

Research Article

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The effect of soil water content and biochar on rice cultivation in polybag

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Abstract: Rice (*Oryza sativa* L.) production is important in the national food of Indonesia. The growth and yield of rice can be increased by the soil water supply and biochar application into the soil in a polybag. Water is a unique material resource that plays a vital role in agriculture. Biochar is a carbon-rich product obtained from biomass and can hold water and nutrients, making them more available to plants. The biochar used in this study was made from rice husks. This study aims to determine the effect of soil water content and biochar application in the soil on the growth and yield of rice in the polybag. The experiment appears to be a randomized multifactorial design with one factor being water content and the other being biochar application rate. A completely random design usually suggests only one factor in the experimental design. The first factor was soil water content consisting of two levels, i.e.: field capacity and soil waterlogging. The second factor was the biochar application consisting of four doses i.e.: 0; 14; 28; and 42 tons/ha. The results of the research showed that rice cultivation with soil waterlogging is better than field capacity on the tillers number, panicle length, and harvest index. Without biochar application was given higher tillers number, but biochar dose of 14 tons/ha produced wider leaf area. There was a significant interaction between soil water content and biochar application on the dry weight of roots, shoots, and grains. The treatment combination of soil waterlogging and biochar dose of 14 tons/ha was most effective at increasing the growth and yield of rice in a polybag.

Keywords: Field capacity; Soil waterlogging; Rice husk biochar; Rice cultivation; Polybag

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1 Introduction

Rice (*Oryza sativa* L.) production is an important national food for Indonesia. The conversion of agricultural land into non-agricultural applications, which is increasingly more difficult to control around urban areas, causes rice fields to be narrower. As a result, the ability of the agricultural field to support national food needs is decreasing. One way to get around the narrowness of agricultural field is by rice cultivation in the polybag (Humaerah 2013). Rice cultivation with polybags around urban areas has been adopted by the many farmers.

Water is a unique material resource that plays a vital role in nature and in agriculture (Mbah 2012). The knowledge about the soil water content at field capacity or soil waterlogging is very important for assessing plant water requirements, irrigation scheduling, and predicting crop responses to irrigation. Field capacity is the amount of water remaining in the soil after all gravitational water has drained. The remaining water is held in micropores via attractive 'capillary' forces or surface tension between the water and soil solids (Elkheir 2016). The definition of soil waterlogging is the condition where the soil is fully saturated with water. In soil waterlogging, the diffusion of gases through soil pores is strongly inhibited by their water content, which fails to match the needs of growing roots (Morales-Olmedo et al. 2015).

At the beginning of plant growth, the need for water is low because the size of the habitus is small, so that the surface area of plants, which is conducting evapotranspiration, is low. Water requirements for plants are highest in periods of maximum vegetative growth. At that time the plant surface area reached its highest level, resulting in the highest evapotranspiration (Pinem and Ichwan 2017). Water-wise rice production is now a primary concern that ensures the saving of a considerable amount of freshwater as well as overcoming water shortages for rice production. By reducing water input, compared with the traditional practice, water-wise rice cultivation sustains rice production without affecting plant and soil parameters (Jahan 2018).

Treatment of alternative wet and dry (AWD) significantly decreased plant height, tiller numbers, panicle numbers, filled grains, yield, and harvest index, but increases unfilled grains compared with other treatments. Soil saturation using 1 cm flooding saves 45% of the water use compared with flooding at 5 cm depth, and showed higher water use efficiency (WUE) but produced a similar rice yield to saturated to 1 cm flooding and 1-3 cm deep flooding. Saturation to 1 cm could be easily implemented by the farmers in rice cultivation without affecting rice production, plant and soil characters (Khairi et al. 2015). The increase in water use was greater than the increase in grain yield in the case of maintaining soil moisture at 100% of saturation before reflooding (Hamoud 2018).

Continuous watering can cause the soil in the polybags to become denser. In anticipation of these conditions a soil conditioner can be added. According to Hilber et al. (2012), biochar is increasingly promoted as a beneficial soil conditioner. However, it may contain residues of polycyclic aromatic hydrocarbons (PAHs) as a result of its production by pyrolysis. According to Milla et al. (2013), the partially burned rice husk (husk biochar) enhances soil water holding capacity.

