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Application of Probit Analysis in Studying Allelopathy Phenomenon

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ABSTRACT

Probit analysis is a type of regression used to analyze the relationship between a stimulus and the quantal response. Allelopathy refers to direct or indirect negative or positive effects of one plant on another through the release of chemical compounds into the environment. This study was carried out to apply probit analysis in investigating the allelopathic effects of the leaves aqueous extracts of apple of Sodom [*Calotropis procera* (Aiton) W.T.] on the inhibition of seed germination of African rattlebox (*Crotalaria saltiana* Andr.)**.** Laboratory experiments were carried out at the Faculty of Agricultural Sciences, University of Gezira, Sudan, in season 2014/15. Ten concentrations (2.3, 4.6, 7.0, 9.3, 11.6, 13.9, 16.2, 18.5, 20.8 and 23.2 g/l) of leaves aqueous extract of apple of Sodom were prepared from a stock solution (50 g / l). A control with sterilized-distilled water was included for comparison. Treatments were arranged in completely randomized design with four replicates. The seeds were examined for inhibition in germination at three days after initial germination. Collected data were transformed using Abbott's formula and subjected to probit analysis procedure ($P \leq 0.5$). The results showed that the leaves aqueous extract of apple of Sadom had allelopathic on seed germination of African rattlebox with a direct positive relationship between concentration (g/l) and inhibition (%). Also, the data indicated that the plotting of corrected inhibition (%) against concentration (%) formed a sigmoid curve. Probit analysis transformed the sigmoid concentration-response curve to a straight line. Hence, the LC_{25} (2.16 g/l), LC_{50} (8.55 g/l) and LC_{75} (33.88 g/l) were accurately estimated. It is concluded that probit analysis is an appropriate procedure to study the allelopathy phenomenon.

Keywords: African rattlebox, Allelopathy, Apple of Sodom, *Calotropis*, *Crotalaria*, Probit

تطبيق تحليل البروبيت في دراسة ظاهرة التضاد الحيوي

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الخلاصة

تحليل الاحتمالية "Probit" هو نوع من الانحدار يستخدم لتحليل العلاقة بين التحفيز والاستجابة الكمية. يشير التضاد الحيوي "Allelopathy" إلى التأثيرات السلبية أو الإيجابية المباشرة أو غير المباشرة لنبات على آخر من خلال إطلاق المركبات الكيميائية في البيئة. أجريت هذه الدراسة من أجل تطبيق تحليل الاحتمالية في دراسة تأثير التضاد الحيوي للمستخلص المائي لأوراق نبات العشر [*Calotropis procera* (Aiton) W.T]

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على تثبيط إنبات بذور نبات صفيرة كبيرة (*Crotalaria saltiana* Andr.). أجريت التجارب المختبرية في كلية العلوم الزراعية ، جامعة الجزيرة ، السودان في موسم 15/2014. تم تحضير 10 تراكيز (2.3 ، 4.6 ، 7.0 ، 9.3 ، 11.6 ، 13.9 ، 16.2 ، 18.5 ، 20.8 ، 23.2 غم / لتر) من المستخلص المائي لأوراق نبات العشر من المحلول الاساس (50 غم / لتر). تم تضمين معاملة السيطرة المحتوية على الماء المقطر المعقم للمقارنة. تم ترتيب المعاملات في تصميم كامل العشوائية بأربعة مكررات. تم فحص البذور للتثبيط في الإنبات في ثلاثة أيام بعد الإنبات الأولي. تم تحويل البيانات المجمعة باستخدام صيغة "Abbott" وأُخضعت لتحليل الاحتمالية "Probit". أظهرت النتائج أن المستخلص المائي لأوراق نبات العشر له تأثيرات تضادية حيوية على إنبات بذور نبات صفيرة كبيرة وكانت هناك علاقة إيجابية مباشرة بين التركيز (غم / لتر) والتثبيط (٪). أيضا ، أشارت البيانات إلى أن رسم التثبيط المصحح (٪) مقابل التركيز (٪) شكل منحنى سيني. حول تحليل الاحتمالية "Probit" منحنى تركيز–استجابة السيني إلى خط مستقيم. ومن هنا ، تم تقدير قيمة التركيز القاتلة؛ LC_{25} (2.16 غم/لتر) و LC_{50} (8.55 غم/لتر) و LC_{75} (33.8 غم/لتر) بدقة. يُستنتج أن تحليل الاحتمالية "probit" هو اجراء مناسب لدراسة ظاهرة التضاد الحيوي.

