



**International Seminar on
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**SOYBEAN ROOT NODULES BEHAVIOR IN VARIOUS TREATMENT OF
ENRICHED BIOCHAR WITH NPK**

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Abstract

Biochar is a highly resistant source of carbon to the decomposition process so it can store carbon in the long term and improve soil characteristics. However, the macro nutrient content in biochar is so low that it has not been able to increase soybean plant production especially its dry weight, thus lowering the root nodules. Therefore, biochar needs to be enriched with nutrients, especially NPK, adjusting the requirement of soybean plants by comparison (8: 5,1: 4) in biochar. The addition of inappropriate nutrients especially nitrogen will also affect the development of root nodules. Therefore, this study aims to determine (1) the effect of enriched biochar on soil properties, (2) the effect of soil properties on soybean root nodules, (3) the best combination of biochar and NPK in improving the development of root nodules. This research was conducted using Factorial Randomized Block Complete Design (RBCD) consisting of two factors with three replications. The first factor (B): Biochar source, consisting of four levels and then the second factor (D): Enriched Biochar Dose, consisting of four levels. Based on the correlation result, the root nodules is significantly influenced by soil pH, total N, N uptake, P uptake, and K uptake in acid soil, while measured physical and biological characteristic have not significantly influence. Total N and N uptake have negative correlation so that its enhancement will reduce development of root nodules. The results show that enriched biochar is not significantly influential in increasing total soil N, while significantly reduces soil pH. In soybean crops, enriched biochar significantly influences N, P, and K uptake, vice versa significantly reduces root nodules at doses greater than 2.5 ton / ha. The best combination was found in 2.5 ton / ha dose of rice straw biochar and wood biochar for optimize development of root nodules.

Keywords: Biochar, NPK, total N, N uptake, root nodules



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1. Background

The content of soil organic matter in most agricultural land in Indonesia is low. This results in a lack of maximum productivity of the land. In general, the addition of organic matter has improved soil productivity, but this success lasted only for a short time, especially in the tropics. This is due to the rapid decomposition process so that organic materials decay and mineralized into CO₂ only in a few growing seasons (Sukartono and Utomo, 2012).

Alternatively, biochar has the potential to outperform organic matter because of its stable nature for millions of years. Biochar is a charcoal produced under high temperature conditions and limited oxygen presence that can be utilized as a soil enhancer with the potential to improve soil characteristics and plant growth (Brantley *et al.*, 2015).

Biochar has many advantages over other organic materials, but the macro nutrient contained so low that it has not been able to meet the needs of soybean crops in increasing dry weight, eventually the development of root nodules also decreased. Bhattarai *et al.* (2015) reported, nitrogen and phosphorus in biochar are very low levels. Referring to the problem, the biochar needs to be enriched with nutrients through the addition of inorganic fertilizers ie N of Urea, P of SP-36, and K of KCl based on the needs of soybean crops by comparison (8% N: 5.1% P₂O₅: 4% K₂O). However, excessive nitrogen fertilization can suppress the development of soybean root nodule. From this, it is necessary to examine how the enrichment effect of various biochar sources with NPK on soil properties, how they affect the development of soybean root nodules, and which combination of the best biochar sources and dosage for the development of root nodules.

2. Methods

2.1 Materials and Tools

Materials used in this study are rice straw, soybean straw, wood, seeds of soybean, Urea, SP-36, KCl fertilizer. The used tools are barrel, and laboratory tools.

2.2 The process of making biochar

Preparation of biochar is done by preparing the pyrolysis tool that is from the barrel that has been open the top. Biochar materials in the form of rice straw, soybean straw and wood are prepared in a dry state to facilitate the burning. Dried rice straw is inserted into the barrel as $\frac{3}{4}$ the vat volume is then compacted until there is no cavity that causes air to enter. Then light a fire in the straw until the fire is completely burned.



