Tropical Agricultural Research Vol. 25 (3): 396 – 402 (2014)

Link Function for Binomial Model in Estimating Knockdown Time (KT_{95} and KT_{50}) of Mosquito Repellents

T.J. Silva*, S. Samita[1] and S.A.S.C. Senanayake[2]

Postgraduate Institute of Agriculture
University of Peradeniya
Sri Lanka

ABSTRACT: *Estimation of knockdown time (KT) is useful in determining bio-effectiveness of mosquito repellents. Knockdown or not knockdown is a binary variable thus, analysis is done by fitting generalized linear models, based on binomial distribution. Use of appropriate link function in fitting a generalized linear model is crucial especially when estimating quantities such as KT_{50} and KT_{95}. This study was done to determine the most appropriate link function in fitting generalized linear models to estimate KT_{50} and KT_{95}. Knockdown activity of metofluthrin 0.005% (w/w) and d-trans-allethrin 0.12% (w/w) was tested under two different physiological conditions (blood fed and sucrose fed) using wild-caught female Culex tritaeniorhynchus mosquitoes from an agro-farming area of the north-western province of Sri Lanka. Coefficient of variation of the observed KT_{50} and KT_{95} was less than 5.5%. Both KT_{50} and KT_{95} values were estimated by fitting altogether 120 binomial distribution-based generalized liner models with three different link functions namely, logit, probit, and complementary log–log. The G2 statistics was used to test the goodness of fit of the models. However, in order to evaluate the accuracy of all estimated KT_{50} and KT_{95} values obtained using the above three link functions, they were compared against corresponding observed values using ANOVA followed by Dunnett mean separation procedure. The probit and logit link functions were found to be appropriate in the estimation of KT_{50}. As the logit link function is commonly used in modeling binary responses, out of the two, logit link function is recommended. Complementary log–log link function was found to be the most appropriate in estimation of KT_{95}. Thus, one link function cannot be recommended in estimating both KT parameters.*

Keywords: *Bio-effectiveness, generalized linear models, tolerance distribution*

INTRODUCTION

The efficacy of an insecticide against particular insect is determined under laboratory and field conditions using various parameters. Out of them estimation of 50% cumulative knock-down (KT_{50}) and 50% lethal dose (LD_{50}) are widely applied parameters. Knockdown is the rapid paralysis of insects causing them to fall down and remain in a state as to be incapable of co-ordinate movements and apparently dead (SLS, 2001). Although KT_{50} is the popular concept in the comparison of knockdown patterns among different mosquito species, KT_{95} indicates the accepted maximum tolerance limit of the target insect species against particular concentration of an active ingredient. The generalized liner model based on

[1] Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka.
[2] Faculty of Medicine, University of Colombo, Colombo, Sri Lanka
* Corresponding Author: janaki152h@yahoo.ca

binomial model with probit procedure is recommended for the calculation of KT values for the testing of the efficacy of mosquito coils (WHO, 2013). Sri Lanka Standards (SLS) 453:2001 section E.5.1 instructs to analyze the obtained knock-down data using PROBIT procedure, either implemented in a computer programme or PROBIT graph paper by plotting proportion of knockdown verses knockdown time in minutes. Bliss (1934) reported using probit link function but logit and complementary log-log link functions have also been used in fitting of binomial models. Although it is well established that quantities such as LD_{50} is estimated by fitting binomial models based on probit link function, no adequate literature is available on appropriate link function for binomial models when establishing KT in general. Estimating quantities KT_{50} and KT_{95} is crucial because bio-effectiveness of mosquito repellent product are adjusted specifically based on KT_{95}. The objective of this study was to recommend the best link function for binomial models when estimating KT_{50} and KT_{95} using three types of link functions namely probit, logit and complementary log-log.

