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EFFECT OF FORMULATED BIOCHAR ON NODULE PRODUCTION, DRY MATTER AND GRAIN YIELD OF BLACK SOYBEAN (*Glycine max* (L.) Merr) IN INDONESIA

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ABSTRACT

Biochar is an important material for soil mineral supply and amendment. Root nodule formation in leguminous plants is also stimulated by biochar. Biochar contains carbon which can be used as an energy source by microbes and contains pores which are suitable for microbial habitat, and it temporarily increases activity of free-living nitrogen-fixing bacteria. Coconut shell biochar can be used as an ameliorant, but the use of single coconut shell biochar can cause a problem such as the use of high dose that will lead into high cost. One of the solutions to solve the problem is to improve coconut shell biochar into formulated biochar (FB) by managing the composition and enrich it with chemical fertilizer's such as dolomite, organic fertilizer compost of sugarcane bagasse and guano. Mixture of formulated biochar (FB) gave better results than single biochar alone on black soybean. When this FB is applied in the field, it can make efficient use of an organic fertilizers in increasing grain yields. This research was conducted to determine the effect of Formulated Biochar (FB) on nodule production, dry matter and productivity of black soybean seeds. The study was conducted for one season in an experiment field in Winaya Mukti University, Sumedang-Indonesia at 850 m above sea level (m ASL). Detam I seed cultivar was used because it has a wide adaptability in Indonesia and has high nutrient composition and yield potential. The experiment was arranged in a randomized block design consisting of 5 treatment doses of FB (0, 0.5, 1.0, 1.5, 2.0 tons ha⁻¹) and replicated 5 times. The responses observed were the number of effective nodules, growth characteristics, production components and productivity of black soybeans. The results showed that the application of FB had a significant effect on increasing the effectiveness of nodule production, dry matter, and productivity of black soybean seeds. The formulated biochar dose of 2.0 tons ha⁻¹ increased the effectiveness of nodule production up to 100% and 44.87% of soybean productivity, while the formulated biochar dose of 1.5 tons ha⁻¹ increased soybean productivity by 38.46% compared to the control. It is therefore concluded that formulated biochar as soil conditioner and soil booster could be used to improve the growth and grain yield of black soybean.

Key words: Black soybean, coconut shell biochar, component yield, dry matter, formulated biochar, grain yield, growth, nodulation

INTRODUCTION

Black soybean (*Glycine max* (L.) Merr.) is known as a natural remedy against lifestyle diseases such as obesity and hyperglycemia. The reason is that the black coat of the seeds contains low fat, flavan-3-ols, polyphenol and are rich in protein [1]. Soy sauce entrepreneurs prefer black soybeans, as their main raw material, because of the high amino acid content of leucine and lysine. These amino acids are soy-breaking enzymes that form a distinctive delicious aroma and taste [2]. Black soybean is considered a healthy food. Therefore, black soybean research is needed because it can be a solution to overcome the need for healthy food. The application of black soybean research has potential to improve food and nutrition security on both local and global scale.

Increasing the production of soybeans is one of Indonesian Government policies in realizing food security and sustainable agriculture. Based on the Indonesian Central Statistics Agency, soybean production in 2018 was 982.598 tons from 680.373 Ha land area, with productivity of 1.44 tons ha⁻¹. This productivity decreased compared to 2017 and 2016 data where soybean productivity was 1.51 tons ha⁻¹ and 1.49 tons ha⁻¹ respectively. Therefore, innovative breakthroughs are needed in the use of superior seeds and manipulation planting media to increase soybean productivity [3]. Black soybeans Detam I variety was used in this study, which was released in Indonesia in 2008 and has a wide adaptability and high productivity. The characteristics of black soybeans Detam I variety are large seeds (14.84 g per 100 grains), high yield potential of on average 2.5 tons ha⁻¹ and containing 45.36% fat and 33.06% protein and has the potential to be developed [4].

