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Influence of photosynthetic bacteria and bio-charcoal on growth, yield and quality of lettuce (*Lactuca sativa*) cv. iceberg chrispiano

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Abstract
A field experiment was conducted to determine the growth, yield and quality of lettuce in response to different doses of Biochar and Photosynthetic Bacteria (PSB). The treatments were arranged in a randomized block design with 10 treatments of different doses of Biochar and PSB with 3 replications. The observations were recorded on growth, yield and quality showed that all the different doses of Biochar and PSB had significant effect on the lettuce compared to the control. Organic Carbon content, Nitrogen, Phosphorous and Potash increased in all the treated plots except in the control. Among the treatments, T_2 [PSB 40ml to 10L water (400 L PSB: 1,00,000 L Water) and Biochar 5 t ha^{-1} (500 g/m^2)] showed the greatest influence on the growth, yield and quality of the lettuce. Hence it is concluded that the application of Biochar and PSB is a promising approach to increase the growth, yield and quality of lettuce.

Keywords: biochar; PSB; lettuce; growth; yield; quality parameters

1. Introduction
The term 'biochar' first appeared in the modern scientific literature in a paper presented by Harshavardhan Bapat and Stanley E. Manahan at the 215th National Meeting of the American Chemical Society in 1998. Biochar is Charcoal used as a Soil conditioner. Like most charcoal, biochar is made from Biomass via Pyrolysis. Biochar can increase Fertility (soil) of Acidic soil (low pH soils), increase agricultural productivity, and provide protection against some foliar and soil-borne diseases. Biochar can improve water quality, reduce soil emissions of greenhouse gases, reduce nutrient leaching, reduce soil acidity, and reduce irrigation and fertilizer requirements.
Photosynthetic bacteria have been around for longer than the Earth's atmosphere could sustain human life. It was only recently though that scientists began to unravel the mystery of how these micro-organisms execute the mechanisms of photosynthesis. Photosynthetic bacteria can be used in a variety of agriculture applications. Currently, some of the most popular usages include rice fields, Allie vegetables, greenhouse cultivation, floriculture, fruit trees, and stock raising (including seafood). In most cases, photosynthetic bacteria and is applied directly onto organic matter added to cropping fields which reduces the time necessary to prepare bio fertilizers. Photosynthetic Bacteria can also be applied as a fertilizer spray or added to the water supply of an agricultural facility.

2. Materials and Methods
2.1 Preparation of PSB
The purple non-sulfur bacterium strain *Rhodopseudomonas palustris,* was purchased from Japan and was mass produced in SHUATS, Department of Horticulture. They were produced in 1 L water bottles where 900 ml of water was added to it, 60 ml of PSB strain and 40 ml of beaten chicken egg was added. Sealing the bottle tight with a cap and mixing it and the keeping it bright area away from sunlight for 2 weeks till the whole pale yellow liquid turn maroon red. The PSB was given at the same time irrigation was given i.e. once a week. It was mixed with the irrigation water and given to each plot separately.

2.2 Preparation of Biochar
Rice husk was purchased from Makino School and a construction made out of oil tins was constructed which had holes all around and a long chimney which is used for turning rice husk into charcoal. A fire was started with a few woods and the chimney was kept over the fire.

Then the rice husk was piled at the side of the chimney not allowing air to pass through the chimneys holes. The rice husk is continuously turned and replied on the chimney till it turns to char. When all the rice husk have turned to char, remove the chimney and with the help of a rake, spread out the rice husk char into evenly distributed thickness and keep on turning and churning the rice husk char to prevent it from turning to ash. This turning and churning of the rice husk char is continued for about 2 to 3 hours, then water is sprayed on the char and the char is left overnight to cool and ready for use. The biochar was incorporated into the soil prior to transplanting.

2.3 Soil sampling

Soil samples were collected from the experimental site before and after the experiment and was kept in a zip lock plastic bags and shipped to a soil laboratory to do analysis. Both Chemical and Mechanical analysis was done.

