

## **Effects of Phosphate Solubilizers and Biochar on Growth and Yield of Tomato (*Solanum lycopersicum*)**

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### **Abstract**

A study was conducted to determine the effects of phosphate solubilizers and biochar on growth and yield of tomato plants. For this purpose, a 4×3 factorial experiment based on Completely Randomized Block Design (RCBD) with 4 replications were conducted in the growth room. In this factorial experiment, 4 levels of phosphate solubilizer bacteria (B1, B2, Bmix and Control) and 3 levels of biochar (Powder, Large Size, and Control) were applied on tomato plants to identify changes in growth parameters and yield. Results were analysed using Genstat Statistical Package (16th Edition). Analysis of Variance (ANOVA) were conducted to test the effects of each factor and their interactions. Subsequently, Tukey test was used to compare the treatments means at  $P < 0.05$ . The results showed that phosphate solubilizers had no significant effects on the growth parameters measured, while biochar treatments significantly affected the total fresh weight of the tomatoes and increased the speed of plant growth in tomatoes (Flowering and fruiting). In addition, there was no interaction between bacterial inoculation and biochar application on the parameters studied. The bacterial inoculation did not significantly affect any parameters studied. This is contrasted by the results from the treatments with biochar application which showed the enhancement of plant growth and yield. The sustainability of the bacterial strains used are suggested to be tested before starting the experiment.

**Keywords:** Biochar, Phosphate solubilizers, Plant growth, Plant yield, Tomato plant.

### **INTRODUCTION**

Tomato (*Solanum lycopersicum*) is a vital vegetable and contains important nutrients for human body such as phosphorus (P), iron (Fe), vitamins A and C (Pastor et al., 2014). With rapid increase in world population, there is a continuously growing demand for food crops including tomato. Since the amount of agricultural land worldwide is almost constant or even decreasing due to climate change, plant production should be increased to fulfil market demand. One of the methods extensively used by farmers is by enhancing the application of chemical fertilizer input. Phosphorus (P) is one of the most important macronutrients required by plants in soil. It plays an important role in many plant activities such as photosynthesis and cell division. However, most of phosphorus applied as fertilizer is in unavailable form for root uptake. Several researchers found that approximately 75-90% of applied P fertilizer is precipitated by calcium (Ca), iron (Fe) and aluminium (Al) metal cations and these insoluble forms are not completely taken up by the plants (Banerjee et al., 2010).

In addition, P fertilizers can contribute detrimental impacts to the environment and produce a huge cost production (Tilman et al., 2001; Sharma et al., 2013). Since the start of the last century, there have been efforts to enhance crops production whilst protecting human health, preserving the natural resources and the environment. These potential approaches will require an application of biological solutions, including phosphate solubilizers and biochar.

Phosphate solubilizers are able to switch organic and inorganic soil P through various mechanisms of solubilisation and mineralisation (Khan et al., 2009; Sharma et al., 2013) into the form which can facilitate uptake by plant roots (Sharma et al., 2013). Walpolo & Yoon

(2012) reported that several main processes which connected to the conversion process are chelation, exchange reactions, acidification of the medium and production of various acids. Some researchers also revised that there are several bacterial strains which can have the highest potential in converting insoluble compounds of phosphorus into available phosphates such as from the genera *Rhizobium*, *Bacillus* and *Pseudomonas* (Rodriguez & Fraga, 1999).

Plant growth promotion and improved yield availability due to inoculation of plants with phosphate solubilizers have been assessed in several studies under glasshouse conditions (Khan et al., 2010; Walpola & Yoon, 2012). Moreover, a stimulating impact on growth of plant has been proved with phosphate solubilizers in several plants such as tomato (Adesemoye et al., 2009), wheat (Babana & Antoun, 2006) and barley (Canbolat et al., 2006).

In recent years, considerable efforts have been made to minimize usage of chemical fertilizer. Biochar application is one of the methods used for soil amendment. Several studies have shown that biochar makes a positive contribution to improving soil fertility and plays a role in carbon sequestration when used as a soil amendment (Park et al., 2013). Therefore, the objective of this study was to determine the effects of phosphate solubilizers and biochar on growth and yield of tomato.

