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An universal tool for the analysis of effectiveness of insecticides

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Abstract: Insecticides have leading role in reduction of population of arthropods, especially vectors of infectious diseases. Efficacy of insecticides is usually proved on biological material and described after adjusting the values of parameters in some “theoretical” function to the experimental results. In the standard approach, such function is described by probit regression. However, in some cases reaction of the population on pesticide differs from those which could be described by the probit regression curve and the results could not be properly assessed. The aim of this study was to elaborate a practical tool for evaluating insecticide/acaricide efficacy, more universal than classical probit calculation. Software performing analysis was created using R 2.13.1 statistical software.

Keywords: efficacy of insecticides, practical tool for the efficacy analysis

INTRODUCTION

Insecticides are basic tools in reduction of arthropod populations, especially vectors of infectious diseases. They are complex substances, in the case of which

even a precise and meaningful knowledge will not allow to predict the effect; therefore, their effectiveness is measured in biological tests. During a typical test on effectiveness, groups of insects are exposed to different concentrations of the insecticide (or different doses are applied on their bodies) for a specified time. Basing on overall reaction or its lack – the insect will either survive the exposure or not – the calculation of the dose that will exterminate 50% of the specimen can be made, which will characterize the sensitivity of the population: LC_{50} (LD_{50} in the case of a dose). The exposure of the insect groups to the same concentration but in different time intervals (value of LT_{50} is calculated) is a variation of this experimental schema. In biological research concerning pesticide (insecticide) effectiveness the linear-regression method is used in log-probit transformation (in other words probit regression), developed by Finney [1]. This method for experimenting on insects was adopted by Bojanowska [2]. It is presented also in detail in the “Pesticide Bioassays with Arthropods” [3].

Nevertheless, the probit regression used for determining the effectiveness of results has their limitations and it is not always possible to evaluate the tested substance properly. The most interesting parameter from practical point of view – lethal time or more particularly – the LT_{50} value, cannot be calculated properly by probit regression. An attempt at adapting the probit method, to the conditions when observations on lethality are carried out on one group of insects, was presented by Throne et al. [4, 5]. However, this was an incomplete solution. It did not take into consideration the cases of “recovery” (in the opinion of the authors they to be rejected), as well as the very fast effect of insecticides when in a very short time it is possible to observe only low lethality rates (under 20%) and very high lethality rates (over 90%).

In the previous papers [6, 7] we described a method for evaluating the effects of pesticides on arthropods which will be an alternative to the classic probit method and its mathematical background. The comparison of the new method and the classical method, as well as depicting the classes of experimental outcomes for which the application of the newly developed method may result in an improved precision of calculations were done.

The aim of this study was to elaborate a practical tool for evaluating insecticide/acaricide efficacy, more universal than classical probit calculation.

MATERIAL AND METHODOLOGY

Software supporting practitioner’s everyday work was created. It consists of two parts. The first one is a database prepared especially using MS Access to

store the results from bioassay data. The second one is a program written using R 2.13.1 [8] statistical software communicating with the database and performing analyses. More about R software can be found on project's webpage [9]. The idea is to perform analysis by applying to the data not only standard probit model, but also different models that may work better for such a kind of data. To achieve this aim, we apply the generalized linear models (GLM) methodology [10]. GLM model has general form $g(\mu) = b_0 + \sum_i b_i x_i$, where $\mu = EY$ and we assume, that

Y has binomial distribution. To specify a generalized linear model, one should give the distribution of dependent variable (binomial in our case) form of linear predictor ($b_0 + b_1 x$, where x is dose or time) and form of link function g . Implemented combinations are listed in Table 1.

Table 1. Models used in the analysis of bioassay data

	Model	Link function	Distribution	Linear predictor	Modeled probability of
(1)	Probit	$\phi^{-1}(\mu)$	<i>Binomial</i>	$b_0 + b_1 \ln(x)$	<i>Insect's death</i>
(2)	Logit	$\ln\left(\frac{\mu}{1-\mu}\right)$	<i>Binomial</i>	$b_0 + b_1 x$	<i>Insect's death</i>
(3)	Clog-clog	$\ln(-\ln(1-\mu))$	<i>Binomial</i>	$b_0 + b_1 \ln(x)$	<i>Insect's death</i>
(4)	Clog-clog-2	$\ln(-\ln(1-\mu))$	<i>Binomial</i>	$b_0 + b_1 x$	<i>Insect's survival</i>
(5)	Inverse	μ^{-1}	<i>Binomial</i>	$1 + b_1 x$	<i>Insect's survival</i>

On the basis of fitted model the program computes LT/LD₅₀ and LT/LD₉₀ points. Mean square error is computed to assess the fit of each model. The best model is chosen using leave-one-out cross-validation criterion.