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When the fire is burning and is not expected to be extinguished, the barrel is closed tightly with the cover. Prohibition is considered complete when the smoke coming out of the closing gap is thinning. The cover is removed and immediately discharged and watered so as not to continue to ash and then sieved using a 2 mm sieve. Furthermore, soybean straw and wood materials are carried out by the same procedure.

2.3 Characteristics of Land Used

Table 1: Characteristics of Land Used

Analysis	Unit	Value	Category ^{*)}
Texture	-	Sandy Clay Loam	-
Sand	%	50,08	-
Silt	%	23,79	-
Clay	%	26,14	-
pH H ₂ O (1:2,5)	-	5,03	Acid
pH KCl (1:2,5)	-	4,89	Acid
WHC	%	6,01	-
Total N (Kedjahl)	g/kg	0,30	Very low
Available P (Bray)	mg/kg	1,86	Very low
Exchangeable K	mg/kg	70	Low
CEC	cmol/kg	8,40	Low
Organic C	g/kg	6,87	Very low
Total Microbe	cfu/gram	109x10 ⁸	-
Total Bacterial	cfu/gram	211x10 ⁹	-
Total Fungi	cfu/gram	289x10 ⁹	-

* Based on Assessment Criteria Results of Soil Analysis, Soil Research Institute (2005)

2.4 Characteristics of biochar

Table 2: Characteristics of Biochar Used

Characteristics of Biochar	Unit	Rice Straw	Soybean Straw	Wood
pH H ₂ O	-	9,96	10,47	8
pH KCl	-	9,18	10,37	7,96
WHC	%	31,87	2,10	32,06
N	%	0,08	0,13	0,05
P ₂ O ₅	%	0,0004	0,0001	0,0002



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Characteristics of Biochar	Unit	Rice Straw	Soybean Straw	Wood
K ₂ O	%	0,50	0,23	0,39
CEC	cmol/ kg	73,2	67,2	32
Organic C	%	54,10	25,78	60,25

2.5 Biochar enrichment process

Biochar which has been made, given N, P, and K nutrients from Urea, SP-36, and KCl fertilizers as needed soybean crop (8: 5,1: 4). Biochar, Urea, SP-36 and KCl are mixed stored in a plastic container to be applied to the growing medium. For Biochar treatment without NPK weighed according to treatment dose without mixing with NPK from inorganic fertilizer.

2.6 Experimental Design

This research uses Factorial Randomized Block Complete Design consisting of two factors with three replications. The first factor (B): Biochar source, consisting of four levels: 1. Biochar without NPK (B0), 2. Rice Straw Biochar + NPK (B1), 3. Soybean Straw Biochar + NPK (B2), 4. Wood Biochar + NPK (B3) and then the second factor (D): Enriched Biochar Dose, consisting of four levels: 1. 0.5 ton / ha (D1), 2. 2.5 ton / ha (D2), 3. 5 Ton / ha (D3), 4. 10 ton / ha (D4). The data obtained will be analyzed using variance analysis or ANOVA and correlation between variables will be processed with SPSS program.

3. Results

Addition of N from Urea fertilizer into biochar increases the nutrient content in biochar thus increasing soil total N. In addition, soil total N may be significantly affected by soil conditions. When viewed from the soil chemicals properties, it turns out nitrogen has a direct relationship with CEC. Total N is influenced by the high ability of the soil to bind the NH_4^+ main cations in the flooded or reductive soils (Triyono *et al.*, 2013). Due to the presence of biochar which is able to increase linearly CEC so significantly increases total N because of its significantly direct relationship. In addition, although the occurrence of hydrogen bonds between the biochar surface and the NO_3^- anions is still possible, the presence of functional groups such as ketones, chromates, and pyrons capable of adsorbing NO_3^- into the biochar through hydrogen bonds reduces leaching (Nguyen *et al.*, 2017).