Soybeans are grown after planting rice using zero tillage or minimum tillage techniques. The condition of rice fields and agricultural land in Indonesia in general has experienced degradation of soil fertility, where the soil pH is acidic and organic matter is less than 2%. Thus, it is necessary to innovate cultivation techniques that are suitable for soybean habitats, such as the application of biochar which is proven to make nitrogen use more efficient, through nitrogen / N₂ fixation activities [5,6].

Coconut shell biochar, is an organic ingredient resulting from pyrolysis. From the research results it is reported that it can improve soil quality physically, chemically, biologically and increase plant growth and yield [6,7,8]. The chemical composition of coconut shell biochar varies depending on the raw material, temperature and the duration of pyrolysis. Coconut shell biochar contains ± 27-36% biochar ingredients.

At a temperature of 420°C, it contains 9.85 m² g⁻¹ BET (theory Surface area Brunauer – Emmett - Teller), Al (66.9 ppm), B (20.69 ppm), Na (1305.68 ppm), Ca (33.84 ppm), Cu (33.84 ppm), Fe (4966.18 ppm), K (9264.71 ppm), Ti (10.24 ppm), Li (1,51 ppm), O₂ (2.5% v / v), Mg (389.12 ppm), Mn (41.47 ppm), P (1266 ppm), S (498.56 ppm), and Zn (41.46 ppm) [9].

Application of coconut husk biochar with various dosage increases the crop growth and yield of maize plant in the form of plant height, leaf area, total dry weight of plants, number of seeds, weight of seeds. In Ultisol, coconut husk biochar can increase soil water content, total pore space, pH, CEC, C, P and K of the soil. Increasing the coconut husk biochar dose into 15 tons ha⁻¹ can increase the nutrient content of P and K plants [8]. Research has found that compared to rice husk biochar, coconut shell biochar is very effective in retaining nitrogen [10]. Soil treated with an equivalent rate of 30-ton ha⁻¹ coconut husk biochar resulted in an increase in maize biomass by 90% and plant N and P concentrations of 0.88 and 0.12%, respectively [11]. Biochar have an effect on soil geomicrobiology by the nitrification process. Biochar effect on crop yields varies in different soils [12].

Biochar applications are generally given in high doses and spread on the ground. This method creates problems because it increases production costs and it has limitation in raw materials. As a solution, it can be done by making an enriched biochar namely formulated biochar (FB). In this study, a multi-function formulated biochar was tested which consisted of FB as a coconut shell biochar, guano fertilizer as a source of phosphate compost of sugarcane. Applying dolomite Ca Mg (CO₃)₂ can supply or provide Ca and Mg nutrients in the soil, increase soil pH and neutralize soil acidity, neutralize toxic compounds in the soil, increase nutrients in the soil, reduce acidity and eliminate Al poisoning. Increasing Ca and Mg will stimulate cell turgor and formation of chlorophyll so that the photosynthesis process can increase. By utilizing the coconut shell biochar formula, it is hoped that many benefits will be obtained, such as soil remediation, improvement of physical soil, chemical and biological properties, and better crop growth and production of various upland crops [12].

The results of several experiments show that the application of biochar makes nitrogen use more efficient, through nitrogen / N₂ fixation activities. Fan Bi [6] states that the combined application of biochar of rice straw 2% (2 mg kg⁻¹ soil) and urea fertilizer (200 mg N kg⁻¹ soil) could enhance the nitrification of vegetable planted soil. A research that combined biochar and biofertilizer showed that only biochar addition (2.5, 8.0 and 16 tons ha⁻¹) affects the amount of residual nitrogen

in the soil after harvest, cation exchange capacity (CEC), acidity (pH), and electrical conductivity (EC) [7]. The combination of biochar (8 tons ha⁻¹) and bio-fertilizer that contains phosphorus and sulfur growth-promoting rhizobacteria have significant interaction for bulk density, cation exchange capacity, and harvest index [7].