MATERIALS AND METHODS

Data Collection

Two types of commercially available (bio-efficacy approved) mosquito coils containing metofluthrin 0.005%w/w and d-trans- allethrin 0.12%w/w as active ingredient were used for the study. A rural area with large paddy fields in Kuliyapitiya of Kurunegala district was selected for the collection of mosquitoes. Cattle baited net trap was used as the sole method of sampling the test mosquitoes. Mosquitoes belonged to *Culex tritaeniorhynchus* (a known vector of Japanese encephalitis in Sri Lanka) found within the cattle traps was used for the study. From these mosquitoes samples of 20 blood fed and 20 sucrose fed mosquitoes were exposed to a coil (without active ingredient) to ensure the suitability of them for the efficacy testing. KT_{50} and KT_{95} was estimated following standard procedure (SLS, 2001) against two active ingredients metofluthrin 0.005%w/w and d-trans- allethrin 0.12%w/w. Thus KT was measured under four conditions viz: (i) metofluthrin– blood fed (ii) metofluthrin-sucrose fed (iii) d-trans-allethrin–blood fed (iv) d–trans-allethrin–sucrose fed under each condition 10 packs were tested. Accordingly three were 40 KT sets.

Model fitting

The general form of the models fitted was $g(\square) = \beta_0 + \beta_1 x$ where $g(\square)$ denotes the link function. Link functions considered in the study were logit, probit, and complementary log–log and they are respectively of the form $ln\left(\frac{p}{1-p}\right)$, $\Phi^{-1}(p)$, $ln\{-ln(1-p)\}$ where p is proportion knockdown and Φ^{-1}indicates inverse cumulative standard normal distribution. The variances of the cumulative distribution functions are not same. In fact the means and the variance of the three distributions, probit, logit and complementary log–log are respectively (0, 1), (0, π^2 /3) and ($-\gamma$, $\pi^2/6$) where γ is the Euler constant (Bilder, 2010; Gourdon & Sebah, 2004).

Models were fitted for the data using above three link functions and thus altogether 120 models were fitted. The goodness of fit of the fitted models was evaluated using G^2 statistics (McCullagh & Nelder, 1989). Mean KT_{50} and KT_{95} values estimated from the fitted models. These estimates were compared with the observed mean KT_{50} and KT_{95} values using one way ANOVA followed by Dunnett mean separation technique using observed mean as the control.

RESULTS AND DISCUSSION

The summary of the goodness of fit (G^2) with 19th degree of freedom (df) for 120 fitted models are represented in Table 1. According to the Table 1, all fitted models were adequate (P>0.05) and thus models with any of the three link functions is able to capturing the variability of the response variable. Thus a model with any of those link functions can be considered in estimating important quantities.

Table1. G^2 for different models fitted for KT under different active ingredients and feed