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Table 3: Correlation of variable soil chemical properties and root nodules

	pH H ₂ O	pH KCl	Organic C	Available P	Total N	Exchangeable K	CEC	Total Root Nodules	Total Effective Root Nodules
pH KCl	,72**								
Organic C	0,17	0,17							
Available P	0,27	0,17	0,10						
Total N	-,39**	-0,22	,29*	-0,05					
Exchangeable K	-0,10	-0,09	,39**	0,20	,41**				
CEC	-0,03	-0,03	,43**	0,13	,36*	,90**			
Total Root Nodules	,59**	,55**	0,17	-0,00	-0,27	-0,23	-0,08		
Total Effective Root Nodules	,59**	,54**	0,19	0,01	-0,21	-0,24	-0,10	,99**	
Weight of Root Nodules	,57**	,52**	0,08	0,07	-,30*	-0,22	-0,11	,98**	,97**

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

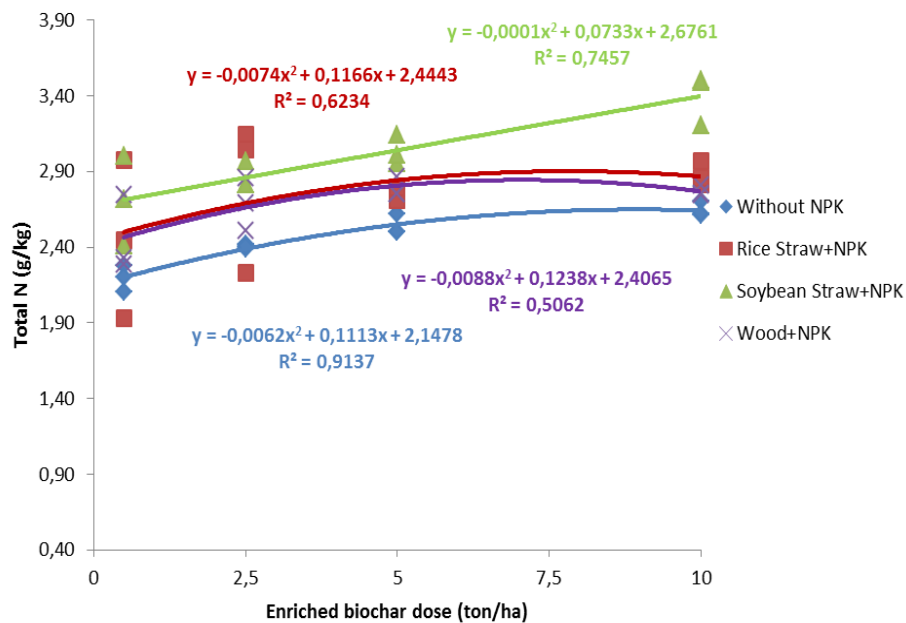


Fig. 1: Influence of Treatment on total N

The increase of nitrogen in the form of ammonium causes reduction of the soil pH. As shown in the significantly opposite relationship. Biochar of soybean straw



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increases total N is highest compared to other biochar, this can be expected because of the contribution of soybean straw biochar which has a higher N content than other biochar sources. The information of nitrogen in what form is dominantly contributed by biochar thus increasing total N still unknown.

Soil pH turns out to have a direct relationship with variables of root nodule. The soil pH response to the enriched biochar treatment showed a decrease causing a decrease in nodule variables. *Rhizobium* is a symbiotic bacterium with soybean plants forming root nodules influenced by P, Ca, Mg and pH (Birnadi, 2014). If the environmental conditions do not support the population of these bacteria decreased so that the formation of nodules also decreased.