The highest grain yield of soybean was produced by 8 tons biochar and inoculated bio-fertilizer, compared to control [7]. At a dose of 40 tons ha⁻¹ biochar, the stability of soil aggregates (> 0.25 mm) in the 0-15 cm soil layer experienced a very sharp increase compared to other treatments, especially macro aggregates with particle sizes greater than > 2 mm. Rapeseed and sweet potato yields were increased by 36.02% and 53.77% respectively at a dose of 40 tons ha⁻¹ biochar [13]. Głodowska et al. [14] stated that biochar from softwood feedstock Pyrovac with Brady japonicum inoculation significantly improved the growth variables of soybean. Pyrovac biochar inoculated with Brady japonicum and watered with complete Hoagland solution also increase root characteristics (nodules per plant, nodule fresh weight, nodule dry weight, and root dry weight). The application of biochar and compost did not affect the agronomic characters and productive nodules of several soybean genotypes [15].

From this study, it appears that there is an opportunity to modify biochar that has not been explored in the form of a package of biochar formula. Formulated biochar has many benefits because it contains several components, such as sugarcane bagasse, dolomite, and guano. Biochar which will be used is sourced from coconut shells. Biochar mixed with dolomite is expected to neutralize soil pH, guano as a source of phosphate, sugarcane bagasse compost to increases the quality of soil.

The present experiment was aimed at evaluating the effects of different levels (0, 0.5, 1.0, 1.5, 2.0 tons ha⁻¹) of biochar formula on nodule growth parameters, and grain yield of soybean. The outcome of this study may provide a better way of increasing nodule growth, component yield, and yield of the black soybean.

MATERIALS AND METHODS

The study was conducted for one season in field experiment starting from April 2019 to August 2019 in Winaya Mukti University, Sumedang- Indonesia at 850 m above sea level (m ASL), characterised by Andisols soil order. Supporting

observations results of climatic conditions such as daily air temperature was around 22 °C -32 °C, humidity during the study around 60% - 70% per month and climate classification is rather wet (52.25 %).

The experiment was arranged as randomized block design and consisted of 5 treatments (0, 0.5, 1.0, 1.5 and 2.0 tons ha⁻¹) dosage of FB and provided with 5 replications. The materials used in this experiment were Detam I seed cultivar, sheep manure 2 tons ha⁻¹, chemical fertilizers used were Urea (45% N) dosage 25 kg ha⁻¹, SP-36 (36% P₂O₅) dosage 75 kg ha⁻¹, KCl (60% K₂O) dosage 100 kg ha⁻¹. FB consists of coconut shell biochar 20%, compost 40%, dolomite 30% and guano fertilizer 10%. Detam I seed cultivar was used because it has a wide adaptability in Indonesia and high nutrient compound and yield potential.

The biochar was produced from coconut shells waste collected from a market in Bandung, Indonesia. The production of biochar was by use of slow pyrolysis method [10]. Coconut shell biochar was prepared through auto thermal shells combustion in a vertical drum (1.5 m high, and 1.5 m diameter). Combustion was conducted for 8 hours and then cooling allowed for 12 hours (one night). Temperatures during the charring process were fluctuated between 200°C to 310°C (average 250°C). After the biochar cooled, it was ground and pulverized until the particles were > 2 mm in size. A biochar formula consists of coconut shell biochar 20%, compost from sugarcane biomass 40%, dolomite 30% and guano fertilizer 10% which were combined evenly using mixer machine. The biochar had a pH of 10.2, a total carbon (C) content of 53.7%, a TN content of 1.2%, a total hydrogen (H) content of 1.2%, a H:C ratio of 0.3, a C:N ratio of 53.5%, a 35.1% ash content.

