MODELLING REPEATED MEASURES DOSE-RESPONSE MORTALITY DATA USING GENERALIZED ESTIMATING EQUATIONS (GEE)

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ABSTRACT

Repeated measures dose-response mortality studies usually involve obtaining responses at different times on the same group of subjects, which often leads to correlation. A commonly used method for correlated dose-response mortality data is the Probit analytical technique which is suitable for data collected at one point in time and not for repeated measures. This study developed a Generalized Estimating Equations (GEE) using logistic regression for estimating the model parameters in repeated measures dose-response mortality data. The GEE model was applied to adult-termites mortality data observed at 6, 12, 18 and 24 hours respectively from an experiment conducted in the Entomology Division of the Nigerian Institute for Oil Palm Research, NIFOR, Edo State, Nigeria. In the experiment conducted, adult-termites were exposed to two plant extracts; *Jatropha Curcas* and *Ricinus Cummunis* at varying concentration levels (10%, 20% and 35%) respectively. The GEE estimated LT_{50} results for each plant extracts at varying concentration levels were given as *J.Curcas* (LT_{50}=12.47hrs, 12.47hrs and 12.47hrs) and *R.Cummunis* (LT_{50}=12.47hrs, 12.47hrs and 12.47hrs) which shows that the potency of the concentration levels is the same considering the time to mortality. Repeated measures logistic regression using GEE has proven to be a robust method in estimating LT_{50} since it consistently gave precise LT_{50} estimates with a smaller confidence interval, thus should be incorporated into studies of this nature as other existing methods for analyzing data from bioassay experiments.

Keywords: Repeated measures, dose-response, correlation, Probit analysis, GEE, Survival data, plant extracts, and mortality.

1.0 INTRODUCTION

Dose-response relationships commonly occur in agricultural research in areas such as plant science, soil science, entomology and animal science e.tc (Ritz et al. 2015; Dungan et al 2001; Turner, et al 1995).

To access selectivity and efficacy of various chemical formulations, a group of dose response is usually individually compared by evaluating the estimated potencies (Seefeldt et al 1995).

In dose response relationships study, when subjects are exposed to a particular stressor and its effect (mortality) from the same experimental unit is observed at different time intervals, say, t_1, t_2, t_3…. t_n. Data collected this way are usually correlated because successive observations are made on the same group of subjects at several concentrations over time (Robertson and Preisler, 1992; Thomsen and Eilenberg, 2000).

Correlated mortality data cannot be analyzed using standard probit analysis technique (Finney, 1964; Finney, 1971) which is the usual way of analyzing data from bioassay experiment (Thorne *et al.,* 1995.). This is because Probit analysis is adequate if the responses are independently, true for data collected at once after a given time point.

When measurements from dose-response mortality studies are correlated in addition to taking interest in the speed of death, there is need for an alternative method that will take care of the correlation in the data set while estimating lethal time. Such method includes the use of Generalized Estimating Equations (GEE) and the Survival Analysis technique.

The Generalized Estimating Equations (GEE) was introduced by Liang and Zeger (1986) as an extension of Generalized Linear Model (GLM) method (McCullagh and Nelder, 1983; McCullagh and Nelder, 1989) to handle correlated data. GLM are a generalization of standard linear regression that allows the response variables to have a distribution other than the normal distribution. The primary difference is that GEE has the ability to account for the within-subject covariance structure for the various types of response data. Zeger and Liang (1986), Ziegler *et al. (*1998). The available covariance structures specify how observations within a subject or cluster are correlated with each other.

In this study, Generalized Estimating Equations (GEE) is used in estimating lethal time (LT_{50}) for correlated termite's dose-response mortality data. Also, its performance using its confidence intervals was shown.

2.0 METHODOLOGY

2.1 DISCRIPTION OF DATA

The data for this study was obtained from a laboratory experiment conducted at the Entomology Division of the Nigerian Institute for Oil Palm Research (NIFOR), Benin City to test the effect of two botanical extracts with a positive control on mortality of adult-soldier termites as part of insect-plant control project.

