applied for the inoculation of a selected strain called *Bacillus megaterium*. Each biochar material was crushed and passed through a 2 mm sieve. Thereafter, in flasks (250 mL) each biochar was soaked individually with fresh Luria-Bertani Broth (LB) medium at a ratio of 1:20 *w*/*v* and sterilized at a temperature of 121 ^{0}C for 20 min. The selected strain (6.6×10^8 CFU per mL) was inoculated into flasks at a ratio of 1:10 *v*/*v* after cooling at room temperature. After 24 h intermittent shaking the respective biochar carrier was washed thrice thoroughly with double distilled (sterilized) water. Immobilized counts (initial population densities) of the *Bacillus megaterium* strain were recorded according to the standard dislodging method (Nasser *et al.*, 2022). Colony forming units (CFU) were determined using the plate count assay technique. Another round of plate count assay was conducted after rape plants harvest (4 weeks of pot experiment) to determine the inoculum abundance.

Glasshouse trail: Soil samples (0-10 cm depth) were randomly collected in triplicates from Changshu Long-term Experimental Station, Jiangsu Province, China. This area is located in the mid-west of the Taihu Lake Region (TLR). Soil samples were transported and air-dried in the glasshouse at the Institute of Urban Environment, Chinese Academy of Science, Xiamen, China. Samples were sieved (2 mm) and basic physiochemical properties were measured. Plant debris was removed manually. The soil particle size was 80.84 % sand, 18.38 % silt, and 0.78% clay. The respective six carriers CCB, WB, RHB, PNB, RSB, and SSB were individually amended into the soil at 3% *w*/*w* thoroughly in cylindrical polyvinyl chloride (PVC) pots containing 4 kg soil per pot. Control treatment (CT) without carrier amendment was also incorporated into each set of batch experiments. Pot trial was conducted in a complete randomized block design (CRBD) in 4 replicates. The soil moisture level was maintained approximately at 60% water holding capacity (WHC). Soil samples were collected with the help of a soil corer after 4 weeks of incubation and frizzed carefully for soil P fractions and stain population densities. Rape seeds were surface sterilized (30% H_2O_2) for 10 min and washed thrice with double distilled water carefully. Ten seeds per pot were sown and thinned to four after 10 days of growth period. The pot experiment was conducted in natural conditions under 12 h natural light, with daytime temperature (25±2 ^{0}C), night-time temperature (20±3 ^{0}C), and relative humidity (70±5%). Pots were irrigated manually with double distilled water on a daily basis when needed.

Plants chemical analysis: The rape plants were harvested after one month of growth period and carefully separated from the soil. Plant samples were washed thrice with double distilled (deionized) water thoroughly to remove adhered soil particles. Delta biomass was recorded on a dry weight basis after oven-drying plant samples at 70 ^{0}C for 72 h. Plant-accumulated P was measured using standard method. The concentration of P was measured using ICP-OES (Perkin-Elmer, Downers Grove, IL, USA). Plant P bioaccumulation (mg pot^{-1}) was determined as P content (mg kg^{-1}) ×Plant biomass (g pot^{-1}).

Soil chemical analysis: Soil pH was determined in a 1:2.5 (*w*/*v*) soil/$CaCl_2$ solution (0.01M) using a pH meter (Accumet, MA, USA). Soil available P concentration was estimated by the addition of 0.5M $NaHCO_3$ (pH 8.5).

Soil P-fractions: Soil P fractions were sequentially measured according to the standard method (Hedley *et al.*, 1982). Briefly, the P fraction (0.5 g air-dried soil) constitutes the five fractions: (a) resin-P was extracted using double deionized water and one anion-exchange resin stripe (Sinopharm Chemical Reagent Co., Ltd); (b) Soil $NaHCO_3$-Pi and $NaHCO_3$-Po were extracted using 0.5 M $NaHCO_3$ (c) NaOH-Pi called moderately labile P pool, (supposedly related with Fe and Al minerals) and NaOH-Po were extracted with 0.1 M NaOH (d) HCl-Pi (non-labile P pool, supposed to be related with Ca minerals) was extracted with 1 M HCl (e) residual-P, exists in soil after above extracts, was digested with H_2SO_4/H_2O_2 at 360 ^{0}C. Two aliquots from $NaHCO_3$ and NaOH extracts were prepared for measuring total P and inorganic P (Pi) concentration. The organic P (Po) in each extract was obtained by the difference between total P and inorganic P (Pi). The P content in each supernatant was determined using the ascorbic acid colorimetric method using an Ultraviolet Spectrometer (UV 2500, Japan).

