The Influence of Urea Modified Lignin from Palm Empty Bunch toward Vegetative Aspects of Lettuce Leaves

Lucy Arianie, Ahmad Mulyadi, Wan Arif Abidin, Indra Johansyah Alam, and Afghani Jayuska

***Abstract*—Modified of urea fertilizer with lignin's palm empty bunch was applied to lettuce growth. This research aims to enhance the utility value of lignin from palm empty bunch, investigate slow release of urea-lignin fertilizer and vegetative aspect of leaves lettuce after period of week. Application of fertilizer was done in two difference places, namely green house laboratory and traditionally environment. Research showed that fertilized using urea fertilizer which modified by 2 % of lignin resulted amount (pc), length (cm) and wide (cm) of lettuce leave is better than fertilized only by urea in laboratory scale. Amount (pc), length (cm) and wide (cm) average of fresh lettuce leaves using urea modified by 2 % lignin resulted 8 pieces, 12 cm and 11 cm respectively on 6 weeks after plantation. Furthermore in traditionally environment, amount (pc), length (cm) and wide average (cm) of fresh lettuce leaves by using urea modified 3% lignin resulted 14 pieces, 25 cm and 21 cm consecutively on 6 weeks after plantation.**

***Index Terms*—Lignin, lettuce, palm empty bunch, urea.**

I. Introduction

Palm empty bunch (PEB) is a solid waste biomass from palm farming. The primary component of palm empty bunch is lignin and cellulose. Lignin is widely used based on structure, chemical characteristics and its abundances [1]. Lignin is a hydrofob polymer fenolic with non toxic fenilpropanoid monomer. On the contrary, urea is nitrogen fertilizers that hydrofil and easily dissolved in water. In general, urea fertilized repeatedly to makes the process of urea absorption by the plants' root is optimal. Modification of urea - lignin fertilizers was done to make slow release urea fertilizer.

II. Method

A. Preparation of Sample

Palm empty bunch was taken from Pabrik Minyak Sawit, PTPN XIII, Kabupaten Parindu, Kalimantan Barat, Indonesia. This sample was prepared by heated under sunlight and milled. All chemical which used are pro analysis grade except urea, then all procedures below was done triplo.

B. Extraction of Lignin

Palm empty bunch (PEB) was dried on the temperature 100 – 105 ^{0}C for 4 hours. Extraction of lignin was done using reflux equipment which 50 g PEB, ethanol : water (1:1 v/v) as solvent, 6% NaOH at 180 ^{0}C for 60 minutes [2]. Ratio of PEB – solvent = 1:10 w/v. This process which called organosolv delignification will create two products namely pulp and black liquor. Lignin extracted by added water into black liquor (water : black liquor = 1:6 v/v). Then, the acidity was done by add 10% H_2SO_4 up to pH 2, heated up to 60 ^{0}C and stirred with magnetic stirrer. The sedimentation process occured for 4 hour. The product was filtered by whatman paper 42. Residu was washed using aquades pH 2 and filtered vaccum again. This step were done for four times. The solid material namely lignin extract, was dried at 60 ^{0}C for 10 hours then stored in dessiccator [3].

C. Purity Analysis

The lignin extract was analyzed of its purity through gravimetric Klason method, by put 0.25 g of dry lignin extract in the beaker glass to the oven tempered 105 ^{0}C for 4 hours. The lignin then dissolved into 15 mL of 72% sulfuric acid. Beaker glass is closed by the watch glasses for 2 hours then diluted by 400 mL of aquades. The reflux process was done up to 4 hours, filtered by filter funnel with fixed weight and residu washed until acid free [4]. The lignin residu was dried in the oven tempered 105 ^{0}C for 4 hours, then kept in the desiccator. This lignin residu called pure lignin and measured. Percentage of the lignin purity was formulated as follows :

$$\%\ \text{of purity lignin} = \frac{\textit{Weight}\ \text{of pure lignin}}{\textit{Weight}\ \text{of dry lignin}} \times\ 100\ \% \qquad (1)$$

D. Infra Red Measurements

The analysis of functional group in lignin extract was determined by infra red spectrometer through pellet KBr method.

E. Tablet of Urea - Lignin

Tablet of urea - lignin made with the total weight of tablet was 850 mg. The process binding urea - lignin was done with the percentage 1, 2, 3, 4 and 5 % of lignin from the total weight of the tablet [5]. Kjehdal method are used to analizing nitrogen concentration of each tablet.

F. Solubility Tablet Analysis

Solubility tablet was analyzed using the modified glass tools that runs the water continually through the tablet.