The two botanical plant extracts used, were from *Jatropha curcas* (Physic seed) and *Ricinus communis* (Castor seed). Cypermethrin 25% EC and water served as positive and negative control respectively. The group of subjects (adult termites) was exposed to the botanicals extracts at three concentration levels: 10%, 20% and 35% (w/v).

A total of three hundred and twenty (320) adult termites were selected from the stock and a total of ten (10) termites were introduced into the each petri dish containing the impregnated filter papers containing botanical extracts at the different concentrations as well as those for the control. Each concentration with different botanical extracts was replicated four times. The response variable was adult-termites mortality observed at 12 hours and 24 hours, after exposure.

There was no death in negative control which consisted of water only and hence will not appear in the analysis. R statistical software version R 3.1.0 was used for the data analysis.

2.2 The Generalized Logistic Regression:

The logistic (logit) regression model which can be generalized for a dependent variable having two more categories is a type of regression analysis used for predicting the outcome of a categorical dependent variable based on one or more predictor variables (McCullagh and Nelder, 1983; McCullagh and Nelder, 1989).

Dose-response mortality data is a set of Bernoulli trials (a special case of Binomial distribution) in which the appropriate GLM to use is the Generalized Logistic Regression. Here, the values of response variable (mortality) are *1* if there is a success and *0* otherwise.

The p.d.f of the binomial distribution $B(n_i, \pi_i)$ is given as:

$$f(y_i) = \binom{n_i}{y_i} \pi_i^{y_i} (1-\pi_i)^{n_i - y_i} \qquad (1)$$

Taking logs of both sides and collecting like terms of equation (1) gives

$$\log f(y_i) = y_i \log\left(\frac{\pi_i}{1-\pi_i}\right) + n_i \log(1-\pi_i) + \log\binom{n_i}{y_i} \qquad (2)$$

Equation (2) has the general form of the exponential family

$$\log f_y(y; \theta, \phi) = \frac{[y\theta - b(\theta)]}{a(\phi)} + c(y, \phi) \qquad (3)$$

When compared, so that

$$\theta = \log\left(\frac{\pi_i}{1-\pi_i}\right), \quad b(\theta) = n_i \log(1-\pi_i), \quad c(y, \phi) = \log\binom{n_i}{y_i}$$

Thus, for the response variable Y_i, and a set of *n* predictor variables (dose or time), X_i we will consider a binary response variable with a logistic transformation or logit function defined by

$$Y_i = log\left(\frac{\pi_i}{1-\pi_i}\right) = \beta_0 + \beta_1 x_1 + \cdots + \beta_n x_n + \in_i \qquad (4)$$

Where π_i is the probability of success, β_0 is the intercept (slope), β_1 is the regression coefficients for each corresponding predictor variable, X_n (dose or time), and $\in_i$ is the error.

2.3 Generalized Estimating Equations (GEE):

The generalized logistic regression under GLM works with the assumptions that the response variables are correlated as with repeated measures data. An extension of GLM to handle such correlation in the data set is the GEE. Thus, the GEE is used to fit a specified model, which in this

case is the generalized logistic regression model to cater for within-subject/within-group correlations.

2.4 Derivation of GEE

Recall that the logistic regression (McCullagh and Nelder, 1989) has the general form of the exponential family as shown in equation (2).

$$\Rightarrow\ f_y\,(y_i;\theta,\phi) = exp\left\{\frac{\left[y_i h(\beta_i' X_i) - b[h(\beta_i' X_i)]\right]}{a(\phi)} + c(y_i,\phi)\right\} \qquad (5)$$

where

$\theta = h(\beta_i' X_i), i = 1, \ldots n\,, \beta_i$ *is a* $(P+1)\times 1$ *coefficient vector*, and ϕ *a scale parameter*.

