Research Article

PROBIT AND LOGIT ANALYSIS: MULTIPLE OBSERVATIONS OVER TIME AT VARIOUS CONCENTRATIONS OF BIOPESTICIDE *METARHIZIUM ANISOPLIAE* STRAIN

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ABSTRACT

A study was done to assess the goodness of fit of the regression lines using the data of silkworm larvae (J_{12} x C_{12} race) killed by various concentrations of *M. anisopliae* and LC_{71} of *Metarhizium. anisopliae* at different time intervals (hr) applying probit and logit function. The data were transformed before analysis using probit and logit transformations of proportion kill and with and without a logarithmic transformation of predictors. Analysis showed that the LC_{50} value were 5.969×10^6, 6.000×10^6, 7.250 and 7.235 spores mL^{-1} for probit, logit, log-probit and log-logit, respectively. The LT_{50} values were 204.247, 204.381, 2.304 and 2.305 hr for probit, logit, log-probit and log-logit, respectively. Significant Chi-square value indicates the necessity of heterogeneity factor for correction of variances under all functions. Residual deviance values were lower at the log-probit (2.826 for concentration and 0.292 for time) and log-logit (2.406 for concentration and 0.440 for time) models with higher p-values ($\geq$ 0.587) compared to probit and logit model. In our study, p-values was higher ($p>0.05$) with lower residual deviance in log transformed data which indicated that the log-probit and log-logit models could best fit to the mortality data of silkworm larvae when the both concentration and time were as predictors. Results indicated that the log-transformation of predictors would be best for describing the mortality values of insects by concentration of *Metarhizium. anisopliae* and under different time values. However, it requires more précised complete datasets and good knowledge of statistics of samples values along with the conversion of results of probit and logit analyses back to original units before coming into concrete application of these analytical inferences into practice.

Key words: Models, Log transformation, regression lines, predictors, LT_{50}, LC_{50}

INTRODUCTION

Probit analysis is used to analyze data from bioassay experiments, such as the proportions of insects killed by several concentrations of an insecticide or at several time intervals at one or more concentrations of an insecticide (Finney, 1964). In probability theory and statistics, the probit function is the inversecumulative distribution function (CDF), or quantile function associated with the standard normal distribution. Logit is another form of transforming binomial data into linearity and is very similar to probit. In entomology, samples of insects are typically exposed to several concentrations of an insecticide to determine the concentration that will kill 50% of the insects within a given time span (Cilek & Knapp, 1993). Effects of time on percentage of kill at one concentration (serial time-mortality data) may be of interest when (a) materials are limited, as might occur in tests of insecticides on field strains where few insects are available, or when testing an experimental pesticide that is available in limited quantities; or (b) when speed of kill is important, as might occur with a pest that lays all of its eggs within a few days (like a short-lived stored-product insect) or in quarantine treatments. Standard probit analysis techniques are not applicable to serial time-mortality data because observations made on the same group of organisms at different times are correlated (Robertson & Preisler, 1992).

Results of probit analyses are reported typically as a concentration or time required to kill a certain proportion of the test insects (for example, LC_{50}). Cilek & Greene (1994) also reported the use of the slope and intercept of the regression line of the probit-transformed data for describing the results. Lampkin and Ogawa (1975) developed a method for calculating the slope and intercept of serial time-mortality data. Reports on results of probit-type analyses should include the standard errors of the slope, intercept, and lethal time or lethal concentration values, and a test for goodness-of-fit. Goodness-of-fit of the regression line is indicated by the chi-square. Any tests comparing slopes, intercepts, or lethal time values should include confidence limits on the estimated statistics.

The results of probit analyses are rarely reported in the original units, that is, proportion of insects killed. A researcher should examine and report the results in the original units because the purpose of a bioassay is to make inferences about the proportions of insects killed by the insecticide, not to make inferences about probits (Finney, 1964). A plot of observed and predicted proportions of insects killed aids in assessing goodness-of-fit of the regression line. Goodness-of-fit also should be assessed by examination of residuals and standardized residuals in the original units, particularly to determine the possible causes of lack of fit when the chi-square is

significant (Robertson & Preisler, 1992). In addition to the probit transformation, the complementary log-log and logit transformations also are used to linearize bioassay data (Robertson &Preisler, 1992). Complementary log-log- and logit-transformed data are converted easily back to the original units. Converting probit-transformed data back to the original units is not straight-forward; the conversion is most easily accomplished using tables (Beyer, 1987) or mathematical computer programs (for example, Mathematica, Wolfram, Champaign, IL). Logistic regression uses categorically explained variable (Kollár, 2014). Preisler & Robertson (1989) described a method to analyze bioassay data when response by the same groups of organisms was determined at several times and at several concentrations of the insecticide (time-dose-mortality data). Also mixed logit models and its variants have supplanted simpler models in many areas of economics, marketing, management, transportation, health, housing, energy, and environmental science (Train, 2003; Jones & Hensher, 2008).

