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Assessing the Comparative Toxicity of *Salvia Officinalis* Extracts and Copper Sulphate on *Melanopsis Nodosa*, a Freshwater Snail

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Abstract

This study investigates the molluscicidal properties of *Salvia officinalis* extracts against *Melanopsis nodosa* snails, with a focus on environmentally friendly alternatives to traditional chemical molluscicides. *Melanopsis nodosa*, a common freshwater snail in Iraq, is an intermediate host for diseases affecting humans and animals. Traditional molluscicides, such as copper sulphate, pose environmental and non-target organism risks. *S. officinalis*, known for various medicinal uses, was explored for its potential molluscicidal activity. Snails were collected from Muhyii Canal, Baghdad, and exposed to different concentrations of *S. officinalis* extracts and copper sulphate in controlled laboratory conditions. The study evaluated mortality rates at 24-, 48-, 72-, and 96-hours exposure, using the WHO method for molluscicide testing and Probit analysis for mortality calculation. Results showed significant molluscicidal activity of *S. officinalis* extracts, with the LC50 values of 15.53 ppm (24 hrs), 6.821 ppm (48 hrs), 4.288 ppm (72 hrs), and 0.0735 ppm (96 hrs). Compared to copper sulphate, the extracts were less effective but still significant, indicating the potential of *S. officinalis* as an environmentally friendly molluscicide. This study contributes to the search for safer molluscicides, highlighting *S. officinalis* extracts' effectiveness against *M. nodosa*. These findings could aid in controlling snail populations, thereby reducing the spread of snail-borne diseases, with lower environmental impact compared to traditional chemical molluscicides. Further research is suggested to understand the exact mechanism of snail mortality caused by these extracts.

1. Introduction

Molluscs including snails as *Melanopsis nodosa* are important organisms in the ecosystem. Also, mollusks were important medically because they are vectors and intermediate hosts for important human and animal diseases [1]. Melanopsis spp. was a common freshwater snail, belonging to the family Melanopsidae (Gastropoda: Caenogastropoda: Cerithioidea). It is widely distributed in Iraq [14, 15, 20, 21]. *Melanopsis nodosa* also was recorded in different regions and locations of Iraq by many authors, of these regions; Al-Swaib marsh in southern Iraq [26], Tigris River at Baghdad capital [27], Chebayish marsh at Thi-Qar province

[23], Rumaitha distract southern of Iraq [22], Gharaf River (branch of Tigris River) [18]; and Kut city at Wassit province [24].

There were many ways to snail control, including chemical, physical, and biological methods. Copper sulphate, Niclosamide, and Carbamates are the most common Molluscicides used to chemically control the snails. Methyl and Methiocarb were found to be moderately toxic to land snails too [17, 18].

Chemical substances used as Molluscicides found to be a hazard to the environment and non-target organisms [3]. Moreover, the disadvantage of these molluscicides is the high concentration (0.5–5%) may kill no target organisms [16]. In addition, the efficacy of these Molluscicids is varying in dry and moist conditions [15].

To overcome these problems, there was a need for new, more effective, and less hazardous molluscicides. In previous studies, it was discovered the promising molluscicidal properties of scharin from *Calotropis procera*, ouabin from *Acokanthera ouabaio* Lewin, cardenolide extracts from *Pergularia tomentosa* Linn and *Nerium oleander* L [14, 2]. Also, *Thevetia peruviana* (which contains cardenolides) was found to be toxic to slugs and snails [19]. Previous studies have shown that *Adenium arabicum* contains cardenolides that cause death to snails [14], thus this study was carried out to discover if *S. officinalis* displayed molluscicidal properties compared with copper sulphates.

2. Experimental Procedure

2.1. Collection of Samples

The freshwater snails *M. nodosa* (L) were collected from a canal named Muhyii Canal (longitude 33º 32′, 83″E and latitude 44º 25′ 37″ N) located in Al-Rasheed district (30km) Baghdad southern, Iraq, through 2018 weekly. Snails were brought to the laboratory, isolated, identified according to stander keys of snails, and acclimatized for a week. The snails were provided with proper food (*Alfa alfa* leaves extracts 10ml per 50L/ day) and ventilation to keep snail's survivals. Healthy snails (20-30 mm shell height and 3-4 gm weight) were selected for the experimentation.

2.2. Preparation of Aqueous Extract

S. officinalis was used as a medicinal plant for different purposes. Stock solution (SS) from the leaves of *S. officinalis* was prepared. After drying, a hand mill was used to dry the leaves in a shade condition (Estrella®, model 41B), sifted by a sieve (mesh size 20 µm) to obtain a fine powder, and kept in a cool dry place. We macerated 5 grams of leaf powder in 1 litter of distilled water for 24hrs to produce a stock solution of 5% then placed in glass flasks. The macerate was filtered in cotton gauze to get crude extracts. From this stock solution, serial of dilutions was made. One gram of copper sulphates ($CuSO_4.5H_2O$) (Riedel-De Haen company) was added to (1 litter) of distilled water to get a stock solution of 1% as a standard control.

2.3. Contact Toxicity and Calculation of Mortality

A serial of 1-10% concentrations was prepared from each stock solution of the *S. officinalis* extracts. All tests were repeated three times at different times. Ten individuals of snails without any food were tested in each replicate and calculated as average. In addition, a stock solution (1g/L) of copper sulphate ($CuSO_4$) made as standard in comparisons. The W.H.O. method (II) for molluscicide testing was followed to monitor the susceptibility of snails and compare its potency with the extracts, exposure, and recovery determined. Different levels of the LC were calculated using probit analysis [13]. Bioassays experiments were conducted in the TBRU (Tropical Biological Research Unit, College of Science) laboratories. Bioassays evaluated by LC10, LC16, LC50, LC84, LC90, and LC100. These parameters were determined for each exposure period (24, 48, 72, 96 hours) in all concentrations. The results were recorded at the end of each 24-hour exposure. The numbers of dead snails were removed and recorded at 24, 48, 72, and 96 hrs after each application. The endpoint of dead individuals was considered when there was no movement, no response to stimulation by the glass rod, no recovery after 24 hrs of putting in clean water, and lack of the ability to adhere [3]. The mortality rate was calculated against *S. officinalis* and $CuSO_4$ as a standard. Probit analysis was used to calculate the mortality and comparison among concentrations.