The first two moments of Y_i is given as

$$E(Y_i) = \mu_i = b'(\theta_i) = b'\big(h(\beta_i' X_i)\big)$$

$$Var(Y_i) = b''(\theta)\, a(\phi) = b''\big(h(\beta_i' X_i)\big)\, a(\phi)$$

We define, $\mu_i = \beta_i' X_i$ if the subject within a group responds independently, then the regression parameters β_i can be estimated by the estimating equations as

$$g(\beta) = \sum_{i=1}^{n} X_i^T \Delta_i \big(Y_i - E(Y_i)\big) = 0, \qquad (6)$$

where $\Delta_i = diag\left(\frac{\partial \theta_i}{\partial \mu_i}\right) = diag\big(h'(\beta' X_i)\big)$

is an $n_i \times n_i$ ''working'' covariance matrix of Y_i, X_i is an $n_i \times (p+1)$ covariate matrix, and Y_i is a $n_i \times 1$ response vector. If $\Delta_i = cov(y_i)$ is correct, then $\hat{\beta}$ is asymptotically unbiased and efficient (i.e it has the smallest variance of all other possible estimations).

Similarly, If $\Delta_i \neq cov(y_i)$, when the subjects within a group do not respond independently, then $\hat{\beta}$ is asymptotically unbiased but not efficient. Thus, the GEEs that can be used to estimate regression parameters is of the form

$$g(\beta_i) = \sum_{i=1}^{n} D_i^T\, V_i^{-1}\big(Y_i - E(Y_i)\big) = 0 \qquad (7)$$

Where

$D_i = \partial\{b_i'(\theta)\}/\delta\beta_{i,}$ the j^{th} row of D_i correspond to $D_{ij} = \partial E\big(Y_{ij}\big)/\partial\beta_i = b''\big(\theta_{ij}\big)\, h'\big(\beta_i' X_{ij}\big) X_{ij}$

$$then, \quad D_i = \begin{pmatrix} D_1^T \\ D_2^T \\ \vdots \\ D_n^T \end{pmatrix} = \begin{pmatrix} b''(\theta_{i1})h'(\beta_1' X_1)X_{1n}^T \\ b''(\theta_{i2})h'(\beta_2' X_2)X_{2n}^T \\ \\ \vdots \\ b''(\theta_{in})h'(\beta_n' X_n)X_{nn}^T \end{pmatrix}$$

$$= \begin{pmatrix} b''(\theta_{i1}) & 0 & \cdots & 0 \\ 0 & b''(\theta_{i2}) & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & b''(\theta_{nn}) \end{pmatrix} \begin{pmatrix} h'(\theta_{i1}) & 0 & \cdots & 0 \\ 0 & h'(\theta_{i2}) & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & h'(\theta_{nn}) \end{pmatrix} \begin{pmatrix} X_1^T \\ X_2^T \\ \vdots \\ X_n^T \end{pmatrix}$$

Therefore, $D_i = B_i \Delta_i X_i$ is an $n_i \times p$ matrix, where $B_i = diag\big(b''(\theta_i)\big)$. Let's define $V_i = B_i^{\frac{1}{2}} R_i(\alpha) B_i^{\frac{1}{2}} / \phi$, where $R_i(\alpha)$ is an $n \times n$ correlation matrix of Y_i, and α is a vector of unknown parameters that defines the correlation matrix. $R_i(\alpha)$ is called the working correlation matrix because consistent estimators can be obtained even when $R_i(\alpha)$ is not correctly specified. V_i is a function of $\beta, \phi \; and \; \alpha$, where β is the parameter of interest, $\phi \; and \; \alpha$ are nuisance parameter.

Thus equation (7), becomes

$$g(\beta_i) = \sum_{i=1}^{k} (B_i \Delta_i X_i)^T \left[\frac{B_i^{\frac{1}{2}} R_i(\alpha) B_i^{\frac{1}{2}}}{\phi} \right]^{-1} \big(Y_i - E(Y_i)\big) \qquad (8)$$