In the Nepalese context, describing of the bioassay data with probit or logit analysis is not reported. However, limited use of probit and logit model was found in socioeconomic analysis. Considering this, the biassay data were analyzed to describe under different conditions of link function to assess the goodness of fit of regression lines using probit or logit transformation of proportion of silkworm larvae (J_{12} x C_{12}) killed by various conidial concentrations of *Metarhizium anisopliae* strain at a specific time or at various times by one conidial concentration.

MATERIAL AND METHODS

Numerical methods

$$p = \frac{e^z}{1+e^z} \dots\dots\dots\dots\dots\dots\dots\dots\dots 1$$

Where p is the proportion of occurrences, $Z = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k Z = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k$and $Z = log\left(\frac{p}{1-p}\right) \dots\dots\dots\dots\dots\dots\dots\dots\dots 2$nverse relation of equation (1) is:

That is, the natural logarithm of the odds ratio, known as the logit. It transforms p which is restricted to the range [0, 1] to a range [−∞, ∞].

Probit regression analysis involves modeling the response function with the normal cumulative distribution function. The probit of a proportion p is just the point on a normal curve with mean 0 and standard deviation 1 which has this proportion to the left of it.

The model can be presented as:

$$\Phi^{-1}(p) = Z = \beta_0 + \beta_1 X_1 + \cdots + \beta_k X_k \dots\dots\dots\dots\dots\dots 3$$

where p is the proportion and Φ^{-1} is the inverse of the cumulative distribution function of the standard normal

$$p = \Phi(Z) = \int_{-\infty}^{z} \frac{1}{\sqrt{2\pi}} e^{\left(\frac{-u^2}{2}\right)} du \dots\dots\dots\dots\dots\dots\dots\dots 4$$

is the cumulative distribution function of the standard normal distribution.

For logistic and probit regression, the binomial, rather than the normal distribution describes the distribution of the errors and will be the statistic upon which the analysis is based. The principles that are used for ordinary linear regression analysis could be adapted to fit both regressions. However, instead of using least square method to fit the model, for logistic and probit regressions, it is more appropriate to use maximum likelihood estimate.

$$L = \prod_{i=1}^{m} p_i^{r_i} (1-p_i)^{n_i - r_i} \dots\dots\dots\dots\dots\dots 5$$

where the *pi* are defined in terms of the parameters β_0……β_k and the known values of the predictor variables. This has to be maximized with respect to the parameters (Shariff et al., 2009).

$$SE = \sqrt{\frac{p(1-p)}{n}} \dots\dots\dots\dots\dots\dots\dots 6$$ dividing the residuals of proportion kill at each X by their standard

where p = observed proportion killed and n = number tested at each X.

Manual analysis

The logit transformation of *p* is defined by $Log\left(\frac{p}{1-p}\right)Log\left(\frac{p}{1-p}\right)$, and the lines

$$Log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 X \ldots\ldots\ldots\ldots\ldots\ldots\ldots 7$$

are fitted, using maximum likelihood. Where β_0 is slope and β_1 is intercept from the regression and X is the concentration or time.

$$Log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 Log_{10} X \ldots\ldots\ldots\ldots\ldots\ldots\ldots 8$$

which is equivalent to assuming a log-normal distribution.

