ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.11 pp 104-112, 2016

Multi-Response Linear Mixed Model (MLMM) for Longitudinal Data: Modelling Agricultural Waste: Plant Rice Straw, Clove Leaf And Water Hyacinth

Solimun[1]*, Adji Achmad Rinaldo Fernandes[1]

[1]Department of Statistics, Faculty of Natural Sciences, University of Brawijaya, Malang, Indonesia & Jl MT Haryono 169 Malang 65145, Indonesia

Abstract : The research objective is to establish a data model of agricultural waste using multi-response mixed model, with the responses are phenolics, flavonoids, and tannins (PFT). In this study, data to be modeled is a secondary data obtained in Sulawesi Province. The samples were washed, dried and extracted by maceration. Chemical materials such as distilled water, CH3OH, Folin Ciocalteu reagent 50%, Na2CO3 solution 3%, chloride aluminium solution 2%, HCl solution, C2H5OH, vanillin solution 4% were used as needed. The result shows the model of agricultural waste using multi-response mixed model. Multi-responses dealing with phenolics, flavonoids, and tannins (PFT), and the single predictors is the level of methanol, and types of plants (plant rice, clove leaf and water hyacid). From the results of the above model predictions show that the pattern of differences between responses (phenolic, flavonid, and tannin) if it is associated with the predictor (methanol), for the three types of plants (plant rice, clove leaf and water hyacid). Response phenolic and flavonid tends to increase with increases in levels of methanol in water plants yacid, but levels go up and down on the crop plant rice and clove leaf. Patterns tend to decline and then increase seen in the response to the three types of plant in tannins response.
Key words: MLMM, Phenolic, Flavonoid, Tannin, Agricultural Waste, Methanol.

Introduction

Longitudinal data is data that has two characteristics which repeated measurements on the same subjects during a certain period and there is a relationship between the studied observations from time to time[1]. In general, studies to determine the explanatory variables that most influence on more than one response using Multivariate Analysis of Variance (MANOVA) model. The correlation between observations within the same unit on longitudinal data lead MANOVA procedure can not be applied, so that appropriate methods for the analysis of longitudinal data with more than one response variable using the Mixed Models[2]. In the longitudinal data is often found to observations that have more than two response variables are interconnected and quantitative. This research will form a model of multi-response longitudinal data on chemical model using the Mixed models[3].

Indonesia is famous for its abundant natural wealth of flora and fauna. North Sulawesi region rich in natural resources with a wealth of biodiversity and have an environment where growth and development are very favorable to the growth and development of flora and fauna. Biodiversity and supportive environment can be managed and * organisms. In North Sulawesi, natural products as the rest or the final part of the process of plant life, such as sticks, leaves, unprocessed and utilized only allowed to fall scattered and scattered to decompose naturally.

According to Wahyuni[4] agricultural waste is a source of organic material available in large quantities and produced continuously, but have not been used optimally. Agricultural wastes generated during the production process in the field, at the time of harvest and agriculture pascapanen.Limbah containing organic compounds containing primary metabolites, such as carbohydrates, fat, protein and other constituent lainnya.Bahan constituent materials are secondary metabolites, such as; bioactive compounds, drug compounds, antioxidant compounds and other secondary metabolites that can serve as a sensitizer. Theorganic agricultural waste is broken down into other forms by way of aerobic and anaerobic. Agricultural waste leaves contain various types of base material or the main source of phenolic components are abundant, it can be recycled into useful materials sensitizer for plant growth, and fertility of the soil by the help of sunlight[4].

The leaves that had parents will be brownish-yellow and will detach and fall from the trees will be scattered on the ground, and the waters will flow. If the plant life on the banks of waterways will lead to accumulation of garbage or waste into organic soil surface plantations or agricultural land, watershed and coastal environments. Wrong attitude and is often done by farmers during the harvest is finished burning of crop residues are considered as agricultural waste. This occur can cause environmental pollution that can damage soil, water and air. Agricultural wastes of different types of leaves when buried above ground level, by the work of microbes in the soil will cause the weathering process takes long waste. The different kinds of leaves is done by chemically treating waste into organic matter that contain natural ingredients sensitizer can be called biosensitizer. Biosensitizer materials can be obtained by extracting different kinds of leaves and analyzed the phytochemical resulting phenolic compounds, flavonoids and tannins by using certain reagents and by the help of ultraviolet light (UV) from sunlight[5].