Biochar is charcoal or biomass that has been burned (pyrolysis) in environmental conditions with no or low oxygen. The scientific agreement states that giving biochar into the soil is expected to improve carbon sustainably and can simultaneously improve soil function (current and future management) while avoiding damaging effects in the short and long term for the wider environment and for both human and animal health (Verheijen 2009). Biochar can be produced from a variety of biomass feedstock but is generally designed only as a product that can be used for soil improvement (McLaughlin et al. 2009) and presents a small survey of measured properties. Explicit terminology is proposed about “resident and mobile carbon and other matter” in biochars intended for addition to soils rather than for use as a fuel. Specific data are presented for commercial lump charcoals and Top-Lit UpDraft (TLUD).

Biochar is produced by thermal decomposition of organic material with a limited supply of oxygen (O_2) and at relatively low temperatures ($< 700^\circ C$) (Lehmann and Joseph 2012) manure or crop residues. The raw materials that can be used to make biochar are biomass waste that is not otherwise utilized, namely: rice husks, corncobs, cocoa pods, hazelnut shells, coffee skins, wood sawdust, eucalyptus oil leaves, wood branches, the waste of animal feed residues, coconut shells, and the like (Widiastuti and Lintang 2017).

The benefits of biochar are determined by two main properties, which are a high affinity for nutrients and persistence in the soil. Biochar provides habitat for microbes in the soil, but it is not consumed and can persist in the soil for hundreds or even thousands of years. Biochar persistence will not upset the carbon-nitrogen balance in the soil but can hold water and nutrients, making them more available to plants. Organic and inorganic fertilizers with biochar can increase productivity, as well as nutrient retention and availability for plant roots (Gani 2009).

Utilization of rice husk for biochar is one of the innovations that can be applied by farmers to overcome problems in agriculture, such as reducing the level of soil acidity, increasing crop productivity, and storing carbon stocks to overcome global environmental problems (Widiastuti and Lintang 2017). Utilization of biochar as a soil enhancer in the first planting season has produced good soil physical properties for the second planting season which directly gives positive results for the growth and yield of lowland rice. The application of a biochar dose of 10 tons/ha can increase rice yield, in the form of grain, by 13.5% compared with the control of 5.11 tons/ha to 5.80 tons/ha (Waty et al. 2014). The use of a biochar dose of 8 tons/ha can give a significant increase in plant height, the number of leaves, leaf area and weight of mustard plants (Musnoi et al. 2017). The combination of rice straw (10 tons/ha) and biochar from rice husks (10 tons/ha), together with the use of chemical fertilizers, makes it possible to increase the weight of grains/panicle for better results compared with only the application of chemical fertilizers (Thavanesan and Seran 2018).

Biochar is able to increase the availability of soil water. The highest availability of pores for water was found in coconut shell biochar by 21.55% and followed by risk husk biochar and the lowest was in wood biochar. The highest percentage of available pore spaces for water was found in the administration of biochar doses of 45 tons/ha and the lowest was followed by biochar doses of 30 and 15 tons/ha (Khoiriyah et al. 2016). The interaction between the biochar dose of 15 tons/ha with 60 mesh particle size can reduce the soil pH by 5.19% (from 7.7 to 7.3) and increase the soil CEC by 32.92% (from 16.37 to 22.25 $cmol^+/kg$). This can also improve the soil organic-C by 33.94% (from 1.09% to 1.46%), and enhance the soil available phosphorous by 277.08% (from 12.61 ppm to 47.55 ppm) (Salawati et al. 2016). The use 75% of biochar (7.5 tons/ha) + 25% straw compost (2.5 tons/ha) produces 29 tillers, 8.23 tons/ha of rice production and is able to provide nutrients in the ultisol soil by increasing pH, N, P, K, Ca, Mg, and S (Herman and Resigia 2018).

Biochar significantly affected the following yield components: the number of tillers, percentage of the productive tillers, number of grains/panicle, panicle density, percentage of filled grain, and weight of 1,000 grains (12). The purpose of this study is to determine the effect of soil moisture content and the application of biochar in the soil on the growth and yield of rice in polybags. Groundwater inundation and biochar dose of 14 tons/ha are expected to provide higher rice growth and yields in polybag cultivation.

2 Materials and methods

2.1 Experiment area

The study was conducted from April to September 2019 in the greenhouse of Agrosop garden, Faculty of Agriculture, Universitas PGRI Yogyakarta having an elevation of 118 m above mean sea level (MSL) in the position 70 33' LS – 8 12' LS and 110 00' BT – 110 50'.

