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Biological studies on the effect of certain inorganic fertilizers with observations on protein electrophoretic pattern of *Biomphalaria alexandrina* snails

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ABSTRACT

The present study deal with three inorganic fertilizers they were; (balanced, high phosphorus and high nitrogen), their (LC50) values were 505.7, 1600 and 9500 ppm, respectively after 24 hours of exposure. Survival rate of B. alexandrina snails group that exposed to sublethal concentrations of balanced fertilizer showed a gradual reduction, that increase with increasing the used concentrations. On the other hand, the application of both high phosphorus and high nitrogen fertilizers had more powerful effect on the treated snails and the declining of survivorship and egg laying capacity was inversely proportional to the concentrations. In addition, SDS–PAGE protein profiles of treated snails revealed that the minimum number of protein bands was noticed in tissue and hemolymph of snails groups that subjected to high phosphorus and high nitrogen fertilizers compared to control protein fractions. The highest similarity indices values (0.50 and 0.42) were obtained when snails exposed to ¼ LC50 and ½ LC50 doses of balanced fertilizers in tissue and hemolymph, respectively after four week.

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INTRODUCTION

Fertilizers like any chemical compounds may cause changes in biological and physiological parameters when used against snails. So that a lot of authors dealing with fertilizers to determining their activity against snails, especially fertilizers that reach water streams during agriculture activities and may kill snails or make their environmental conditions unsuitable for their life El- Sayed, Ragab and Shoukry, and Ismail [13,21,16].

Sodium Dodecyle Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) of whole cell proteins proved a useful technique for identification of isolates complex and easy to perform without complicated or expensive equipment Esteban *et al*. [14].

Proteins play very important role for overall growth, development and reproduction of the animals. The depletion, destruction and degeneration of protein metabolites in the cleavage stage of experimental groups of snails correlated with the depletion of negatively charged protein fractions were detected by SDS-PAGE. This technique was used by several authors to detect the variation in protein patterns of different snail species Ragab, Ismail, Mohamed *et al*., Mahobiya and Bhide and Osman *et al*. [20,16,18,17,19]. Therefore the present study aimed to evaluate the effect of three inorganic fertilizers on B. alexandrina snails using SDS-PAGE technique.

MATERIALS AND METHODS

Experimental Materials:

Fertilizers are purchased from Misr el dawliya for Agricultural and Industrial Development Company. Three types of complex mineral nitrogen, phosphorus and potassium (N:P:K) fertilizers as following: balanced content

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fertilizers (N:P:K, 20:20:20); high phosphorus content fertilizers (N:P:K, 5:40:5) and high nitrogen content fertilizers (N:P:K, 35:5: 5).

Experimental animals:

B. alexandrina snails (9-11mm shell diameter) used in this work were obtained from Schistosome Biological Supply Program (SBSP) in Theodor Bilharz Research Institute (TBRI), Egypt. They maintained under laboratory conditions (temperature 25 ±2°C - pH 7-7.7) for three weeks. The snails were provided daily with fresh lettuce leaves as a food source El-Nahas and El-Deeb [12].

Bioassay test:

Amounts of the dry powder from each fertilizer type were weighed separately and then were added to 1000 ml of dechlorinated tap water to make up the desired weight/volume concentrations. A series of concentrations that would permit the computation of LC_{50} and LC_{90} were prepared. Three replicates, 10 snails/L were used. Another 3 replicates in dechlorinated water were used as control. Exposure and recovery periods were 24 hours. Then, snails' mortality was recorded El-Deeb [10] and Ismail [16]. Computation of LC_{50} and LC_{90} values and slope function were determined utilizing the statistical program SPSS version 17 (SPSS, Inc., Chicago, IL) SPSS [24] for windows.

Effect of three tested fertilizers on survival rate and egg laying capacity of B. alexandrina:

B. alexandrina snails were exposed to different sublethal concentrations ($^1/_{10}$ $LC_{50,}$ ¼ LC_{50} and ½ LC_{50}) of each tested fertilizers. For each concentration, thirty adult snails (9-11 mm) were used in three replicates, 10 snails /L. A set of untreated snail was maintained in clean dechlorinated tap water as a control group. The exposure period continued for 12 weeks at room temperature (22±2˚C). Renewing the experimental solution was changed once weekly. All egg masses laid by the exposed and control snails were collected and counted weekly. Also, the percentage of survival snails (L_x) was calculated weekly, (Mx) represents the mean number of eggs/snail/week. (L_xM_x) refers to the reproductive rate in each week while ($\sum L_xM_x$) is the total reproductive rate at the end of the experiment El-Deeb and El-Nahas [11].

Electrophoretic analysis:

SDS-PAGE was performed according to the protocol of Boswell *et al*. [3] to separate digestive gland and hemolymph total proteins of *B. alexandrina* snails. Total protein were separated on 8% resolving gel and 3.75% stacking gel using electrophoresis apparatus (Bio-Rad USA vertical minigel, double side). Snails of (9-11 mm) were exposed to sublethal concentrations ($^1/_{10}$ $LC_{50,}$ ¼ LC_{50} and ½ LC_{50}) of each tested fertilizers, separately for successive 4 weeks.

