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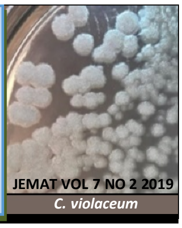

Grubb's Test for the Presence of Outlier in the Four-parameter Logistic Model Used in Obtaining the IC_{50} Value for *Salvia officinalis* Extract Against *Aeromonas hydrophila*

Rusnam[1]* and Gunasekaran, B.[2]

[1]Department of Agricultural Engineering, Faculty of Agricultural Technology, Andalas University, Padang, 25163, Indonesia.
[2]Faculty of Applied Sciences, UCSI University Kuala Lumpur (South Wing), No.1, Jalan Menara Gading, UCSI Heights 56000 Cheras, Kuala Lumpur, Malaysia.

*Corresponding author:
Prof Rusnam
Department of Agricultural Engineering,
Faculty of Agricultural Technology,
Andalas University,
Padang, 25163, Indonesia.
Email: rusnam_ms@yahoo.com

HISTORY

Received: 25th August 2019
Received in revised form: 14thSeptember 2019
Accepted: 11th October 2019

KEYWORDS

Aeromonas hydrophila
Salvia officinalis
four-parameter dose response curve
outlier
Grubb's test

ABSTRACT

The current trend in treating fish infection is to reduce the dependency to antibiotics. Active compounds from plants are being intensively studied as potential antibacterial to treat or to prevent infections caused by fish pathogens. A nonlinear regression exercise using the four-parameter dose response variable slope of the inhibition curve of the bacterium *Aeromonas hydrophila* using solvent extracts from the plant *Salvia officinalis* is checked for the presence of an outlier [at 95 or 99% of confidence). A potential outlier in a nonlinear regression is actually an extreme data point that is most certainly too extreme. This is normally done using the Grubb's test, which is the focus of this study. Grubb's statistical tests for the residuals indicated that the nonlinear regression model i.e. the four-parameter logistic model is adequate in finding the IC_{50} value as there was no outlier present.

INTRODUCTION

Just about the most harmful fish pathogenic agents is Aeromonas hydrophila, a known reason behind motile aeromonad septicaemia in numerous stream fishes as well as being thought to be caused by method of accidental scratches [1–5]. Instances of this ailment especially on fish species are documented in certain great deal places from the United states of America to south East Asia. Species of fish influenced by the bacteria are numerous and include hybrid striped bass, channel cat fish, Goldfish (*Carassius auratus*), Tilapia (*Tilapia nilotica*), Snakehead fish (*Ophiocephalus striatus*), American eel (*Anguilla rostrata*), Carp (*Cyprinus carpio*), Chinook salmon (*Oncorhynchus tshawytscha*) and Rainbow trout (*Oncorhynchus mykiss*) amongst them [6–10]. The bacterium is a Gram-negative rod-shaped and comes from the family Aeromonadaceae. It comes with a great specific polar flagellum that is unbelievably motile, plus it's found in diverse environment such as soil, in sewer, and also in brackish water. The bacterial virulence components consist of its capability to produce a number of tandem-like invasion on the bacterial system, which includes adhesions, the development of cytotoxins, enzymes like lipases, and the continuing development of a dense biofilm [11–14].

A previous study shows the inhibition of the bacterium *Aeromonas hydrophila* using solvent extracts from *Salvia officinalis* [15]. A nonlinear regression exercise using the four-parameter logistics equation gave the IC_{50} value of 21.92 mg/mL (95% confidence interval from 20.86 to 23.03). Nevertheless, in nonlinear regression the residuals of the curve must be distributed normally, the variance equal (homoscedastic) and randomness is assured [16,17]. Also, the residuals must be tested for the presence of outliers [at 95 or 99% of confidence). This is normally done using the Grubb's test, which is the focus of this study.

METHOD

Data were acquired from the works of Rusnam [18]. Initial outcomes demonstrated that the residuals followed the normally distribution. Visible remark of the data revealed that the third data point was most likely an outlier, and Grubb's test is going to be employed to evaluate this [19].