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Stopwatch was used to investigate the time of tablet solubility. Rate of water flow is fixed 0.15 m^2/second [5], [6].

G. Germination of Seed

Various consideraton in choosing lettuce as sample plant because the main product of lettuce is its leaves. Lettuce seeds was got from plant traditional market. Before it is planted, the seeds of lettuce are germinated. The plantation into the 5 Kg of plastic bag was done after the plant reach the age of two weeks after the seeds are germinated.

H. Fertilization and Grooming of Plant

Lettuce's fertilizing was done two weeks after planting. Three fertilizer tablets were inserted into the hole soil which its depth is 5 cm and the distance between hole and the lettuce's stem is 5 cm. The grooming covers watering and weeds preventing. Lettuce's watering was done twice a day, in bright weather. If it is raining, the watering will not be done. Weeds' prevention was done mechanically by pulling it out and repeatedly done once a week. Lettuce planting done in two methods, firstly in modified green house laboratory and the last is traditional environment.

I. Number, Length and Wide of Leaves Measurement

The number of leaves measurement was done manually. The measurement of leaves length and wide done by leaf area meter and manually with milimeter block paper.

III. Result and Discussion

A. Extraction of Lignin

Extraction of lignin is similar to organosolv hydrolysis that aims to delignification of black liquor selectively. The principal of organosolv hydrolysis is hydrolyzed hemicelluloses simultaneously and dissolution of lignin using organic solvent that produce residue of cellulose. The average of black liquor and crude fiber that produced from this process were 233.3 mL and 44.34 g respectively [5].

The process of lignin extraction was done by adding aquades into black liquor with ratio black liquor : aquades = 1 : 6 (v/v) meant to enhance the form of lignin sediment. The average of extract lignin that produced was 0.837 and purity 81%.

B. Infra Red Lignin Analysis

Studied using ^{13}C showed that *p*-hydroxyphenyl α-prophenol (trans-*p*-coumaryl alcohol), guaiacyl α-prophenol (trans-*p*-conyferil alcohol) and syringil α-prophenol (trans-*p*-cinapyl alcohol) [1], which symbolized with H, G and S respectively are precursor and units of lignin framer.

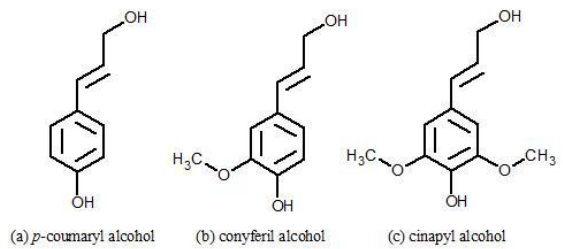

Fig. 1. Monomer of lignin (modified from Fengel and Wegener, 1995)

The characteristics of extract lignin depends on the plant's species, geographic location and delignification process. Wood lignin generally contains monomer unit Guaiasil (G) or Guaiasil – Siringil (G-S) [7]. Infra red spectrum of lignin and its explanation can be seen on Fig. 2 and Table I. Extract lignin consist of G monomer that explained at 1271.09 cm^{-1} and around ~850 cm^{-1}.

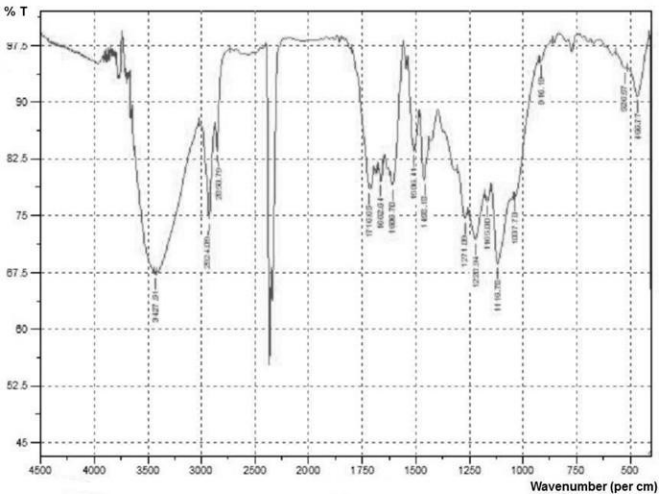

Fig. 2. Spectrum of infra red extract lignin

TABLE I: Comparison of Infra Red Bandth of Standard and Extract Lignin [5], [7]