Digestive gland sampling:

Snails from each treated and control groups were washed with water then dried. Snail's shell was gently crushed between two glass slides and digestive gland was dissected out from 3-5 snails and pooled in 1 ml Ependorf tube to which tissue- extracting buffer (Tris-buffer saline, 50 mM Tris-HCL, pH 7.5 containing 75 ml NaCl) was added in a ratio of 1:10 w/v Bradford [4]. Homogenization was carried out in Ependorf tube using small glass road, freezing-thawing method was performed to facilitate homogenization process. Centrifugation was carried out at 10.000 rpm for 15 min at 4 °C.

Hemolymph sampling:

All hemolymph samples from each experimental group were centrifuged at 5000 rpm for 5 min at 4°C to pellet hemolymph and other particulate materials Dikkeboom *et al*. [9]. The pellet was discarded and cell-free hemolymph was mixed with sample buffer in a ratio of 1 part of lymph: 1 part sample buffer. Samples were boiled for 5 min at 100 °C in a water bath.

Gel analysis:

Similarity of the polypeptide profile between the different groups was assessed from Dice similarity coefficient Dice [8] which is still valid and used to solve many genetic problems in animal and plant species Ravelo *et al*. [22]. Similarity equation is **(S = 2a / 2a + b + c)** where **(**S) is the degree of identity, **(**a) is the number of common shared bands in two compared samples, (b) is the number of excess bands in the first compared sample and (c) is the number of excess bands in the second compared sample. An "S" value of 1.0 denotes complete identity in the electrophoretic profile of both groups, while a value of < 1.0 indicate a variation in the polypeptide profile between the two compared samples.

Statistical analysis:

The survival rate was analyzed by Chi-square values of contingency tables using the statistical program SPSS version 17 (SPSS, Inc., Chicago, IL) SPSS [24] for windows.

Results:

The calculated half lethal concentrations (LC_{50}) values were 505.7, 1600 and 9500 ppm for balanced, high phosphorus and high nitrogen fertilizers, respectively after 24 hours exposure (Table 1).

Survivorship and egg-laying capacity of B. alexandrina snails exposed to the tested fertilizers (N:P:K):

Data in table (2) revealed that the survival rate of *B. alexandrina* snails group that exposed to balanced fertilizer showed a gradual reduction, that increase with increasing in the used concentrations. Also, they had greatly impaired in their fecundity efficiency throughout the whole experimental weeks, where the reduction % of total reproductive reaching 40.85% and 55.25% for snails that exposed to ¼ LC_{50} and ½ LC_{50}, respectively. The application of high phosphorus fertilizer and high nitrogen fertilizer had more powerful effect on the treated adult snails with all sublethal concentrations and the declining of survivorship and egg laying capacity was inversely proportional to the concentrations. Data showed a reduction % in the total reproductive rate reaching 16.4 %, 82.58 % and 100% for the experimental groups treated with ($^1/_{10}$ LC_{50}, ¼ LC_{50} and½ LC_{50}) of high phosphorus fertilizer (Table 3). Meanwhile, the reduction % of total reproductive rate reaching 87.78% for snails exposed to $^1/_{10}$ LC_{50} of high nitrogen fertilizer and 100% for snails exposed to the rest concentrations (Table 4).

Electrophoretic separation of tissue soluble protein from digestive gland:

Two bands (8.27 and 5.22 K.Da) were common in control and in all treated snail groups, except snail groups that subjected to $^1/_{10}$ LC_{50} of both high phosphorus (160 ppm) and high nitrogen (950 ppm) fertilizers separately, where their snail's digestive homogenate of each group revealed two protein fractions of molecular weight (219.93 and 88.98 K.Da) and (88.98 and 6.06 K.Da) respectively, and there was no similarity between control and two treated groups, since the similarity index was (0.0). Meanwhile, the snails exposed to ($^1/_4$ LC_{50}) of balanced fertilizer was shared control group in three bands (20.81, 8.68 and 5.73 K.Da) and the highest value of similarity index (0.50) was observed with this treated group. In addition, bands (6.06, 6.23 and 6.06 K.Da) were characterized for snail groups that exposed to ($^1/_{10}$ LC_{50}, $^1/_4$ LC_{50} and $^1/_2$ LC_{50}) of high nitrogen fertilizer, respectively (Table, 5 and Fig. 1: A).

Electrophoretic separation of protein from hemolymph:

Results in table (6) and fig. (1: B) showed that all treatments were share control group in two bands (7.69 and 4.31 K.Da) except the two snail groups that exposed to (160 ppm and 800 ppm) of high phosphorus fertilizer share control group in only one band (4.02 and 4.09 K.Da), respectively. The numbers of bands change from treated group to another when compared to control, results recorded that snails treated with (50.57 ppm) of balanced fertilizer got the same number (8 bands) of control group; however the similarity between this treatment and control was (0.25). The lowest number of bands was observed in treated snails that exposed to (800 ppm) of high phosphorus fertilizer was two bands (176.14 and 4.09 K.Da). Protein patterns of snail's hemolymph that exposed to three sublethal concentrations of high nitrogen fertilizer expressed decrease in number of protein bands (4 bands for each group) when compared to control (8 bands), also they have the same similarity indices value (0.33) with control.