Estimation of strain population densities: Estimation of *Bacillus megaterium* population densities in carrier applied treatments were quantified according to the modified method Zhao *et al.* (2021) up to four weeks. Briefly, flask (250 mL) contained sterile water (100 mL), 10 g fresh soil (10 g) were intermittently shacked and subsequently sonicated in bath sonicator (40 KHZ, 220V). Liquid suspensions were serially diluted (10-fold) and aliquots of 100µl were spread on petri plates (LB) containing antibiotics such as ampicillin, cycloheximide and kanamycin. Plates were incubated at 28^{0}C for 20 h for colony formation units. Four colonies (total 96 colonies) were randomly picked from each plate for estimation of plate count assay. In addition, PCR was conducted to ensure strain survival in amended soil through 16s DNA sequencing.

Statistical analysis: We determined a change that represented a delta between treatments and control to account for the variation in soil and plant data. One-way ANOVA in SPSS 11.5 (SPSS Inc., Chicago, IL, USA) was applied for analysis of the effect of carrier on soil and plant data. Means were contrasted by Tukey's HSD test for comparing samples among treatments. Graphs were plotted using Sigma plot 12.5 (Systat Software, Inc., San Jose, CA USA).

RESULTS: **Soil pH and P concentration:** The physiochemical properties of tested soil and six biochars used as carriers are shown in table 2. Soil pH decreased significantly (P≤ 0.05) in carrier-amended soil compared to non-amended control. Carrier SSB showed the highest surface area (12.88 m^2 g^{-1}) and pore size (7.47 nm) as compared with other carriers. Soil pH declined significantly

(P≤ 0.05) in carrier-amended treatments as compared to non-amended control (table 3). Total P content (7.61 mg kg^{-1}) was highest in SSB-amended soil as compared with other carriers. However, in the remaining treatments, the order of increment in soil total P concentration was RSB>PNB>WB>RHB>CCB as compared with non-amended control (table 3).

Parameters	pH	BET surface area (m^2 g^{-1})	Pore Volume (cm^2 g^{-1})	Pore Size (nm)	C (%)	N (%)	H (%)	Total P (mg kg^{-1})	Available P(mg kg^{-1})
Soil	6.55	ND	ND	ND	3.05	0.27	1.16	5.59	2.51
RHB	8.94	4.81	0.0043	4.44	49.9	0.63	2.79	9.04	4.63
RSB	10.2	5.72	0.0109	5.62	46.8	2.04	2.55	28.48	18.81
SSB	9.55	12.88	0.0143	7.47	65.0	1.04	2.80	40.13	24.39
WB	8.92	9.32	0.0048	2.13	77.5	0.34	4.21	4.01	0.71
CCB	10.1	5.46	0.0043	3.17	74.1	0.55	3.68	9.08	2.96
PNB	9.33	4.24	0.0036	4.44	70.6	1.45	3.22	17.78	4.03

Table 2: Basic physiochemical properties of the tested soil and biochar.
An abbreviation represents RHB: rice husk biochar, RSB: rice straw biochar, SSB: Soybean straw biochar, WB: Wood biochar, CCB: corn cob biochar, PNB: peanut shell biochar, ND: No data.

Treatments	pH	C (%)	N (%)	P (%)	Total P (mg kg^{-1})
CK	7.04 ±0.24 a	3.03± 0.11b	0.27± 0.10 b	0.17± 0.15 b	5.59±0.51b
CCB	6.84 ±0.21b	4.80 ± 0.31b	0.69± 0.18 b	0.19± 0.13 b	6.65± 0.19b
PNB	6.99± 0.18 b	6.85 ±0.23 b	1.46± 0.12 b	0.38± 0.15 b	7.01± 0.98b
RHB	6.94± 0.16 b	4.35 ±0.24 b	0.68± 0.13 b	0.18± 0.11 b	6.83±0.19 b
RSB	6.84± 0.15 b	4.73 ± 0.21 b	1.22± 0.32 a	0.84± 0.21 a	7.11 ±0.96 a
SSB	6.95± 0.12 b	6.41 ± 0.24	1.05± 0.25 b	0.62± 0.23 b	7.61±1.06 b
WB	6.84±0.16 b	4.93 ± 0.21 a	0.34± 0.14 b	0.15± 0.13 b	6.98 ±0.18b

Table 3. Concentration of various elements in carrier amended soil.