2.4. Statistical Analysis

Results were analysed using Bio-Stat v 5 software; all data were subjected to probit analysis according to Finney, using the probit equation bellow:

$$\mathbf{Y = \phi^{-1}(p)} \text{ ……… (1)}$$

Where Y' is the probit transformed value (5 used to be added to avoid negative values in hand calculation), p is the proportion (p = responders/total number) and inverse Φ(p) is the 100*p% quantile from the standard normal distribution.

Also, the concentrations were converted to Log10 by the bellow equation:

$$\mathbf{Log(10)\ x = \log(x) / \log(10)} \text{ ……… (2)}$$

Where log(x) is the natural logarithm of x and log(10) is the natural logarithm of the base 10.

3. Experiments of the Exposure

According to the Kolmogorov-Smirnov test, the mortality values of the snail *M. nodosa* exposed to *S. officinalis* extracts were followed to the normal distribution ($P>0.05$).

The results of this study showed that the lowest mortality recorded in 24hrs of exposure was zero (probit value 0.0001), while the highest mortality recorded in 24hrs of exposure was five (probit value 0.1642). Compared with $CuSO_4$, the lowest mortality was 2 (probit 0.0295) and the highest mortality was killing off all snails of the experiment (probit 1). There were significant differences ($P<0.001$) in mortality caused by *S. officinalis* following the concentrations used in this experiment, but no significant differences ($p>0.001$) in the $CuSO_4$ experiment (Table 1). The concentration chosen in this work to get the LC50 value was suitable as we note from the probit analysis results which showed that the values of probit for *S. officinalis* extracts concentrations were graduated from 3.2965-151.3557 for *S. officinalis* and 0.0842-0.5216 for $CuSO_4$ (Table 2).

The results of the study showed that the LC 50 of *S. officinalis* against the snail *M. nodosa* was 15.53ppm. While $CuSO_4$ was 0.212ppm. LCL (lowest confidence level), UCL (upper confident level), and other LC different levels (10, 16, 84, 90, and 100) were tabulated in (Table 3).

The results of this work showed that increasing in *M. nodosa* mortality percentage was followed by an increase in *S. officinalis* extract and $CuSO_4$ concentrations (Figure 1).

Table (1): Experimental mortality and probit of *M. nodosa* snail exposed to *S. officinalis* extract for 24hr according to Finney Method (Lognormal Distribution).

Log10 [Dose or (Stimulus)]	Actual Percent (%)	Probit Percent (%)	N (total sample)	R (mortality)	E(R) error of mortality	Difference	Chi-square
			***Salvia officinalis* extracts**				
0.	0.0083	0.0001	30	0.25	0.0024	0.2476	25.7839
0.301	0.0083	0.0017	30	0.25	0.0501	0.1999	0.7971
0.4771	0.0083	0.0073	30	0.25	0.2195	0.0305	0.0043
0.6021	0.0333	0.0182	30	1.	0.5472	0.4528	0.3748
0.699	0.0333	0.0344	30	1.	1.0309	-0.0309	0.0009
0.7782	0.0667	0.055	30	2.	1.649	0.351	0.0747
0.8451	0.0667	0.0791	30	2.	2.3735	-0.3735	0.0588
0.9031	0.1	0.1059	30	3.	3.1769	-0.1769	0.0098
0.9542	0.1333	0.1345	30	4.	4.0346	-0.0346	0.0003
1.	0.1667	0.1642	30	5.	4.9263	0.0737	0.0011
			Chi-square 27.1057				

Degrees Of Freedom 8
p-level 0.0007

$CuSO_4$							
-1.	0.0667	0.0295	30	2.	0.8852	1.1148	1.4038
-0.699	0.3667	0.4525	30	11.	13.5759	-2.5759	0.4888
-0.5229	0.8	0.82	30	24.	24.6004	-0.6004	0.0147
-0.3979	0.9667	0.9505	30	29.	28.5144	0.4856	0.0083
-0.301	0.9917	0.9868	30	29.75	29.6027	0.1473	0.0007
-0.2218	0.9917	0.9964	30	29.75	29.891	-0.141	0.0007
-0.1549	0.9917	0.999	30	29.75	29.9687	-0.2187	0.0016
-0.0969	0.9917	0.9997	30	29.75	29.9905	-0.2405	0.0019
-0.0458	0.9917	0.9999	30	29.75	29.997	-0.247	0.002
0.	0.9917	1.	30	29.75	29.999	-0.249	0.0021

Chi-square 1.9245
Degrees Of Freedom 8
p-level 0.9832

Alpha value (for confidence interval) 0.001

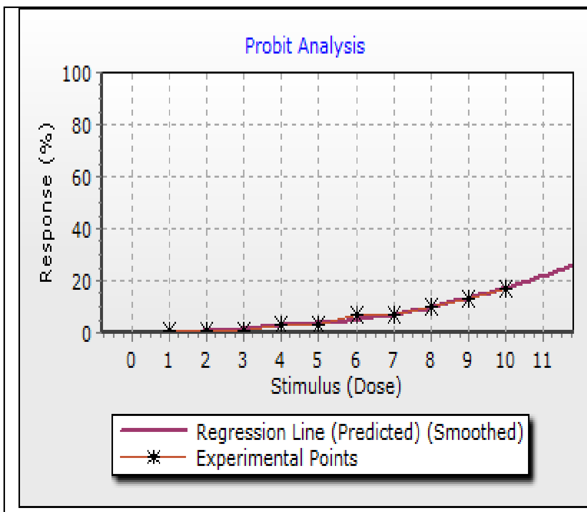
Table (2): Comparative between *S. officinalis* and $CuSO_4$ observed and expected of concentrations (Stimulus) percentile according to regression analysis.