Total nitrogen content: Estimated by alkaline permanganate method (Subbiah and Asija, 1965) [17] where the sample was digested in potassium permanganate and sodium hydroxide and titration was potassium hydroxide.

Total phosphorous content: Soil sample was digested with sodium bicarbonate and carbon black. Then para nitro phenol indicator was added. Shake the flask and phosphorous was determined photo metrically (Bini Das and Bindi, 2014) [3].

Total potassium content: It was determined using flame-photometer from the extract obtained by digestion with ammonium acetate (Bini Das and Bindi, 2014) [3].

pH estimation: A ratio of 1:2.5 soil water suspension was taken and using glass electrode pH meter we measure the value (Bini Das and Bindi, 2014) [3].

E.C. estimation: The soil Sample was mixed with distilled water and a Conductive meter (Systronics) was used to obtain the E.C. value (Bini Das and Bindi, 2014) [3].

Organic Matter Estimation: The soil sample is first weight at the initial part, then it is exposed to 440˚ C in a muffle furnace to burn out the organic matter. The soil is then weighed again after cooling down and the weight is taken (ASTM D 2974 – Standard test methods for moisture, ash and organic matter of peat and organic soils).

2.4 Experimental Design

The experiment was laid out in Randomized block design and it included 10 treatments, each treatment had a different combination of Biochar and PSB with 3 replication having one control in each (Table 1). Each plot was given an early dose of FYM of 2 kg which is incorporated into the soil, to support the nutrient requirement of the seedlings.

Table 1: Treatment Details

T_0	**Control (No treatments only FYM)**
T_1	PSB 20ml to 10 L water (200 L: 1,00,000 L) and Biochar 10 t ha^{-1} (1 kg)
T_2	PSB 40ml to 10 L water (400 L: 1,00,000 L) and Biochar 5 t ha^{-1} (500 g)
T_3	PSB 60ml to 10 L water (600 L: 1,00,000 L) and Biochar 7 t ha^{-1} (700 g)
T_4	PSB 20ml to 10 L water (200 L: 1,00,000 L) and Biochar 5 t ha^{-1} (500 g)
T_5	PSB 40ml to 10 L water (400 L: 1,00,000 L) and Biochar 7 t ha^{-1} (700 g)
T_6	PSB 60ml to 10 L water (600 L: 1,00,000 L) and Biochar 10 t ha^{-1} (1 kg)
T_7	PSB 20ml to 10 L water (200 L: 1,00,000 L) and Biochar 7 t ha^{-1} (700 g)
T_8	PSB 40ml to 10 L water (400 L: 1,00,000 L) and Biochar 10 t ha^{-1} (1 kg)
T_9	PSB 60ml to 10 L water (600 L: 1,00,000 L) and Biochar 5 t ha^{-1} (500 g)

2.5 Observations

The Observations were recorded on plant height, number of leaves head girth, head diameter, total soluble solids and vitamin C:

Plant height was recorded by a measuring scale and number of leaves of the lettuce was counted and recorded at 20, 40 and 60 days after transplanting (DAT). The head girth of the lettuce was recorded with the help of a string wrapped around the lettuce once then it was measured with a scale at 2 intervals i.e. 40 and 60 DAT. The lettuce head diameter was measured at the time of harvest by cutting the head into half horizontally then taking two points of the lettuce and measuring it in a straight line. Total soluble solids was done with the help of a hand refractometer. The estimation of Vitamin C was done using 2, 6-, Dichlorophenol indophenols dye (AOAC, 1968) [1]. Each data was subjected to statistical analysis.

3. Result and discussion

The lettuce result of the growth, yield and quality was taken and presented in table 3.