Introduction

Probit analysis is a type of regression used to analyze the relationship between a stimulus (concentration) and the quantal (all or nothing) response. The idea of probit analysis was originally proposed since 1934 by Bliss [1]. He offered the idea of transforming the sigmoid concentration-response curve to a straight line. The statistical theory and techniques using probit analysis for analyzing data from concentration-quantal response experiments were further developed and discussed in details by Simon [2]. Probit analysis is widely used to analyze data obtained from the bioassays that are generally in percent response at the corresponding concentrations. The response is always binary by nature (yes/no) and the relationship between the response and the concentration is always sigmoid. Probit analysis acts as a transformation from sigmoid to linear and then runs a regression on the relationship. Once a regression is run, the output of the probit analysis could be used to compare the amount of chemical required to create the same response in each of the various chemicals. There are many endpoints used to compare the differences of concentration response. However, LC_{50} value is the most widely used outcome of the concentration-response experiments. LC_{50} is the lethal concentration that is required to kill 50 % of the population. There are several computer programs such as SPSS, SAS, Minitab, etc. that are faster and more accurate in estimating the critical LC_{50} value.

Allelopathy refers to direct or indirect negative or positive effects of one plant on another through the release of chemical compounds into the environment [3]. These chemical compounds, known as allelochemicals, are released from plant parts by exudation from roots, leaching and volatilization from stems and leaves, or decomposition of plant material in both natural and agricultural systems [4, 5]. The allelochemicals can reduce cell division or the levels of auxin that induces the growth of shoot and roots [6]. Allelochemicals such as phenolic compounds inhibit root and shoot length [7]. Growth inhibition caused by these allelochemicals may probably be due to its interference with the plant growth processes [6]. Allelochemicals released to the environment can either inhibit shoot and/or root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship, thereby destroying the plant's source of a nutrient. Understanding well the mechanism of allelopathic interactions between weeds and crops will enable to come up with proper and effective management ways to prevent further infestations. Several statistical analysis procedures were applied to analyze data in studying allelopathy phenomenon, such as descriptive analysis, analysis of variance, correlation and regression.

Apple of Sodom [*Calotropis procera* (Aiton) W.T.], a member of family Asclepiadaceae, is a xerophytic perennial shrub. It is native to tropical and subtropical Africa, Asia and common in the Middle East. It grows on a variety of soils, from fine to coarse texture, with varying degrees of salinity [8]. The widespread and persistent occurrence of apple of Sodom near the agricultural fields causes some adverse effect on the cultivated crops through allelopathic interaction [9]. Thus, the plant has received much attention from researchers due to its allelopathic behavior and has extensively been used for the control of many plants [10]. African rattlebox (*Crotalaria saltiana* Andr.), belonging to the family Fabaceae, is a large and diverse group of the sub-family papilionoidae (legumes) that

largely occur in Africa [11]. This plant is sometimes useful for nitrogen fixation by its root nodules [12]. The plant is common throughout central and northern Sudan [13, 14].

Despite that the idea of probit analysis procedure was proposed and developed since 1934 and the term allelopathy was first used in 1937, however, there is no application of probit analysis in studying the allelopathy phenomenon. Therefore, this study was carried out to apply probit analysis in investigating the allelopathic effects of the leaves aqueous extracts of apple of Sodom on the inhibition of seed germination of African rattlebox.

Materials and Methods

1. Experimental site

A series of germination tests were conducted in the Biology laboratory at the Faculty of Agricultural Sciences (FAS), University of Gezira (UofG), Sudan in June, 2018. The laboratory has an average temperature range between 26 - 28°C and the relative humidity ranges between 65 and 68 %.

