

Potential of Various Trees Litter Containing Tannin on Agroforestry System as Nitrification Inhibitor for Increasing Nitrogen Fertilizer Efficiency for Soybean

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Abstract: Soybean plants production in agroforestry system in Gunung Gajah forest were very low since the percentage of empty pods relatively high. The case presumably about soil fertility mainly is nitrification activity. The research aim is to study about the potential of trees litter containing tannin as nitrification inhibition for increasing nitrogen fertilizer efficiency. The research was conducted by survey (about trees characteristics, micro climate, soil properties, and farmers practice in soybean cultivation) and experiment method (variety, N, P, Mo, and Mg fertilizer). The yield of the research are: nitrogen in the soil is low since nitrification and oxidation process by bacteria, and leaching; The trees litter potential for inhibiting nitrification process are litter from: Mahogany, Teak, Jackfruit, Manggo, and Breadfruit (the sequence also exhibiting the sequence of litter quality as nitrification inhibition). By N, P, Mo, and Mg fertilizations the number of empty pods of soybean plant decreases so the plant seeds production obtained 0.86 ton ha⁻¹.

Key words: Agroforestry, soybean, fertilizer, nitrification activity.

1. Introduction

The Indonesian need of soybean grain not enough yet since the plant production still low. The effort of increasing plant production in agroforestry system is one of alternative way, because the decreasing of farming land area to other functions. Several soybean varieties like Kaba and Pangrango are potentially cultivated in agroforestry systems based on teaks and pines with 50 kg fertilizer N ha⁻¹ [1]. The agroforestry farmers at Gunung Gajah forest less interested in soybeans cultivation, because they have relatively high empty pods. This problem should be solved through research on the potential and constraints (land and trees) the area for soybean cultivation.

Obstacles cultivation in agroforestry systems is the interaction between trees and crops in the form of

competition for light, water, and nutrients [1-3]. Empty pods means plants have certain constraints in the seed filling process. Constraints are most likely derived from soil fertility instead of light or water. Soybean is C₃ plants which relatively low light tolerant, as mesophytes which relatively low of water needs, thus this crop suitable as understorey crop in agroforestry system. The process of seed formation and seed filling of soybean plants requires the availability of high N and P. Nitrogen is needed because the soybean plant soybean seed protein content is relatively high (34.1 g 100 g⁻¹ seed). In addition, soil N in agroforestry systems can undergo immobilization in connection with the rate of decomposition of organic matter [4]. Seed yield, seed filling period (SFP), and accumulation of N in soybean plants closely related. The phosphorus element is needed in the formation of energy substance (ATP), nucleic acids, and also involved in

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the formation of nodules on legume plants. Generally contain of P element on the land forest area is between too low and low (8%-11%) [1].

Based on the description above, the empty pod problem of soybean plants in agroforestry system needs to be solved. The method to solve the problem has to be easy, cheap, and environmentally approach. What is organic material from several types of agroforestry crops can be used as an alternative material to inhibit the nitrification process in soil. How do to manage those plant materials, what technology which biological and sustainable approach purpose to controlling the rate of nitrification. Is the soybean plant cultivated in agroforestry system requires the input of N, P, and micronutrients (Mn and Mo) fertilizer.

2. Materials and Methods

The research was conducted from July until October 2009 and 2010, at forest area Cawas, Klaten regency, Jawa Tengah province, Indonesia (7°45'877" S and 110°39'954" E). The analysis of litter and soil quality (chemical, biological, and physical properties) was done in the laboratory. The first research method is survey with observations about the characteristics of trees, microclimate, soil characteristics, and habits of agroforestry farmers in soybean cultivation. Tree and shrub litter collected by net mounted under the canopy (each of 15 trees in three types of age) for 6 months (the first three months was in the rainy season and the second one during the dry season), then weighed and analyzed the quality of litter (done in 2009). The second research method is experiment by randomized completely block (RCB) arrange in split plot design. The main plot are varieties (Kaba and Grobogan) while the sub plots are fertilizer (N: 0, 25, 50, and 75 kg ha⁻¹, P: 0, 50, 100, and 150 kg ha⁻¹).

The expected information from the research are: (1) General condition (geographic position, elevation, climate and hydrological characteristics); (2) Soil quality and land characteristics (slopes, texture, pH,

organic matter, N, P, K, Ca, Mg, Mn, Mo, Bo and Fe content), total microbes and N fixing microbes colony, (3) Trees characteristics mainly about litter (quality of the litter which are lignin and polyphenol content and number of litters), (4) Microclimate (light penetration and temperature under the tree canopy), and (5) Growth and yield of soybean.