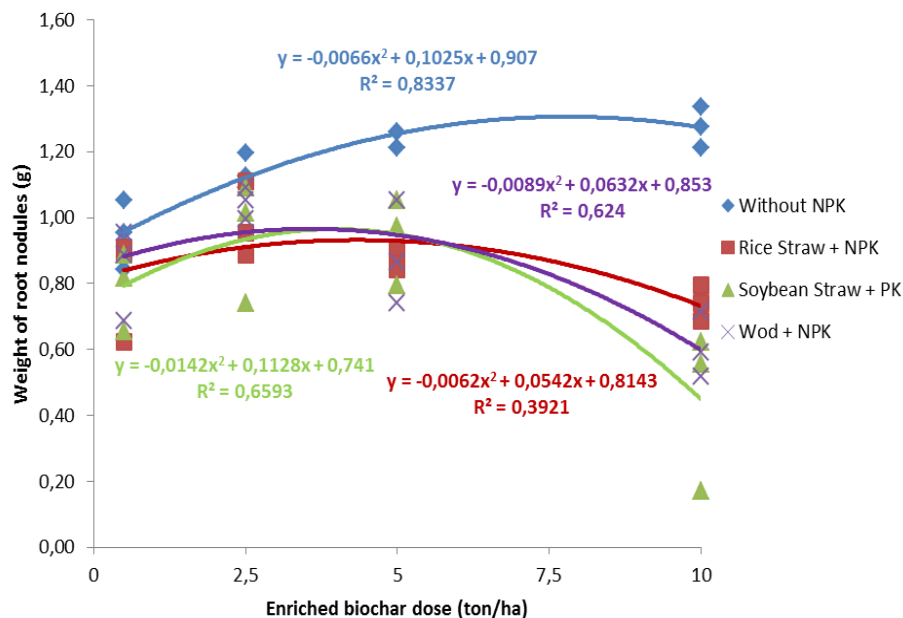


Fig. 2: Influence of Treatment on Weight of Root Nodules

In addition to pH, total N appears to have an opposite relationship with the weight of the root nodule. The total N response due to the treatments showed an increase causing the weight of the reduced nodule. Soil nitrogen in the form of nitrate absorbed by the plant is reduced nitrate reductase which requires an electron donor ie NADPH from photosynthesis results. Described in Kumalasari *et al.* (2013), high nitrate content will require a large electron donor resulting in a competition to obtain NADPH between nitrates and CO₂ in photosynthesis, so that the photosynthesis of the required sugars of nodules will decrease and their impact to the weight drop of the root nodule.



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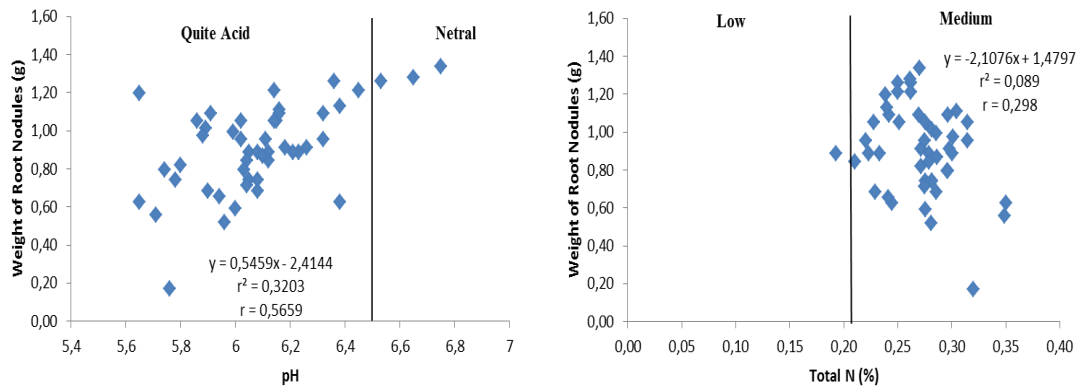


Fig. 3: Influence of pH and Total N Status on Weight of Root Nodules

The pH status has a positive correlation that increases the weight of the root nod to neutral status. Total N due to treatment, dominantly has medium status with a significantly weak relationship. The negative relationship pattern is decreasing the weight of root nodules after total N increased by more than 0.25%.