Plants require food or (plant nutrient) called plant nutrients, which are different to men who can use organiksebagai ingredients of foodstuffs, whereas in plants using inorganic materials to obtain energy for pertrumbuhannya.Keberadaanunsur nutrients in nature, which can be absorbed by plants very mempengaruhiproduksi a plant, such as carbon, hydrogen, and oxygen obtained from air and water is an essential element for plant growth. The elements needed by plants consist of macro nutrients, which has six (6) types of elements, namely; nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) while the micro-nutrients, which has seven (7) types of elements, namely; boron (B), copper (Cu), zinc (Zn), molybdenum (F), chlorine (Cl), manganese (Mn) and iron (Fe). According foth (1984) the availability of nutrients in the soil is used by plants depend on factors compound or humus and soil pH. The pH value (acidity) of the soil is an important factor in influencing the solubility of an element in the soil. If the soil has a high acidity will affect the availability of some nutrients, which can lead to increased solubility of aluminum (Al) in the soil, which would result in toxic for plants. Chandler and Silva[5] states that concentrations of nitrogen, phosphorus and potassium in soybean leaves will be reduced by the presence of increasing concentrations of aluminum. While Foth[6] stated that aluminum poisoning will reduce the absorption of nutrients, one of which is the element iron (Fe). The element iron plays an important role in the system of enzymatic synthesis of chlorophyll. In case of iron deficiency will cause chlorosis symptoms on plant leaves will yellow light, and will be visible on the younger leaves. In the area between leaf veins will largely be unaffected and leaf veins remain dark, a condition called chlorosis[6]. This research wants to develop the modeling of agricultural waste, especially in plants rice straw, clove leaf and water hyacinth. The research objective is to establish a data model of agricultural waste using multi-response mixed model, with the responses are phenolics, flavonoids, and tannins (PFT).

Theoretical Review

Longitudinal data is data that has two characteristics which repeated measurements on the same subjects during a certain period and there is a relationship between the studied observations from time to time. Research data in which the number of time units for each unit of the same cross-sectional data are called balance longitudinal data[7]. The framework of balanced longitudinal data show the same measurement unit (fixed) and the number of observations for the cross-sectional units (subjects) is the same in all subjects was observed.

Verbeke and Molenberghs[7] explains that in practice, the longitudinal data using linear regression function on each subject (subject-specific). This method is called with two-stage analysis.

a. First Stage

At this stage, response variables Y_{ij} indicates that the response to be observed, for the i-th individual, at time j-th, where i = 1, ..., S, and j = 1, ..., n_i

Models in the first stage is defined as:

$$\mathbf{Y_i} = \mathbf{Z_i}\boldsymbol{\beta}_i + \boldsymbol{\varepsilon}_i \,, \qquad (1)$$

$$\mathbf{Y_i} = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \vdots \\ y_{n_i} \end{bmatrix}_{n_i x1}, \mathbf{Z_i} = \begin{bmatrix} 1 & t_1 & t_1^2 & \cdots & t_1^r \\ 1 & t_2 & t_2^2 & \cdots & t_2^r \\ 1 & t_3 & t_3^2 & \cdots & t_3^r \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 1 & t_{n_i} & t_{n_i}^2 & \cdots & t_{n_i}^r \end{bmatrix}_{n_i xq},$$

$$\boldsymbol{\beta}_i = \begin{bmatrix} \beta_{1i} \\ \beta_{2i} \\ \beta_{3i} \\ \vdots \\ \beta_{qi} \end{bmatrix}_{qx1}, \text{ and } \boldsymbol{\varepsilon}_i = \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \\ \vdots \\ \varepsilon_{n_i} \end{bmatrix}_{n_i x1}$$

where:

$\mathbf{Y_i}$: response variable
$\mathbf{Z_i}$: predictor variable
$\boldsymbol{\beta}_i$: subject-specific coefficient
$\boldsymbol{\varepsilon}_i$: error model, $\boldsymbol{\varepsilon}_i \sim N(\mathbf{0}, \sigma^2 \boldsymbol{I}_{n_i})$
$\boldsymbol{I}_{n_i}$: identity matrix

b. Second Stage

In the second stage, the regression model used to explain the diversity of each patient associated with regression coefficients $\boldsymbol{\beta}_i$ specific subject, with the form:

$$\boldsymbol{\beta}_i = \mathbf{K_i}\, \boldsymbol{\beta} + \mathbf{b_i} \qquad (2)$$

where:

$\mathbf{K_i}$: predictor variable
$\boldsymbol{\beta}$: unknown parameter regression
$\mathbf{b_i}$: residual, $\mathbf{b_i} \sim N_q(\mathbf{0}, \mathbf{D})$
$\mathbf{D}$: covariance matrix of response variable

According to Verbeke and Molenberghs[7], the combination of the two-stage analysis into a single statistical model, which combines $\boldsymbol{\beta}_i$ in equation (2) into equation (1) will be obtained Mixed Model as follows:

$$\mathbf{Y_i} = \mathbf{X_i}\, \boldsymbol{\beta} + \mathbf{Z_i}\mathbf{b_i} + \boldsymbol{\varepsilon}_i \qquad (3)$$

The model assumes the vector of repeated measurements followed the linear regression model with population-specific parameter, β (same for all subjects) and subject-specific parameters b_i assumed to be random so-called random effects.