Thus, equation (8) can be expressed in matrix form as

$$= \sum_{i=1}^{n} \left[\begin{pmatrix} b''(\theta_{i1}) & 0 & \cdots & 0 \\ 0 & b''(\theta_{i2}) & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & b''(\theta_{nn}) \end{pmatrix} \begin{pmatrix} h'(\theta_{i1}) & 0 & \cdots & 0 \\ 0 & h'(\theta_{i2}) & \cdots & 0 \\ \vdots & \vdots & \vdots & \\ 0 & 0 & \cdots & h'(\theta_{nn}) \end{pmatrix} \begin{pmatrix} X_1^T \\ X_2^T \\ \vdots \\ X_n^T \end{pmatrix} \right]^T$$

$$\begin{pmatrix} \sqrt{b''(\theta_{i1})} & 0 & \cdots & 0 \\ 0 & \sqrt{b''(\theta_{i2})} & \cdots & 0 \\ \vdots & \vdots & & \vdots \\ 0 & 0 & \cdots & \sqrt{b''(\theta_{nn})} \end{pmatrix}^{-1} \begin{pmatrix} r_{11}/\phi & r_{12}/\phi & \cdots & r_{1n}/\phi \\ r_{21}/\phi & r_{22}/\phi & \cdots & r_{2n}/\phi \\ \vdots & \vdots & & \vdots \\ r_{n1}/\phi & r_{n2}/\phi & \cdots & r_{nn}/\phi \end{pmatrix}^{-1}$$

$$\begin{pmatrix} \sqrt{b''(\theta_{i1})} & 0 & \cdots & 0 \\ 0 & \sqrt{b''(\theta_{i2})} & \cdots & 0 \\ \vdots & \vdots & \vdots & \\ 0 & 0 & \cdots & \sqrt{b''(\theta_{nn})} \end{pmatrix}^{-1} \begin{pmatrix} Y_{i1} - E(Y_{i1}) \\ Y_{i2} - E(Y_{i2}) \\ \\ \vdots \quad \vdots \\ Y_{nn} - E(Y_{nn}) \end{pmatrix}$$

When all responses within clusters are independent,

$$R_i(\alpha) = \begin{pmatrix} 1 & 0 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & 1 \end{pmatrix}$$

Then, equation (7) is equal to (6).

2.5 GEE Working Correlation Matrix:

According to (Zuur *et al.*, 2009; Liang and Zeger, 1986), choices for the correlation structure within GEE include the following: Independent, Exchangeable, Autoregressive AR(1), Unstructured correlation etc. One main feature of the GEE model is that the estimators are robust to departures from the true correlation patterns. Although, a loss in estimator efficiency can occur but this loss decreases as the sample becomes larger.

The GEE model works best if the numbers of observations per subject is small and the number of the subjects is large. Also, it is useful in longitudinal studies if the measurements are taken the same time for all subjects. However, the exchangeable correlation matrix was adopted since it is assumed that the correlation is different for each pair made.

2.6 Choosing the Correlation Structure in GEE:

The Quasi-likelihood Information Criterion (QIC) which is an extension of the Akaike Information Criterion (AIC) which applies to model fit by GEE was used to find an acceptable working correlation structure (Hardin and Hilbe, 2003).

$$QIC = -2Q(\mu; I) + 2trace(A_I^{-1}V_R) \quad (9)$$

Where, I is the independent covariance structure used to calculate the quasi-likelihood $\mu_i = g^{-1}(X_i\beta_i)$ and $g^{-1}(.)$ is the inverse link function for the the model (logit). A_I^{-1}, is the variance inverse matrix under the assumption of independence model. V_R is the robust variance estimator obtained from a general working covariance structure R. The model with the smaller statistic was preferred.

2.7 Covariances of $\boldsymbol{\beta_i}$:

In GEE (Liang and Zeger, 1986) we have both model-based and empirical covariance's produced as shown in equation (6) and (7).

2.7.1 The Model-based Estimate:

The model-based estimator of the covariance matrix of β_i is given by

$$Cov(\beta_i)_n = \sum_n(\beta_i) = I_0^{-1} \quad (10)$$

$$I_0 = \sum_i^n \frac{\partial \mu_i'}{\partial \beta_i} V_i^{-1} \frac{\partial \mu_i}{\partial \beta_i}$$

$$I_0 = X^T V^{-1} X \quad (11)$$

From the above, $Cov(\beta_i)_n$ consistently estimates $cov(\beta_i)$ if the mean model and the working correlation are correct.