Table 4: Correlation of soil physical and biological properties and root nodules

	WHC	Bulk Density	Particle Density	Porosity	Microbe	Bacteria	Fungi	Total Root Nodule	Total Effective Root Nodules
Bulk Density	,52**								
Particle Density	0,05	0,01							
Porosity	-0,17	-,65**	,412**						
Microbe	-0,07	0,08	0,04	-0,20					
Bacterial	0,22	-0,01	0,03	-0,08	0,02				
Fungi	0,01	0,01	0,28	0,02	0,19	,32*			
Total Root Nodule	0,14	0,15	-0,13	-0,20	0,05	-0,03	-0,11		
Total Effective Root Nodules	0,16	0,15	-0,09	-0,17	0,05	-0,03	-0,07	,99**	
Weight of Root Nodules	0,16	0,14	-0,14	-0,20	0,03	-0,04	-0,12	,98**	,97**

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).



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Root nodules was not significantly related to the physical and biological properties of the soil as measured in this study. The addition of biochar is known to be good for microbial habitats if the dose increases, allowing higher microbial populations, both bacteria and fungi. Allegedly, biochar is known to improve the physical properties of the soil, but it takes a long time, because the research conducted only takes 3 months so that the impact is the physical properties have very low variance. Biological properties have very high variance, so that causes its effect is not significant to root nodules.

Table 5: Correlation of nutrient uptake and root nodules

	pH H ₂ O	Total N	N uptake	P uptake	K uptake	Total root nodules	Total effective root nodules
Total N	-,389**						
N uptake	-,423**	,585**					
P uptake	-0,251	,316*	,739**				
K uptake	-,322*	,559**	,903**	,721**			
Total root nodules	,593**	-0,266	-,507**	-,377**	-,447**		
Total effective root nodules	,590**	-0,211	-,449**	-,350*	-,410**	,990**	
Weight of root nodules	,567**	-,301*	-,521**	-,339*	-,470**	,979**	,967**

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

The addition of Nitrogen to the biochar from Urea (46% N) was able to increase the N uptake of soybean plants higher than without enrichment. Nitrogen in plants is needed for protein formation and vegetative growth of soybean crops. Nitrogen in the tissues is necessary in the formation of amino acids that will form proteins thereby increasing the yield of soybean crops (Tairo and Ndakidemi, 2014). The effect of increasing the dose rate can provide a noticeable increase in N uptake at a rate of 5 ton / ha compared to uncooperative biochar, which significantly increases the absorption of soybean plant N.

Wood biochar is higher in increasing N uptake than soybean straw and rice straw biochar. The water content of biochar wood is capable of higher dissolving nitrogen, so it is easily absorbed by plants although the initial level is much lower than the other two biochar. In addition, N uptake is also significantly affected by P and K uptake as shown in the correlation table. High P and K uptake will increase N uptake. This may explain the rooting system factor, if rooting is deep then N uptake will



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increase. The effect of wood biochar showed the best P and K absorption response compared to other biochar. P and K are known to increase the growth of plant roots better, thus functioning optimally in the absorption of N elements. Explained in Manshuri (2012), root growth is essential for optimal absorption of nutrients.