AI	Pack no	Feed	Probit	Logit	Clog–log	AI	Pack no	Feed	Probit	Logit	Clog–log
DT	1	BF	5.2303 (0.9992)	2.1639 (1.0000)	3.7693 (0.9999)	MT	1	BF	2.3184 (1.0000)	3.4197 (1.0000)	5.7782 (0.9984)
	2	BF	2.1639 (1.0000)	3.3891 (1.0000)	4.3410 (0.9998)		2	BF	1.9535 (1.0000)	2.6837 (1.0000)	1.9450 (1.0000)
	3	BF	11.9712 (0.8869)	13.7972 (0.7954)	9.3732 (0.9668)		3	BF	8.2593 (0.9839)	10.8180 (0.9298)	10.3368 (0.9441)
	4	BF	6.6822 (0.9957)	8.2702 (0.9837)	6.2366 (0.9973)		4	BF	3.3030 (1.0000)	3.5562 (1.0000)	14.3968 (0.7601)
	5	BF	9.7264 (0.9594)	11.0366 (0.9226)	6.8717 (0.9949)		5	BF	4.9822 (0.9994)	4.9073 (0.9995)	5.0586 (0.9994)
	6	BF	7.9745 (0.9869)	9.8577 (0.9564)	6.9496 (0.9945)		6	BF	3.1933 (1.0000)	3.9097 (0.9999)	12.9433 (0.8415)
	7	BF	4.2677 (0.9998)	5.3684 (0.9990)	3.1086 (1.0000)		7	BF	6.3792 (0.9969)	4.9090 (0.9995)	16.4541 (0.7004)
	8	BF	4.5799 (0.9997)	6.2611 (0.9972)	5.3419 (0.9991)		8	BF	3.9876 (0.9999)	5.0892 (0.9994)	9.6096 (0.9619)
	9	BF	3.7274 (0.9999)	5.0256 (0.9994)	2.6521 (1.0000)		9	BF	4.8244 (0.9996)	5.5641 (0.9988)	15.3288 (0.7015)
	10	BF	5.6724 (0.9986)	7.3829 (0.9919)	4.7328 (0.9996)		10	BF	7.3320 (0.9922)	5.2610 (0.9992)	15.3979 (0.7046)
	1	SF	5.3533 (0.9991)	5.4383 (0.9990)	11.1028 (0.9203)		1	SF	8.0030 (0.9866)	10.0305 (0.9522)	10.7950 (0.9305)
	2	SF	1.6766 (1.0000)	2.5060 (1.0000)	4.3410 (0.9999)		2	SF	4.5714 (0.9997)	5.5577 (0.9988)	10.7930 (0.9306)
	3	SF	3.8297 (0.9999)	5.0283 (0.9994)	3.9363 (0.9999)		3	SF	7.0976 (0.9937)	9.4034 (0.9662)	11.3571 (0.9112)
	4	SF	2.3637 (1.0000)	2.6843 (1.0000)	3.9933 (0.9999)		4	SF	2.1640 (1.0000)	2.2762 (1.0000)	6.1370 (0.9976)
	5	SF	2.7312 (1.0000)	3.6469 (0.9999)	3.4862 (1.0000)		5	SF	4.6195 (0.9997)	6.4318 (0.9967)	5.8370 (0.9983)
	6	SF	2.7312 (1.0000)	3.6469 (0.9999)	3.4862 (1.0000)		6	SF	4.8686 (0.9995)	6.0856 (0.9977)	11.3985 (0.9097)
	7	SF	2.7312 (1.0000)	3.6469 (0.9999)	3.4862 (1.0000)		7	SF	6.8218 (0.9951)	8.0381 (0.9863)	3.4579 (1.0000)
	8	SF	3.8297 (0.9999)	5.0283 (0.9994)	3.9363 (0.9999)		8	SF	5.9376 (0.9981)	5.5777 (0.9988)	19.9068 (0.5809)
	9	SF	5.3533 (0.9991)	5.4383 (0.9990)	11.1028 (0.9203)		9	SF	2.3184 (1.0000)	3.8791 (0.9999)	6.3330 (0.9970)
	10	SF	1.6766 (1.0000)	2.5060 (1.0000)	3.6447 (0.9999)		10	SF	7.4209 (0.9916)	9.2105 (0.9698)	12.3395 (0.8706)

Active ingredient (AI), Blood fed (BF),Complementary log-log (clog-log), d-trans-allethrin (DT) metofluthrin (MT), Sucrose fed = (SF). The values in parenthesis are the significant probability levels (p).

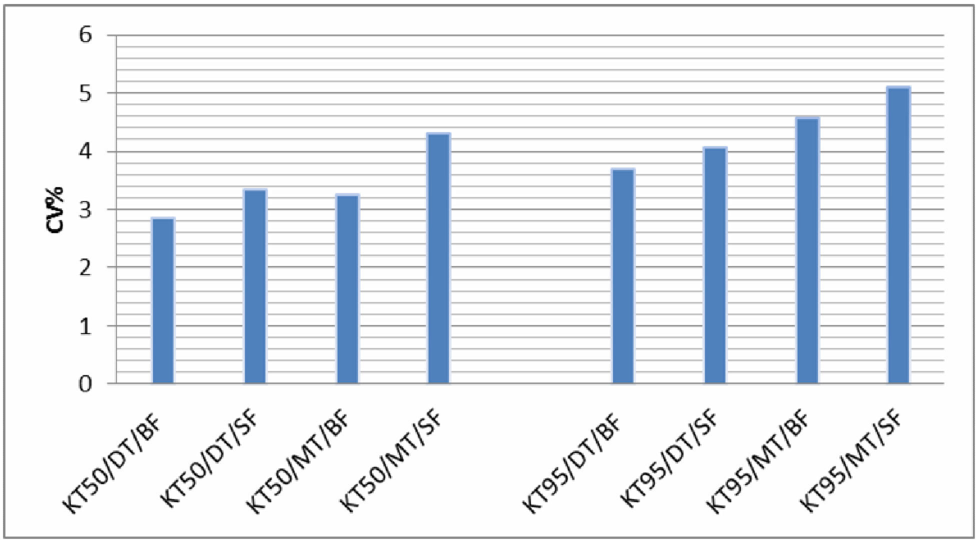

Fig. 1. Coefficient of variability of observed KT_{50} and KT_{95}

The coefficient of variability (CV) of the observed KT_{50} and KT_{95} are shown in Fig 1. All CVs are below 5.5%, indicating that data have been generated under well controlled conditions and thus even a minor effect can be detected. The CV of the blood fed mosquitoes was relatively lower than that of the sucrose fed mosquitoes. The CV of the KT_{95} is higher than that of KT_{50} , for a given feed type and for a given active ingredient.