The delta concentration of inorganic P fractions in carrier-amended soil is shown in figure 2. Concentration of Pi significantly increased by 24%, 62%, 20%, 32%, 20%, and 25 % with the amendment of RSB, SSB, RHB, PNB, WB, and CCB in sequentially extracted 0.5M $NaHCO_3$ (Ca-P). Similarly, the concentration of Po significantly enhanced by 140%, 412%, 21%, 221%, 50%, and 20% with amendment of RSB, SSB, RHB, PNB, WB, and CCB as compared with non-amended control. The highest increment in Pi and Po concentration (62% and 412%) was revealed in SSB carrier-amended soil (figure 2A). The concentration of Pi in 0.1 M NaOH extracted soil non-significantly increased with carrier RSB, SSB, RHB, PNB, WB, and CCB amendments. However, the concentration of Po significantly (P≤ 0.05) increased by 66%, 75%, 61%, 21%, 79%, and 33% with the amendment of RSB, SSB, RHB, PNB, WB, and CCB (figure 2B). The highest increment in 0.1 M NaOH extracted Pi and Po concentration (22% and 75%) was revealed in SSB-amended soil (figure 2B). Similarly, 1.0 M HCl extracted Pi concentration significantly (P≤ 0.01) increased by 48%, 107%, 38%, 45%, 32%, and 29%, particularly in SSB treatment as compared with non-amended control (figure 2C). Residual P concentration significantly (P≤ 0.05) increased by 112%, 150%, 62%, 87%, 75% and 75% in carrier RSB, SSB, RHB, PNB, WB, and CCB amended soil. SSB amendment significantly increased (150%) residual P fraction as compared with other carriers (figure 2D). The concentration of total P significantly (P≤ 0.05) increased by 53%, 68%, 28%, 23%, 21%, and 20% particularly in SSB-amended soil as compared with other carriers (figure 2E). The extraction efficiency was calculated and ranged from 93-100%.

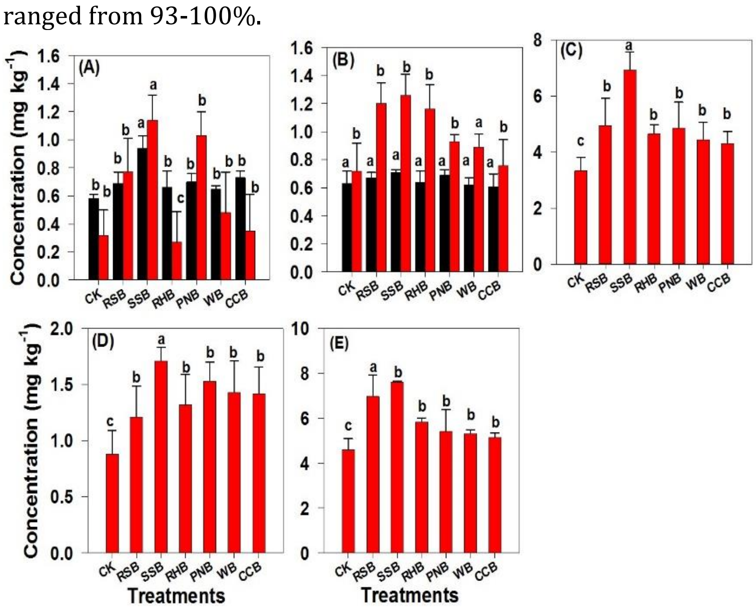

Figure 2: Sequentially extracted soil P-fractions affected by various carrier A) 0.5 M $NaHCO_3$ Pi (black color bars) and Po (red color bars), B) 0.1 M NaOH Pi (Fe/Al-P) (black color bars) and Po (red color bars), C) 1.0 M HCl Pi, D) P residual and E) P total. Error bars represent standard deviation (n= 4). Different letters indicate significant difference (P≤ 0.05) between treatments, while similar letters indicate non-significant difference.

Rape biomass production and P uptake: Rape plant biomass significantly increased in carrier SSB followed by RSB, PNB, and RHB addition. Shoot biomass significantly improved by 96%, 97%, 167%, 185%, 235%, and 304%, with CCB, WB, RHB, PNB, RSB, and SSB respectively. Similarly, root biomass increased by 71%, 78%, 114%, 128%, 228% and 230% in the carrier amended soil. The highest increment in rape shoots and root biomass (304% and 230%) was recorded in the SSB amendment. The decreased rape biomass production, P uptake, and accumulated values were observed in CCB and WB treatments (figure 3A). Roots P bioaccumulation was significantly enhanced by 21%, 30%, 38%, 40%, 63%, and 74% with amendments of WB, CCB, PNB, RHB, RSB, and SSB as compared with non-amended control soil. Similarly, the above-mentioned carriers soil amendments enhanced the stem bioaccumulation of P by 29%, 30%, 64%, 78%, 82%, and 111%. Leaf bioaccumulation of P was significantly increased by 20%, 21%, 37%, 46%, 70%, and 75% with amendment of WB, CCB, PNB, RHB, RSB, and SSB as compared with non-amended control (figure 3B).