## RESEARCH METHOD

### Seeds Germination

Tomato seeds from 'Moneymaker' variety were chosen for this experiment. The seeds were germinated by using a propagator. Propagator lid was removed. Warm water was added to tray to expand pellets. Excess water was poured off when pellets fully expanded to about 2.5cm (1") tall. Approximately, 2-3 seeds were sowed per pellet. Lid was removed when most seedlings were appeared and kept in a warm place. Weaker seedlings were removed to leave one per pot.

### Biochar and Soil Preparation

Biochar was prepared by mashing and sieving with two particular sizes which are powder size (212 µm) and large size (2 mm). Topsoil was used in this experiment. The dry weight of each pack of topsoil used was calculated. Three topsoil samples were taken from each pack used. Fresh weight and dry weight of each sample were taken. The samples which were used for dry weight calculation have been put in the oven (75°C) for 24 hours.

### Pot Preparation

The topsoil was placed in pots (unit) (13.2 cm x 10.4 cm), with filter paper on the bottom inside of each pot. An amount of biochar was calculated which was needed to add 10% of the soil in each pot. The calculation was done on fresh weight basis. Soil and biochar were well-mixed. Biochar was added to the pot corresponding to the treatment. A hole was made and the peat block with seedling was slotted in. Then, a little amount of soil was firmed on it. The plants were applied daily with 50 mL deionized water and left for seven days before adding bacteria. The pots were located in a growth room.

### Preparation and Application of Inocula

Four phosphate solubilizer bacterial strains, namely Bacterium 1 (SR1), Bacterium 2 (ST12), Bacterium mix (SR1 + ST12) and uninoculated control (deionized water) were utilized in this study. For each bacterium, cells were scraped from the surface of nutrient

agar plates by using a sterile loop. The sterilized loop full of bacterium was added to the reagent bottles containing sterilized nutrient broth. Then, the bottles were shaken for 5 days until the broth was cloudy with the cells. After shaking for 5 days, falcon tubes were prepared by filling with 35 – 40 mL the cloudy media. The bacterial cells were then harvested by centrifugation at 4100/4200 rpm for 5 minutes in the falcon tubes.

After centrifuging, the supernatant was thrown and a small volume of deionized water added and mixed with the remaining bacterial cells in the falcon tubes. Then, the falcon tubes were vortexed. The liquid was then added to the flask corresponding to the bacteria. A volume of deionized water which is needed to the flask was added. Approximately, 10 mL of live bacterial cells were applied to each pot. The same amount of deionized water was applied to the uninoculated treatments. The bacterial population was further confirmed using dilution series and plating onto nutrient agar (NA).

### **Growth Stages Determination**

Growth stages of plant were determined by taking dates of each activity including pot transplanting, bacteria inoculating, first flowering, first bearing fruit and fruit ripening stages. The duration (days) between the stages was also calculated.

### **Tomato Fresh Weight Determination**

Tomatoes were harvested as they turned red colour. This was a continual process. All the red tomatoes from each individual plant were weighed. The weight and total numbers of the tomato that correspond to each individual plant were recorded.

### **Soil pH Determination**

Soil from each pot was well mixed. Soil pH (1:2.5; fresh soil: deionized H<sub>2</sub>O) was determined by using a pH meter.

### **Soil Dry Weight and Moisture Content Determination (after experiment)**

Dry weight of soil from each pot was determined. The fresh soil of each sample was then put in the oven (60°C) for 24 to 48 hours. After getting the dry weight, soil moisture content was calculated.