According to Hedeker and Gibbons[8], the excess Mixed Model in longitudinal data modeling are: (1) Model the evolution time response on the subject clearly, (2) More flexible in terms of repeated measurements, by not requiring the same number of observations for each subject and time can be a continuous value, (3) Specifications of covariance are more flexible structure in repeated measurements, and (4) The model can be expanded into a higher level, repeated measurements of each subject in the group.

Materials and Methods

In this study, data to be modeled is a secondary data obtained from Rorong[9]. Sulawesi Province. The samples were washed, dried and extracted by maceration. Chemical materials such as distilled water, $CH3OH$, Folin Ciocalteu reagent 50%, $Na2CO3$ solution 3%, chloride aluminium solution 2%, HCl solution, $C2H5OH$, vanillin solution 4% were used as needed. The implementation was conducted for eight (8) months from February to September 2011. The research was conducted in the laboratory of Advance Chemistry of FMIPA Sam Ratulangi University and the Laboratory of Research and Industry Standardization of North Sulawesi Province.

The method has been used for longitudinal data and multi-responses using Multi-responses Linear Mixed Model (MLMM). Thiebaut[10] defines the Mixed Models in birespon variables with Gaussian mixture model of the component is random, order to-1 from the auto-regressive, AR (1) and remnant components. The expansion of the model into a multi-response model, suppose $\mathbf{Y_i} = \begin{bmatrix} Y_{1i} \\ Y_{2i} \\ \vdots \\ Y_{ki} \end{bmatrix}$, is the response vector for i[th] subject, with Y_{ki} is the measurement vector n_{ki}, then k (k = 1,2 ,...,.) with $n_{1i} = n_{2i} = n_{ki}$. Mixed Models in multi-response variables that can be used are as follows:

$$\mathbf{Y_{1i} = X_{1i}\beta_1 + Z_{1i}b_{1i} + W_{1i} + \varepsilon_{1i}} \qquad (3)$$
$$\mathbf{Y_{2i} = X_{1i}\beta_2 + Z_{1i}b_{1i} + W_{2i} + \varepsilon_{2i}} \qquad (4)$$
$$\vdots$$
$$\mathbf{Y_{ki} = X_{1i}\beta_k + Z_{1i}b_{1i} + W_{ki} + \varepsilon_{ki}}$$

where

$\boldsymbol{\varepsilon_{ki}} \sim \mathrm{N}\,(\mathbf{0}, \boldsymbol{\sigma^2_{\varepsilon_k}}\mathbf{I_{n_i}}),\ \mathbf{b_{1i}} \sim \mathrm{N}(\mathbf{0}, \mathbf{G_1}),\ \mathbf{W_{ki}} \sim \mathrm{N}(\mathbf{0}, \mathbf{R_{ki}})$

$\mathbf{X_{1i}}$: predictor variable for fixed effect

$\boldsymbol{\beta_k}$: fixed effect

$\mathbf{Z_{1i}}$: predictor variable for random effect

$\mathbf{b_{1i}}$: random effect

$\mathbf{I_{n_i}}$: identity matrix

$\boldsymbol{\varepsilon_{ki}}$: error model

$$\boldsymbol{\sigma^2_{\varepsilon_k}}\mathbf{I_{n_i}} = \begin{bmatrix} \sigma^2_{\varepsilon_1} & 0 & 0 & 0 \\ 0 & \sigma^2_{\varepsilon_2} & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & \sigma^2_{\varepsilon_i} \end{bmatrix} : \text{error covariance matrix}$$

$$\mathbf{W_{ki}(t)} : \begin{bmatrix} w_{1i}(t) \\ w_{2i}(t) \\ \vdots \\ w_{ki}(t) \end{bmatrix} \text{covariance function}$$

Suppose that in a longitudinal data set has three response variables at three time iteration:

$$\mathbf{w_{ki}(t)} = \mathbf{R_i} \otimes \boldsymbol{\rho}$$

$$\mathbf{W_{ki}(t)} = \begin{bmatrix} \sigma^2_{w_1} & \sigma_{w_1w_2} & \sigma_{w_1w_3} \\ \sigma_{w_1w_2} & \sigma^2_{w_2} & \sigma_{w_2w_3} \\ \sigma_{w_1w_3} & \sigma_{w_2w_3} & \sigma^2_{w_3} \end{bmatrix} \quad \begin{bmatrix} 1 & \rho & \rho^2 \\ \rho & 1 & \rho \\ \rho^2 & \rho & 1 \end{bmatrix}$$