Percentile	Probit (Y)	Log10[Dose (Stimulus)]	Standard Error	Dose (Stimulus)	Standard Error	LCL	UCL
***Salvia officinalis* extracts**							
1	2.6732	0.5181	0.1912	3.2965	1.4985	0.8292	4.657
5	3.3548	0.7615	0.0784	5.7739	1.0483	3.4875	7.0782
10	3.7183	0.8913	0.0601	7.7854	1.08	6.2136	10.6844
16	4.0056	0.9939	0.0912	9.8602	2.0867	7.9804	18.1832
20	4.1585	1.0485	0.1151	11.1819	2.9996	8.822	24.9427
25	4.3258	1.1083	0.1435	12.8307	4.3161	9.748	35.5884
30	4.476	1.1619	0.1699	14.5174	5.8262	10.6127	49.1959
40	4.7471	1.2587	0.219	18.1424	9.54	12.2999	88.7703
50	5.	1.349	0.2655	22.3371	14.525	14.0637	154.5417
60	5.2529	1.4394	0.3125	27.5016	21.5453	16.0497	269.5605
70	5.524	1.5362	0.3632	34.3689	32.2126	18.4667	489.9449
75	5.6742	1.5898	0.3914	38.8868	39.9808	19.9515	682.4773
80	5.8415	1.6495	0.4228	44.6209	50.6284	21.7409	987.4234
84	5.9944	1.7042	0.4515	50.602	62.6125	23.5132	1,384.4572
90	6.2817	1.8068	0.5056	64.0877	92.6486	27.2323	2,612.8642
95	6.6452	1.9366	0.5742	86.4144	150.5611	32.7756	5,838.5098
99	7.3268	2.18	0.7029	151.3557	366.8505	46.347	26,392.3893
$CuSO_4$							
1	2.6732	-1.0747	0.0692	0.0842	0.0135	0.0573	0.107
5	3.3548	-0.9587	0.0549	0.11	0.0139	0.0813	0.1334
10	3.7183	-0.8968	0.0476	0.1268	0.0139	0.0978	0.1503
16	4.0056	-0.8479	0.0421	0.1419	0.0138	0.113	0.1653
20	4.1585	-0.8219	0.0394	0.1507	0.0137	0.1219	0.174
25	4.3258	-0.7934	0.0365	0.1609	0.0135	0.1325	0.1842
30	4.476	-0.7679	0.0341	0.1707	0.0134	0.1426	0.194
40	4.7471	-0.7217	0.0302	0.1898	0.0132	0.1625	0.2135
50	5.	-0.6787	0.0275	0.2096	0.0133	0.1829	0.2343
60	5.2529	-0.6356	0.0257	0.2314	0.0137	0.2049	0.2584
70	5.524	-0.5895	0.0252	0.2573	0.015	0.2299	0.2888

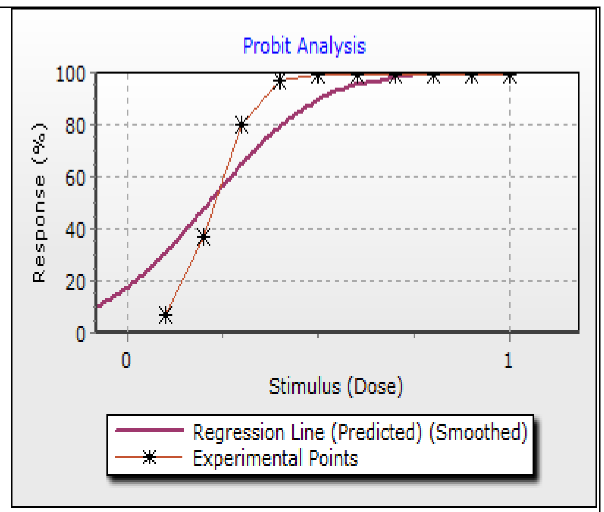
75	5.6742	-0.5639	0.0256	0.2729	0.0161	0.2444	0.308
80	5.8415	-0.5355	0.0266	0.2914	0.0179	0.2609	0.3318
84	5.9944	-0.5094	0.0279	0.3094	0.0199	0.2765	0.3558
90	6.2817	-0.4605	0.0314	0.3463	0.025	0.307	0.4074
95	6.6452	-0.3987	0.0369	0.3993	0.034	0.3484	0.4863
99	7.3268	-0.2827	0.0496	0.5216	0.0597	0.4375	0.6845

Table (3): LC levels of *S. officinalis* extract and $CuSO_4$ against *M. nodosa* snail exposed for 24hr.

***Salvia officinalis* extracts**			
LC10	8.0285	LC50 LCL	12.561
LC16	9.6774	LC50 UCL	18.4995
LC50	**15.5302**	LC50 Standard Error	0.8725
LC84	21.3831	Beta	0.1709
LC90	23.032	Intercept	2.3465
LC100	24.3095	Beta Standard Error	0.0837
$CuSO_4$			
LC10	-0.0789	LC50 LCL	0.069
LC16	-0.0147	LC50 UCL	0.3571
LC50	**0.213**	LC50 Standard Error	0.0416
LC84	0.4408	Beta	4.3902
LC90	0.505	Intercept	4.0647
LC100	0.5547	Beta Standard Error	0.8729



***Salvia officinalis* extracts**



$CuSO_4$

Figure (1): Regression line and experimental point described the effect of *S. officinalis* and $CuSO_4$ concentrations that caused *M. nodosa* mortality within 24hrs of exposure**.**

4. Experiment with 48hrs Exposure

The results of this study showed that the lowest mortality recorded in 48hrs of exposure was 7 (probit value 0.1758), while the highest mortality recorded in 48hrs of exposure was 20 (probit value 0.5890). Compared with $CuSO_4$, the lowest mortality was 2 (probit 0.0295) and the highest mortality was killing all the experiment snails (probit 1). No significant differences (P>0.001) between mortality following the concentrations of *S. officinalis* and $CuSO_4$ used in this experiment (Table 5).

The concentration chosen in this work to get the LC50 value were suitable as we note from the results of probit analysis which showed that the values of probit for *S. officinalis* extracts concentrations were graduated from 0.0622 - 656.2968 and 0.0485-0.5216 for $CuSO_4$ (Table 6).

The results of the study showed that the LC 50 of *S. officinalis* against the snail *M. nodosa* was 6.82ppm and 0.213ppm of $CuSO_4$. LCL (lowest confidant level), UCL (upper confidant level), and other LC different levels (10, 16, 84, 90, and 100) were tabulated in (Table 7).

The results of this work showed that increasing in *M. nodosa* mortality percent was followed by an increase in *S. officinalis* extract and $CuSO_4$ concentrations (Figure 2).