Land preparation and tillage were done manually using a hoe at two weeks before planting. The land was made into an experimental plot measuring 125 cm x 150 cm. A total of 25 plots were formed to matched the total number of treatments. The distance between the treatment repetition is 50 cm and the distance between the experimental plots is 30 cm. Planting hole depth was 5 cm, with planting distance of 30 cm x 25 cm and the plant population was 5 plants per plot for the sample. FB was applied to the plots one week before planting with the amount of the dose that were tried and then added 2 tons ha⁻¹ sheep manure. FB was applied on the root zone (inserted into the planting hole with a depth of ±5 cm). Urea of 25 kg tons ha⁻¹, SP 36 of 75 tons ha⁻¹ and KCl of 100 tons ha⁻¹ were applied into the hole plant with a distance of 5 cm from the plant. Those inorganic fertilizer were given after the plants age were 10 days after planting (DAP). Plant maintenance includes weed control that was carried out once a week by manual destruction and watering is

carried out once a day every morning until field capacity water. Pest and disease control was carried out once a week before pests and diseases attack. Harvest was done when all the leaves fall off, the pods are brown and dry with hard seeds.

The data that were observed in this study are the number of effective nodules, shoot root ratio, dry matter production, component yield and grain yield. Each data has its own observation time. The number of effective nodules and shoot root ratio were observed 45 days after planting (DAP). Dry matter production, component yield and grain yield were observed 90 days after planting (DAP). Data were analyzed using ANOVA (Analysis of Variance) and continued with DMRT (Duncan's Multiple Range Test) α 5 %.

Component yield (number of pods, weight of seed per plant, weight of 100 seeds, weight of seed per plot and yield of black soybean) were measured in this study. Measurement of number of pods were done by calculating the average number of seeds per pod that were obtained from 5 plant sample that counted manually. Weight of seed per plant, weight of seed per plot and weight of 100 seeds were measure using electric scales. Weight of seed per plot are the mean weight of seed taken from entire plant population per plot. Yield of black soybean per hectare were calculated using the formula: 1 hectare / plot area x yield per plot. The seeds that were counted are seeds that have been dried in the sun with a moisture content of 14% that were measured using a grain moisture meter tool.

RESULTS AND DISCUSSION

Effective Nodule Production and Dray matter

Soil conditions before the study are consisted of Andosols order, low pH (5.9 in H₂O, 6.0 in water), medium C-organic level (2.12%), medium N-total (0.26%), medium C/N ratio (12), medium P₂ O₅ (35.00 mg 100 g⁻¹), low K₂O (15.21 mg 100 g⁻¹), high P available Bray 1 (150 mg kg⁻¹). The soil cation composition of Na-dd was low (0,19 cmol kg⁻¹), Ca-dd was high (13.76 cmol kg⁻¹), Mg-dd was high (1.14 cmol kg⁻¹), K-dd was low (0.2 cmol kg⁻¹) and CEC is classified as medium (24,43 cmol kg⁻¹). Rainfall rate are around 4.-131,5 mm per month. Based on the environmental conditions, it turns out that Detam I black soybean plants thrive in this environment. Application of FB showed influence on the number of effective nodules production, shoot root ratio and dry matter as showed Table 1.

In Table 1, The experiment showed that FB can increase growth components such as the number of effective nodules, shoot - root ratio, and biomass weight. The highest number of effective nodules was obtained at FB doses of 1.5 tons ha⁻¹ and

2.0 tons ha⁻¹ which were 78.20% and 100%, respectively, compared to control. The highest of shoot ratio and biomass weight was shown at FB dose of 2.0 tons ha⁻¹ dosage FB.

Component Yield and Grain Yields

Table 2 shows that Formulated Biochar could increase the component yield (number of pods, weight of seed per plant, weight of 100 seeds, weight of seed per plot and yield of black soybean). The highest was obtained by 2.0 tons ha⁻¹ dosage FB for all component yields. Formulated biochar different dosage had different effect a on grain yields. The highest grain yields were obtained at 2.0 tons ha⁻¹ dosage FB that increase 44,87 % compared to control.

Two main factors that influence plant growth and grain yield are abiotic and biotic factors. Abiotic factors include climatic factors such as rainfall, humidity, temperature, wind and light penetration, physical factors such as soil texture, soil color, soil bulk density, space pore, infiltrations, permeable of soil and soil chemistry such as the availability of nutrients, pH and C-organic of soil. Biotic factors including plant factors, pests and soil microbes such as soil respiration, the number of soil microorganisms and the population of soil biota. A different factor in this study was the provision of biochar from FB. The addition to this FB is dolomite, organic biomass of sugarcane plant and guano fertilizer. This enriched material is applied to the rootzone or in every plant hole so that its use can be streamlined.