2.2 Production and application of biochar

The biochar used in this study was made from rice husks. The biochar characterization was done using a simple pyrolysis apparatus made from a modified drum as a biochar reactor, with fresh rice husk from rice milling being put in the reactor. Burning start from the bottom of the reactor with the average temperature of 225°C in the reactor. After 5 hours, the husks changed into biochar.

During the pyrolytic production of biochar, polycyclic aromatic hydrocarbons (PAHs) can form and are present on the surface of biochar. The pyrolysis process is the key factor responsible for the yield of PAHs in biochar. Slow pyrolysis and longer residence time result in lower PAHs yields than fast pyrolysis and shorter residence time. Temperature is also another significant determinant affecting the formation and yield of PAHs. Low molecular weight PAHs are usually formed at low temperatures (< 500°C) whereas the high molecular weight PAHs commonly appear under high temperatures (> 500°C). Analytical methods for the extraction of PAHs from biochar mainly include Soxhlet extraction and accelerated solvent extraction (Wang et al. 2017).

Rice husk biochar had higher concentrations of PAHs (64.65 mg/kg) than wood biochar (9.56 mg/kg), and both

soil types contained quantifiable levels of PAHs. However, soil that had contained biochar for 3 years had significantly higher levels of PAHs (1.95 mg/kg) compared to unamended soil (1.13 mg/kg) (Quilliam et al. 2013).

The chemical content of biochar was not analyzed by the researchers. The researchers used the results of chemical data analysis by Bakri (2009), which indicated that the most dominant chemical component contained in the rice husk ash produced SiO₂ (72.28%), and incandescent lost compounds (21.43%), while the percentage of CaO (0.65%), Al₂O₃ (0.37%), and Fe₂O₃ (0.32%) compounds were classified as very low.

The biochar needed (g/polybag) for each treatment can be calculated. The formula used to calculate the soil dry weight per hectare (kg/ha) = soil area of one ha (m²) x soil tillage depth (m) x soil bulk density of alluvial (kg/m³). If it is known that the soil area of one hectare is 10,000 m², soil tillage depth of 0.2 m, and bulk density of 1.4 kg/m³, then the soil dry weight per hectare is 2,800,000 kg/ha. The doses of biochar application consisted of four levels i.e.: 0; 14; 28 and 42 tons/ha (or 0; 1,400; 28,000; and 42,000 kg/ha). If it is known that the dry weight per polybag is 15 kg/polybag and the soil dry weight per hectare is 2,800,000 kg/ha, so the doses of biochar application per polybag (kg/polybag) can be calculated by the following formula =

$$\frac{\text{Soil dry weight per polybag (kg/polybag)} \times \text{doses of biochar (kg/ha)}}{\text{Soil dry weight per hectare (kg/ha)}}$$

Based on this formula, so the doses of biochar application for each treatment i.e.: 0; 0.075; 0.150; and 0.225 kg/polybag, respectively, which was the same as 0; 75; 150; and 225 g/polybag.

2.3 Experiment design

This experiment was arranged in a completely randomized factorial design (CRD), repeated three times. The first factor was soil water content, which consisted of two types of soil conditions: field capacity and soil water-logged. The second factor was the application of biochar, which consisted of four doses: 0; 14; 28; and 42 tons/ha (or 0; 75; 150; and 225 g/polybag). This research consisted of eight treatment combinations and was repeated three times, where each repetition consisted of three samples so that it consisted of 72 polybags.

2.4 Soil media preparation

The soil dry weight of 15 kg, to which was added 25 g of TSP fertilizer and the dose of biochar, according to the treatment, and then mixed evenly. The soil media mixture was put in a polybag. The polybag had a diameter of 40 cm and a height of 30 cm. The filling of soil media mixture was conducted on the 72 polybags. According to Humairah (2013), the diameter of the pot affects the number of panicles produced by rice plants. The diameter of 40 cm produces more panicles than the diameter of 30 cm.

2.5 Ciherang variety

Rice yield could be increased by the use of a superior rice variety. Ciherang is a new superior variety that can adapt well to the environment to ensure better plant growth, high production, and good quality as well as delicious and fluffier rice flavors so that it can be accepted by the market.