Table (5): Experimental mortality and probit of M. nodosa snail exposed to S. officinalis extracts for 48hrs according to the Finney Method (Lognormal Distribution).

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent (%)	N	R	E(R)	Difference	Chi-square
***Salvia officinalis* extracts**							
0.	0.2333	0.1758	30	7.	5.2732	1.7268	0.5654
0.301	0.3	0.2798	30	9.	8.3943	0.6057	0.0437
0.4771	0.3	0.3521	30	9.	10.5622	-1.5622	0.231
0.6021	0.3667	0.407	30	11.	12.2105	-1.2105	0.12
0.699	0.3667	0.451	30	11.	13.53	-2.53	0.4731
0.7782	0.4333	0.4874	30	13.	14.6225	-1.6225	0.18
0.8451	0.5	0.5183	30	15.	15.549	-0.549	0.0194
0.9031	0.5667	0.545	30	17.	16.3491	0.6509	0.0259
0.9542	0.6333	0.5683	30	19.	17.0499	1.9501	0.223
1.	0.6667	0.589	30	20.	17.6709	2.3291	0.307
Chi-square	2.1887						
Degrees Of Freedom	8						
p-level	0.9747						
$CuSO_4$							
-1.	0.0667	0.0295	30	2.	0.8852	1.1148	1.4038
-0.699	0.3667	0.4525	30	11.	13.5759	-2.5759	0.4888
-0.5229	0.8	0.82	30	24.	24.6004	-0.6004	0.0147
-0.3979	0.9667	0.9505	30	29.	28.5144	0.4856	0.0083
-0.301	0.9917	0.9868	30	29.75	29.6027	0.1473	0.0007
-0.2218	0.9917	0.9964	30	29.75	29.891	-0.141	0.0007
-0.1549	0.9917	0.999	30	29.75	29.9687	-0.2187	0.0016
-0.0969	0.9917	0.9997	30	29.75	29.9905	-0.2405	0.0019
-0.0458	0.9917	0.9999	30	29.75	29.997	-0.247	0.002
0.	0.9917	1.	30	29.75	29.999	-0.249	0.0021
Chi-square	1.9245						
Degrees Of Freedom	8						
p-level	0.9832						
Alpha value (for confidence interval) 0.001							

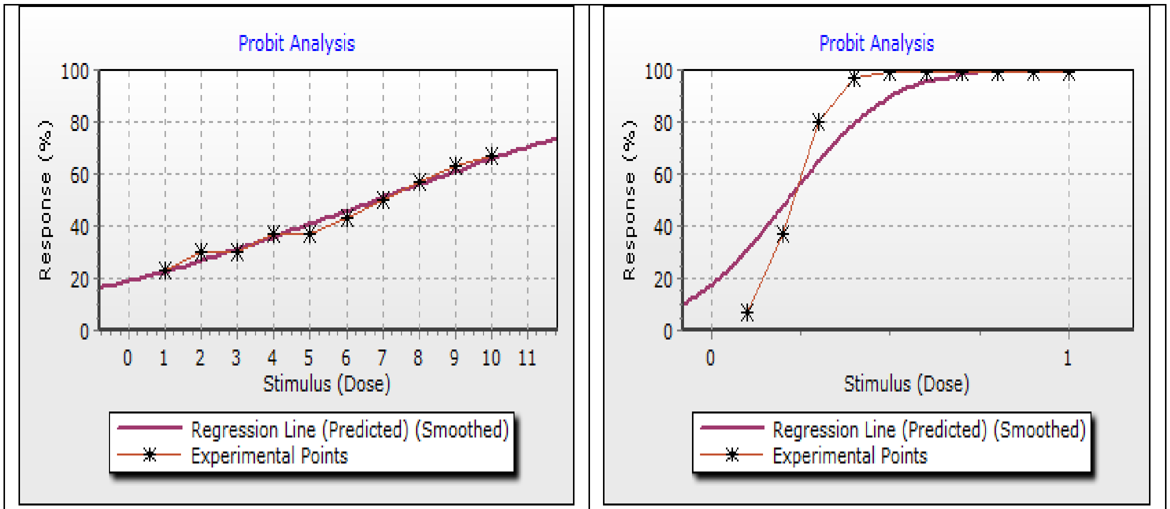
Table (6): Dose (Stimulus) Percentile

Percentile	Probit (Y)	Log10[Dose (Stimulus)]	Standard Error	Dose (Stimulus)	Standard Error	LCL	UCL
***Salvia officinalis* extracts**							
1	2.6732	-1.2062	0.5339	0.0622	0.0972	0.0019	0.2407

5	3.3548	-0.617	0.3707	0.2416	0.2321	0.0219	0.6226
10	3.7183	-0.3027	0.2847	0.4981	0.3504	0.0795	1.0384
16	4.0056	-0.0543	0.2179	0.8824	0.4616	0.2188	1.5645
20	4.1585	0.0779	0.1833	1.1965	0.5201	0.3736	1.9538
25	4.3258	0.2225	0.1468	1.6693	0.575	0.6664	2.507
30	4.4760	0.3524	0.1164	2.2511	0.6103	1.1087	3.1689
40	4.7471	0.5867	0.0761	3.8614	0.6805	2.6000	5.1699
50	**5.0000**	**0.8054**	**0.0799**	**6.3889**	**1.1813**	**4.7818**	**9.8314**
60	5.2529	1.0241	0.1203	10.5707	2.9668	7.4490	22.0724
70	5.5240	1.2585	0.1776	18.1328	7.6219	11.2543	55.8936
75	5.6742	1.3883	0.2114	24.4518	12.3788	14.0088	94.4368
80	5.8415	1.5329	0.2500	34.1135	20.7371	17.8084	170.0207
84	5.9944	1.6652	0.2857	46.2568	32.6751	22.1296	291.7345
90	6.2817	1.9136	0.3536	81.9546	74.3525	33.1669	807.0366
95	6.6452	2.2278	0.4403	168.9781	202.2244	55.1387	2,934.5252
99	7.3268	2.8171	0.6041	656.2968	1,236.9837	142.2819	33,193.6366
$CuSO_4$							
1	2.6732	-1.0747	0.0692	0.0842	0.0135	0.0573	0.107
5	3.3548	-0.9587	0.0549	0.11	0.0139	0.0813	0.1334
10	3.7183	-0.8968	0.0476	0.1268	0.0139	0.0978	0.1503
16	4.0056	-0.8479	0.0421	0.1419	0.0138	0.113	0.1653
20	4.1585	-0.8219	0.0394	0.1507	0.0137	0.1219	0.174
25	4.3258	-0.7934	0.0365	0.1609	0.0135	0.1325	0.1842
30	4.476	-0.7679	0.0341	0.1707	0.0134	0.1426	0.194
40	4.7471	-0.7217	0.0302	0.1898	0.0132	0.1625	0.2135
50	**5.**	**-0.6787**	**0.0275**	**0.2096**	**0.0133**	**0.1829**	**0.2343**
60	5.2529	-0.6356	0.0257	0.2314	0.0137	0.2049	0.2584
70	5.524	-0.5895	0.0252	0.2573	0.015	0.2299	0.2888
75	5.6742	-0.5639	0.0256	0.2729	0.0161	0.2444	0.308
80	5.8415	-0.5355	0.0266	0.2914	0.0179	0.2609	0.3318
84	5.9944	-0.5094	0.0279	0.3094	0.0199	0.2765	0.3558
90	6.2817	-0.4605	0.0314	0.3463	0.025	0.307	0.4074
95	6.6452	-0.3987	0.0369	0.3993	0.034	0.3484	0.4863
99	7.3268	-0.2827	0.0496	0.5216	0.0597	0.4375	0.6845