Thiebaut[10] describes the marginal Mixed models in longitudinal multi-response data are given:

$$\mathbf{Y_{1i}} \sim N(\mathbf{X_{1i}\beta_{1i}}, \mathbf{Z_{1i}G_1Z^T_{1i}} + \mathbf{R_i} + \Sigma_{1i})$$
$$\mathbf{Y_{2i}} \sim N(\mathbf{X_{1i}\beta_{2i}}, \mathbf{Z_{1i}G_1Z^T_{1i}} + \mathbf{R_i} + \Sigma_{2i})$$
$$\vdots$$
$$\mathbf{Y_{ki}} \sim N(\mathbf{X_{1i}\beta_{ki}}, \mathbf{Z_{1i}G_1Z^T_{1i}} + \mathbf{R_i} + \Sigma_{ki})$$

If $\boldsymbol{\alpha}$ is variance component as variance parameter in $\mathbf{V_i} = \mathbf{Z_iG_iZ_i^T} + \mathbf{R_i} + \boldsymbol{\Sigma}_i$, then $\boldsymbol{\alpha}$ is thus composed of q(q+1)/2 distinct elemen in $\mathbf{G}$ and all parameter in $\boldsymbol{\Sigma}_i$. Thus $\boldsymbol{\theta} = (\boldsymbol{\beta}', \boldsymbol{\alpha}')'$ is parameter estimates of marginal model $\mathbf{Y_i}$, and $\Theta : \Theta_\beta \times \Theta_\alpha$ as parameter space for $\boldsymbol{\theta}$, then $\mathbf{G}$ and all $\boldsymbol{\Sigma}_i$ as semi definit positive matrix.

According Verbeke and Molenbergh[7], the classical approach to obtain a conclusion based on the expected value of fixed effects parameters β obtained by maximizing the marginal likelihood function:

$$L_{ML}(\theta) = \prod_{i=1}^{s} \left\{ (2\pi)^{-\frac{n}{2}} |\mathbf{V}_i(\boldsymbol{\alpha})|^{-\frac{1}{2}} \exp\left(-\frac{1}{2} (\mathbf{Y}_i - \mathbf{X}_i\boldsymbol{\beta})' \mathbf{V}_i^{-1}(\boldsymbol{\alpha}) (\mathbf{Y}_i - \mathbf{X}_i\boldsymbol{\beta}) \right) \right\} \qquad (6)$$

Based $\boldsymbol{\theta}$ and assuming $\boldsymbol{\alpha}$ is known. *Maximum Likelihood* (ML) estimation for fixed effect $\boldsymbol{\beta}$, obtained by maximizing (6), conditional on variance components of $\boldsymbol{\alpha}$ is:

$$\widehat{\boldsymbol{\beta}}(\boldsymbol{\alpha}) = \left(\sum_{i=1}^{S} \mathbf{X}_i' \mathbf{W}_i \mathbf{X}_i \right)^{-1} \sum_{i=1}^{S} \mathbf{X}_i' \mathbf{W}_i \mathbf{y}_i \qquad (7)$$

Where $\mathbf{W_i} = \mathbf{V_i}^{-1}(\boldsymbol{\alpha})$, as covariance matrix for $\widehat{\boldsymbol{\beta}}$ as follow:

$$var(\widehat{\boldsymbol{\beta}}) = \left(\sum_{i=1}^{s} \mathbf{X}_i' \mathbf{W}_i \mathbf{X}_i \right)^{-1} \left(\sum_{i=1}^{s} \mathbf{X}_i' \mathbf{W}_i var(\mathbf{Y}_i) \mathbf{W}_i \mathbf{X}_i \right) \left(\sum_{i=1}^{s} \mathbf{X}_i' \mathbf{W}_i \mathbf{X}_i \right)^{-1} \left(\sum_{i=1}^{s} \mathbf{X}_i' \mathbf{W}_i \mathbf{X}_i \right)^{-1} \qquad (8)$$

Then $\widehat{\boldsymbol{\beta}}(\boldsymbol{\alpha}) \sim N(\boldsymbol{\beta}, var(\widehat{\boldsymbol{\beta}}))$.

Solve the equations such as likelihood function can also use the Newton-Raphson iteration method. Newton-Raphson iteration method is a method for determining the value of a parameter estimator is repeated until converges at a certain value. $T_1 = (T_{11}, T_{12}, \ldots, T_{1k})$ is a k-dimensional vector statistic obtained from the trial solution. Tr is a vector view of the rth iteration. $\hat{\theta} = (\hat{\theta}_1, \ldots, \hat{\theta}_k)$ is the maximum likelihood estimator. Where ε_r is the error in the rth iteration is obtained from the difference between Tr and $\hat{\theta}$. To achieve a convergent then the value of $\hat{\theta}$ if $\varepsilon_r \leq 10^{-6}$ for $r \to \infty$ ([8]).