Bandth (cm^{-1})		Expalanation
Extract Lignin	Standard lignin	
1716.65	1738 – 1709	C = O unconjugated keton in carbonil or ester
1662.64	1675 – 1655	C = O in aril – keton conjugated
1606.70	1593 – 1605	Aromatic vibration
1506.41	1505 – 1515	Aromatic vibration
1460.11	1460 – 1470	C – H deformation (in CH_2 or CH_3)
1458.18	1422 – 1430	Aromatic vibration and CH deformation
	1365 – 1370	CH aliphatic in CH_3
	1325 – 1330	Ring S and G.
1271.09	1266 – 1270	Ring G and C = O
1220,94	1221 – 1230	C – C, C – O, C = O
1165.00	1166	Specific for HGS lignin
	1140	C- H aromatic
1116.78	1125 – 1128	Specific for unit S; secondary alcohol and C = O
1037.70	1030 – 1035	C – H aromatic, C – O in primary alcohol
916.19	915 – 925	C- H (in aromatic ring)
~ 850	853 – 858	C- H in position 2,5,6 (Unit G)
	834 – 835	C – H in position 2 and 6 unit S and all unit unit H
	817 – 832	C – H in position 2,5,6 (Unit G)

C. Lignin – Urea Tablet and Its Solubility

Extract lignin from black liquor of palm empty bunch material is mixed by urea powder fertilizer and formed into tablet which its total weight reach 850 mg. Process of the binding lignin is done by the percentage 1, 2, 3, 4 and 5% from the total weight of tablet. The modification of commercial urea powder with lignin (variation percentage) is done to reduce the speed of urea dissolving. Solubility tablet was analyzed using the modified glass tools that runs the water continually through the tablet and rate of water flow is 0.15 m^2/second [5]. In this case, the tablet that has higher time is considered to have low speed solubility. Fig. 3

demonstrated urea tablet and urea – lignin tablet. It can be seen that the more lignin bound to urea tablet, the more brown of tablet.

Fig. 3. Tablet of urea-lignin fertilizer.

Lignin – urea bound is able to increase the tablet's solubility time. Table II shows that urea tablet fertilizer bound with lignin 4% produces optimum tablet solubility time was 12.39 minutes. Meanwhile, the urea tablet fertilizer that is used to control fertilizer produces the tablet solubility time for 2.22 minutes.

TABLE II: SOLUBILITY TIME OF TABLET FERTILIZER

Treatment	Average of solubility time (minute)
Urea	2.22
Urea-lignin 1%	1.36
Urea-lignin 2%	1.77
Urea lignin 3%	7.15
Urea-lignin 4%	12.39
Urea-lignin 5%	9.85

TABLE III: TOTAL NITROGEN IN TABLET FERTILIZER

Tablet fertilizer treatment	**The average of total nitrogen (g)**
Urea	44.63
Urea-lignin 1%	40.94
Urea-lignin 2%	31.24
Urea-lignin 3%	25.03
Urea-lignin 4%	17.46
Urea-lignin 5%	12.03

Reference [6] said, the use of lignin as the additional material in the urea tablet fertilizer has some advantages as follow :

1) Increase the economic value from the palm empty bunch waste by isolating lignin.
2) Lignin has a character which cannot be dissolved into water. If it is mixed with urea, the urea solubility will decrease and it will also decrease the nitrogen release so it can increase the efficiency in using urea fertilizer. It is worthed that plant will be able to absorb more nitrogen and grow optimally.
3) Lignin has the natural characteristics as the adhesive among the fiber, it means the bound power between urea and lignin will be stronger. When the mix of urea and lignin is formed into a tablet by using compression, lignin has a function to help the particle stick in the mix so the tablet fertilizer that made is stable. The urea lignin tablet fertilizer that produced is not fragile, not easily to crack and not easily soluble in water.
4) Lignin has non-toxic characteristic; it means lignin does not have bad impact to the environment. Lignin also can be found in the soil that comes from the residue of dead plant and unite with the soil. This means the use of lignin as the additional material in urea fertilizer does not affect the level of land fertility and plant's growth.

D. Analysis of Nitrogen Concentration in Total Tablet

The analysis of total nitrogen in each tablet produces various number. It shown in the Table III. It is assumed by the high weight of mass molecule lignin influenced the total weight of the tablets when it is weighted. Hygroscopic character of urea influence as well [5].

E. Fertilization and Fertilizer Aspect

The application of urea modified by lignin is done in lettuce which considered as a short term plant. It is applied repeatedly from 2009 to 2011 due to optimum observation.

This research using the lettuce's seeds branded 'panah merah' that assumed it has the same genetic factor and the process of photosynthetis is influenced by land, sunlight energy and fertilizer factors.