Table (7): LC levels of S. officinalis extract and $CuSO_4$ to the snail M. nodosa depending on regression statistics.

***S. officinalis* extract**			
LC10	-3.1738	LC50 LCL	4.7054
LC16	-0.9768	LC50 UCL	8.9376
LC50	**6.8215**	LC50 Standard Error	**0.6367**
LC84	14.6198	Beta	0.1282
LC90	16.8168	Intercept	4.1253
LC100	18.5189	Beta Standard Error	0.0521
$CuSO_4$			
LC10	-0.0789	LC50 LCL	0.069
LC16	-0.0147	LC50 UCL	0.3571
LC50	**0.213**	LC50 Standard Error	0.0416
LC84	0.4408	Beta	4.3902
LC90	0.505	Intercept	4.0647
LC100	0.5547	Beta Standard Error	0.8729



Salvia officinalis **extracts**	**$CuSO_4$**

Figure (2): Regression line and experimental point described the effect of *S. officinalis* and $CuSO_4$ concentrations caused *M. nodosa* mortality within 48hr exposure**.**

5. Experiment with 72hr Exposure

The results of this study showed that the lowest mortality recorded in 72hrs of exposure was 21 (probit value 0.6438), while the highest mortality recorded in 72hrs of exposure was 27 (probit value 0.8605). Comparing with $CuSO_4$, the lowest mortality was 2 (probit 0.0295) and the highest mortality was killing of all snails of the experiment (probit 1). No significant differences (P>0.001) among mortality following the concentrations of *S. officinalis* and $CuSO_4$ used in this experiment (Table 8).

The concentration chosen in this work to get the LC50 value were suitable as we note from the results of probit analysis which showed that the values of probit for *S. officinalis* extracts concentrations were graduated from 0.0002-12,626.0322 and 0.0485-0.5216 for $CuSO_4$ (Table 9).

The results of the study showed that the LC 50 of *S. officinalis* against the snail *M. nodosa* was -4.2889 ppm and 0.1737ppm for $CuSO_4$. LCL (lowest confidence level), UCL (upper confidant level), and other LC different levels (10, 16, 84, 90, and 100) were tabulated in (Table 10).

The results of this work showed that increasing in *M. nodosa* mortality percent was followed by an increase in *S. officinalis* extract and $CuSO_4$ concentrations (Figure 3).

Table (8): Mortality and probit analysis of *M. nodosa* snail exposed to *S. officinalis* extracts for 72hr depending on Finney Method (Lognormal Distribution).

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent (%)	N	R	E(R)	Difference	Chi-square
			S. officinalis				
0.	0.7000	0.6438	30	21.	19.3148	1.6852	0.1470
0.301	0.7000	0.7202	30	21.	21.6071	-0.6071	0.0171
0.4771	0.7333	0.7609	30	22.	22.8272	-0.8272	0.0300
0.6021	0.7667	0.7877	30	23.	23.6303	-0.6303	0.0168
0.699	0.7667	0.8072	30	23.	24.2154	-1.2154	0.0610
0.7782	0.8000	0.8223	30	24.	24.6681	-0.6681	0.0181
0.8451	0.8333	0.8344	30	25.	25.033	-0.033	0.0000

0.9031	0.8333	0.8445	30	25.	25.3357	-0.3357	0.0044
0.9542	0.9000	0.8531	30	27.	25.5923	1.4077	0.0774
1.	0.9000	0.8605	30	27.	25.8138	1.1862	0.0545
Chi-square 0.4264							
Degrees Of Freedom 8							
p-level 0.9999							
			$CuSO_4$				
-1.	0.0667	0.0371	30	2.	1.1144	0.8856	0.7038
-0.699	0.4667	0.5373	30	14.	16.1186	-2.1186	0.2785
-0.5229	0.8667	0.8834	30	26.	26.5034	-0.5034	0.0096
-0.3979	0.9917	0.9757	30	29.75	29.2709	0.4791	0.0078
-0.301	0.9917	0.995	30	29.75	29.8504	-0.1004	0.0003
-0.2218	0.9917	0.9989	30	29.75	29.968	-0.218	0.0016
-0.1549	0.9917	0.9998	30	29.75	29.9927	-0.2427	0.002
-0.0969	0.9917	0.9999	30	29.75	29.9982	-0.2482	0.0021
-0.0458	0.9917	1.	30	29.75	29.9995	-0.2495	0.0021
0.	0.9917	1.	30	29.75	29.9999	-0.2499	0.0021
Chi-square 1.0098							
Degrees Of Freedom 8							
p-level 0.9982							