2. Materials collection

Leaves of mature plants of apple of Sodom were collected from the Experimental Farm of the FAS in March, 2018. The leaves were transferred to the Biology laboratory of the FAS. Then, the leaves were washed with sterilized distill water and air dried on bench for 21 days at room temperature and in a dark room to avoid the direct sun light that might cause undesired reactions. The dried leaves were then crushed into powder and kept in brown bottles till used. The seeds of African rattlebox (*Crotalaria saltiana* Andr.) that have a germination percentage of 95-100% were collected from the Experimental Farm of the FAS, Gezira state, Sudan, in season 2017/18. The seeds were surface sterilized by 1% (v/v) solution of sodium hypochlorite (NaOCl), with continuous agitating for 3 min to reduce fungal infections. Subsequently, the seeds were washed with sterilized distill water for several times and stored at room temperature till used.

3. Preparation and calculation of the actual concentration of the leaves aqueous extract

Fifty grams, initial weight (IW), of leaves powder of apple of Sodom were placed in a conical flask, sterilized distill water was added to give a volume of 1000 ml, and then the flasks were shaken for 24 hours at room temperature (26±2°C) by an orbital shaker (160 rpm). The aqueous extract of the leaves was filtered by a muslin cloth, the leachate was dried, and the final weight (FW) of the filter or the weight of the precipitation (cake) was calculated by a sensitive balance. The final volume (FV) of the water extract for the apple of Sodom leaves was calculated by a measuring cylinder. The actual concentration (AC) of the aqueous extract of the leaves was calculated using the following equation:

$$AC\ (g/l) = \frac{(IW-FW)\times 1000}{FW} \quad (1)$$

4. Bioassay experiment procedure

Ten concentrations (n) of the leaves aqueous extract were prepared by sequential dilution of the stock extract with sterilized-distilled water to give 2.3, 4.6, 7.0, 9.3, 11.6, 13.9, 16.2, 18.5, 20.8 and 23.2 g/l. A control with sterilized-distilled water was included for comparison. Seeds (100 seeds) of African rattlebox were placed on a Glass Fiber Filter Paper (GFFP) placed in a glass Petri-dish (GPD) of 9 cm internal diameter. Each GPD was moistened with 30 ml of apple of Sodom aqueous extract, sealed with parafilm, covered with black polyethylene bag, and incubated at 28°C in the dark. The treatments were arranged in completely randomized design with four replicates (r). The seeds were examined for germination at three days after initial germination. The percentage of the inhibition of seed germination was calculated using the following equation:

$$Inhibition\ (\%) = \frac{Total\ number\ of\ seeds - number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100 \quad (2)$$

5. Probit analysis procedure

Probit analysis was used to analyse data from bioassay experiment, *i.e.* the portions of seeds of African rattlebox inhibited by several concentrations of apple of Sodom. Results from probit analysis were reported typically as a concentration required inhibiting a certain portion of the test seeds (LC_{10}, LC_{50} and LC_{90}); the slope and intercept of the regression line of the probit transformed data were also reported. Complementary probit transformed data were converted back to the concentration. In this study, probit analysis was achieved by conducting regression analysis, as follows:

Step 1: Correction of inhibition (%) using Abbott's formula

The inhibition (%) was corrected using Abbott's formula. It is given by:

$$Corrected\ inhibition\ (\%) = \frac{X-Y}{X} \times 100 \quad (3)$$

Where:
X is the % survivorship of the control group (germination % in the control treatment).
Y is the % survivorship in the experimental group (germination % in the concentration treatment).
Step 2: Plotting of inhibition (%) against concentration (g/l)

Corrected inhibition (%) was plotted against concentration (%) to explore relationship between them.

Step 3: Transformation of data

The concentration (g/l) was transformed to $\log_{10}$ concentration, (independent variable, X) and the corrected inhibition (%) was transformed to probits (dependent variable, Y) by using Finney's table [15], (Table-1).