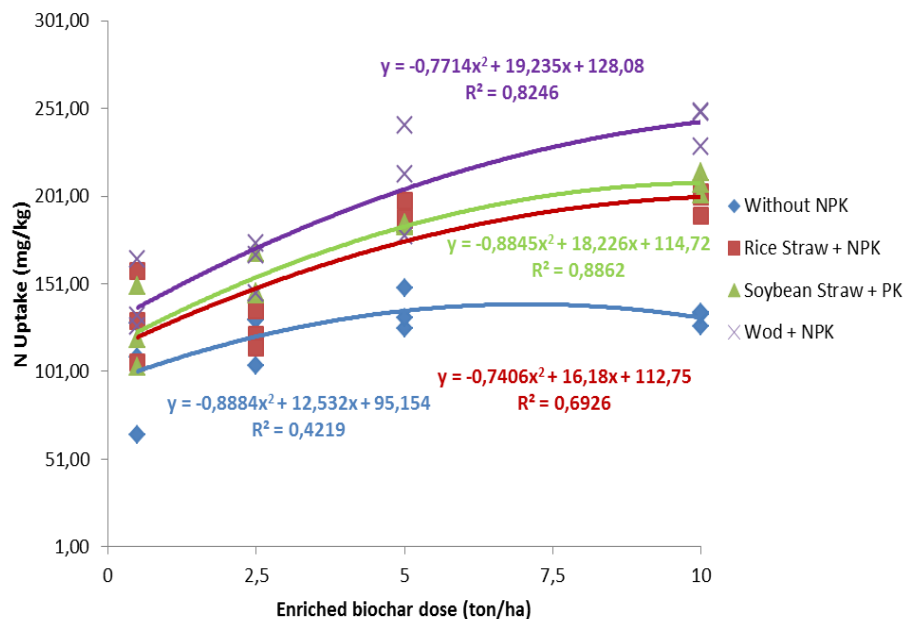


Fig. 4: Influence of Treatment on N uptake

The N uptake has an opposite relationship with the variables of the root nodule. The effect of treatment on N uptake significantly increases to a dose of 10 tons / ha. This leads to an increase in N uptake, eventually decreasing the number of root nodules primarily. Amir *et al.* (2015) states, nitrate has the ability to negate the change of root hair necessary for the entry of bacteria, thus reducing the number of nodules. High N uptake causes a decrease in the number of root nodules, the number of effective root nodules and weight of nodules as shown by very significant negative correlation values. Kumalasari *et al.* (2013), nitrates increase the absorption of roots of soil nitrogen thereby inhibiting the transcription of nitrogenase genes. This transcription inhibits the biosynthesis of the enzyme nitrogenase so that its activity decreases in the free nitrogen inhibiting and decreases the percentage of the effective root nodule.

P and K uptake also have an opposite relationship with the variables of the root nodule. This can be expected because of P and K contribution to the improvement in absorption of N. Mulyadi (2012) mentions, P elements play a role in



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the synthesis of ATP and NADPH as energy supply of nodule root formation. However, with the presence of NADPH, nitrate also requires a large electron donor so that N uptake is increased and decreases the varicella variables as described.