3. Results and Discussion

3.1 Characteristic and Quality of Soil

The physical fertility on the research area is low to moderate level. Soil texture is dominated by dust, soil layer (A and B) is shallow potentially inhibit the development of plant roots. Soil texture is silt loam that means low water infiltration capacity and high surface runoff. This indicates that soil aggregation processes in the region not yet obtained the optimum. The organic matter and C organic at low level associated with vegetation density is low to moderate with the litter contains high lignin. The soil reaction (pH) of the research area is slight acid while the rock matter is Karst (lime). This is due to high rainfall and relatively steep slope, so the alkaline washing process is more intensive. Slightly acidic soil reaction resulted in a high cation exchange capacity, while soil base saturation is low due to the high content of H⁺. The effect of low number of cation base is low of saturation base. The influence of low pH is to nitrification process so the soil suitable for nitrifying bacteria. The highest nitrifying bacteria population occurs at pH 6.6 to 8.0. Under pH of 5.0 the nitrification rate is decrease, but nitrifying bacteria and NO₃⁻ still there at pH under 4.5 [5]. Phosphor fixation by Al only occurs at the acid soil like this research soil site. The majority of P fertilizer which is not absorbed by plant not lost due to leaching process. This element becomes stable P nutrient as Al-P and Fe-P (at soil pH < 5.5) or Ca-P (at soil pH > 6.5) which are no available for plant. Fixation of P fertilizer can also occur if the ions released from the outskirts of Al-silicate crystals which then reacts with

the anion of phosphorus into phosphorus-hydroxyl [6].

The available of the main macro nutrient (N, P, and K) and secondary macro nutrient (Ca, Mg, dan Na) at the soil research site is low and medium respectively due to light soil texture and low organic matter. Clay fraction of the soil and organic matter as colloidal soil there as negative charge those they can bind nutrient elements. The soil content of Mg and Na in medium level classification related with the lime rock matter (Karst). This situation resulted that soybean crop nutrient needs at the study area cannot be met from within the soil nutrient content, so the need for the provision of nutrients through fertilizers. The absorption of N, P, and K for soybean plant is 90, 8, and 36 kg ha⁻¹ respectively for reaching 1.5 ton ha⁻¹ seed yield [7]. Based on the statement and the availability nutrient element in the soil, the need of nutrient for soybean plan can be calculated (Table 1).

3.2 Soil Biology Properties

Soil biology properties such as: total number of microbe colonies, Azotobacter, NH₄⁺ and NO₂⁻ oxydized bacteria, Fungi, Bacteria, and Actinomycetes (Table 2). Number of the microbe colonies show that the soil at the research site has relatively high biological fertility (number of colony more than 1 × 10⁶ cfu g⁻¹ soils). But C organic and organic matter of the soil is low due to the influence of several factors to the decomposition process of organic matter (litter),

such as the litter quality and microclimate. The erosion can influence the organic matter contain at the steep slope location.

NH₄⁺ Oxidizing Bacteria: NH₄⁺ oxidizing in the research site soil was medium level, about 13-16 cfu 10⁴ g⁻¹ soil. The number of NH₄⁺ oxidizing bacteria was high, and available of N was low because the N elements oxidized to NO₃ (easy to leached). The application of organic matter with high C/N in the soil can be inhibited nitrification process since NH₄⁺ from organic matter and NH₄⁺ mineralization in the soil will be immobilized by organic matter decomposing heterotrof microbe, so there is no NH₄⁺ residue for nitrification process [8, 9]. The natural inhibiting nitrification process those are: (1) difference of affinity between the plants, heterotrof microbe and bacterial nitrification which compete for obtaining NH₄⁺; (2) root exudates which are allelopathic for nitrifying bacteria; (3) spatial separation between the source of NH₄⁺; (4) organism compete for obtaining NH₄⁺ especially between bacterial nitrification and the source of NH₄⁺ [10].

NO₂⁻ oxidizing bacteria (Nitrobacter): Population of the NO₂⁻ oxidizing bacteria was very low if compare than NH₄⁺ oxidizing bacteria. In the process of hydroxylamine oxidation (NH₂OH) to nitroxil (HNO), some of NH₄⁺ will be loss as nitrous oxide gas (N₂O) and it makes the substrate of NO₂⁻ oxidizing bacteria always low [9]. Accumulation of the NO₂⁻ just occur in

Table 1 The nutrient available and necessary of soybean plant.

Element	Need (kg ha ⁻¹)	Available (kg ha ⁻¹)	Gap (kg ha ⁻¹)	Fertilizer (kg ha ⁻¹)
N	90	69	21	90 (Urea)
P	8	6	2	9 (SP 36)
K	36	10	26	98 (KCl)

Table 2 Soil biological properties.