Table 1-Table of transformation of percentages to probits, Finney's table

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
-	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Step 4: Calculation of simple linear regression equation

The simple linear regression equation is:

$$Y = \alpha + \beta X \quad (4)$$

where:
Y: Probit value
X: Log_{10} concentration
α: intercept
β: regression coefficient, the slope

The simple linear regression equation was calculated as follows:
The regression coefficient, i.e. the slope value, was calculated by the following equation:

$$\beta = \frac{\sum_{i=1}^{n} X_i Y_i - \frac{\sum_{i=1}^{n} X_i \sum_{i=1}^{n} Y_i}{n}}{\sum_{i=1}^{n} X_i^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}} \quad (5)$$

The intercept value was calculated by the following equation:

$$\alpha = \overline{Y} - \beta \overline{X} \quad (6)$$

Step 5: Testing the significance of the regression coefficient

To test the significance of the regression coefficient, i.e. the slope (β), analysis of variance was conducted by testing the following hypotheses:
Null hypothesis: $H_0: \beta = 0$
Alternative hypothesis: $H_a: \beta \neq 0$

The analysis of variance depends on studying and partitioning the sum squires (SS) of the total to its basic components, regression sum squares, and error sum squares which were computed as follow:

$$SS.Total = \sum Y_i^2 - \frac{(\sum y_i)^2}{n} \quad (7)$$

$$SS.Regression = \frac{\left(\sum_{i=1}^{n} X_i Y_i - \frac{\sum_{i=1}^{n} X_i \sum_{i=1}^{n} Y_i}{n}\right)^2}{\sum_{i=1}^{n} X_i^2 - \frac{\left(\sum_{i=1}^{n} X_i\right)^2}{n}} \quad (8)$$

$$SS.Erorr = SS.Total - SS.Regression \quad (9)$$

Degree of freedom for total =n-1 (10)

Degree of freedom regression =1 (11)

Degree of freedom error= DF for total- DF for regression (12)

$$Mean\ squares\ for\ regression = \frac{SS\ for\ regression}{Degree\ of\ freedom\ for\ rgression} \quad (13)$$

$$Mean\ squares\ for\ error = \frac{SS\ for\ Error}{DF\ for\ error} \quad (14)$$

$$F - cal\ for\ regression = \frac{MS\ for\ regression}{MS\ for\ error} \quad (15)$$

F-tab was obtained from F-table (F – Distribution).

The calculation processes of the analysis of variance were summarized in the ANOVA table.

Step 6: Coefficient of simple determination (r^2)

The coefficient of simple determination was calculated as follows:

$$r^2 = \frac{\text{SS.Regression}}{\text{SS.Total}} \quad (16)$$

Step 7: Calculation of the lethal concentrations

The LC_{25}, LC_{50} and LC_{75} were calculated by substituting Y by 2.5, 5 or 7.5 in the regression equation (4), respectively. Then, the estimated log concentration was converted back (the antilog) to concentration.

6. Statistical analysis

Step 1: Correction of inhibition (%) using Abbott's formula

The anticipated natural inhibition (%) was corrected using Abbott's formula (3) as shown in Table- 2.

E.g. Inhibition (%) and corrected inhibition (%) for the concentration 2.3 g/l were calculated as follows:

$$Inhibition\ (\%) = \frac{100-95.75}{100} \times 100 = 4.3 \quad (2)$$

$$Corrected\ inhibition\ (\%) = \frac{98.5-95.75}{98.5} \times 100 = 2.8 \quad (3)$$

Table 2-Concentration (g/l) of the leaves aqueous extract of apple of Sodom, inhibition (%), and correction of inhibition (%) of the seed germination of the African rattlebox using Abbott's formula

Concentration (g/l)	Total number of seeds	Number of germinated seeds	Inhibition (%)	Corrected inhibition (%)
0.0	100	98.50	1.5	0
2.3	100	95.75	4.3	2.8
4.6	100	90.50	9.5	8.1
7.0	100	75.50	24.5	23.4
9.3	100	50.75	49.3	48.5
11.6	100	34.75	65.3	64.8
13.9	100	20.75	79.3	79.0
16.2	100	10.50	89.5	89.3
18.5	100	6.00	94	93.9
20.8	100	4.00	96	95.9
23.2	100	1.75	98.3	98.3

Step 2: Plotting of inhibition (%) against concentration (g/l)

Corrected inhibition (%) was plotted against concentration (g/l) as shown in Figure-1.