2.7.2 Empirical-sandwich Estimate:

The empirical or robust estimator of the covariance matrix of β_i is given by

$$Cov(\beta_i)e = \sum_e (\beta_i) = I_0^{-1} I_1 I_0^{-1} \quad (12)$$

So that from equation (7),

$$I_1 = \sum_{i=1}^n \frac{\partial \mu_i}{\partial \beta_i} V_i^{-1} Cov(y_i) V_i^{-1} \frac{\partial \mu_i}{\partial \beta_i} \quad (13)$$

I_1 can also be written as

$$I_1 = D^T V^{-1} (y_i - \mu_i)(y_i - \mu_i)^T V^{-1} D \quad (14)$$

Here $Cov(\beta_i)_e$ is a consistent estimator of $Cov(\beta_i)$ even if the working correlation is mis-specified, i.e. $cov(y_i) \neq \sum_i$. In computing $\sum_e$, β_i and ϕ are replaced by estimates, and $cov(y_i)$ is replaced by the estimate $(y_i - \mu(\beta_i))(y_i - \mu(\beta_i))'$.

The robust or model-based standard errors is used in estimating the GEE model for large sample size regardless of the true form of $cov(y_i)$. If smaller it could be rather noisy.

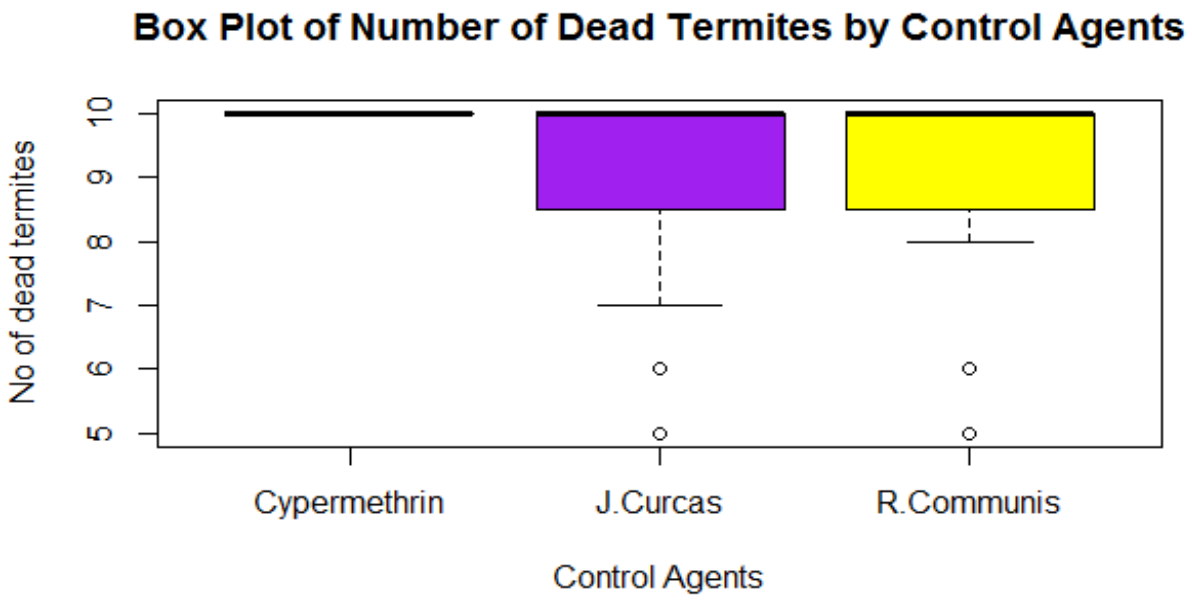
REMARK:

When within-subject correlations are not strong, (Zeger 1988) suggests that the use of GEE with empirical estimator is highly efficient.

3.0 RESULTS AND DISCUSSION

Descriptive Graphical Display

Fig 1: Boxplot for Number of dead termites/group with the Control Agents.