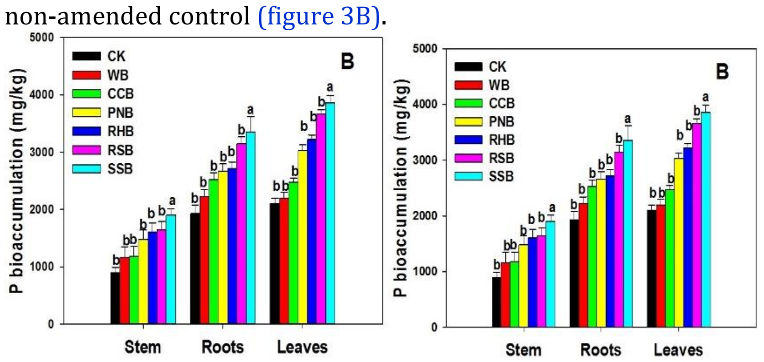

Figure 3: (A) Effects of carriers on *Brassica napus* L. plant biomass and (B) P bioaccumulation in stem, roots, and leaves in carrier amended and control treatments. Error bars represent standard deviations (n=4). Different letters indicate significant difference (P≤ 0.05) between treatments, while similar letters indicate non-significant difference.

Strain population abundance: The effectiveness of all six carriers is shown in a heat map (figure 4). For initial strain counts (F=111.65, p<0.001) and after 4-weeks counts (F=39.02, p<0.001), in six carriers statistically significant result was investigated. Present study showed that in SSB treatment the count of viable strain cell was highest (2271%) ($1.5×10^8$ CFU g^{-1}), followed by RSB (728%) ($1.2×10^8$ CFU g^{-1}) and other carrier treatments with counts lower than $1×10^8$ CFU g^{-1} (figure 4). It was obvious that the inoculum abundance was significantly or slightly increased towards the fourth week except WB treatment which cell counts declined by up to 14.6% after four weeks of incubation.

DISCUSSION: Soil pH is considered a key factor for plant growth and nutrient uptake, especially P. Generally, with the addition of biochar soil pH increases however, in the current study, soil pH declined significantly (P≤ 0.05) in carrier-amended soil as compared to non-amended control. This decline in soil pH in the carrier's amended soil may be due to the effect of the inoculated *Bacillus megaterium* strain. The concentration of the soil total P, in the TLR is extremely low (3.01 mg kg^{-1}) and is the key limiting factor for crop plant growth and development in this region.

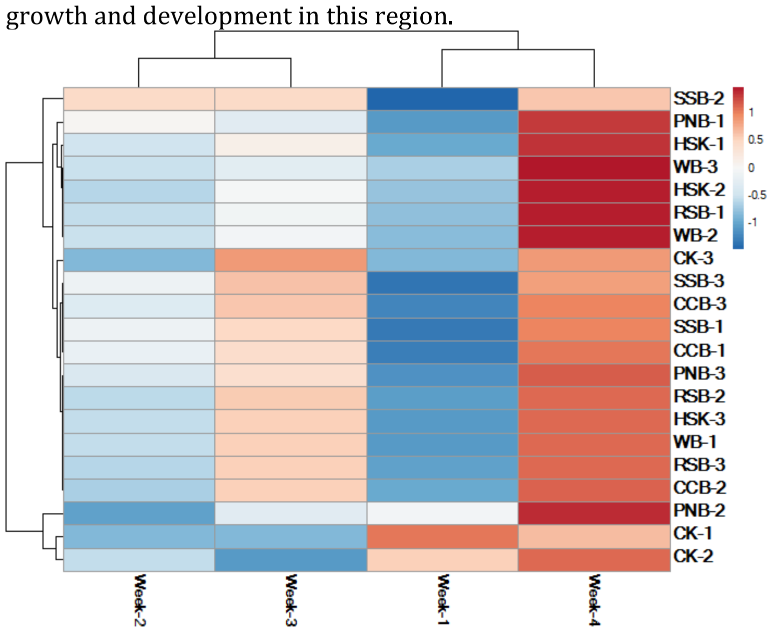

Figure 4: Heat map represents *B. megaterium* KU647234 (CFU g-1) abundance in carriers amended soil.