Transect	∑ microbial colony	Azbt	NH ₄ ⁺ OB	NO ₂ ⁻ OB	Fungi	Bact	Actinom
1	111. 10 ⁵	7	16	10	24	18	11
2	121. 10 ⁵	5	15	8	17	14	14
3	124. 10 ⁵	7	13	8	22	14	13
Average	119.10 ⁵	6.7	14.7	12	21	15.3	12.7

Analysed by Soil Biology Laboratory, Soil Department, Faculty of Agriculture, University of Brawijaya.

Explanation: Colony unit: cfu 10⁴ gram⁻¹ soil, OB: oxidizing bacteria, Azbt: azotobacter, Bact: bacteria, Actinom: actinomycetes.

the soil with high pH only because NO_2^- oxidizing bacteria (*Nitrobacter*) more susceptible to ammonia and high pH compare than NH_4^+ oxidizing bacteria [5]. The addition by 1.4 ppm NH_4^+ to the 9.5 soil pH will be inhibiting *Nitrobacter* growth but no influence for *Nitrosomonas*. The inhibitor substance there is no NH_4^+ but by free ammonia (NH_3) formation. If ammonia decreased by oxidation process, *Nitrobacter* will be active again [5].

Bacteria and fungi: Fungi as heterotroph microbe is the most active as litter decomposer at the first process, then the second is bacteria, and then actinomycetes. Bacteria have high responds to simple substances such as starch and sugar, while fungi and actinomycetes will be dominant in organic matter rich cellulose or resistance substances [11]. Vegetations on the research area are dominated by perennial plant so the organic matter (litter) contains cellulose and resistance substances such as lignin and polyphenol. The case as previous mention is influencing the number of bacteria which smaller than fungi.

Actinomycetes: Bacteria are very responsive to the supply of simple compounds, whereas fungi and actinomycetes will be dominant if there are rich organic matter resistant compounds. So if the surface forest land is covering by litter, the activity of fungi is higher than others. Bacteria will play dominant when organic materials mixed in the soil, either due to soil cultivation, earthworm activity, as well as the distribution of roots. Litter on the ground will decompose more slowly than those buried in the soil. Nutrient element from litter mineralization process on soil surface would be easier lost by runoff and evaporation rather than embedded in the soil surface. Litter is buried in the soil will be more moist and covered by a variety of biota, resulting in faster decomposition [11]. The number of actinomycetes colony in the research site than other microbes is the lowest. This is because actinomycetes play a role in the decomposition of organic material such as chitins and phospholipids resistant, so it is more dominant at

the end of decomposition after the substrates which easy decompose depleted [11]. Bacteria and fungi are able to decompose cellulose by extracellular enzyme, thus by that way break down cellulose outside microbial cell into monomer or oligomer of glucose units [12].

3.3 Trees

Kinds of the trees and litters: The kind of trees at the research site there are: mahogany (*Swietenia mahogany*), jackfruit (*Artocarpus integrus*), mango (*Mangifera indica*), breadfruit (*Artocarpus communis*), gmelina (*Gmelina arborea*), petai (*Parkia speciosa*), and teak (*Tectona grandis*), where gmelina is the most dominant tree. Tree litter collected both dry and rainy season showed that quality varied according to the diversity of trees (Tables 3 and 4). Rate of the litter decomposition is more determined by lignin content than C, N ratio [13]. Several kinds of the litter have low C/N but phenol content is too high so the rate of N mineralization too late for supplying the need of plant. The same case occurs if lignin content of the litter > 20%-25% [11]. The lignin-polyphenols ratio and N has higher correlation with N mineralisation than polyphenol content or polyphenols and N litter [13]. Polyphenols is phenol polymer which soluble in water that can bind with plant proteins and enzymes degrading biota. Lignin is a component of the cell wall formed by the condensation of free radicals, cinnamyl alcohols, is *trans*-coniferyl alcohol, *trans*-sinapyl alcohol and *trans-p*-coumaryl alcohol [5, 13]. The base framework of lignin composer is phenylpropane unit containing a ring of 6-C aromatic benzene (phenol) and a chain 3-C linear side. Because the bond between lignin structures is very strong and varied then only a few microbial genera are able to describe the lignin through the production of extracellular enzymes phenoloxidase. The microbes are white root fungus (Basidiomycota), Agaricaceae, Hymenochaetales, Corticiaceae, Polyporaceae and Thelephoraceae, and several species of Ascomycota,

Table 3 The trees litter quality (1).

Trees	Polyphenol (%)	Lignin (%)	Ash (%)	Sellulose (%)
Mahogany	26.17	18.64	4.01	16.39
Jackfruit	14.18	21.47	6.63	27.62
Mango	25.13	16.92	4.11	17.34
Breadfruit	6.71	16.04	12.01	33.9
Gmelina	7.75	17.43	0.92	18.65
Petai	16.96	22.38	8.31	27.21
Teak	9.44	30.66	1.13	17.81

Analyzed by di Soil Biology Lab, Department of Soil Science, Faculty of Agriculture, University of Brawijaya, Indonesia, 2009.