The growth of vegetative and production of plant depend on the interaction among the plant and environment condition where the plant grows. Condition of the environment is divided into climate, soil and other organism. These factors can limit or support the plant growth and production. Moreover, high production can be done by regulate the environment factors. One of the efforts to regulate the environment is by adding the fertilizer for vegetative growth.

Leaves is the main organ where the process of photosynthesis takes places. Reaction of photosynthesis illustrated as follows that Fig. out elements that affect its process:

$$6CO_2 + 6H_2O + \text{sunlight energy} \rightarrow C_6H_{12}O_6 + 6O_2$$

TABLE IV: THE INFLUENCE OF FERTILIZING TREATMENT TOWARD LETTUCE'S LEAVES AT FIRST CONDITION

Fertilizing treatment	at first condition 6 WAP*, average leaves :		
	Amount (pc)	Length (cm)	Wide (cm)
1. Without fertilizer	6	5	5
2. Urea	7	10	10
3. Urea - lignin 1%	8	10	10
4. Urea - lignin 2%	8	12	11
5. Urea - lignin 3%	8	10	10
6. Urea - lignin 4%	8	8	8
7. Urea - lignin 5%	8	7	9

*WAP = Weeks after planting

TABLE V: THE INFLUENCE OF FERTILIZING TREATMENT TOWARD LETTUCE'S LEAVES AT SECOND CONDITION

Fertilizing treatment	at second condition 6 WAP*, average leaves :		
	Amount (pc)	Length (cm)	Wide (cm)
1. Without fertilizer	8	10	10
2. Urea	12	18	10
3. Urea - lignin 1%	12	18	13
4. Urea - lignin 2%	12	20	17
5. Urea - lignin 3%	14	25	21
6. Urea - lignin 4%	15	24	18
7. Urea - lignin 5%	14	22	19

*WAP = Weeks after planting

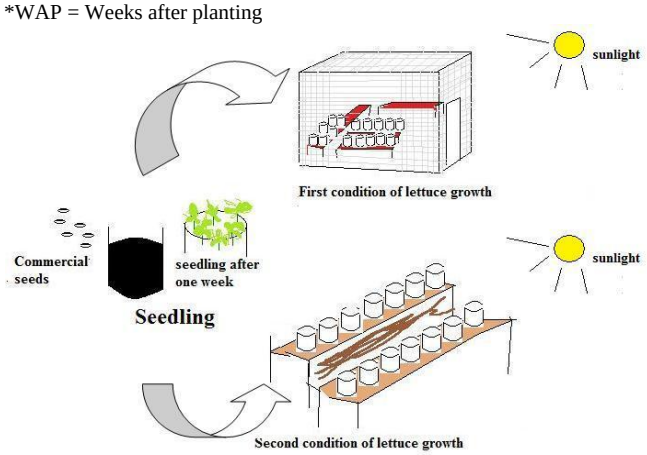

Fig. 4. Ilustration of urea modified lignin which applied to lettuce.

Fig. 5. Lettuce's leaves which fertilized using urea – lignin fertilizer in the second condition.

Stem of lettuce placed in the main centre and its leaves spread into stem caused the distribution of sunlight widespread throughout the leaves. Uniforms distribution of sunlight among the leaves will encourage leaves to photosynthesis.

The observed shows that the growth, numbers, length and wide of lettuce's leaves in modified green house laboratory are better by giving the urea tablet fertilizer modified by 2% lignin than using only the urea tablet fertilizer. Conversely, lettuce planting in traditional environment with urea tablet fertilizer which modified by 3% of lignin given better growth than planting in laboratory scale. It reach 21 cm of wide leaves average and 25 cm of length. It is clearly displayed in Table IV and Table V.

Fertilizing used urea fertilizer which modified by 2% and 3% of lignin are the optimum condition that can be seen on Table IV and Table V. Interestingly, there were significant differences between first and second condition of lettuce growth. It can be seen on Table 5 that second condition gives higher length and wide of leaves. It maybe caused by sunlight at the second condition optimally received by lettuce during its growth. Reference [8] shows that growth and production of plant, cannot be denied, depends on photosynthesis energy from sun. Closed green house tightly gives limited fresh air infiltration and carbon dioxide levels become reducing and affect for plant growth [8]. After all, it is assumed that urea fertilizer modified by 2-3 % lignin contains carbon (in the form of carbohydrate and chlorophyl) more than control lettuce.

IV. Conclusions

This research must be tested repeatedly before applied to major plant cause there are lots of factor that influence vegetative factor of plant. Moreover, urea characteristics which higroscopics needs special handling when tablet binding.

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