For probit, results of experiment are analyzed by fitting the line,

$$\Phi^{-1}(p) = \beta_0 + \beta_1 X \Phi^{-1}(p) = \beta_0 + \beta_1 X \ldots\ldots\ldots\ldots\ldots\ldots 9$$

Where p is the proportion of larvae killed and X is the corresponding concentration or time. This is equivalent to assuming a normal distribution. Then a comparison is made with

$$\Phi^{-1}(p) = \beta_0 + \beta_1 Log_{10} X \ldots\ldots\ldots\ldots\ldots\ldots 10$$

Analysis by computer program

To examine the goodness of fit of equations fit to both data describing the concentration required to kill the silkworm larvae treated with different concentration of *M. anisopliae* and data describing the time required to kill the silkworm larvae treated with $LD_{71}$of *M. anisopliae,* the spreadsheets, Stat Ggraphic Centurian and GenStat computer programs were used. The data obtained from the laboratory experiment were recorded and managed by using spreadsheets. The data were transformed before analysis with the probit or logit transformations of proportion of kill and with or without a logarithmic transformation of X. The data subjected to a probit transformation of proportion of kill and logarithmic transformations of X were referred to as log-probit transformed. Likewise, the data subjected to a logit transformation of proportion of kill and logarithmic transformations of X were referred to as log-logit transformed. The concentrations of each larval parasitoid and time values were transformed using log transformation at the base of 10.

Since there was no natural mortality, the correction for natural mortality was not done. Probit, logit, log-probit and log-logit regression lines were compared by their LC_{50}, slopes, residual deviance value, standardized residual. A chi-square test was used to test for heterogeneity within the data. Since heterogeneity was significant in our study a heterogeneity factor was included to calculate variances and confidence limits.

RESULTS AND DISCUSSION

Goodness of fit, LC_{50} and LT_{50}

In the log likelihood ratio test for lethal concentration (LC_{50}), the chi-square values were found as 28.253, 28.144, 34.222 and 34.642 for probit, logit, log-probit and log logit, respectively with $p<0.05$ for all the models (Table 1). In addition, the LC_{50} was observed 5.969×10^6, 6.000×10^6, 7.250 and 7.235 spores mL^{-1} for probit, logit, log-probit and log logit, respectively.

Table 1. Chi-square test and LC_{50} for probit, logit, log-probit and log-logit model for *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race)

Models	Chi-square	p-value	LC_{50}(Spores mL^{-1})	Confidence limits (L, U)* for LC_{50}
Probit	28.253	0.000	5.969×10^6	(4.279×10^6, 9.007×10^6)
Log-probit	34.222	0.000	7.250	(6.633, 8.250)
Logit	28.144	0.000	6.000×10^6	(4.258×10^6, 9.147×10^6)
Log-logit	34.642	0.000	7.235	(6.649, 8.165)

* L = Lower limit, U = Upper limit

Similarly, in the logliklihood ratio test for lethal time (LT_{50}), the chi-square values were found as 42.930, 42.594, 43.625 and 43.477 for probit, logit, log-probit and log logit, respectively with $p<0.05$ for all the models (Table 2). In addition, the lethal time (LT_{50}) was observed 204.247, 204.381, 2.304 and 2.305 hour for probit, logit, log-probit and log logit, respectively.

Table 2. Chi-square test and LT_{50} for probit, logit, log-probit and log-logit model for LC_{71} *M. anisopliae* at different time (hr) intervals in silkworm larvae (J_{12} x C_{12} race)

Models	Chi-square	p-value	LT_{50} (Hr)	Confidence limits (L, U)* for LT_{50}
Probit	42.930	0.000	204.247	(192.061, 220.223)
Log-probit	43.625	0.000	2.304	(2.275, 2.342)
Logit	42.594	0.000	204.381	(192.076, 220.280)
Log-logit	43.477	0.000	2.305	(2.275, 2.342)

* L = Lower limit, U = Upper limit

Because the p-value is less than 0.05 in both cases, that term is statistically significant at the 95.0% confidence level. A significant chi-square indicates that the data are heterogeneous and should be corrected using a heterogeneity factor or that an alternative transformation would be more appropriate for the data (Finney, 1964; Rangaswamy, 2005).

If the intent of the bioassay is to determine the concentration or time that is required to kill a certain proportion of the insects, then the researcher want to choose a model that also minimizes the confidence limits on that lethal concentration or time value (Throne et al., 1995). The analyzed results indicates that the log-transformation of predictor data has minimum confidence limits in both log-probit and log-logit model. Therefore, the log-probit and log-logit model could be used to determine the concentration or time that is required to kill a 50% proportion of the larvae over the probit or logit transformation. However, the best results would be obtained when the transformed data will be converted back to the original units (Throne et al., 1995).