The growth of black soybeans is shown in Table 1. Soybeans are included in the Leguminosae group, where they are uniquely able to perform N₂ efficiency, through N₂ fixation in symbiosis with Rhizobium bacteria which stimulates the formation of effective nodules. The provision of FB has a good effect on the growth of soybean plants (number of nodules effective, shoot ratio and weight of biomass), best shown by 1.5 tons ha⁻¹ and 2.0 tons ha⁻¹ dosage FB compared to control. This is possible that the dosage is sufficient to meet the plant needs. In Table 2, application of FB at dosage of 1.5 tons ha⁻¹ and 2.0 tons ha⁻¹ was able to effectively increase the number of nodules at 78.20% and 100% compared to control. The results of this study are supported by Husna [16] who stated that the use of biochar can increase the viability of microbial inoculants free from toxic elements for up to six months. Coconut shell biochar is the most suitable carrier for indigenous phosphate solubilizing microorganism because it has the highest viability and pH of 7.01 and 26.86% humidity compared on rice husk, coconut shell, oil palm empty bunch and corncob.

The survival of bacteria over time is greatly affected by the chemical and physical properties of biochar. *Bradyrhizobium japonicum* (*B. japonicum*) has the capacity to supply soybean N₂ needs and significantly reduce N₂ fertilizer application. The biochar-based inoculant can ensure efficient soybean nodules by maintaining a high population of *B. japonicum* for five months. The survival of bacteria can be supported for approximately nine months by suitable conditions that are created from hard and soft wood biochar. It is thought that the chemical composition and porosity of biochar are factors that contribute to the viability of bacteria, and pH is an important factor that limits the viability of bacteria [14]. Biochar application with compost can increase the number of effective nodules per plant and growth plant of *Glycine max.* while organic fertilizers can increase the number of nodules per plant [17].

Table 2 shows that Biochar solid inoculant has a positive effect on root characteristics, soybean growth characteristics, plant growth metrics, and plant chemical composition supplied from N-free nutrients, while nutrient uptake is highly dependent on fertilization [14]. Likewise, according to Senevirathne [17], biochar that is formulated with compost materials can increase the number of nodules and plant biomass in large doses, unlike this study where low doses of 2.0 tons ha⁻¹ with rootzone application can increase the number of effective nodules up to 100%. This is because the FB contains dolomite material, which can increase soil pH and neutralize soil acidity. Biochar has the effect of neutralizing the pH which was initially slightly acidic to neutral, but to get a neutral pH on acidic soil requires repetition every third season [18]. The application of the dolomite dosage at about 2 tons ha⁻¹ and 4 tons ha⁻¹ can increase height and dry weight of soybean and also increase N and P uptake of soybean [19].

The effect of soil porosity depends on the particle size, biochar distribution and other components. The addition of other components such as dolomite, compost and guano fertilizer, can neutralize soil acidity, neutralize toxic Al and Fe compounds in the soil, increase nutrients in the soil, accelerate the stimulation of plant root growth, increase the population of microorganisms in the soil, increase the amount of chlorophyll, increases land productivity and yield quality. The effect of adding dolomite and biochar can raise low pH to optimal pH, increase EC, nutrient availability, and KTK. Variations in the effect of biochar on plant growth depend on the characteristics of biochar, namely the raw materials used and the pyrolysis process, the ratio of biochar to the planting medium, and other additional components. Application of 25% biochar showed a lower or the same yield as control, application of 50% biochar could increase the growth to 36.4%, while application of 100% biochar showed plant growth would be the same as control

[20]. Biochar treatment with inoculated bio-fertilizer affected acidity (pH), electrical conductivity (EC), and after harvest amount of residual nitrogen in the soil. The biochar and bio-fertilizers can improve the grain yield of soybean to 51% relative to the control [7]. The application of biochar does not have an effect on growth and yield, but the differences in soybean genotype give different effects, this is because of the dominant effect of each genotype [15].