RESULTS

Figure 1 presents a printout from the database interface. Database allows to store not only results, but a lot of information about assay conditions, analyzed toxin and inspected insects. Database design takes into account that more than one series of measurements can be done during an assay.

The screenshot shows a software interface for managing experimental data. At the top, there is a dropdown menu for 'Choose the experiment'. Below it, fields for 'No of experiment' (with a '000' button) and 'Date of experiment (RRRR-MM-DD)' (with '1996-11-19') are visible. The 'Biocide' section includes fields for Name (filled with 'Jezycie propoksur95'), Producer, Distributor, and Formulation. To the right, there are columns for 'active substance', 'concentration', and 'unit', with 'propoksur' entered in the first row. The 'Environment' section has a dropdown for 'Environment' (set to '1'), a 'Field' checkbox, and input fields for Humidity (80%), Temperature (22°C), and Light (16L8N). There are also checkboxes for 'Application to insects', 'Dose', 'Concentration' (0.1%), and 'Application'. A 'Notes' text area is on the right. At the bottom, there are three small tables: 'No of series' (with 'numer seri' and value 1), 'Time/dose' (with 'czas/dawk' and values 10, 15, 20, 25, 30, 40, 60), and 'No of replicates' (with 'Numer powtorzen' and values 1, 2, 3). Buttons for 'Create series', 'Create', 'Delete series', and a refresh icon are at the bottom right.

Figure 1. Database main window.

Software performing analysis was created using R 2.13.1 statistical software. It can communicate with the database described above. After running the program, user is asked to locate the database file. Then, in the main interface appropriate experiment for which analysis should be performed can be pointed. Results are printed in a new window of the default (on a particular computer) internet browser. After running the program, four R packages (*tcltk*, *RODBC*, *drc*, *R2HTML*) are loaded. If these packages are not installed, user will be asked to do so.

Table 2 presents example data: males of German cockroaches from field strain were exposed to deltamethrin by contact method.

Table 2. Experimental data: mortality rates of male German cockroaches from field strain exposed to deltamethrin

Time	Percent of killed insects	Time	Percent of killed insects
15	3.3%	130	36.7%
20	10.0%	140	36.7%
25	20.0%	150	50.0%
30	16.7%	180	63.3%
40	20.0%	210	63.3%
50	26.7%	240	80.0%
60	23.3%	270	80.0%
70	20.0%	300	83.3%
80	33.3%	330	83.3%
90	26.7%	360	86.7%
100	36.7%	1440	90.0%
120	36.7%		

Table 3 shows results of computation performed on these data. MSE and LOOC MSE are mean square error and mean square error obtained using leave-one-out cross-validation method. LT_{50} and LT_{90} are times in which 50% and 90% of insects are killed, respectively.

Table 3. Results obtained on data from Table 2

Model	MSE	LOOCV MSE	LT_{50}	LT_{90}
(1)	0.0065	0.0076	134	718
(2)	0.0066	0.0080	177	436
(3)	0.0061	0.0072	149	609
(4)	0.0046	0.0056	167	454
(5)	0.0089	0.0099	140	1260

It may be seen, that the best fit is obtained for model (4). LT_{50} for model (4) is 20% higher than this obtained from model (1) (probit) and LT_{90} value is 58% higher.

DISCUSSION

In our previous papers [6, 7] we showed, that application of models alternative to the probit regression can significantly improve fit to the data. We found also, that differences between estimated LT_{50} points were not very big on average, but substantial differences were quite often. Large differences in resulting LT_{50} point were observed in experiments in which large differences in fit were observed, thus use of alternative models may be advisable. We also paid attention to the experiments where the assumptions of the probit method are not met. In such situations, the automatic use of the standard probit model can lead to an underestimation of the LT_{50} value, but the functions at work are considered resistant to these problems. Our program implements more robust methodology and its application may be useful in every day practitioner's work.

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