The goodness of fit of the data for LC_{50} was also depicted in the graphical features plotting probit and logit values versus concentration of *M. anisopliae.* The examination of fitted regression lines compared with the transformed observations (Figure 1) indicated that the log-probit and log-logit model had result a more or less linearity of the data for response of *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race). It can also be best describe and fit to the data of the response of *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race).

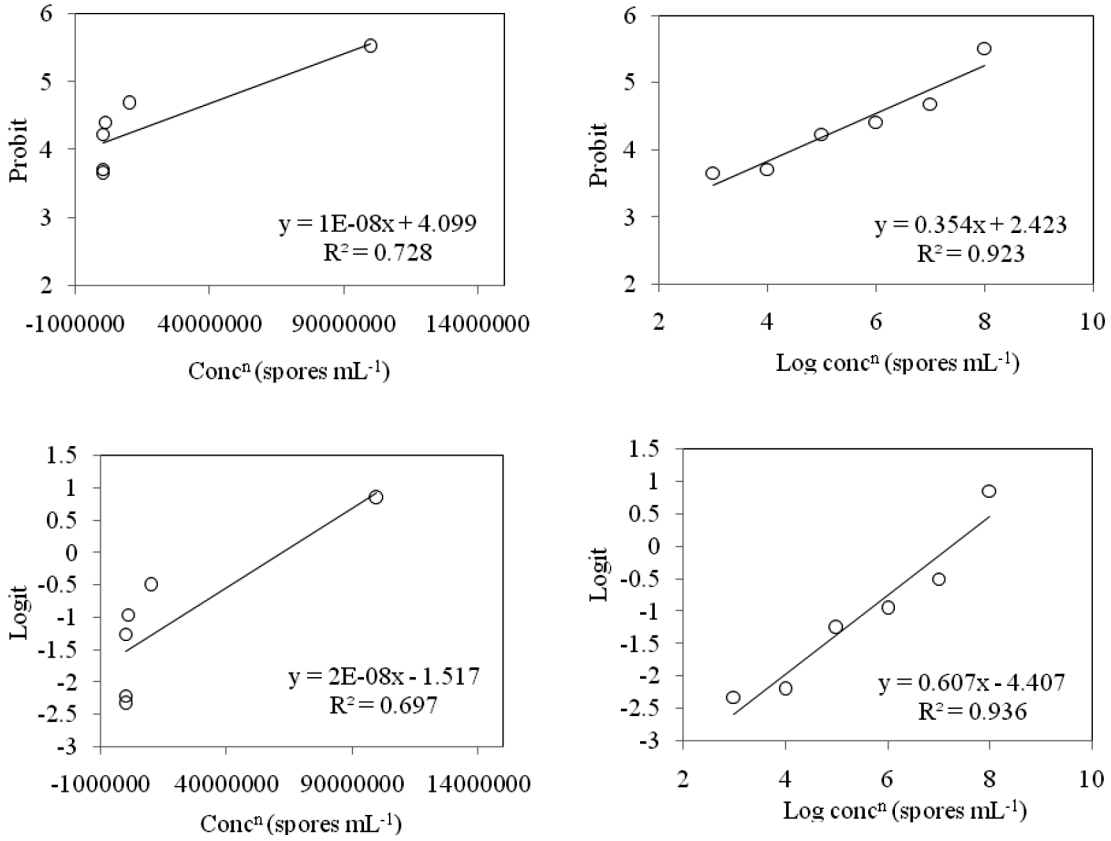

Figure 1. Observed (open circles) and predicted (line) probit, log-probit, logit and log-logit transformations of proportion of silkworm larvae killed at different concentrations (spores mL^{-1}) of *M. anisopliae* strain

Similarly, the goodness of fit of the data for LT_{50} was also depicted in the graphical features plotting probit and logit values versus time value for the response of LC_{71} *M. anisopliae.* The examination of fitted regression lines compared with the transformed observations (Figure 2) indicated that the log-probit and log-logit model had resulted more precision in linearity of the data for response of LC_{71} *M. anisopliae* under different time values (hr) in silkworm larvae (J_{12} x C_{12} race). It can also be best described and fitted to the data of the response of LC_{71} *M. anisopliae* under different time values (hr) in silkworm larvae (J_{12} x C_{12} race).