The Ciherang variety has a growing season of 116 to 125 days. Plant are erect, with a plant height of 107 to 115 cm. They have productive tillers of 14 to 17 stems, with an average and potential rice yield of 6.0 and 8.5 tons/ha. They are resistant to brown plant hopper biotype 2 and 3, and resistant to bacterial leaf pest's strains III and IV. Ciherang was suitable for planting in the lowland irrigated rice fields (Anonymous 2009).

2.6 Nurseries and seedlings

The rice variety used is Ciherang. The rice seedlings were grown in plastic germination tubs, with dimensions of 25 cm x 30 cm x 10 cm (length x width x height) that were filled with soil media. The seeds were spread out on the surface of soil media. Then, seeds were covered slightly with soil. The soil media was watered until field capacity. The seeds germinated in about 4 days after sowing.

2.7 Planting and plant spacing

Before planting, the soil media in the polybag was watered to field capacity. Then, the seedlings were transplanted into the polybags at 10 days after planting (DAP). The plant number per polybag consisted of three clumps. Each polybag has three holes and one hole is planted with two seedlings. The planting system used the equilateral

triangle model. The plant spacing between holes in the polybag was 25 cm x 25 cm.

2.8 Soil water content

The treatment of field capacity and soil waterlogging was begun from planting to 30 days. After that, there was no difference in the watering treatment in the polybags. For the soil waterlogging treatment, the water was as high as 3 cm from the soil media surface in the polybag. Any decrease in the water volume in the polybags was added by watering. For the field capacity treatment, the soil moisture was maintained through watering of the soil media in the polybag.

2.9 Observation parameters

Observations on the growth and yield of rice consisted of the tillers' number (stems), leaf area (dm²/clump), dry weight of shoot (g/clump), root (g/clump), grains (tons/ha), panicle length (cm), and harvest index. The leaf area was measured with a portable pallet leaf area meter (model CI-202). The dry weight of roots, shoots, and grains was measured using a digital analytical balance (ACIS AD-i Series). The panicle length (cm) was measured using a ruler. The harvest index was measured with the formula: a ratio of economic yield (grain dry weight) and biological yield (the dry weight of grains, leaves, stem, roots). The dry weight grains per hectare (tons/ha) were measured using the formula = the dry weight grains per clump (g) * the plant number per hectare (clumps). The plant number per hectare, with a plant spacing of 25 x 25 cm², is 160,000 clumps.

2.10 Statistical analysis

Data were analyzed by analysis of variance (ANOVA) at P-value 0.05 (Gomez and Gomez 1984). The analysis of data was performed using the IBM SPSS Statistics 23 software. Duncan's new multiple range tests (DMRT) at P-value 0.05 was used to determine the differences between treatments.

3 Results and discussion

3.1 Tiller number, leaf area, panicle length, and harvest index

The results of the analysis of variance on the tiller number, leaf area, panicle length, and harvest index showed no significant interaction occurred between the treatment of soil water content and biochar application. The treatment of soil water content significantly affected the tiller number, panicle length, and harvest index. The biochar application significantly affected the tiller number and leaf area. A comparative test between the averages of treatments, based on DMRT at P-value 0.05, for the tiller number, leaf area, panicle length, and harvest index is presented in Table 1.

3.2 The water effect

The soil water content significantly affected the tiller number, panicle length, and harvest index, but there was no significant effect on the leaf area. Rice planted in waterlogged soil had a higher number of tillers, panicle length and harvest index compared to rice grown in soil at field capacity. The Ciherang variety is more suitable to grow in waterlogged soil because it forms more tillers. The water content in the field capacity was not enough for optimal growth of the Ciherang variety. According to Subari et al. (2012), rice plants are able to grow well in waterlogged soil, because it has the ability to oxidize its root area through

a network of parenchyma that can diffuse oxygen to the root area. Oxygen from the leaf flows through a process of diffusion to the roots and stems through the cortex. The existence of this process in rice plant is able to meet the oxygen needs for root respiration even in waterlogged soil conditions.

The occurrence of water deficit at the reproductive stage affects rice grain quality, but it could not be stated that water stress improves or decreases rice grain quality (head rice ratio, amylose content, protein content, and gel consistency). Soil water deficit has significant implications on rice grain quality (Bleoussi et al. 2016). The rice grown in soil at field capacity was not optimal, because water shortages often occur due to watering delays. Soil water content in the root zone affects all aspects of plant growth including the physiological and biochemical processes. The water will further cause part of the stomata to close and inhibit the entry of CO₂ and further inhibit the photosynthesis process. The low carbohydrate production will decrease the growth and yield of rice.