Grubb's' Statistic

Data deformation by an individual data point whether it is an averaged value, or a single data point can result in gross error in the fitting of a nonlinear curve. Looking for outlier is thus a fundamental part of curvefitting. Grubb's test is utilized to identify outlier in univariate setting and the data is presumed to be normally distributed [19]. The test can be applied to the maximal or minimal observed data from a Student's t distribution (**Equation 1**) and to test for both data simultaneously (**Equation 2**).

$$G_{\min}=\frac{\bar{X}-\min(X)}{s}$$

$$G_{\max}=\frac{\max(X)-\bar{X}}{s} \quad \textbf{(Eqn. 1)}$$

$$p_G=2n.p_t\left(G\frac{\sqrt{n(n-2)}}{n-1},n-2,1\right)$$

$$G_{\text{all}}=\frac{\max(\bar{X}-\min(X),\max(X)-\bar{X}}{s}$$

$$p_G=n.p_t\left(G\frac{\sqrt{n(n-2)}}{n-1},n-2,2\right) \quad \textbf{(Eqn. 2)}$$

RESULTS AND DISCUSSION

The statistics often used in nonlinear regression depends on the usage of residuals data, which are the difference between predicted and observed values. Statistical tests ought to be performed to analyze for the adequacy of the residuals in randomness, does not contain outlier, following normality and do not display autocorrelation. Typically, the greater the distinction between the predicted and observed values, the less well off the model [20]. The Grubb's' test handles one outlier at a particular time. The outlier is then removed, and the test is repeated until it is found that no outliers are spotted. As a general rule sample sizes of six or fewer distort the test since it will frequently be assigned most of the points as outliers. In addition, multiple iterations change the probabilities of detection.

Table 1. Residual data from the four-parameter dose response variable slope regression of the inhibition curve of the bacterium *Aeromonas hydrophila* using solvent extracts from *Salvia officinalis.*

	Residuals
	0.070977
	0.033491
	-0.19754
	0.163402
	-0.09834
	0.061865
	-0.10659
	-0.04197
	0.056905
	0.057797
Mean	0.00000025
Standard Deviation	0.1080535

Grubb's' test was applied to the residual data (**Table 1**), and the Grubb's' test statistic identifies the largest absolute deviation from the sample mean in units of the sample standard deviation. The critical value of Z was 2.2899478331. When the test statistic ***g*** is greater than the critical assigned value, an outlier identity can then be assigned to that corresponding value. The Grubb's' test did nor indicates the presence of any outlier. Residuals are very important in assessing the health of a curve from a particular used model. Mathematically, residual for the i^{th} observation in a given data set can be defined as follows;

$$e_i=y_i-f\left(x_i;\hat{\beta}\right) \quad \textbf{(Eqn. 3)}$$

where y_i denotes the i^{th} response from a given data set while x_i is the vector of explanatory variables to each set at the i^{th} observation corresponding values in the data set.

A potential outlier is actually an extreme data point that the researcher tags as unlikely in view of a number of specific demands. Considerably more specifically, an outlier in a sample can be an extreme value that is most certainly too extreme. By way of example, the most is regarded as an outlier only when it's statistically too large for that distribution on the maximum in the population model [21].A straightforward approach to label prospective outliers in measurements is to apply boxplot, while a bit more sophisticated technique is also employed including the Chauvenet's criterion in engineering and the 3-sigma criterion along with the Z-score in chemometrics. Despite the fact that these techniques are pretty straight forward and rapid, a much more appropriate method to utilize statistical test for outlier recognition. Specific tests range from the Dixon's Q-test or Grubb's' ESD-test for one outlier.

The leading constraint of the Grubb's test is usually that the thought quantity of outliers, k, must be explained exactly. If k is not explained effectively, this might distort the findings of the tests. In the case outliers are numerous or the precise quantity of outliers are not identified, the Rosner's generalized Extreme Studentized Deviate or ESD-test is recommended. [22]. This is because the presence of more than one outlier can skew the results from the Grubb's test and when this happen, the Ferguson's test based on sample skewness is more robust against the masking effect than the Grubb's test [23].

CONCLUSION

To conclude, the Grubb's statistical tests for the residuals indicated that the nonlinear regression model i.e. the four-parameter logistic model is adequate in finding the IC_{50} value as there was no outlier present.

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