The predicted line fits the first and last data points in the log-probit and log-logit plot. Fig. 1 & 2 well compared transformed data to non-transformed data and found that log transformed of data well fitted to the probit and logit model as it has higher R^2 values (R^2>0.92). Similar results and suggestion was also given by Throne *et al*. (1995).

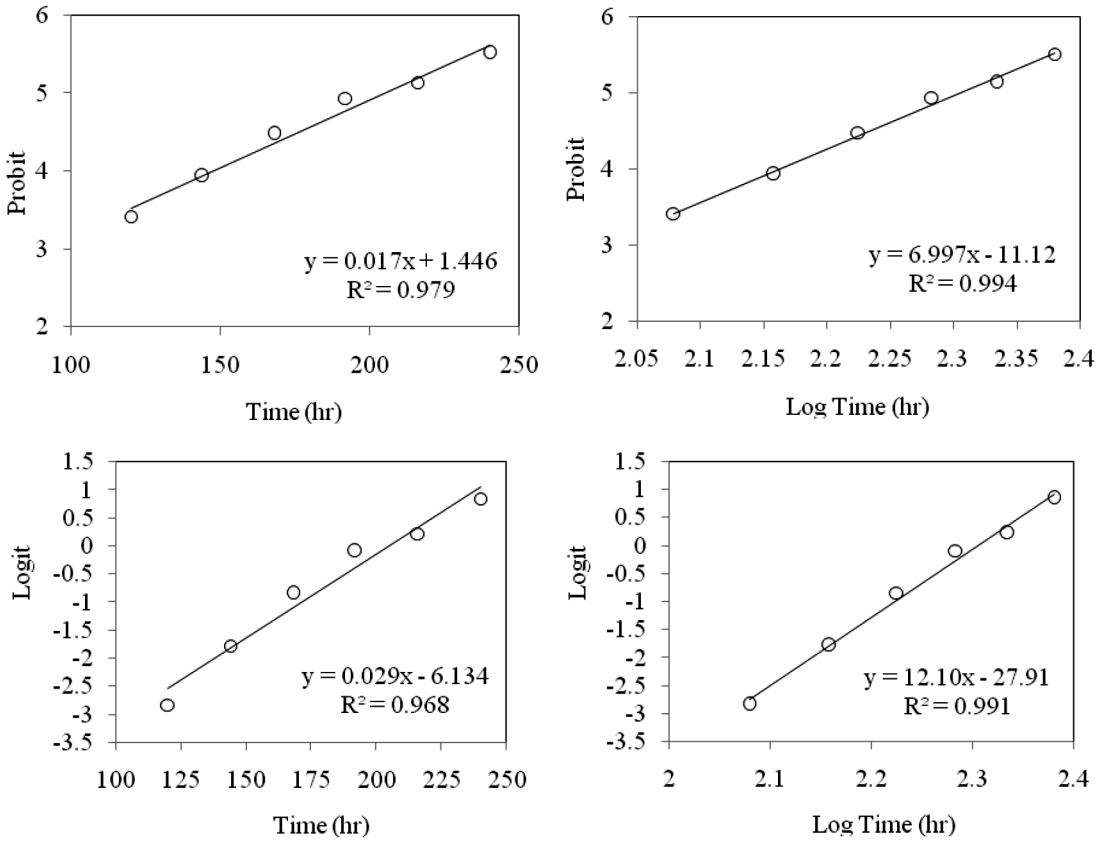

Figure 2. Observed (open circles) and predicted (line) probit, log-probit, logit and log-logit transformations of proportion of silkworm larvae killed at time (hr) by LC_{71} of *M. anisopliae* strain

Analysis of deviance

The analysis of deviance for the probit, logit, log-probit and log-logit model for *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race) was worked out (Table 3) and found that the deviance value for the models probit, logit, log-probit and log-logit was 28.253, 28.144, 34.221 and 34.642, respectively on 1 degree of freedom with $p<0.05$. This indicates that the models were found significant at 95% confidence level under both transformations. Similarly, residual deviance value for probit, logit, log-probit and log-logit model fit to the data was 8.975, 8.904, 2.826 and 2.406, respectively on 4 degree of freedom with p value 0.066, 0.063, 0.587 and 0.661 (non-significant at 5% α level), respectively for probit, logit, log-probit and log-logit model.