Data was compared between the treated plots and the control to find out the best possible combination of PSB and Bio-char (Fig1 and 2). The findings are as given below:

Plant Height

In the present study, the maximum plant height 17.43 cm was observed in T_2 followed by T_8 and T_5 i.e. 17.37 cm and 17.20 cm respectively which are statistically at par with the maximum plant height. The minimum plant height of 14.78 cm is seen with Control. This increment of plant height may be due to good amount of bio char which stimulated shifts in microbial populations towards beneficial plant growth promoting rhizobacteria or fungi, due to either chemical or physical attributes of the biochar and increase in biomass. Similar findings were found by Graber *et al.* (2010) [6] in Pepper, Carter *et al.* (2013) [4] in lettuce and William *et al.* (2015) [21] in okra, beans and coriander. The amount of PSB added promote the growth of the bacteria of azotobacter, rhizobium, and actinomyces and restrain the growth of fungi in the soil, promote the plant resistance against disease and improve output, increase the content of chlorophyll and promote growth. Similar findings were observed by Ke *et al.* (2005) [13] in cucumber, Jun-lin *et al.* (2012) [12] in eggplant and Yali *et al.* (2014) [23] in Chinese cabbage.

Number of leaves

Significantly the maximum number of leaves of 17.72 cm was observed in T_2 followed by T_8 at 18.28. However, minimum number of leaves of 15.78 cm is observed with Control. This may be due to the amount of bio char that help the biomass increase, improving soil total nitrogen and phosphorus contents. Similar findings were reported by Carter *et al.* (2013) [4] in lettuce, William *et al.* (2015) [21] in okra, beans and coriander and Trupiano *et al.*, (2017) [18]. The amount of PSB added to the soil, increase the population of beneficial micro-organism, promote the plant resistance against disease and improve output. Similar findings were observed by Ke *et al.* (2005) [13] in cucumber and Li-na (2012) [14] in cucumber.

Head diameter

Significantly the maximum head diameter of 12.88 cm was observed in T_2 followed by T_5 with 12.65 and T_8 with 12.64. The minimum was observed in Control with a value of 11.76.

Head Girth

Significantly the maximum head girth of 40.44 cm was observed with T_2 followed by T_8 at 39.72 cm. per plant. However, minimum head girth (36.93 cm) is observed with Control.

Yield

The maximum yield is observed in T_2 with 19.44 t/ha followed by T_8 and T_5 with 19.41 t/ha and 18.72 t/ha respectively which are also at par with the maximum yield. The minimum yield was recorded in Control with 11.39 t/ha. This may be due to the amount of bio char that allowed the plant to penetrate the soil deeper, decrease the acidity of soil pH, stimulate beneficial bacteria population, give good drainage, reduce phytotoxic, increase nitrogen use efficiency and lesser nitrate leaching. Similar findings were found by *Graber et al.* (2010) [6] in pepper, Jia *et al*. (2012) [9] in maize, Vinh *et al.* (2014) [19] in rice, DaWei *et al* (2016) [5] in tomato and pepper and Trupiano *et al.*, (2017) [18]. The amount of PSB added also promotes nutrients absorption for plants and promote plant resistance against diseases. Similar findings were observed by Guifu *et al.* (1988) [7] in rice, Wididana *et al.* (1993) in garlic, onion, tomato and watermelon, Daly M. J. *et al*., (2008) [15] in onion, peas and sweet corn, Jun *et al.* (2002) [11] in tomato, Xiao-ping *et al.* (2003) [22] in soybean, Jun-lin *et al.* (2012) [12] in eggplant, Jihui *et al.* (2013) [10] in hot pepper and Yali *et al.* (2014) [23] in non-heading Chinese cabbage.

TSS

The significantly higher total soluble solids 4.73 ^{0}Brix was recorded in T_2 followed by T_8 with 4.2 ^{0}Brix. The minimum TSS content is recorded under Control with 2.73 ^{0}Brix.