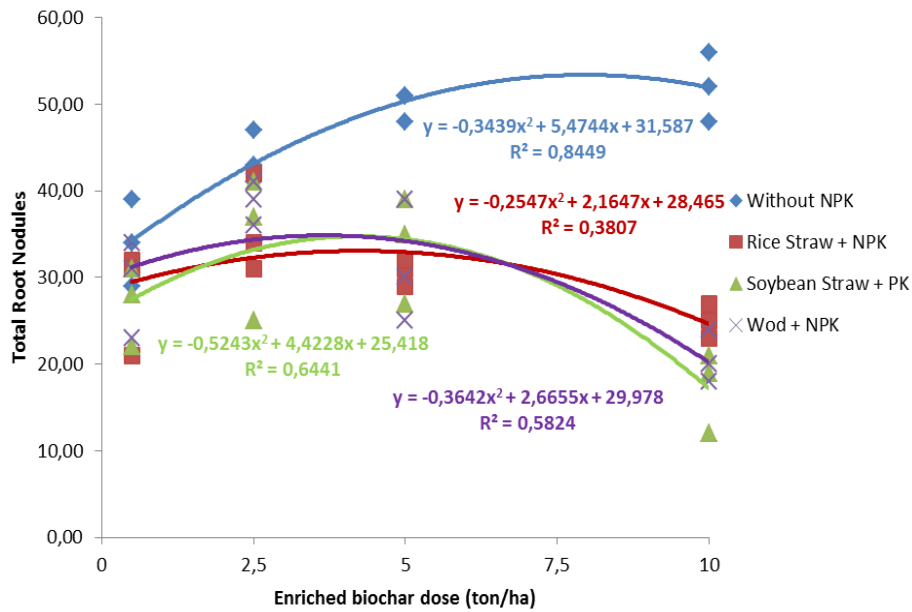


Fig. 5: Influence of Treatment on Total Root Nodules

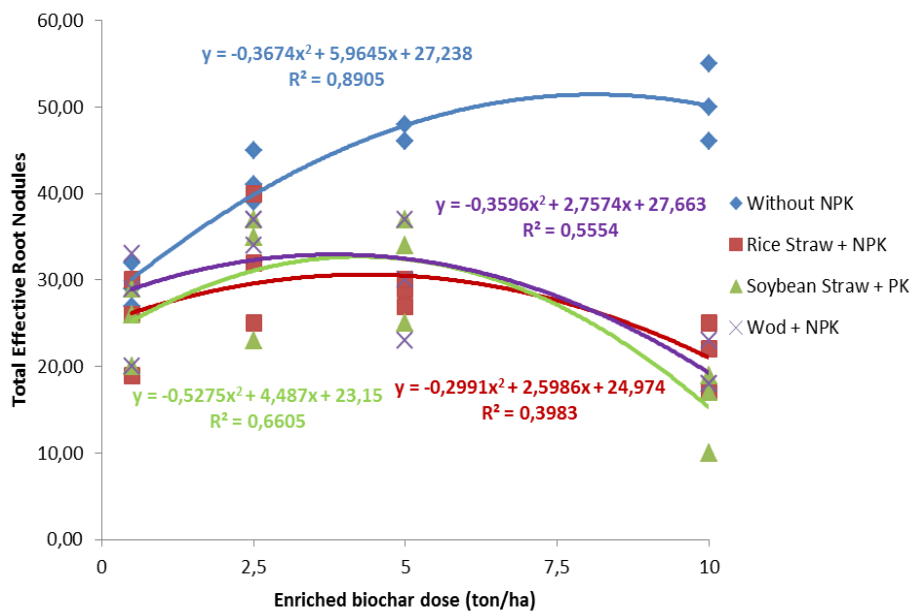


Fig. 6: Influence of Treatment on Total Effective Root Nodules



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The development of root nodules is best in treatment with a dose of 2.5 tons / ha. Explained Saragih *et al.* (2016), sufficient nitrogen will stimulate plants to provide photosynthesis for growth and activity of root nodules. A higher dose will increase the N uptake of the plant so that the nodules decrease. The mechanism of effective reduction of root nodules is explained by Kumalasari *et al.* (2013), nitrate when adsorbed into a root nodule is reduced to nitrite and then forming a NO compound that prevents leghemoglobin binding O₂ so that the enzyme decreases its activity. Generally the formation of root nodules will fail if the nitrogen content in the soil is more than 100 kg (Saragih *et al.*, 2016). In this study, nodule is still formed until the dose of urea more than 100 kg, although only decreased the amount formed, can be explained because in the presence of biochar can suppress the occurrence of nitrification because it contains potassium capable of being inhibitor enzyme nitrite oxidoreductase.

From the above discussion, the best treatment in terms of their effects on soil properties, plant uptake and root nodules is generally biochar rice straw and biochar wood enriched at a dose of 2.5 tons / ha. This consideration is due to the formation of a more optimal nodule at that dose, so the need for synthesis of N fertilizer is less due to biochar as a carrier.

4. Conclusion

The development of root nodules in acid soil is significantly influenced by chemical properties like soil pH, total N, N uptake, P uptake, and K uptake. The enriched biochar is not significantly influential in increasing total soil N, while significantly increases N uptake. This enhancement reduces development of root nodules at doses greater than 2.5 ton / ha. So that, the best combination was found in 2.5 ton / ha dose of rice straw biochar and wood biochar for optimize development of root nodules.

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