Table 3. Analysis of deviance for probit and log-probit model for *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race)

Models	Analysis of deviance			
	Source	**Deviance**	**df**	**p-value**
Probit	Model	28.253	1	0.000
	Residual	8.795	4	0.066
	Total	37.048	5	
Log-probit	Model	34.221	1	0.000
	Residual	2.826	4	0.587
	Total	37.047	5	
Logit	Model	28.144	1	0.000
	Residual	8.904	4	0.063
	Total	37.048	5	
Log-logit	Model	34.642	1	0.000
	Residual	2.406	4	0.661
	Total	37.048	5	

Likewise, the analysis of deviance for the probit, logit, log-probit and log-logit model for LC_{71} *M. anisopliae* at different time (hr) intervals in silkworm larvae (J_{12} x C_{12} race) was also worked out (Table 4). Under this analysis, the deviance value for the models probit, logit, log-probit and log-logit was 42.930, 42.594, 43.625 and 43.477, respectively on 1 degree of freedom with $p<0.05$. This indicates that the models were found significant at 95% confidence level under both transformations. Similarly, residual deviance value for probit, logit, log-probit and log-logit model fit to the data was 0.987, 1.323, 0.292 and 0.440, respectively on 4 degree of freedom with p value 0.911, 0.857, 0.990 and 0.979 (non-significant at 5% α level), respectively for probit, logit, log-probit and log-logit model.

Under both conditions, the residual deviance value was found higher in probit and logit model (with $p>0.05$) than in log-probit and log-logit model. Because the p-value for the model in analysis of deviance table is less than 0.05, there is a statistically significant relationship between the variables at the 95.0% confidence level. In addition, the p-value for the residuals is greater than or equal to 0.05, indicating that the model is not significantly worse than the best possible model for this data at the 95.0% or higher confidence level.

Table 4. Analysis of deviance for probit and log-probit model for LC_{71} *M. anisopliae* at different time (hr) intervalsin silkworm larvae (J_{12} x C_{12} race)

Models	Analysis of deviance			
	Source	**Deviance**	**df**	**p-value**
Probit	Model	42.930	1	0.000
	Residual	0.987	4	0.911
	Total	43.917	5	
Log-probit	Model	43.625	1	0.000
	Residual	0.292	4	0.990
	Total	43.917	5	
Logit	Model	42.594	1	0.000
	Residual	1.323	4	0.857
	Total	43.917	5	
Log-logit	Model	43.477	1	0.000
	Residual	0.440	4	0.979
	Total	43.917	5	

Nylor (1964) reported that the choice of the complementary log-log, logit or probit transformation had little effect on goodness of fit to several biological datasets. However, the analysis of silkworm larvae mortality data indicate that, for insect bioassay data, the choice of transformation may have a greater effect on goodness of fit as

indicated by the deviance analysis and plots of transformed data and predictor values. Since the residual deviance is small ($p>0.05$), this indicates a no significant lack of fit for the log-probit and log-logit model. Cook et al. (2001) reported that the smaller the deviance, the better the fit of the logistic model. A large value for the deviance is an indication that there is a significant lack of fit for the logistic model and some other model may be more appropriate.

Standardized residual

Standardized residual would be obtained when the difference of observed and predicted values divided by the standard errors. It was obtained for probit, logit, log-probit and log-logit models for *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race) (Table 5). In most of the concentrations results, contrasting mathematical sign for standardized residual was obtained in all models. It was smaller for the log-transformed model compared to non-transformed model. At 10^8 Spores mL^{-1} treatment, the standardized residual was highest and negative (-2.030 and -1.95, respectively for probit and logit) but for the same concentrations when log transformed, it was highest and positive (1.57 and 1.43, respectively for probit and logit). This indicates that the observed was smaller than the predicted in case of probit and logit transformation where as vice-versa was in log-transformed model. The smallest positive standardized residual was at 10^5Spores mL^{-1} treatment with 0.32 and 0.30 for probit and logit model, respectively. For the same concentrations at log-transformed condition, it was positive and smallest (0.27 and 0.43, respectively for log-probit and log-logit). In both conditions, the predicted values were smaller compared to observed values. At the smallest concentration i.e. 10^3Spores mL^{-1}, the standardized residual was found -1.88, -1.89, 0.82 and 0.66, respectively for probit, logit, log-probit and log-logit model. At this time, the observed was found smaller than the predicted in probit and logit model where as predicted was smaller than observed in log-probit and log-logit model.