Water is a raw material for photosynthesis, solvents and biochemical reactions, and transport medium compounds. Water provides turgor for cells for cell division and enlargement and maintains constant plant temperature. Water deficit causes biochemical processes in the body of the plant to be disrupted. The available water content in the soil will cause the pressure of plant cell turgor to be more maintained so that cell photosynthetic activity is better and more carbohydrates are produced. Carbohydrates are used as an energy source for the division of plant cells, improving tiller number, panicle length, and harvest index.

Table 1: The Effect of Soil Water Content and biochar application on the Tillers Number, Leaf Area, Panicle Length and Harvest Index

Treatment	Tiller number (stem/clumps)	Leaf area (dm ² /clumps)	Panicle length (cm)	Harvest index
Soil water content				
Field capacity	13.06 b	24.47 a	18.74 b	0.29 b
Soil waterlogging	15.15 a	24.36 a	21.91 a	0.35 a
Doses of biochar (g/polybag)				
0	17.03 p	25.59 p	19.87 p	0.31 p
14	13.80 q	27.76 p	20.43 p	0.32 p
28	13.00 q	24.59 p	20.45 p	0.32 p
42	13.31 q	20.12 q	20.53 p	0.32 p
Interaction between treatment	(-)	(-)	(-)	(-)

Remarks: Number in the same column followed by the same characters are not significantly different based on DMRT at P-value 0.05. (-) = No interaction between the soil water content and biochar application

3.3 The biochar application

Biochar application significantly affected the tiller number, and leaf area, but did not significantly affected the panicle length, and harvest index. The treatment without the application of biochar (0 tons/ha) to the soil had a higher number of tillers. The tiller number was inhibited by biochar application. This was different from the observed leaf area, which showed no significant differences between biochar doses of 0, 14, and 28 tons/ha. However, the biochar dose of 42 tons/ha resulted in a decreased leaf area. The biochar application exceeds the optimal limit causing narrowing of the leaves.

The application of biochar decreased soil bulk density, soil strength, exchangeable Al, and soluble Fe and increased porosity, available soil water content, C-organic, soil pH, available P, CEC, exchangeable K, and Ca (Masulili et al. 2014). Application of biochar beyond the optimal limit actually causes the shoots, roots, and grain to decrease. The biochar application of 14 tons/ha in the waterlogged soil is the optimal dose. The biochar application could have functioned as a soil amendment and improve the soil's physical properties such as increasing soil pH. The increase of soil pH results in a favourable environment for rice's roots to development. The near-neutral pH conditions result in the optimum availability of nutrients that facilitated higher phosphate absorption in addition to other nutrients. Phosphate is needed for roots

development as the nutrient source for a plant that substitutes inorganic fertilizer role and can be categorized as a chemical function, although this function has not yet been properly implemented by biochar.

3.4 The dry weight of shoots, roots, and grains

Analysis of variance on the dry weight of shoots, roots, and grains showed a significant interaction between the soil water content and biochar application. Comparative test results between treatment interactions based on DMRT at P-value 0.05 on the dry weight of shoots, roots, and grains are shown in Table 2.

The combination treatment between the soil water content and biochar application had a significant effect on the dry weight of shoots, roots, and grain. The rice plants growing in the waterlogged soil, combined with the biochar doses of 0 and 14 tons/ha, give a higher dry weight of shoots, roots, and grain than in biochar doses of 28, and 42 tons/ha (Table 2). The dry weight of shoots, roots, and grains were lower in rice plants in the soil at field capacity with the biochar application doses of 0; 14; 28; or 42 tons/ha. The biochar application in the field capacity soil cannot increase the growth and yield of rice.

The nutrient absorption process can occur well if the Cihorang grows in the waterlogged soil. The turgor pres-

Table 2: The Effect of Treatment Interactions between Soil Water Content and Biochar Application on the Dry Weight of Shoots, Roots, and Grains

Combination of treatment	Shoot dry weight (g/clumps)	Root dry weight (g/clumps)	Grains dry weight (tons/ha)
A ₁ + B ₀	28.83 d	14.61 cd	2.66 c
A ₁ + B ₁	30.33 d	15.08 cd	3.04 c
A ₁ + B ₂	32.81 d	14.81 cd	3.14 c
A ₁ + B ₃	28.31 cd	13.88 d	2.80 c
A ₂ + B ₀	51.48 a	24.67 a	6.57 a
A ₂ + B ₁	51.30 a	24.85 a	6.62 a
A ₂ + B ₂	42.81 b	19.77 b	5.34 b
A ₂ + B ₃	39.39 bc	17.65 bc	4.74 b
Interaction between treatment	(+)	(+)	(+)