Alpha value (for confidence interval) 0.001

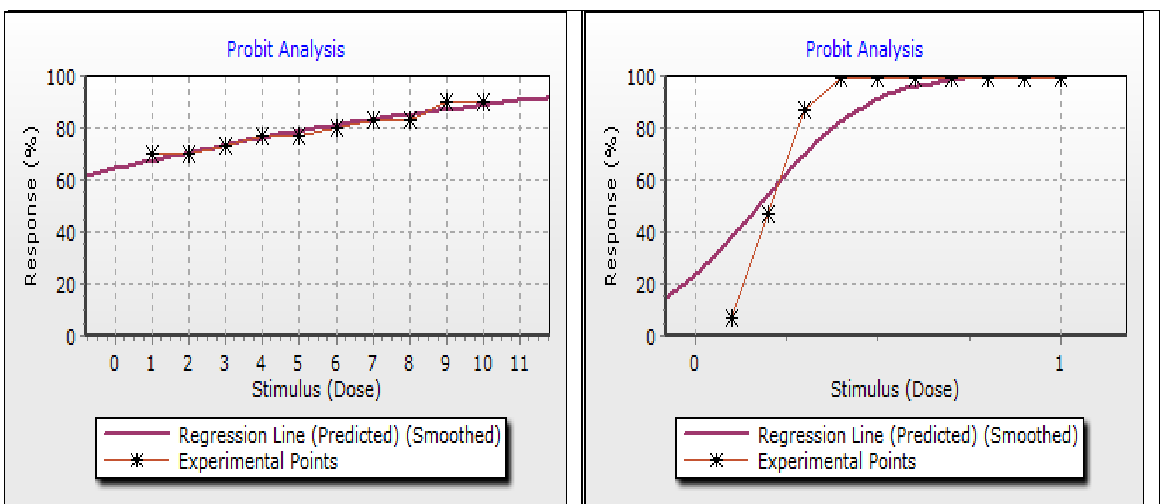
Table (9): Dose (Stimulus) Percentile.

Percentile	Probit (Y)	Log10[Dose (Stimulus)]	Standard Error	Dose (Stimulus)	Standard Error	LCL	UCL
				S. officinalis			
1	2.6732	-3.7769	3.4175	0.0002	0.2185	0.	0.0119
5	3.3548	-2.8219	2.677	0.0015	0.3581	0.	0.043
10	3.7183	-2.3126	2.2824	0.0049	0.4664	0.	0.0852
16	4.0056	-1.91	1.9707	0.0123	0.575	0.	0.1466
20	4.1585	-1.6957	1.805	0.0202	0.6429	0.	0.1957
25	4.3258	-1.4613	1.6238	0.0346	0.7265	0.	0.2687
30	4.476	-1.2509	1.4614	0.0561	0.8109	0.	0.3575
40	4.7471	-0.871	1.1689	0.1346	0.9882	0.	0.6003
50	5.	-0.5166	0.8974	0.3043	1.1823	0.0003	0.9801
60	5.2529	-0.1622	0.6292	0.6883	1.3845	0.0055	1.624
70	5.524	0.2176	0.3539	1.6504	1.4987	0.1208	2.947
75	5.6742	0.428	0.224	2.6794	1.4442	0.6012	4.5415
80	5.8415	0.6624	0.1717	4.5964	1.8645	2.3677	11.1495
84	5.9944	0.8767	0.2618	7.5292	4.8179	4.4492	47.2499
90	6.2817	1.2793	0.54	19.0249	30.2426	8.8945	1,164.2612
95	6.6452	1.7886	0.9241	61.4661	254.3973	18.4901	77,527.2262
99	7.3268	2.7437	1.6588	554.1876	12,626.0322	68.2934	217,303,668.407
				$CuSO_4$			
1	2.6732	-1.0869	0.069	0.0819	0.0131	0.0556	0.1036
5	3.3548	-0.9776	0.0547	0.1053	0.0133	0.0777	0.1272
10	3.7183	-0.9194	0.0474	0.1204	0.0132	0.0927	0.1422
16	4.0056	-0.8733	0.042	0.1339	0.013	0.1065	0.1555
20	4.1585	-0.8488	0.0393	0.1416	0.0128	0.1145	0.1632
25	4.3258	-0.822	0.0364	0.1507	0.0127	0.124	0.1722
30	4.476	-0.7979	0.0341	0.1592	0.0125	0.133	0.1808
40	4.7471	-0.7545	0.0303	0.176	0.0123	0.1506	0.198

50	5.	-0.714	0.0277	0.1932	0.0123	0.1685	0.2163
60	5.2529	-0.6734	0.0261	0.2121	0.0128	0.1876	0.2374
70	5.524	-0.63	0.0258	0.2344	0.0139	0.2091	0.264
75	5.6742	-0.6059	0.0263	0.2478	0.015	0.2215	0.2808
80	5.8415	-0.5791	0.0274	0.2636	0.0166	0.2355	0.3015
84	5.9944	-0.5546	0.0288	0.2789	0.0185	0.2487	0.3224
90	6.2817	-0.5086	0.0323	0.3101	0.0231	0.2743	0.3671
95	6.6452	-0.4503	0.038	0.3546	0.031	0.3088	0.4351
99	7.3268	-0.3411	0.0507	0.4559	0.0534	0.382	0.6038

Table (10): LC levels of S. officinalis extract to the snail M. nodosa depending on regression statistics.

		S. officinalis	
LC10	-19.1932	LC50 LCL	-7.4442
LC16	-15.9172	LC50 UCL	-1.1335
LC50	**-4.2889**	**LC50 Standard Error**	**0.9494**
LC84	7.3395	Beta	0.086
LC90	10.6155	Intercept	5.3688
LC100	13.1536	Beta Standard Error	0.0586
		$CuSO_4$	
LC10	-0.1351	LC50 LCL	0.0214
LC16	-0.0672	LC50 UCL	0.3261
LC50	**0.1737**	**LC50 Standard Error**	**0.044**
LC84	0.4147	Beta	4.1496
LC90	0.4826	Intercept	4.279
LC100	0.5352	Beta Standard Error	0.8718



S. officinalis	**CuSO4**

Figure (3): Regression line and experimental point described the effect of *S. officinalis* and $CuSO_4$ concentrations caused *M. nodosa* mortality within 72hr exposure**.**

6. Experiment with 96hrs Exposure

The results of this study showed that the lowest mortality recorded in 96hrs of exposure was 28 (probit value 0.8971), while the highest mortality recorded in 96hrs of exposure was 29.75 (probit value 0.9914). Comparing with $CuSO_4$, the lowest mortality was 2 (probit 0.0295) and the highest mortality was killing of all snails of the experiment (probit 1). No significant differences (P>0.001) among mortality following the concentrations of *S. officinalis* and $CuSO_4$ used in this experiment (Table 11).