Table 5. Standardized residual for probit and log-probit model for *M. anisopliae* concentrations (Spores mL^{-1}) in silkworm larvae (J_{12} x C_{12} race)

Model							
Probit		Logit		Log-probit		Log-logit	
Concentrations (Spores mL^{-1})	Standardized residual	Concentrations (Spores mL^{-1})	Standardized residual	Log-concentrations (Spores mL^{-1})	Standardized residual	Log-concentrations (Spores mL^{-1})	Standardized residual
10^8	-2.03	10^8	-1.95	8	1.57	8	1.43
10^7	1.83	10^7	1.85	7	-1.14	7	-1.14
10^6	1.08	10^6	1.06	6	-0.6	6	-0.47
10^5	0.32	10^5	0.30	5	0.27	5	0.43
10^4	-1.68	10^4	-1.69	4	-0.35	4	-0.29
10^3	-1.88	10^3	-1.89	3	0.82	3	0.66

Similarly, the standardized residual was also calculated for probit, logit, log-probit and log-logit models for LC_{71} *M. anisopliae* at different time (hr) intervals in silkworm larvae (J_{12} x C_{12} race) (Table 6). In almost all time interval results, similar pattern of mathematical sign for standardized residual values were obtained in all models. It was smaller for the log-transformed model compared to non-transformed model. At 120 hr treatment, the standardized residual was higher and negative (-0.62 and -0.80, respectively for probit and logit) but for the same concentrations when log transformed, it was smaller and negative (-0.09 and -0.31, respectively, for probit and logit model). This indicated that the predicted values were higher compared to observe ones in both cases. The smallest positive standardized residual was at 168 hr treatment with 0.41 and 0.48 for probit and logit model, respectively. For the same concentrations at log-transformed condition, it was positive and smallest (0.12 and 0.24, respectively for probit and logit model). That is, the observed was higher than the predicted values in both conditions. At the highest duration i.e. 240 hr after placement, the standardized residual was found -0.47 and -0.52 for probit and logit model, respectively and was -0.03 and -0.13 for the log-probit and log-logit model, respectively. At this time, the predicted values were found higher compared to observe in both probit and log-probit transformation. The highest positive standardized residual was found at 192 hr in all models which indicates that the observed was highest than the predicted.

Table 6. Standardized residual for probit and log-probit model for LC_{71} *M. anisopliae* at different time (hr) intervals in silkworm larvae (J_{12} x C_{12} race)

Model							
Probit		Logit		Log-probit		Log-logit	
Time (hr)	Standardized residual	Time (hr)	Standardized residual	Log-time (hr)	Standardized residual	Log-time (hr)	Standardized residual
120	-0.62	120	-0.80	2.08	-0.09	2.08	-0.31
144	-0.20	144	-0.22	2.16	-0.19	2.16	-0.16
168	0.41	168	0.48	2.23	0.12	2.23	0.24
192	0.74	192	0.79	2.28	0.44	2.28	0.50
216	-0.30	216	-0.33	2.33	-0.37	2.33	-0.41
240	-0.47	240	-0.52	2.38	-0.03	2.38	-0.13

The residuals of the transformed data are useful to determine whether the model fits the transformed data based on the presence of systematic trends in the pattern of the residuals, but are not useful for determining which of the transformation results in an equation which best describes the original data. Residuals of data converted back to the original units are comparable among transformations and can be used to help determine which transformation best describes the original data. Converting the data back to original units make all the residuals on the same scale and can be compared (Throne et al., 1995). But, the standardized residual of probit, logit, log-probit and log-logit models were not back transformed to original units. So that, for choosing the best describing models for original data, it is necessary to convert the standardized residuals value back to original units and can be easily compared under same scale of data. Preisler (1988) suggested that standardized residuals lying more than ±2 SD from zero indicate possible lack of fit. For more reliability of results, it would require to have SD of individual series data for all standardized residuals.

CONCLUSION

The best fitted regression lines in the entomological data could be obtained when the predictor values are log-transformed. Occurrence of significant Chi-square indicates that the observed and predicted values did not agree, therefore, the heterogeneity factor had to be used to correct the variances. In addition, log-transformation results in smaller residual and standardized residual values indicating the closeness between observed and predicted values. Thus, we have to do log-transformation of the predictor (either dose or time) values to obtain more precise result from bioassay experiments.

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