Remarks: Number in the same column followed by the same characters are not significantly different based on DMRT at P-value 0.05, A₁ = Field capacity, A₂ = Soil waterlogging, B₀ = 0; B₁ = 14; B₂ = 28; and B₃ = 42 tons/ha. (+) = Significant interaction between the soil water content and biochar application

sure of plant cells can be maintained properly if the water is available in the soil. The process of photosynthesis can occur more smoothly and produce maximum carbohydrates if the water needs of rice plants are properly met. Carbohydrates are used as an energy source to improve the dry weight of stem, leaf, and root tissue. Some of the remaining carbohydrates, which are stored in the body of rice plants in the leaves, stems, and roots, are then transferred to the seeds during grain filling in the generative phase.

Figures 1, 2, and 3 show the effect of the interaction between the soil water content and the biochar application on the dry weight of shoots, roots, and grains. Based on Figures 1, 2, and 3 waterlogged soil without the biochar application or with application doses of 14 tons/ha of biochar gave higher growth and yield of rice than the biochar application doses of 28 and 42 tons/ha on the dry weight of shoots, roots, and grains. The interaction between field capacity and all doses of biochar given lower dry weight of shoots, roots, and grains.

3.5 Water and crop performance

Rice that grows in standing water is higher than when it grows in rice fields. Rice leaves appear greener when flowering in ground water puddles than rice in paddy fields. Rice plants flower faster in puddles of ground water than in field capacity. Effect of soil water content on plant height and greenish leaves can be seen in Figure 4.

The effect of water content on the performance of root density in rice plants that grow in waterlogged soil was compared with rice grown in soil at field capacity. Noticeably denser roots occurred with biochar applications of 0 or 14 tons/ha. The root density correlated with the dry weight of roots and then supported the growth and yield of the rice plants. There did not appear to be any difference in the root length between the rice plant that grew in the waterlogged soil compared with the plants grown in soil at field capacity. The root is an important organ for plants and it is directly related to the soil conditions. Root density of rice affects the ability of rice plants to absorb nutrients in the soil to support overall growth. Nutrients are needed by rice plants to support biochemical pro-

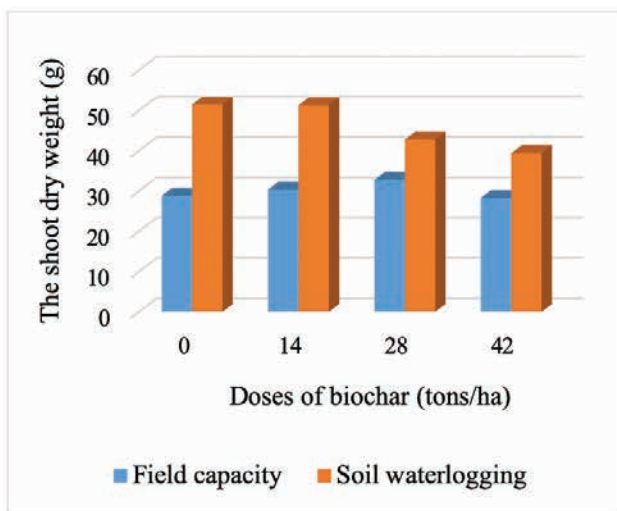


Figure 1: The Interaction between Soil Water Content and Biochar Application on the Shoots Dry Weight

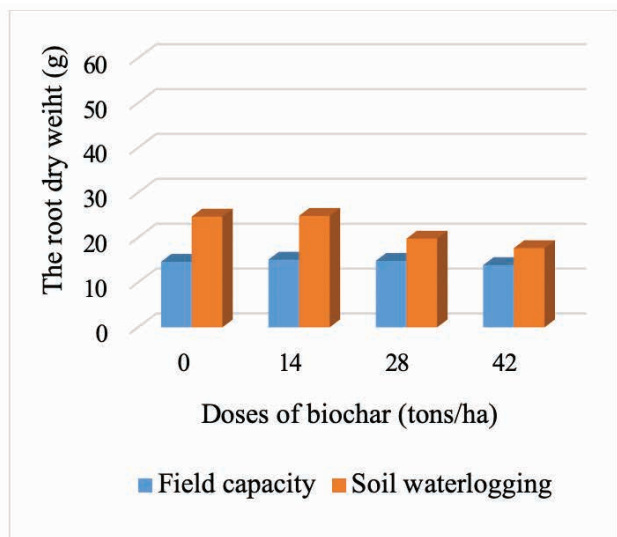


Figure 2: The Interaction between Soil Water Content and Biochar Application on the Roots Dry Weight