The application of biochar can overcome the main limitations of plant cultivation in Albic soils compared to straw, such as increasing soil pH, nitrogen and phosphorus elements and reducing soil density. The use of straw and straw biochar for 2 years had a positive effect on soybean agronomic properties and nutrient absorption (nitrogen, phosphorus, and potassium). The optimal treatment dose for Albic soil is 30g kg⁻¹ biochar per polybag per year which increases soybean yield. [21]. This indicates that the low dose root zone is an efficient innovation that has environmentally friendly and sustainable agriculture principles.

The FB contains dolomite material, guano material and sugarcane compost. Guano material act as a source of phosphate. Addition of dolomite affect in the number of pods per plant of Alfalfa.[22]. The combination of phosphate and other fertilizer give a significant effect on the number of pods per polybag and the number of filled pods of soybean, with TSP + dolomite giving the best result compared to control [23]. The application of sugar cane compost + N fertilizer, in various doses were given the same impact as those given with sugarcane compost only or N fertilizer only on the growth of soybean plants (plant height, number of leaves, flower to pod percentage) and component yield (number of pods/plant, filled pod percentage, dry pod weight/plant, number of seeds/plant) [24]. This finding concludes that formulated biochar as soil conditioner and soil boosters could be used to improve the growth and grain yield of black soybean to improve food and nutrition security both on a local and global scale.

CONCLUSION

Application of FB gave significant effect on increasing the active nodules production, growth, component of yields and yield. The high grain yield, dry matter and effective increase in nodule production (100 %) were obtained by the application of 2.0 tons ha⁻¹ of FB. The grain yield was increased to 38. 46% and 44.87%, respectively, at 1.5 tons ha⁻¹ and 2.0 tons ha⁻¹ dosage FB compared to control. This finding concludes that enriched biochar as soil conditioner and soil booster could be used to improve the growth and grain yield of black soybean.

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Author contribution

Tien Turmuktini is the main researcher, did data analysis and interpretation, drafted and finalized this manuscript. Rudy Irawan and Yana Taryana collected agronomy data, R. Wahyono Widodo did data analysis, Yenny Muliani and Endang Kantikowati observed pest control, and Tualar Simarmata gave guidance in this experiment. All were key in finalizing this manuscript.

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Table 1: Response of FB on effective nodule production, shoot root ratio and dry matter production of black soybean

Treatment FB ton ha ⁻¹	Effective nodule production	Increased number of effective nodule (%)	Shoot root ratio	Dry matter (g)
A: control	23.09 a	0	4.18 a	23.05 a
B: 0.5	24.05 a	4.34	4.59 ab	23.15 a
C: 1.0	30.01 a	56.71	5.56 bc	23.19 a
D: 1.5	40.09 b	78.20	6.52 bc	25.14 a
F: 2.0	46.03 b	100	6.86 c	41.65 b

Note. Followed by the same letter within each column were not significantly, different at the 5% level by DRMT Duncan's test

FB = Formulated Biochar

Table 2: Effect of FB on component yields and grain yield of black soybean

Treatment FB (ton ha ⁻¹)	Number of pod	Weight seed per plant (g)	Weight of 100 seed (g)	Weight of seed per plot (g)	Grain Yields (ton ha ⁻¹)	Increased of Grain Yield (%)
A: control	34.06 a	8.65 a	11.73 a	146.66 a	0.78	0
B: 0.5	34.27 a	8.97 a	11.80 ab	171.37 b	0.91	16.66
C: 1.0	37.50 a	9.70 a	11.99 b	186.88 bc	1.00	28.20
D: 1.5	40.40 a	10.01 a	12.76 bc	203.05 cd	1.08	38.46
F : 2.0	49.20 b	14.31 b	13.41 c	212.53 d	1.13	44.87

Note. FB = Formulated Biochar

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