Estimation of variance components using the Maximum Likelihood method produces the expected value is not valid, so use REML estimators (Diggle[10]).

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{b} + \mathbf{W} + \boldsymbol{\varepsilon}, \qquad (9)$$

S is a combination of subject-specific regression model (4) and (5), where $\mathbf{Y}$, $\mathbf{b}$, and $\boldsymbol{\varepsilon}$, and the matrix $\mathbf{X}$ is obtained from the stacking vectors $\mathbf{Y_i}$, $\mathbf{b_i}$, $\boldsymbol{\varepsilon}_i$, and $\mathbf{X_i}$ matrix, while $\mathbf{Z}$ is diagonal-block by $\mathbf{Z_i}$ on the main diagonal and zero otherwise, while $\mathbf{W}$ is the realization of order-1 vector of auto-regressive of $\mathbf{W_i}$. $\mathbf{Y}$ with $\sum_{i=1}^{S} n_i$ dimension, and is denoted by n.

Thus $\mathbf{Y} \sim \mathbf{N}(\mathbf{X}\boldsymbol{\beta}, \mathbf{V}(\boldsymbol{\alpha}))$, $\mathbf{V}(\boldsymbol{\alpha})$ is a diagonal matrix with the Vi-block on the main diagonal and zero otherwise.

REML estimators for the variance components $\boldsymbol{\alpha}$ of obtained by maximizing the likelihood Error contrast $\mathbf{U} = \mathbf{A'Y}$, where A is any matrix of Likelihood Function Error Contrasts U = A full order with columns orthogonal to the column matrix $\mathbf{X}$. $\mathbf{U} \sim N(\mathbf{0}, \mathbf{A'V(\alpha)A})$, does not depend on $\boldsymbol{\beta}$. According Harvile (1974) in Verbeke and Molenberghs[7], Contrasts Error Likelihood function can be written as:

$$L(\alpha) = \pi^{-\frac{n-p}{2}} \left| \sum_{i=1}^{s} \mathbf{X}_i'\mathbf{X}_i \right|^{\frac{1}{2}} \left| \sum_{i=1}^{s} \mathbf{X}_i'\mathbf{V}_i^{-1}\mathbf{X}_i \right|^{-\frac{1}{2}} \prod_{i=1}^{s} |\mathbf{V}_i|^{-\frac{1}{2}} \exp\left\{ -\frac{1}{2} \sum_{i=1}^{s} (\mathbf{Y}_i - \mathbf{X}_i\hat{\boldsymbol{\beta}})'\mathbf{V}_i^{-1}(\mathbf{Y}_i - \mathbf{X}_i\hat{\boldsymbol{\beta}}) \right\} \qquad (10)$$

Where $\hat{\boldsymbol{\beta}}$ obtained in equation (7). REML estimator for $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ obtained by maximizing the likelihood function of REML as follow:

$$L_{REML} = \left| \sum_{i=1}^{S} \mathbf{X}_i'\mathbf{W}_i(\boldsymbol{\alpha})\mathbf{X}_i \right|^{-1/2} L_{ML}(\boldsymbol{\theta}) \qquad (11)$$

For $\boldsymbol{\beta}$ dan $\boldsymbol{\alpha}$ parameter simultaneously, where $\mathbf{W}_i = \mathbf{V}_i^{-1}(\boldsymbol{\alpha})$ and L_{ML} $(\boldsymbol{\theta})$ as function of *Maximum Likelihood* in equation (6).

Result and Discussion

Firsty, selection the fixed effect. The test results of selected fixed effect that affect the response variables in three plants (Plant Rice Straw, Clove Leaf And Water Hyacinth) in Agricultural Waste.

Table 1: Selection of Fixed Effect

Fixed Effect	**AIC**	**P-value of Parameter Est.**		
		Methanol (predictors)	**Methanol (predictors)^2**	**Methanol (predictors)^3**
Methanol (predictors) Linear	2227.1	0.001*		
Methanol (predictors) Quadratic	2212.3	0.014*	0.001*	
Methanol (predictors) Cubic	2233.7	0.027*	0.202	0.399

* Significant at 5% level of significance

From table 2 shows that the smallest value of Akaike Information Criterion (AIC) indicate the selected fixed effect is methanol (predictors) quadratic. This also indicate that the component of quadratic trend of methanol (predictors) are significant at 5% significance level. Second, selection the random effect. Tentative model for the longitudinal multi-response data show that the random effects included intercept and random effects coefficient of linear methanol (predictors). The results of random testing selection effects are presented in Table 3