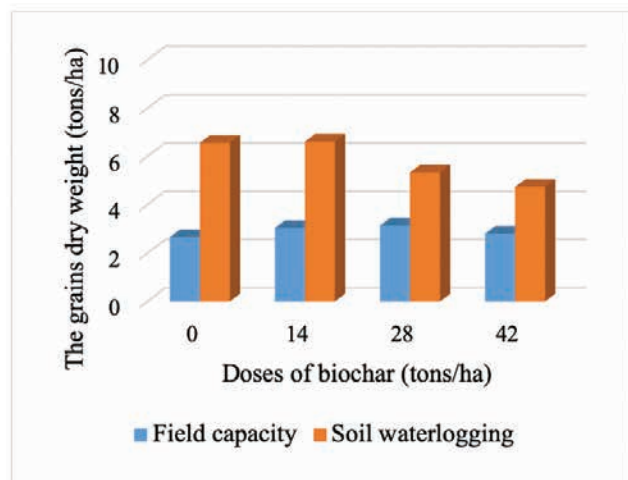


Figure 3: Interaction between Soil Water Content and Biochar Application on the Grains Dry Weight

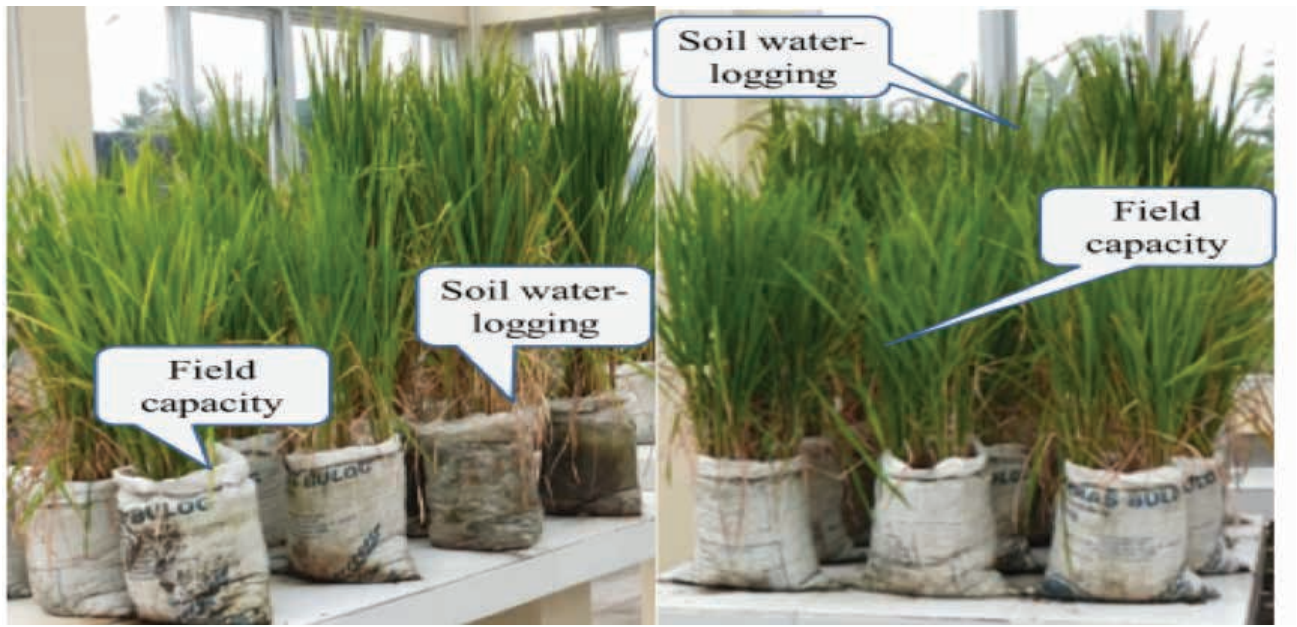


Figure 4: The Effect of Soil Water Content on the Plant Height and Greenish Leaves Performance