The concentration chosen in this work to get the LC50 value were suitable as we note from the results of probit analysis which showed that the values of probit for *S. officinalis* extracts concentrations were graduated from 0.0006- 8.9385 and 0.0485-0.5216 for $CuSO_4$ (Table 12).

The results of the study showed that the LC 50 of *S. officinalis* against the snail *M. nodosa* was 0.0735 ppm and 0.1364 ppm for $CuSO_4$. LCL (lowest confidant level), UCL (upper confidant level), and other LC different levels (10, 16, 84, 90, and 100) were not calculated (Table 13).

The results of this work showed that increasing in *M. nodosa* mortality percent was followed by an increase in *S. officinalis* extract and $CuSO_4$ concentrations (Figure 5).

Table (11): Mortality and probit analysis of *M. nodosa* snail exposed to *S. officinalis* extracts for 96hrs depending on Finney Method (Lognormal Distribution).

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent (%)	N	R	E(R)	Difference	Chi-square
			S. officinalis				
0.	0.9333	0.8971	30	28.	26.9126	1.0874	0.0439
0.301	0.9333	0.9453	30	28.	28.3597	-0.3597	0.0046
0.4771	0.9333	0.9639	30	28.	28.9164	-0.9164	0.029
0.6021	0.9667	0.9736	30	29.	29.2089	-0.2089	0.0015
0.699	0.9667	0.9796	30	29.	29.3875	-0.3875	0.0051
0.7782	0.9667	0.9836	30	29.	29.5068	-0.5068	0.0087
0.8451	0.9917	0.9864	30	29.75	29.5916	0.1584	0.0008
0.9031	0.9917	0.9885	30	29.75	29.6546	0.0954	0.0003
0.9542	0.9917	0.9901	30	29.75	29.703	0.047	0.0001
1.	0.9917	0.9914	30	29.75	29.7411	0.0089	0.
Chi-square 0.0941							
Degrees Of Freedom 8							
P-level 1.							
			$CuSO_4$				
-1.	0.2	0.1394	30	6.	4.1816	1.8184	0.7908
-0.699	0.5667	0.7275	30	17.	21.8262	-4.8262	1.0672
-0.5229	0.9917	0.9444	30	29.75	28.3329	1.4171	0.0709
-0.3979	0.9917	0.9891	30	29.75	29.673	0.077	0.0002
-0.301	0.9917	0.9977	30	29.75	29.9318	-0.1818	0.0011
-0.2218	0.9917	0.9995	30	29.75	29.9845	-0.2345	0.0018
-0.1549	0.9917	0.9999	30	29.75	29.9962	-0.2462	0.002
-0.0969	0.9917	1.	30	29.75	29.999	-0.249	0.0021
-0.0458	0.9917	1.	30	29.75	29.9997	-0.2497	0.0021
0.	0.9917	1.	30	29.75	29.9999	-0.2499	0.0021
Chi-square 1.9402							
Degrees Of Freedom 8							
p-level 0.9828							

Alpha value (for confidence interval) 0.001

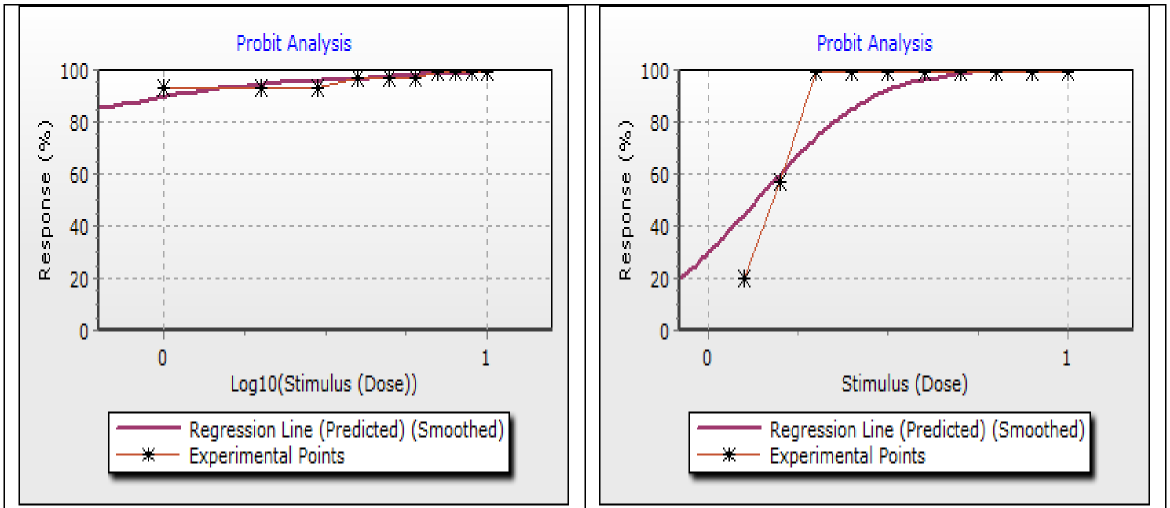
Table (12): Dose (Stimulus) Percentile.