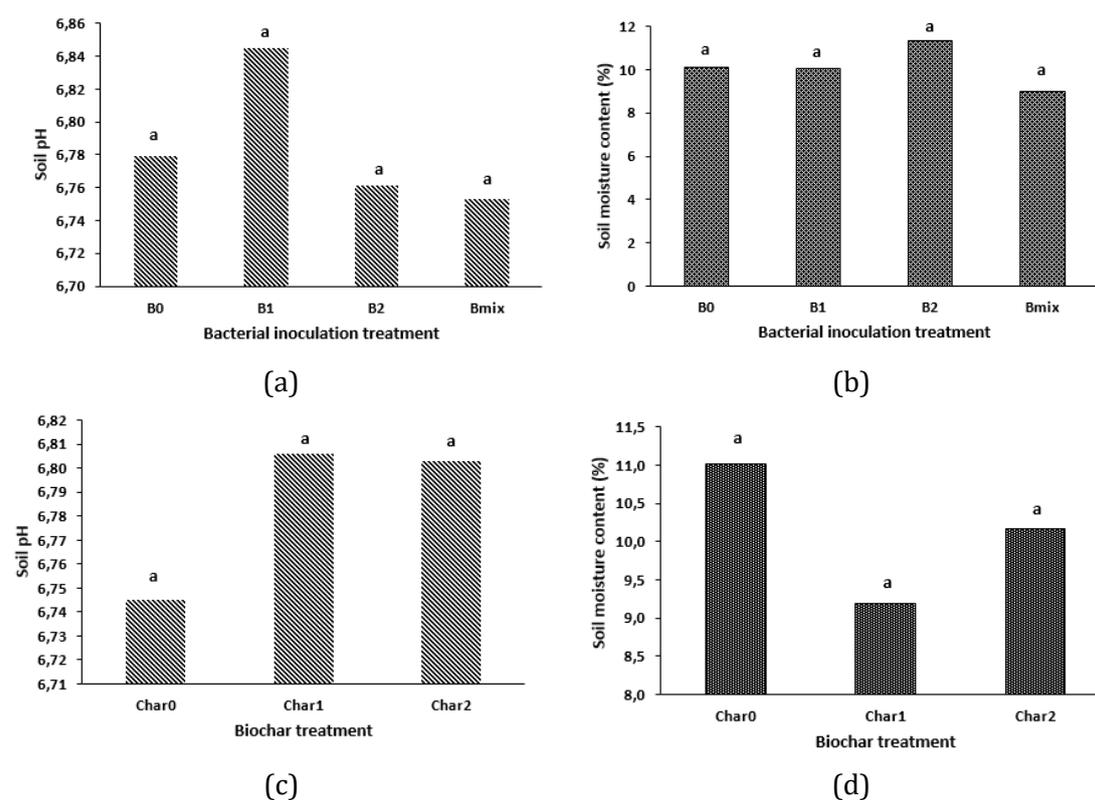
### **Statistical Analysis**

This study was conducted using three different bacterial isolates which are capable of solubilizing phosphates and one control treatment where no bacteria was inoculated. Treatments with biochar application which were powder and large sizes were also used in this experiment in a factorial arrangement. This factorial experiment (4 bacterial treatments x 3 biochar treatments) with 4 replications was laid out based on Randomized Complete Block Design (RCBD). The data collected were analysed using Genstat (16th Edition). Tukey test ( $P < 0.05$ ) was used to compare the treatments means.

## **RESULTS**

### **Soil pH and Soil Moisture Content**

The effect of bacterial inoculation and char application on soil pH and soil moisture content was shown below (See Figure 1). The soil pH and soil moisture content were unaffected by bacterial treatments and char application. There was no interaction between bacteria and biochar.



**Figure 1.** Effect of bacterial inoculation on soil pH (a) and soil moisture content (b); and then effect of biochar inoculation on soil pH (c) and soil moisture content (d).

\*Means value in a graph followed by same letter(s) are not significantly different using Tukey's Test at 5%.

### Total Fresh Weight of Tomatoes

The effect of bacterial inoculation and char application on total fresh weight of tomatoes was shown below (See Table 1). Data were analysed by two-way ANOVA. The total fresh weight of tomatoes was unaffected by bacterial treatment. However, biochar application as a single factor was significantly affected the total fresh weight of tomatoes. There was no interaction between bacteria and biochar.

**Table 1.** Two-way Analysis of variance (ANOVA)

Source of variation	Degree of freedom	Mean square
Blocks	3	1428.5 **
Bacteria	3	62.9
Char	2	854.2 *
Bacteria*Char	6	47.0
Residual	32	214.7
Total	46	

\* Significant difference at 5% level.