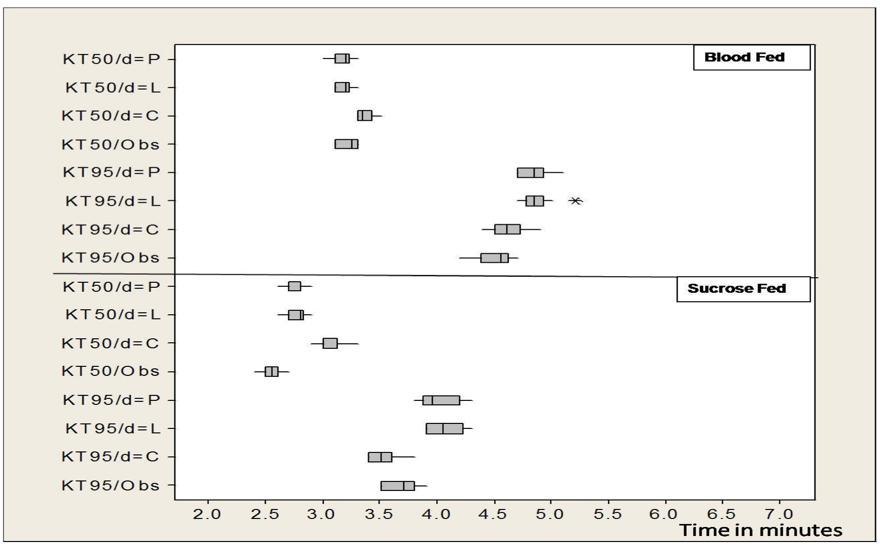

Fig.2a. Box and whisker plots of medians of the estimated KT_{50} and KT_{95} valves of the three different link functions against *0.12%w/w d–trans–allethrin* for blood fed

and sucrose fed mosquitoes considering median of the observed KT_{50} and KT_{95} as the control. (n=10) (P=probit, L=logit, C=complementary log–log, obs=observed)

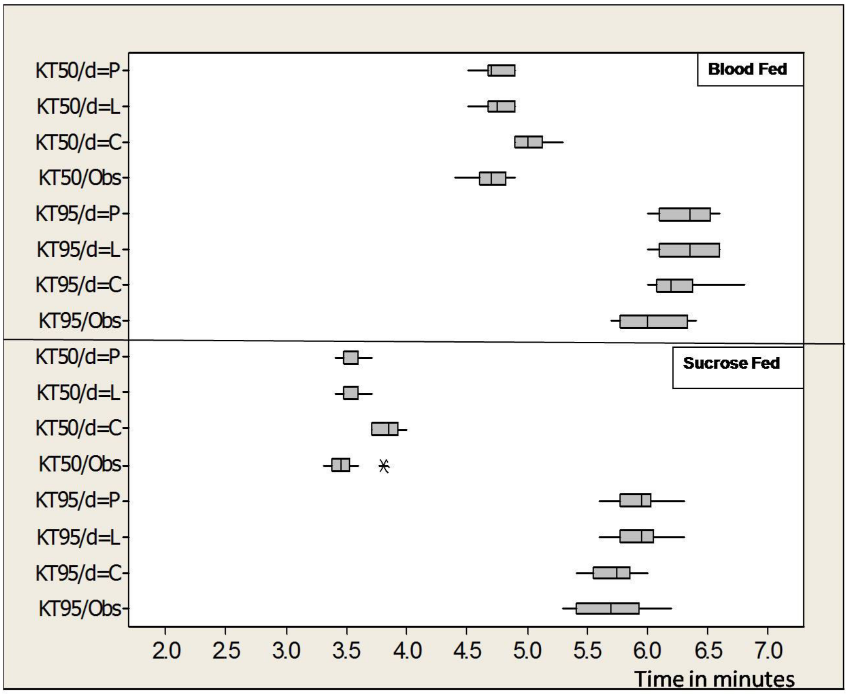

Fig.2b. Box and whisker plots of medians of the estimated KT_{50} and KT_{95} valves of the three different link functions against 0.005% w/w *metofluthrin* for blood fed and sucrose fed mosquitoes considering median of the observed KT_{50} and KT_{95} as the control. (n=10) (P=probit, L=logit, C=complementary log–log, obs=observed)