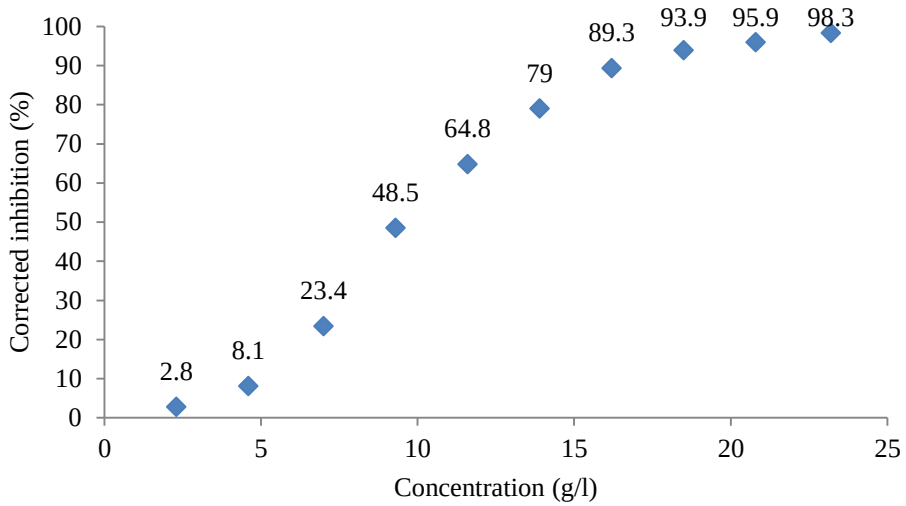

Figure 1-Plotting corrected inhibition against concentration (%).

Step 3: Transformation of data

Concentration (g/l) was transformed to $\log_{10}$-concentration (X) and corrected inhibition (%) was transformed to probit (Y), as shown in Table-3.

E.g. at the concentration 9.3 g/l, the corrected inhibition (48.5 % ≈ 49 %) was transformed to probits (4.97) by using Finney's table, as shown in Table-1.

Table 1-Table of transformation of percentages to probits, Finney's table

%	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
-	**0.0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Table 3-Transformation of concentration to $\log_{10}$ concentration (g/l) and corrected inhibition (%) to probits using Finney's table.

Log_{10} concentration (X)	Probit (Y)
0.362	3.12
0.663	3.59
0.845	4.26
0.968	**4.97**
1.064	5.39
1.143	5.81
1.210	6.23
1.267	6.55
1.318	6.75
1.365	7.05

Probit was plotted against $\log_{10}$ concentration (Figure-2).

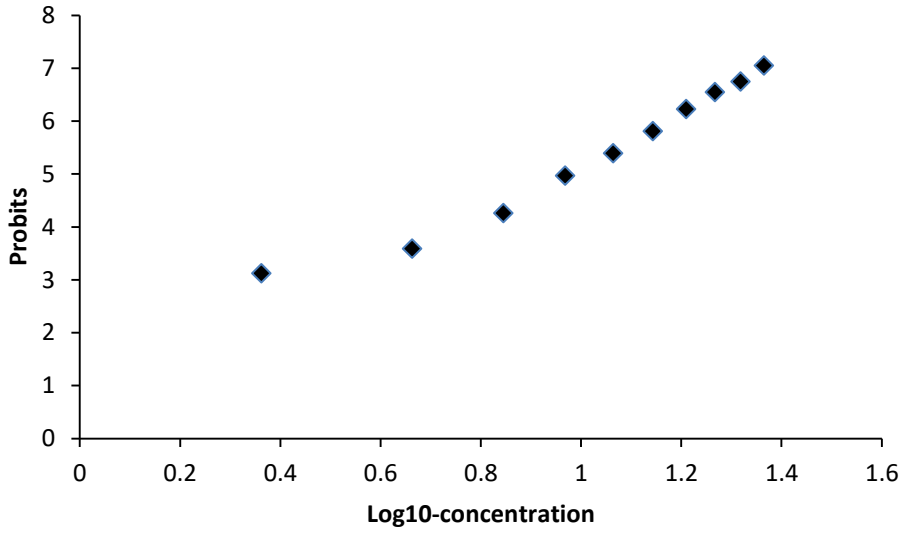

Figure 2-Plotting probits against $\log_{10}$ concentration.