Cochrane Q test for three or more matched pairs suggests that the three control agents are significantly different from each other (Q test statistics was 16.384 with a p-value = 0.0257).

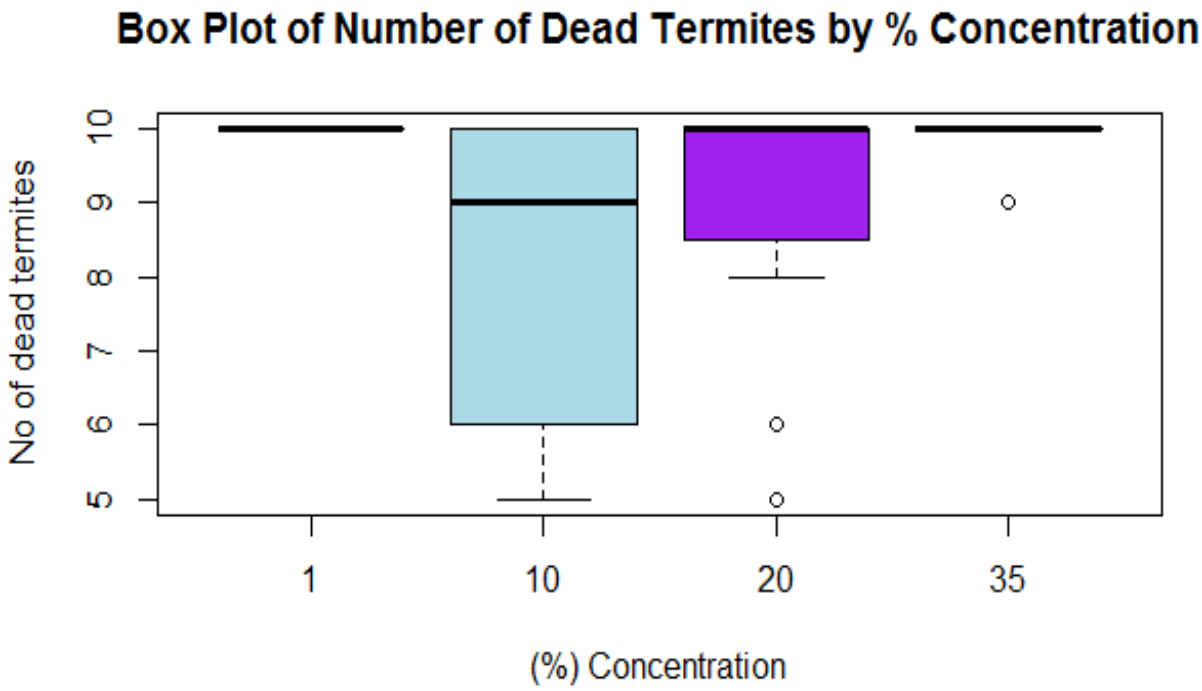


Fig 2: Box plot for the number of dead termites/group by concentration levels.

The Cochrane Q test for the four different concentration levels was 4.398 (p-value = 0.00121). Hence, the four concentration levels are significantly different from each other in achieving 50% mortality.

3.2 GEE Analysis Results

Since insect mortality may vary with time, other factors inclusive, a more meaningful approach using GEE was to estimate the time (LT_{50}) it takes for 50% of the group of test organisms to die at differing dose levels. The different correlation structures for the model fitting processes were chosen to reflect the nature of the data. The results of parameter estimates using the standard logistic regression and that of logistic regression model using GEE are given in Table 1 as well as the standard errors for the given variance estimator.

In two out of GEE-type cases in Table 1, the estimates of β (which is the estimated amounts by which the log odds of the response variable would increase if the covariates were a unit higher) are in agreement except for the independent and unstructured working correlation structure. The same applies to the standard errors for the working correlation fitting methods. Also, comparing results of the estimates β and its standard error for the ordinary logistic regression and that of

logistic regression using GEE shows that GEE is more efficient in estimating the model parameters as it gives population standard error estimates which are generally bigger.