Table 4 The trees litter quality (2).

Trees	C Org. (%)	Total N (%)	C/N	OM (%)	(Plp + lgn)/total N
Mahogany	46.08	0.90	51.50	79.71	50.05
Jackfruit	32.65	0.10	32.50	56.48	35.97
Mango	38.31	1.27	30.50	66.27	33.33
Breadfruit	36.06	1.37	26.50	62.39	16.61
Gmelina	38.03	1.99	19.0	65.79	12.66
Petai	36.22	1.99	18.0	62.66	19.84
Teak	37.11	1.44	32.0	64.20	35.40

Analyzed by di Soil Biology Lab., Department of Soil Science, Faculty of Agriculture, University of Brawijaya, Indonesia, 2009.

Xylariaceae [11]. If the litter with high phenol and/or lignin contains used as green manure, mineralization is too slow so it is not effective for seasonal crop. In contrast to perennial crops or forest trees, the slow release of N is actually beneficial in the long run because N mineralization results will be spared from leaching and denitrification [11].

The presence and activity of soil microbes are dependent on one another, vary in different places and times and greatly influenced by soil management practices (which can affect the group or whole of soil biota [14]. Further research proved that low in NO_3^- at the climax ecosystem not only caused by the *allelochemical inhibitor* but also to gain competition for obtaining NH_4^+ between nitrifying bacteria and organic matter decomposer heterotroph microbes and or extensive root diversity at natural ecosystem [5].

The application of organic matter with high C/N into the soil may indirectly inhibit the nitrification

because NH_4^+ from organic matter mineralization and NH_4^+ in the soil will immobilized by organic matter decomposer heterotroph microbes thus leaving no substrate NH_4^+ for nitrification process [8, 9]. The litter decomposition rate is determined by its qualities those are: soluble carbohydrate contain, amino acids, active polyphenols, lignins, and C element⁻¹ [13]. High litter quality (fast mineralization) is the litter with $\text{C/N} < 25$, lignin $< 15\%$ and polyphenol $< 3\%$ [15]. The criteria to be met a commercial nitrification inhibitor, they are: (a) no poison to plant and other living bodies, (b) inhibit NH_4^+ oxidation to NO_3^- via inhibition of nitrosomonas activity, (c) not interfere NO_2^- oxidation process by Nitrobacter, (d) can be distributed evenly along the nitrogen fertilizer so it is always in contact with fertilizer N in the soil, (e) have stable inhibitory properties are stable up to several weeks, and f) cheaper price [16].

Based on the content of polyphenols and lignin, plant litter that existed at the study site can all be used as nitrification inhibitors. Gmelina and petai litter are not recommended for inhibiting nitrification process due to the $\text{C/N} < 25$. Judging from the balance of the content of polyphenols, lignin, and C/N, then the sequence of potential litter as a nitrification inhibitor is Mahogany > Teak > Jack Fruit > Mango > Breadfruit (Table 5).

Soybean crop. Growth of soybean plants below both *Eucalyptus* or *Gmelina* stands were not significantly different. The empty pods (seedless pod) as main problem still there but the pod fills are also formed so the grain yield was obtained 0.86 ton ha^{-1} (the highest yield was 1.6 ton ha^{-1}). This potential can

Table 5 The trees litter containing nitrification inhibitor.

Trees	Polyphenol (%)	Lignin (%)	C/N
Mahogany	26.17	18.64	51.50
Jackfruit	14.18	21.47	32.50
Mango	25.30	16.92	30.50
Breadfruit	6.71	16.04	26.50
Teak	9.44	30.66	32.00

Analyzed by Soil Biology Lab., Department of Soil Science, Faculty of Agriculture, University of Brawijaya, Indonesia, 2009.

still be improved given the weight of empty pods are still relatively high (almost equal to the weight of pod fill). Other than that the average national soybean crop production ranges 1.1 tons ha⁻¹. Through the effort to reduce the number of empty pods increased soybean production can be achieved.

4. Conclusions

The content of N elements in the soil was low as a result of nitrification process and oxidation by bacteria and leaching. Nitrification inhibitors litter is litter tree Mahogany, Teak, Jackfruit, Mango and Breadfruit. The sequence also shows the order of mention of the potential as a nitrification inhibitor. Soybean cultivation in Gung Gajah Forest as agro forestry system needs macro nutrients intake. N with urea fertilizer recommendation of 90 kg ha⁻¹, P with SP36 as much as 9 kg ha⁻¹ and K with KCl at 98 kg of ha⁻¹. The empty pods (seedless pod) as main problem still there but the pod fills are also formed so the grain yield was obtained 0.86 ton ha⁻¹ (the highest yield was 1.6 ton ha⁻¹).

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