\*\* Significant difference at 1% level.

Table 2 shows the means of total fresh weight of tomatoes between bacterial inoculation (a) and biochar (b) treatments.

**Table 2(a).** Means of total fresh weight of tomatoes among bacterial inoculation treatments.

Treatment	Total fresh weight (g)
B0	51.2
B1	46.2
B2	50.1
Bmix	50.8

**Table 2(b).** Means of total fresh weight of tomatoes among biochar treatments.

Treatment	Total fresh weight (g)
Char0	41.3
Char1	52.4
Char2	55.0

### Number of Days between Key Tomato Growth Stages (Inoculating to Flowering, Flowering to Fruiting, Fruiting to Ripening)

The effect of bacterial inoculation and biochar application on number of days between key tomato growth stages was shown below (Table 3). Data were analysed by two-way ANOVA. The total number of days between different stages in tomato fruit development were unaffected by the bacterial treatments. However, biochar amendment resulted in faster flowering and fruiting (Table 3b), but not in quicker ripening. Further analysis for the flowering and fruiting stages using Tukey test showed that there was significant difference between treatments with biochar application and treatments without biochar application. On the other hand, there was no interaction between bacteria and biochar treatments.

**Table 3(a).** Effects of bacterial inoculation on number of days between key tomato growth stages

Treatment	Number of days between growth stages			Total
	Inoculating bacteria until flowering	Flowering until fruiting	Fruiting until ripening	
B0	44.3 <sup>a</sup>	10.0 <sup>a</sup>	39.8 <sup>a</sup>	94.1
B1	45.1 <sup>a</sup>	11.3 <sup>a</sup>	44.7 <sup>a</sup>	101.1
B2	39.0 <sup>a</sup>	13.7 <sup>a</sup>	44.2 <sup>a</sup>	96.9
Bmix	38.7 <sup>a</sup>	14.0 <sup>a</sup>	42.3 <sup>a</sup>	95.0

**Table 3(b).** Effects of biochar application on number of days between key tomato growth stages

Treatment	Number of days between growth stages			Total
	Inoculating bacteria until flowering	Flowering until fruiting	Fruiting until ripening	
Char0	52 <sup>a</sup>	16 <sup>a</sup>	43 <sup>a</sup>	111
Char1	36 <sup>b</sup>	11 <sup>b</sup>	41 <sup>a</sup>	88
Char2	37 <sup>b</sup>	10 <sup>b</sup>	44 <sup>a</sup>	91

\*Means in a column followed by different letter (s) are significantly different and same letter (s) are not significantly different using Tukey's Test at 5%.

## DISCUSSION AND CONCLUSION

The results of this study showed that the association of phosphate solubilizers with the tomato plants did not significantly affect any of the growth parameters studied. These results might due to the sustainability of the strains used was lost throughout the experiment. However, biochar is known as a microhabitat for microorganisms. Large surface area of biochar seems likely to benefit microhabitat for microorganisms. Previous researchers also proved that biochar has potential as microhabitat (Schnee et al., 2016).

Biochar amendment was significantly affected the total fresh weight of the tomatoes. In addition, soil amendment with biochar significantly increased the speed of plant development (Flowering and fruiting). Previous researchers also reported that charcoal amendments can increase seed germination, crop yields and crop quality (Rondon et al., 2007). Some of the previous researchers also proved that biochar can contribute a greater water holding capacity when it has been added to light-textured soil (Glaser et al., 2002).

Biochar amendment did not significantly affect the soil analyses in this study. However, several studies have shown that biochar makes a positive contribution to improving soil fertility and plays a role in carbon sequestration when used as a soil amendment (Woolf et al., 2010; Park et al., 2013). In addition, there was no interaction between bacterial inoculation and biochar application.

The use of biological inoculants such as phosphate solubilizers and biochar application appear to be a promising alternative to chemicals. However, results of this study showed that the association of these phosphate solubilizer strains with the plants did not significantly affect any of the growth parameters studied. On the other hand, soil amendment with biochar application significantly affected the total fresh weight of tomatoes and increased the speed of plant development. These results might be due to the biochar is known to improve the yields and quality of the crops.

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