The highest total P concentration in SSB-amended soil might be due to increased P supplement from straw material or solubilization of mineral-bound P by PSB on biochar carrier (da Silva Carneiro *et al.*, 2021; Raymond *et al.*, 2021). As a result, the P concentration value reached the threshold that was considered the optimal level of P nutrition to rape plant growth (Yan *et al.*, 2022).. In the current study, soil amendment with carrier improved rape plant growth and biomass production. Present results showed that the addition of all carriers constantly improved the rape biomass which may associate with higher P uptake. These beneficial outcomes suggested a positive feedback between biochar and inoculum where higher plant available P and P turnover, which subsequently stimulated rape increased P uptake and transport from roots to other tissues via the xylem channels for satisfying rape plant requirements. However, contrasting results obtained from previous study Gaskin *et al.* (2010) revealed that decreased crop yield and biomass production with amendments of biochar. Ibrahim *et al.* (2021) revealed that *Brassica oleracea* L. plant biomass increased with various amendment rates (2%, 4%, and 6%) of peanut shell biochar. In addition, our result showed that Soybean straw biochar (SSB) was the best combination that increased the rape plant biomass and P bioaccumulation as compared with other carriers. The increased biomass, P bioaccumulation and P uptake may be associated with the increased surface area, pore size of the respective biochar, and abundance of PSB.

In the current study, the effectiveness of all six carriers was confirmed, particularly for SSB which proved to be the best carrier that maintained the highest population densities of *Bacillus megaterium* KU647234 during week-4 survival outcomes as shown in a heat map. The abundance of PSB population densities was linked positively to pore size and BET surface area of respective biochar. After week 4, we noticed that Ca-P concentration was one of the chemical variables that positively affected introduced strain abundance in carrier-amended soil. Choudhary *et al.* (2021) revealed that the high surface area of each biochar potentially increases the chemical sequestration of nutrients, which was the main reason for improving nutrient status and maintaining higher microbial abundance. There are many mechanisms involved in the abundance of the introduced strain in carrier-amended soil. One possible mechanism may be the porous structure of biochar. The biochar pore size was strongly associated with the initial and week-4 survival of the introduced strain, which was consistent with the findings of (Głodowska *et al.*, 2016). Furthermore, biochar pours structure plays a significant role in providing safer habitat for pre-established bacteria and protecting them from desiccation and predation from indigenous soil microbes (Ajeng *et al.*, 2020). In the present study, the SSB biochar with a mean pore size of 7.47 nm was within the optimal range, which might contribute to the high survival of *B. megaterium* in amended soil. Another mechanism might be the pH of biochar. Biochar pH is one of the most vital parts that alter the living conditions of microorganisms in the pore space and thus microbial abundance (Lehmann *et al.*, 2011). The previous study Hale *et al.* (2015) revealed the effect of biochar pH on the initial abundance of *Enterobacter cloacae* UW5 cells. However, in the current study contrasting results were obtained, there was no significant relationship between biochar pH and initial strain population densities, as well as population density after 4-week integration into the soil, implying *B. megaterium* strain might be more reactive to other factors than biochar pH. A study indicated that variation in the C: N ratio could affect the structure and composition of the soil microbial community (Muhammad *et al.*, 2014). In P-deplete soil, larger inoculum abundance with lower C: N in RSB carrier was obtained implying higher mineralization potential, eventually changing P nutrient composition that fulfilled the necessity of microbial growth (Pereira *et al.*, 2020). This suggested that phytate acting as a substrate of P solubilization would maintain the coupled inoculum active and abundance in soil. In the current study, with C: P ratio and Ca-P, our findings revealed that week-4 abundance of inoculum was positively linked with total P, confirming high microbial P turnover and suggesting strain *B. megaterium* as inorganic P solubilizing bacteria that efficiently mobilized insoluble P and formulate it bio-available for rape plant uptake. Biochar provide a safe habitat for microbes to survive and hot spots for microbial movements. The increased surface area and pore volume of the amended carrier might be linked with increased survival of the added strain after 4 weeks of the rape growth period.

CONCLUSION: In conclusion, by evaluating the suitability of various biochars as carriers for rape plant growth and inoculum survival, our results showed that straw material (RSB, and SSB) had a more stimulatory effect on *Brassica oleracea* plant biomass and P uptake. The characteristics of biochar particularly pore size, BET, and surface area were noticed positively correlated with strain abundance. We concluded that the best carrier was SSB which maintained the higher strain population densities. In addition to better understand the relationship between SSB, soil P and rape plant P uptake, further research is needed in field trial in complex environment.

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LIFE SCIENCE REPORTING: In the current article no life science threat was reported.

ETHICAL RESPONSIBILITY: This article is not submitted in whole or in parts to another journal for publication purpose.

INFORMED CONSENT: The author(s) have reviewed the entire article and approved the final version before submission.

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