Table 1: Results of the parameter estimates and standard errors of the regression analysis of the insect-mortality data using different user-defined model fitting processes for GEE and GLM.

Model Fitting	GLM		Type=Exchangeable (geeglm)		Type=AR1 (geeglm)		Type=Independence (geeglm)		Type=Unstructured (geeglm)	
Parameter	*Estimate*	*Standard Error*	*Estimate*	*Standard Error*	*Estimate*	*Standard Error*	*Estimate*	*Standard Error*	*Estimate*	*Standard Error*
Intercept	*-20.8733*	*1981.0538*	*-4.69e+01*	*9.93e+06*	*-4.69e+01*	*9.93e+06*	*-4.62e+01*	*1.14e+07*	*-4.56e+01*	*1.73e+07*
Time(hrs)	*1.6519*	*165.0878*	*3.89e+00*	*8.27e+05*	*3.89e+00*	*8.27e+05*	*3.84e+00*	*9.52e+05*	*3.76e+00*	*1.44e+06*
Conc.	*0.048*	*0.0339*	*4.80e-02*	*6.95e-02*	*4.80e-02*	*6.95e-02*	*4.80e-02*	*6.95e-02*	*2.68e-02*	*6.69e-02*

Table 2: Estimated unstructured correlation coefficients within mortality-responses from adult-termites, $R(\alpha_{ii})$.

Mortality	α_{i1}	α_{i2}	α_{i3}	α_{i4}
α_{i1}	1	0.7792	0.779	0.304

α_{i2}	1	0.925	0.440
α_{i3}		1	0.440
α_{i4}			1

3.3: Lethal Time Estimates (LT_{50}) from Repeated measures Logistic Regression using GEE:

Since mortality may vary with time (other factors inclusive), hence the need to estimate the time it takes for 50% of the test organisms to die as a function of % concentration. The estimated LT_{50} together with their confidence intervals for the different concentration levels for control agents are summarized in Table 3 below.

As shown in the given table, its lethal time (LT_{50}) ranged between 12.47 hours to 12.47 hours for extract J.Curcas; 12.47 hours to 12.47 hours for extract R.Cummunis. Similarly, the LT_{50} values for the different concentration levels ranged between 12.47 hours to 12.47hrs for 10% concentration; 12.47hours to 12.47 hours for 20% concentration; and 12.47hours to 12.47hours for 35% concentration. From Table 3 below, the % concentration for all extracts have the same potency alongside the control.

Table 3: LT_{50} Estimates from repeated measures logistic using GEE

Control Agents	Conc. (%)	LT_{50} (hrs)	Lower 95% CI	Upper 95% CI
J.curcas	10	12.47	0.548	24.4
J.curcas	20	12.47	0.548	24.4
J.curcas	35	12.47	0.548	24.4
R.cummunis	10	12.47	0.548	24.4
R.cummunis	20	12.47	0.548	24.4
R.cummunis	35	12.47	0.548	24.4
cypermethrin	1	12.47	0.548	24.4

CONCLUSION

This research work contributes immensely by giving a detailed insight in using GEE to fit a logistic regression model, a statistical method in analyzing repeated measures dose-response mortality data. A comparison of GLM and repeated measures logistic regression using GEE was also shown from the analysis results indicating the efficiency in using GEE.

The analysis results showed that the percentage (%) concentration of the different plant extracts were not significantly different from each other for achieving 50% insects mortality (LT_{50}) using GEE. The lethal time estimated corresponds to different extracts as well as its concentration levels. As shown from the results of this method, all concentration levels of the plant-extracts showed high toxicity level (potency) in achieving 50% mortality of each sampled insect's population.

RECOMMENDATION

The implications of this study are that there is need to improve on the way repeated measures mortality data is being analyzed by adopting or using the Generalized Estimating Equations (GEE) instead of the usual Analysis of variance (ANOVA) or MANOVA. In addition, the kind of the information obtained from such study will serve as a guide in analyzing and interpreting results from repeated measures data.

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