Table 3: Selection of Random Effect with fixed effect methanol (predictors) Quadratic

Random Effect	**-2 *Res Log Likelihood***	**$-2\ln\lambda_N$**	**P-value**
Intercept, Slope Methanol (predictors), Slope Methanol (predictors)^2	Not convergence		
Intercept, Slope Methanol (predictors)	2188.8	93.3	0.001*
Intercept Only	2095.5	93.3	0.001*
No Random Effect	2188.8		

* Significant at 5% level of significance

The results of random effects selection in Table 3 shows a significant p-value on the model, indicate that the random intercept and random slope methanol (predictors) linear include in the selection. So the final mixed model is for methanol (predictors) quadratic fixed effect, and methanol (predictors) linear fixed effect.

Final Mixed Models in multi-response variables include fixed effects methanol (predictors) quadratic with random effects methanol (predictors) linear. Table 4 below shows the parameter estimates for selected model

Table 4: Parameter Estimation of Fixed Effect

Response	Parameter	Estimate	Std.Er	$t_{observed}$	P-value
Phenolics	Intercept	14.082	0.934	15.08	0.000*
	t	-1.603	0.654	-2.45	0.026*
	t^2	0.077	0.016	4.80	0.000*
Flavonoids	Intercept	1160.970	114.850	10.11	0.000*
	t	-82.624	37.735	-2.19	0.044*
	t^2	2.369	2.622	0.90	0.367
Tannins	Intercept	17.140	1.980	8.66	0.000*
	t	0.938	0.870	1.08	0.297
	t^2	-0.050	0.043	-1.16	0.245

* Significant at 5% level of significance

Table 4 shows that the fixed effects model with linear methanol (predictors) significant at all four response variables. The final model of multi-response Mixed Model substitude by the parameter estimators marginal fixed effects model to obtain the final model of multi-response Mixed Model following equation (19):

(a) $Y_{1i} = (14.082 + b_{01i}) - (1.603 + b_{11i})\ t + 0.077\ t^2$
(b) $Y_{1i} = (1160.9 + b_{02i}) - (82.62 + b_{12i})\ t + 2.369\ t^2$
(c) $Y_{1i} = (17.140 + b_{03i}) + (0.938 + b_{13i})\ t - 0.050\ t^2$ (19)

Where t indicates predictor variables (percent of methanol). Based on the parameter estimation of fixed and random effect, subject specific prediction are shown in graph below (dot shows observation value, and line shows prediction value):