Step 4: Calculation of the simple linear regression equation

The regression coefficient, i.e. the slope, value was calculated as follows:

$$\beta = \frac{58.653 - \frac{10.205*53.72}{10}}{11.330 - \frac{(10.205)^2}{10}} = 4.184 \quad (5)$$

The intercept value was calculated as follows:

$$\alpha = 5.372 - 4.184 * 1.021 = 1.009 \quad (6)$$

The simple linear regression equation was:

$$Y = 1.099 + 4.184X \quad (4)$$

or

$$Probit = 1.099 + 4.184\ log_{10} concentration$$

Step 5: Testing the significance of the regression coefficient

The significance of the regression coefficient was tested as follows:

$$SS.Total = 305.260 - \frac{(53.72)^2}{10} = 16.676 \quad (7)$$

$$SS.Regression = \frac{\left(\mathbf{58.653} - \frac{\mathbf{10.205*53.72}}{10}\right)^2}{\mathbf{11.330} - \frac{(\mathbf{10.205})^2}{10}} = 16.014 \quad (8)$$

$$SS.Erorr = SS.Total - SS.Regression$$

$$SS.Erorr = 16.676 - 16.014 = 0.662 \quad (9)$$

Degree of freedom for total =10-1=9 (10)

Degree of freedom regression =1 (11)

Degree of freedom error= 9- 1=8 (12)

$$Mean\ sqares\ for\ regression = \frac{16.014}{1} = 16.014 \quad (13)$$

$$Mean\ sqares\ for\ error = \frac{0.662}{8} = 0.083 \quad (14)$$

$$F - cal\ for\ regression = \frac{16.014}{0.083} = 192.940 \quad (15)$$

F-tab values (5.5914 and 12.2466) were obtained from the F-table at alpha = 0.05 and 0.01, respectively.

The calculation processes of the analysis of variance are summarized in the ANOVA table (Table- 4).

Table 4-ANOVA table

SOV	DF	SS	MS	F-cal	F-Tab	
					0.05	0.01
Regression	1	14.014	16.014	192.940 **	5.3177	11.2586
Error	8	0.662	0.083			
Total	9	16.676				

Step 6: Coefficient of simple determination (r^2)

The coefficient of simple determination was calculated as follows:

$$r^2 = \frac{14.014}{16.676} = 0.9603 \quad (16)$$

Step 7: Calculation of the lethal concentration

LC_{25} =

$$2.5 = 1.099 + 4.184 Log_{10} concentration$$

$$Log_{10}\ concentration = \frac{2.5 - 1.099}{4.184} = \underline{0.335}$$

Concentration = Antilog of 0.335 = 2.16

LC_{50} =

$$5 = 1.099 + 4.184\ Log_{10} concentration$$

$$Log_{10}\ concentration = \frac{5 - 1.099}{4.184} = \underline{0.932}$$

Concentration = Antilog of 0.932 = 8.55

LC_{75} =

$$7.5 = 1.099 + 4.184\ Log_{10} concentration$$

$$Log_{10} concentration = \frac{7.5 - 1.099}{4.184} = \underline{1.530}$$

Concentration = Antilog of 1.888 = 33.88

The calculation processes of the statistical analysis are summarized in Table-5.

Table 5-Summary of the calculation processes of the statistical analysis.