Vitamin C

The maximum value is recorded in T_2 with 2.54 mg/100g followed by T_8 2.52 mg/100g. However, the minimum value is observed in Control with 2.37 mg/100g. T_1, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 were found to be statistically at par with T_2. From the experimental findings, treatment T_2 had the best performance with 2.53 mg/100g per plant is observed in. Similar findings were observed by Jun *et al*. (2002) [11] in cucumber, Shujie *et al.* (2009) [16] in Chinese chive and Yali *et al.* (2014) [23] in non-heading Chinese cabbage.

Soil analysis

The soil analysis result showed that after the experiment the soil showed a significant increase in Organic carbon, Nitrogen and Potash. Phosphorous showed some increase in some treatments while pH remained relatively the same in all the treatments before and after the experiment. Electric conductivity results are seen to be lower in the soil after the experiment have been carried out. (Table 1 and 2)

Cost: Benefit Analysis

The careful analysis of the cost of cultivation and return on per hectare basis is given in the table 4. It is evident from the table that the highest cost benefit ratio 1: 2.29 was recorded in T_2 followed by T_8 with a ratio of 1:2.21. However all the other treatment were also significantly superior over Control with 1:1.53.

4. Conclusion

On the basis of the present investigation, it can be concluded that the combination of Bio-char and PSB in treatment T_2 is found to perform the best in terms of plant height (17.43 cm), number of leaves (17.72 cm), head diameter (12.88 cm), head girth (40.44 cm) yield (19.44 t/ha), TSS (4.73 ^{0}Brix), vitamin C (2.54 mg/100g) and a benefit: cost ratio (1: 2.29).

Table 2: Chemical Analysis of Soil

Treatments	Organic Carbon (%)	Nitrogen (kg/ha)	Phosphorous (kg/ha)	Potash (kg/ha)	pH	E.C.
Before the Experiment	0.37	83.25	18.0	257.6	7.5	0.70
After the Experiment						
T_0	0.41	92.25	18.0	268.8	7.1	0.64
T_1	0.44	90.0	18.0	268.8	7.4	0.66
T_2	0.46	103.5	22.5	280	7.3	0.62
T_3	0.43	96.75	22.5	280	7.1	0.62
T_4	0.44	99.0	18.0	268.8	7.3	0.64
T_5	0.45	101.25	22.5	291.5	7.3	0.63
T_6	0.43	96.75	18.0	280	7.4	0.65
T_7	0.44	99.0	22.5	291.	7.3	0.62
T_8	0.46	103.5	18.0	268	7.5	0.63
T_9	0.42	94.5	22.5	280	7.3	.064
F- test	S	S	NS	NS	NS	NS
S. Ed. (±)	0.01	0.73	2.20	12.18	0.26	0.06
C.D. (0.05)	0.01	1.53	4.61	25.58	0.54	0.13

Table 3: Growth, head yield and quality of lettuce as affected by various treatments

Treatments	Plant Height (cm)	No. of Leaves	Head Diameter (cm)	Head Girth (cm)	Yield (t/ha)	TSS (^{0}Brix)	Vitamin C (mg/100g)
T_0	14.78	15.78	11.76	36.93	11.39	2.73	2.37
T_1	16.33	17.72	12.48	39.18	17.85	3.63	2.47
T_2	17.43	18.72	12.88	40.44	19.44	4.73	2.54
T_3	15.97	16.72	12.15	38.16	15.96	3.07	2.43
T_4	16.19	16.77	12.25	38.48	17.60	3.4	2.43
T_5	17.20	17.94	12.65	39.73	18.72	4.17	2.51
T_6	15.22	16.67	12.06	37.88	15.07	2.97	2.5
T_7	16.57	17.78	12.58	39.49	18	4.13	2.5
T_8	17.37	18.28	12.64	39.72	19.41	4.2	2.52
T_9	15.19	16.5	12.03	37.79	13.65	2.83	2.4
F- test	S	S	S	S	S	S	S
S. Ed. (±)	0.12	0.09	0.06	0.24	0.37	0.10	0.09
C.D. (0.05)	0.24	0.18	0.14	0.50	0.78	0.21	0.20