Comparison of estimated median values of KT_{50} and KT_{95} using three different link functions are given in Figs. 2a and 2b. Fig. 2a corresponds to active ingredient *0.12%w/w d–trans–allethrin and* Fig. 2b corresponds to active ingredient 0.005% w/w *metofluthrin.* Observed KT_{50} and KT_{95} values were considered as controls. From the Figs. 2a and 2b, it is apparent that KT_{50} and KT_{95} values are well separated as expected.

Table 2. Results of the mean comparison

KT	Active Ingredient	Fed status	p value	F	Link function	Dunnett test
KT_{50}	DT	BF	<0.0001	14.96	Probit	
					Logit	
					Complementary log–log	***
		SF	<0.0001	13.8	Probit	
					Logit	
					Complementary log–log	***
	MT	BF	0.0005	7.47	Probit	
					Logit	
					Complementary log–log	***
		SF	0.0465	2.07	Probit	
					Logit	
					Complementary log–log	
KT_{95}	DT	BF	<0.0001	13.58	Probit	***
					Logit	***
					Complementary log-log	
		SF	<0.0001	13.64	Probit	***
					Logit	***
					Complementary log-log	
	MT	BF	<0.0001	14.96	Probit	***
					Logit	***
					Complementary log-log	
		SF	0.1903	2.11	Probit	
					Logit	
					Complementary log-log	

*** There is a significant mean difference when compared with the observed values.

According to the Table 2, means of estimated KT_{50} under four different conditions i.e. DT/BF, DT/SF, MT/BF, MT/SF using three different link functions are different (P<0.005) except for MT/SF (P=0.0465). Further, Dunnett mean separation revealed that mean of estimated KT_{50} using complementary log-log link function was significantly different from the observed mean $KT_{50.}$

With estimated KT_{95} under same four conditions using three different link functions also shows that there is a significant mean difference (P <0.0001) except for condition MT/SF (P=0.1903). According to Dunnett mean separation results the means of estimated KT_{95} using probit and logit link functions were significantly different from the observed mean $KT_{95.}$

CONCLUSION

The estimate of KT values is comparatively an easy practice but not routinely applied for monitoring of susceptibility except in the specialized entomological laboratory testing (in the field and under insectary conditions). To achieve the recommended accuracy and precision of KT of specific vector mosquitoes, it is necessary to have both specialized entomological skills and the appropriate statistical procedures. The KT_{95} indicates the accepted maximum tolerance limit of the target insect species against to a particular concentration of an active ingredient and it is more sensitive to the development of insecticide resistance. Therefore the accurate estimation of KT_{95} is important in early detection of insecticide resistance. In general, KT values are estimated by fitting binary regression models with probit link function. However, from this study it can be concluded that complementary log-log link function is more appropriate to estimate KT_{95} for *C. tritaeniorhynchus*. Both probit and logit link functions are appropriate in the estimation of KT_{50} for the same mosquito population. However, out of the two, logit link function is recommended due to the reasons mentioned earlier. Therefore a single link function is not recommended for calculation of KT values under different conditions.

REFERENCE

Bliss, C.I. (1934). The Method of Probits, Science, *79*, 38-39.

Bilder, C. R. (2010). Generalized linear models. Available at Statistics. unl. Edu / faculty / bilder / stat875 / schedule_new/chapter3.doc.

Gourdon, X. and Sebah, P. (2004). The Euler Constant: (γ) gamma. Available at http://numbers.computation.free.fr/Constants/Gamma/gamma.html.

McCullagh P. and Nelder J. A. (1989). Generalized Linear Models, Second Edition, Chapman and Hall, London.

SLS (2001). Specification for Mosquito coils. (453:2001) section E.5.1.

WHO (2013). Test procedures for insecticide resistance monitoring in malaria. Available at http://apps.who.int/iris/bitstream/10665/80139/1/9789241505154_eng.pdf.