Percentile	Probit (Y)	Log10[Dose (Stimulus)]	Standard Error	Dose (Stimulus)	Standard Error	LCL	UCL

			S. officinalis				
1	2.6732	-3.2184	3.9756	0.0006	2.8581	0.	0.0267
5	3.3548	-2.6077	3.3213	0.0025	2.5852	0.	0.0588
10	3.7183	-2.282	2.9725	0.0052	2.4516	0.	0.0898
16	4.0056	-2.0246	2.6971	0.0094	2.3519	0.	0.1255
20	4.1585	-1.8876	2.5505	0.013	2.3008	0.	0.1501
25	4.3258	-1.7377	2.3902	0.0183	2.2466	0.	0.1825
30	4.476	-1.6031	2.2465	0.0249	2.1995	0.	0.2177
40	4.7471	-1.3602	1.9872	0.0436	2.1179	0.	0.2994
50	5.	-1.1336	1.7457	0.0735	2.046	0.	0.4039
60	5.2529	-0.907	1.5047	0.1239	1.9783	0.	0.5462
70	5.524	-0.6641	1.2475	0.2167	1.9097	0.	0.7583
75	5.6742	-0.5295	1.1056	0.2955	1.8724	0.	0.9123
80	5.8415	-0.3796	0.9486	0.4172	1.8297	0.0002	1.1255
84	5.9944	-0.2426	0.8063	0.5721	1.7864	0.0009	1.3721
90	6.2817	0.0149	0.546	1.0348	1.6719	0.0149	2.0543
95	6.6452	0.3406	0.2677	2.1906	1.4373	0.3845	4.3079
99	7.3268	0.9513	0.5531	8.9385	14.7186	4.4833	660.0365
			$CuSO_4$				
1	2.6732	-1.2217	0.0782	0.06	0.0109	0.0386	0.0782
5	3.3548	-1.1002	0.0619	0.0794	0.0114	0.0562	0.0983
10	3.7183	-1.0354	0.0537	0.0922	0.0114	0.0685	0.1112
16	4.0056	-0.9842	0.0475	0.1037	0.0114	0.08	0.1228
20	4.1585	-0.9569	0.0444	0.1104	0.0113	0.0868	0.1296
25	4.3258	-0.9271	0.0412	0.1183	0.0112	0.0948	0.1375
30	4.476	-0.9003	0.0385	0.1258	0.0112	0.1026	0.1452
40	4.7471	-0.852	0.0344	0.1406	0.0111	0.1179	0.1607
50	5.	-0.8069	0.0314	0.156	0.0113	0.1336	0.1774
60	5.2529	-0.7618	0.0298	0.1731	0.0119	0.1505	0.1969
70	5.524	-0.7135	0.0296	0.1934	0.0132	0.1698	0.2219
75	5.6742	-0.6867	0.0302	0.2057	0.0143	0.181	0.2378
80	5.8415	-0.6569	0.0315	0.2204	0.016	0.1937	0.2576
84	5.9944	-0.6296	0.0332	0.2346	0.018	0.2058	0.2777
90	6.2817	-0.5784	0.0373	0.264	0.0227	0.2294	0.3212
95	6.6452	-0.5136	0.0439	0.3065	0.031	0.2616	0.3887
99	7.3268	-0.3921	0.0585	0.4054	0.0548	0.3311	0.5613

Table (13): LC levels of S. officinalis extract to the snail M. nodosa depending on regression statistics.

	S. officinalis		
LC10	-	LC50 LCL	0.
LC16	-	LC50 UCL	0.4039
LC50	**0.0735**	**LC50 Standard Error**	**2.046**
LC84	-	Beta	1.116
LC90	-	Intercept	6.2651
LC100	-	Beta Standard Error	0.4499
	$CuSO_4$		
LC10	-0.1909	LC50 LCL	-0.0251
LC16	-0.119	LC50 UCL	0.2978
LC50	**0.1364**	**LC50 Standard Error**	**0.0466**
LC84	0.3917	Beta	3.9165
LC90	0.4636	Intercept	4.4659
LC100	0.5194	Beta Standard Error	0.8287



S. officinalis	$CuSO_4$

Figure (4): Regression line and experimental point described the effect of *S. officinalis* and $CuSO_4$ concentrations that caused *M. nodosa* mortality for 96hr exposure**.**

7. Discussion

Mollusks were considered as important intermediate host of human parasitic diseases [9]. Our study established that *S. officinalis* extracts have a molluscicidal activity against the target snails. These findings were in agreement with results obtained by references [4, 5].

We have used copper sulfate as a standard toxic material that caused death to the snail. This step was dependent on the findings of previous studies that reported the toxicity of copper to snails. It noted that the Cu (II) induced oxidation of Quinone and hydroquinone in the target cell. Also, Cu can be deposited as insoluble intracellular membrane-bound granules in the hepatopancreas of terrestrial invertebrates [6]. Adewunmi et al. noted that Cu has a high rate of bioaccumulation in the tissues of freshwater snails [7]. Hoang and Rand demonstrated that the toxicity of Cu carbonate to snails is distanced through many biological and chemical reactions. Also, they noted that the Cu was found to have accumulated in the soft tissue of the snails [8].

S. officinalis extracts were found to be potent substances to kill the snail *M. nodosa*. These findings were supported by another study that found that the ethanolic extracts of *S. officinalis* L. Leaves were found to be the most potent molluscicidal activity against *L. auricularia* snail. In another study, the lethal concentration of ethanolic extract of *S. officinalis* L. at 24hrs:12mg/L, 48hrs:10mg/L [10].

The mortality rate of *M. nodosa* caused by the S. *officinalis* extracts is due to the presence of some substances that cause the kill snails as terpenes [11] and monoterpenes [12]. Generally, the mechanism by which these extracts killed snails is not exactly known and requires further biochemical studies for elucidation.

In this work, the contact LC50 values of *S. officinalis* aqueous plant extracts for 24hr, 48hr, 72hrs, and 96hrs exposure were 15.53, 6.821, 4.288, and 0.073 ppm respectively. The contact LC50 values of $CuSO_4$ for 24hr, 48hr, 72hrs, and 96hrs exposure were 0.213, 0.213, 0.1737, and 0.1364 ppm respectively. These results agreed with our previous findings that reported the LD50 of *S. officinalis* and Copper sulphate to *B. truncatus* were (20 and 2.2 g/L) respectively. The study showed that the extracts of *S. officinalis* were less effective than $CuSO_4$. The results showed that the toxicity of extracts was dose and time-dependent [2]. Also, these results agreed with our previous study that found the LC50 of *T. vulgaris* and Copper sulphate to *B. truncatus* were (18.7, and 2.2 g/L) respectively. The study showed that *T. vulgaris* extracts were less effective than $CuSO_4$ [3]. The results showed that the toxicity of extracts was concentration and time-dependent.

8. Conclusions

In conclusion, the obtained results of this study concluded that S. officinalis extract showed molluscicidal activity against M. nodosa snails and thus able assistance to control it.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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