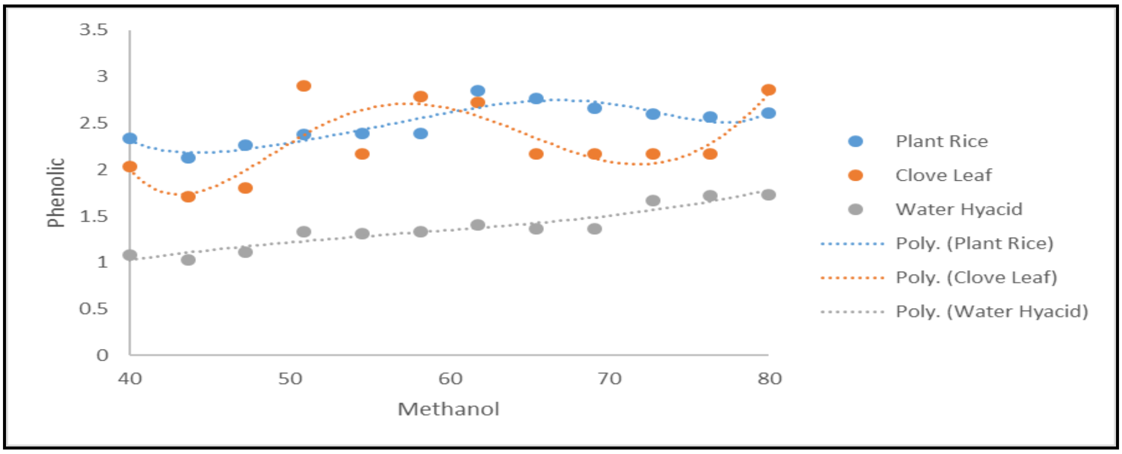

Figure 1: Phenolic Prediction

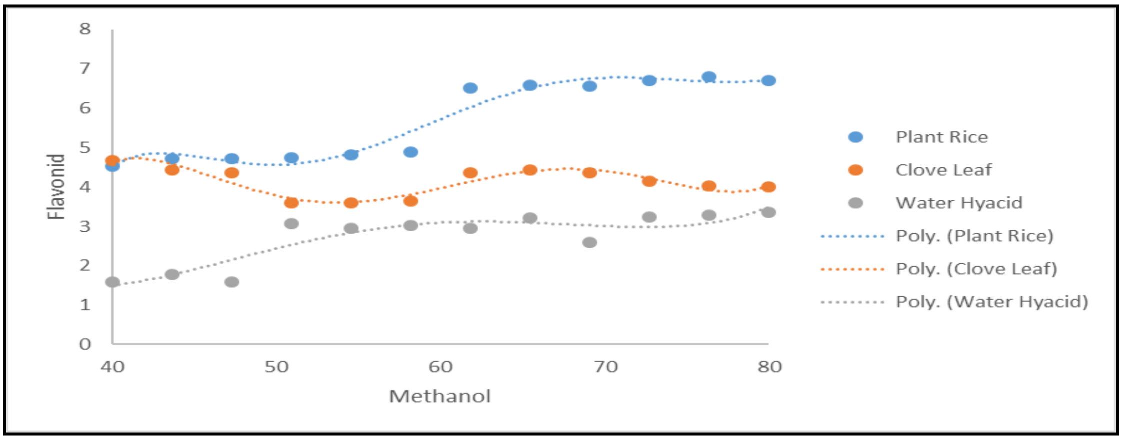

Figure 2: Flavonoid Prediction

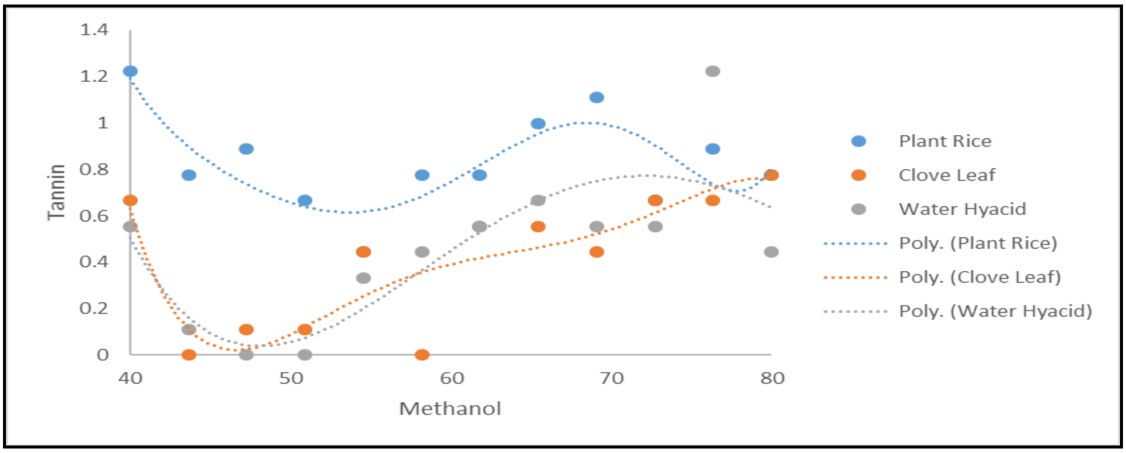

Figure 3: Tannin Prediction

Goodness of fit Mixed Model in equation (19) is obtained by calculating the value adjusted R-square values obtained for 0.8174. So it can be concluded that the variable responses of BMI, LEDs, monocytes and levels Supar 81.74% can be explained by the model include methanol (predictors) quadratic fixed effect, and methanol (predictors) linear random effect.

From the results of the above model predictions show that the pattern of differences between responses (phenolic, flavonid, and tannin) if it is associated with the predictor (methanol), for the three types of plants (plant rice, clove leaf and water hyacid). Response phenolic and flavonid tends to increase with increases in levels of methanol in water plants yacid, but levels go up and down on the crop plant rice and clove leaf. Patterns tend to decline and then increase seen in the response to the three types of plant in tannins response.

Some previous researchers reported that phenolic compounds have the ability to reduce a metal ion present in the oxidized state. Goodman and Cheshire (1982) has been studying ion reduction of molybdenum (Mo) by phenolic compounds. From the results of the study note that most of the ions MoO42-in solution will be reduced by the phenolic compounds into Mo5 +. Both ion MoO42-and Mo5 +, both adsorbed by the phenolic compounds through the exchange mechanism ion.Selanjutnya Goodman and Cheshire[12] states that the phenolic compounds can reduce V + 5 into V + 4 and Hg + 2 to Hgo. In the end, Goodman and Cheshire[12] concluded that the interaction of phenolic compounds with dissolved agent, not only result in a reduction of the species in the form of anions into a cation, but also the reduction of the cation into a cation.