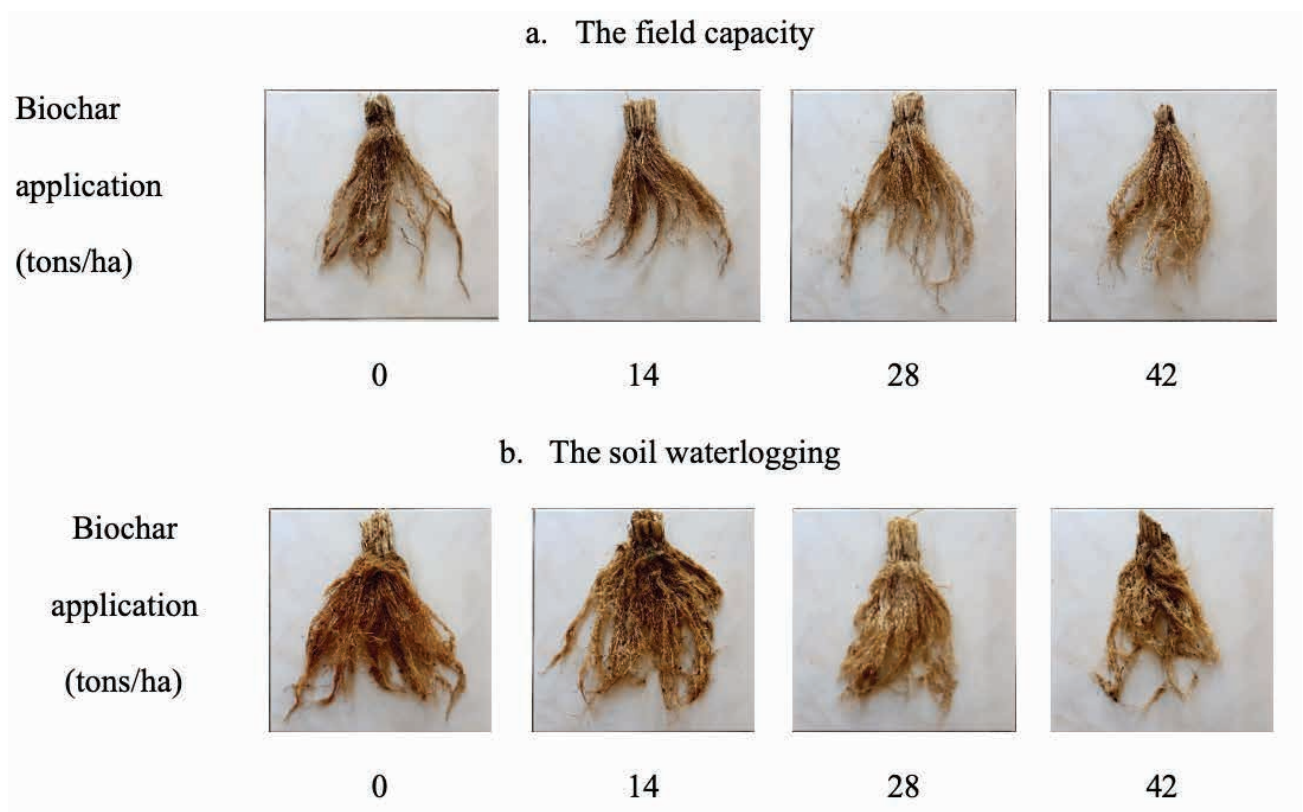


Figure 5: The Effect of Soil Water Content on the Root of Rice Performance in the four doses of biochar application

cesses in plants. The effect of soil water content on the performance of lowland rice roots can be seen in Figure 5.

4 Conclusion

Based on the results of the analysis and discussion it can be concluded that rice cultivation with waterlogged soil is better than rice grown in soil at field capacity in terms of the tiller numbers, panicle length, and harvest index. The treatment without biochar application gave higher tiller numbers, but the biochar dose of 14 tons/ha produce more leaf area. There was a significant interaction between soil water content and biochar application on the dry weight of roots, shoots, and grains. The treatment combination of the waterlogged soil and a biochar dose of 14 tons/ha was more effective in increasing the growth and yield of rice grown in a polybag. Rice cultivation in polybags with sufficient water supply can be done in and around the urban areas.

Conflict of interest: Authors declare no conflict of interest.

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