Concentration (g/l)	Corrected inhibition (%)	Log_{10} concentration (X)	Probit (Y)	X*Y	X^2	Y^2
2.3	2.8	0.362	3.12	1.129	0.131	9.734
4.6	8.1	0.663	3.59	2.380	0.440	12.888
7.0	23.4	0.845	4.26	3.600	0.714	18.148
9.3	48.5	0.968	4.97	4.811	0.937	24.701
11.6	64.8	1.064	5.39	5.735	1.132	29.052
13.9	79.0	1.143	5.81	6.641	1.306	33.756
16.2	89.3	1.210	6.23	7.538	1.464	38.813
18.5	93.9	1.267	6.55	8.299	1.605	42.903
20.8	95.9	1.318	6.75	8.897	1.737	45.563
23.2	98.3	1.365	7.05	9.623	1.863	49.703
$\sum$		10.205	53.72	58.65	11.33	305.26
$\overline{X}$		1.021	5.372			

Results and Discussion

The results showed that the leaves aqueous extract of apple of Sodom inhibited the seed germination of the African rattlebox with a direct positive relationship between concentration (g/l) and inhibition (%) (Table-2). Also, the results indicated a natural inhibition (%) in the seed germination of African rattlebox, as anticipated. Hence, before proceeding to conduct probit analysis, the inhibition (%) was corrected using Abbott's formula (Table-2). Plotting of corrected inhibition (%) against concentration (%) formed a sigmoid curve (Figure-1). Transformations of concentration to log_{10} concentration and corrected inhibition to probit are shown in (Table-3). Plotting probits against log_{10} concentration straightened the cumulative distribution line and the curve was transformed to more accurately describe the data (Figure-2).

The regression coefficient and the intercept values were 4.184 and 1.009, respectively. Hence, the simple linear regression equation was $= 1.099 + 4.184X$, *i.e.* $Probit = 1.099 * 4.184\ log_{10}concentration$. Since the f-calculated value for regression was greater than the f-tabulated value at the levels of significance of 0.05 and 0.01, the regression coefficient was significantly different from zero. Therefore, the regression coefficient was included in the equation of simple linear regression. The value of coefficient of simple determination was 0.9603, i.e. 96.03% of the changes in the dependent variable y (inhibition) were caused by the independent variable x (concentration). The LC_{25}, LC_{50} and LC_{75} were 2.16, 8.55 and 33.88 g/l, respectively.

Gulzar and Siddiqui [10] carried out an experiment to explore the effects of the aqueous extract of apple of Sodom on the seed germination of *Brassica oleracea*. Seeds of *B. oleracea* were steeped in solutions containing concentrations (20%, 40%, 60% and 80%) of leaves aqueous extract of apple of Sodom. The results revealed that the higher concentrations (60% and 80%) of the aqueous extract significantly reduced seed germination in comparison to the untreated control. The inhibitory effect was increased with the increase in the concentration of the aqueous extract. Gulzar and Siddiqui [10] concluded that the delayed germination and low germination rate of the *B. oleracea* after treatment by the aqueous extracts occurred because the extracts might damage the membrane system of the seeds.

The various concentrations of the leaves aqueous extracts of apple of Sodom had varying degrees of inhibition on the seed germination of mustard plant (*Brassica nigra*). The inhibition of seed germination was concentration-dependent. Suppression in the seed germination as a result of allelochemical stress might be attributed to inhibition of some physiological processes, such as water uptake, gibberellic acid activity, cell division, and elongation during germination process [16, 17].

The leaves aqueous extract of apple of Sodom at 5- 60 % has allelopathic effects on the seed germination of several crops such as barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), cucumber (*Cucumis sativus* L.), fenugreek (*Trigonella foenum-graecum* L.), alssana (*Senna occidentalis* L.), tomato (*Lycopersicon esculentum* Mill.) and eggplant (*Solanum melongena* L.). The germination percentage was reduced with further increase in concentration of the aqueous extract [18, 19]. The delay in seed germination and the reduction in germination index might be due to the presence of water-soluble inhibitors in the apple of Sodom extract [9].

Conclusions

The results revealed the effects of the leaves aqueous extract of apple of Sodom allelopathic on seed germination of African rattlebox, with a direct positive relationship between concentration (g/l) and inhibition (%). Also, the data indicated that plotting of corrected inhibition (%) against concentration (%) formed a sigmoid curve. Probit analysis transformed the sigmoid concentration-response curve to a straight line. Hence, the LC_{25} (2.16 g/l), LC_{50} (8.55 g/l) and LC_{75} (33.88 g/l) were accurately estimated. Therefore, probit analysis is appropriate in studying the allelopathy phenomenon.

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