Analysis of the concentration of phenolic compounds was conducted to determine the potential biosensitizer in an extract. Total phenolic compounds in extracts of plants or in the agricultural waste, is determined by the ability of phenolic compounds in the extract that can react with the acid-fosfotungstat fosfomolibdat the Folin-Ciocalteu reagent yellow will change color to blue (Suryanto[13]). According to Markham[14] is estimated at about 2% of the production of carbon as a result of photosynthesis in plants is converted to a compound or compounds turunannya.Sebagian flavonoid compounds are also derived from flavonoid.Jadi tannin flavonoid phenols is one of the largest nature, since flavonoids contained in all green plants. Suryanto[13] flavonoids have an effect on the health of the skin that can prevent bleeding as well as a natural antibiotic, such as found in the leaves of grass tiger (Lantana camara L) .Senyawa flavonoids can be extracted using hot water or ethanol produces a red color indicating the presence of flavonoids as a result of the reduction by hydrochloric acid and magnesium.

Tannin is divided into two classes of compounds and each class of compounds can react differently to the color of the compound ferriklorida ($FeCl_3$) 1%. Tannin hydrolyzed class would generate a blue-black color and condensed tannins will produce a blackish green. At the time of the addition is expected FeCl3akan compounds react with one hydroxyl group contained in tannin, the reaction products that can ultimately cause the color. $FeCl_3$ widespread use of compounds to identify phenolic compounds, including tannins.

Conclusion

The result shows the model of agricultural waste using multi-response mixed model. Multi-responses dealing with phenolics, flavonoids, and tannins (PFT), and the single predictors is the level of methanol, and types of plants (plant rice, clove leaf and water hyacid). From the results of the above model predictions show that the pattern of differences between responses (phenolic, flavonid, and tannin) if it is associated with the predictor (methanol), for the three types of plants (plant rice, clove leaf and water hyacid). Response phenolic and flavonid tends to increase with increases in levels of methanol in water plants yacid, but levels go up and down on the crop plant rice and clove leaf. Patterns tend to decline and then increase seen in the response to the three types of plant in tannins response.

References

1. Rutledge, J.H. 1995. *Multi-response Nonlinear Mixed Effects Models for Longitudinal Data Analysis.* http://www.dtic.mil/cgi-bin/GetTRDoc?Location=U2&doc=GetTRDoc. pdf&AD=ADA294598
2. Khutoryansky, N. M. 2003. *Parameter Estimation For Incomplete Bivariate Longitudinal Data In Clinical Trials.* Novo Nordisk Pharmaceuticals, Inc., Princeton, NJ.
3. Weiss, R. E. 2005. *Modeling Longitudinal Data*. Springer Texts in Statistic. New York.
4. Wahyuni, S. 2009. *Biogas*. Penebar Swadaya, Jakarta.
5. Chandler, J. V., and L. Silva., 1974, Experiments on The Effects of Soil Acidity Season and Spacing on Yields of Soybean Under Humid Tropical Condition. *Proceeding at the Workshop on Soybeans for Tropical and Subtropical Conditions*, Intsoy Pulications
6. Foth,H.D., 1984. *Fundamental of Soil Science*. John Willey & Sons. New York.
7. Verbeke, G. dan G. Molenberghs. 2000. *Linear Mixed Model for Longitudinal Data*. Springer Series in Statistics. New –York:Springer –Verlag.
8. Hedeker, D. dan R. D. Gibbons. 2006. *Longitudinal Data Analysis.* John Wiley & Sons. New York.
9. Rorong. J.A. 2012. Phytochemical Analysis Of Water Hyacinth (Eichhornia crassipes) OF Agricultural Waste As Biosensitizer For Ferri Photoreduction. *Agrivita* 34(2), 152-160.
10. Thiebaut, R., H. Jacqmin-Gadda, G. Chene, C. Leport, dan D. Commenges. 2002. *Bivariate Linear Mixed Models Using SAS Proc MIXED*. Journal of Computer Methods and Programs in Biomedicine, Volume 69, pp. 249 – 256.
11. Diggle, P. J., P. J. Heagerty, K. Y. Liang, dan S. L. Zeger, 2002. *Analysis Of Longitudinal Data*. Second Edition. Oxford University Press Inc., New York.
12. Goodman, B. A . and M V Chesshire. 1982. Reduction of Molybdate by Soil Organic Matter : EPR evidence for formation of Both Mo(V) and Mo(III). *Nature*, 299 : P: 618-620.
13. Suryanto, E., 2008. Singlet Oxygen Chemical: Sensitizer, Illumination and Reactivity Against Unsaturated Fatty Acid, *J. Chemistry Progress*. FMIPA of Sam Ratulangi University Manado. ISSN: 1979-5920. Volume 1 (2): 117-124.
14. Markham,K.R., 1988. *How to Identify Flavonoids*. Petone New Zealand.

*****