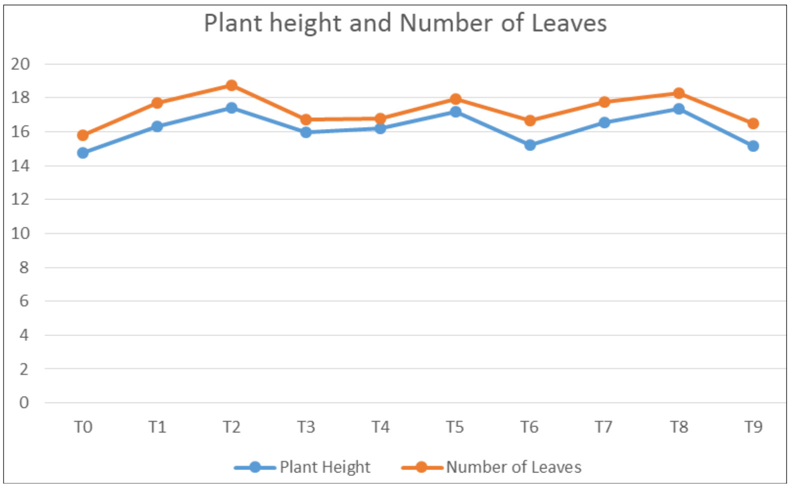

Fig 1: Plant height and Number of Leaves at 60 DAT

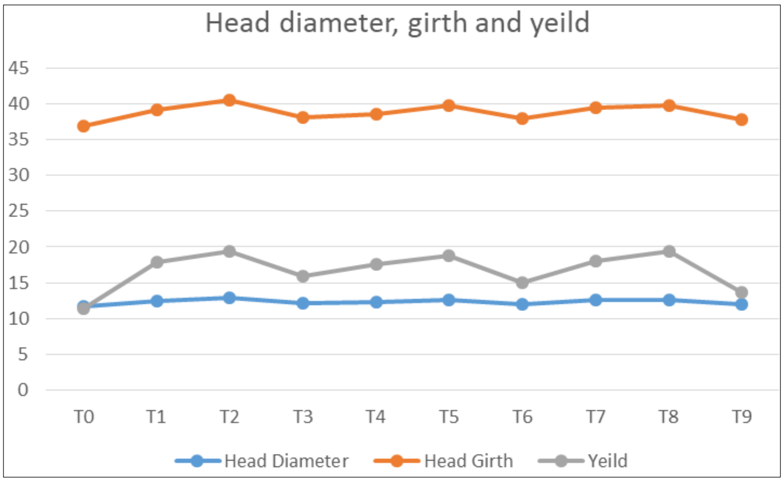

Fig 2: Head diameter, Head girth and Yeild of lettuce at the time of harvesting at 60 DAT

Table 4: Benefit: Cost analysis

Treatment combination	Cost of cultivation (Rs./ha)	Yield (T/ha)	Sale rate (Rs./Plant)	Gross return (Rs./ha)	Net Return (Rs./ha)	Benefit:Cost ratio
T_0	74,405	11.39	10	1,13,900	39,495	1.53
T_1	83,405	17.85	10	1,78,500	95,095	2.14
T_2	84,905	19.44	10	1,94,400	1,09,495	2.29
T_3	89,905	15.96	10	1,59,600	69,695	1.78
T_4	80,905	17.60	10	1,76,000	95,095	2.18
T_5	85,905	18.72	10	1,87,200	1,01,295	2.18
T_6	91,405	15.07	10	1,50,700	59,295	1.65
T_7	81,905	18.00	10	1,80,000	98,095	2.20
T_8	87,405	19.41	10	1,94,100	1,06,695	2.21
T_9	88,905	13.65	10	1,36,500	47,595	1.54

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