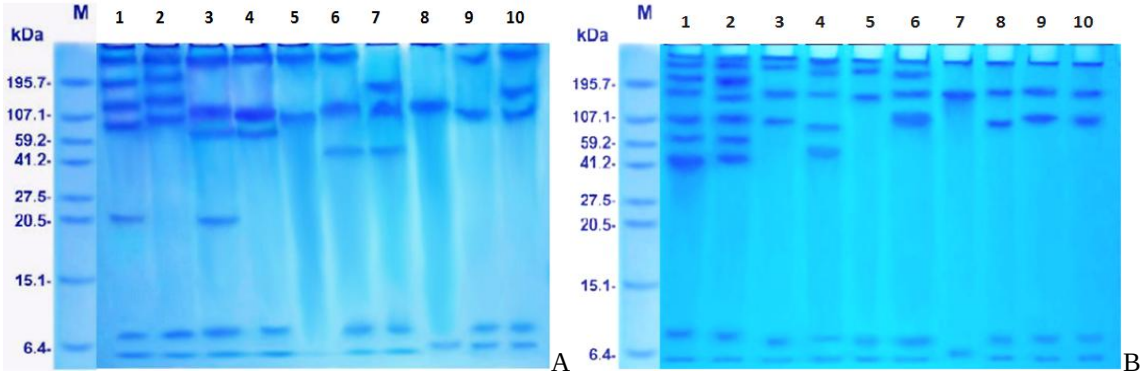

Fig. 1: SDS-PAGE proteins patterns of (A) digestive gland tissue, (B) hemolymph of *Biomphalaria alexandrina* snails groups exposed to sublethal concentrations of each composite fertilizer type after 4 weeks of exposure period. M= Marker 1 = control.
2, 3 & 4 = $^1/_{10}$ LC_{50}, ¼ LC_{50} & ½ LC_{50} of N: P: K (20:20:20) balanced fertilizer.
5, 6 & 7= $^1/_{10}$ LC_{50}, ¼ LC_{50} & ½ LC_{50} of N: P: K (5:40:5) high phosphorus fertilizer.
8, 9&10= $^1/_{10}$ LC_{50}, ¼ LC_{50} & ½ LC_{50} of N: P: K (35:5:5) high nitrogen fertilizer.

Table 1: Probit analysis of toxic effect of three composite fertilizers (N:P:K) on adult *Biomphalaria alexandrina* snails after 24 hours of exposure.

Tested Fertilizers	LC_{50} (ppm)	Confidence limit of LC_{50} ppm	LC_{90} (ppm)	Slope	$^1/_{10}$ LC_{50} (ppm)	$^1/_4$ LC_{50} (ppm)	$^1/_2$ LC_{50} (ppm)
N:P:K 20:20:20 Balanced	505.7	390.31 - 625.33	851.2	1.89	50.57	126.4	252.8
N:P:K 5:40:5 High phosphorus	1600	1356.56 - 1843.4	2309.3	1.45	160	400	800
N:P:K 35:5:5 High nitrogen	9500	8891.4 - 10108.5	11273.3	1.16	950	2375	4750

Table 2: Survivorship and fecundity of adult *Biomphalaria alexandrina* snails after exposure to sublethal concentrations of balanced fertilizers (N:P:K, 20:20:20).

Weeks	Control			$^1/_{10}$ LC_{50} (50.57 ppm)			$^1/_4$ LC_{50} (126.4 ppm)			$^1/_2$ LC_{50} (252.8 ppm)		
	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X
1	1.00	----	----	1.00	---	---	1.00	---	---	1.00	---	---
2	1.00	0.75	0.75	1.00	1.05	1.05	1.00	---	---	1.00	---	---
3	1.00	2.85	2.85	0.96	2.15	2.15	0.96	2.10	2.02	1.00	1.00	1.00
4	1.00	0.35	0.35	0.96	---	---	0.93	0.20	0.18	1.00	---	---
5	1.00	1.52	1.52	0.96	3.25	3.12	0.93	0.13	0.12	0.86	1.61	1.38
6	1.00	3.88	3.88	0.96	2.18	2.10	0.93	0.46	0.43	0.86	---	---
7	1.00	3.41	3.41	0.96	5.87	5.64	0.93	3.93	3.65	0.80	0.54	0.43
8	1.00	1.70	1.70	0.96	3.93	3.78	0.93	2.13	1.98	0.70	5.75	4.02
9	0.96	0.31	0.29	0.96	---	---	0.93	0.13	0.12	0.70	---	---
10	0.96	0.37	0.35	0.93	---	---	0.90	0.64	0.57	0.63	0.5	0.31
11	0.93	0.06	0.062	0.86	---	---	0.80	0.45	0.36	0.60	---	---
12	0.80	1.00	0.80	0.80	---	---	0.66	---	---	0.53	---	---
ΣM_X		16.2			18.8			10.17			9.40	
ΣL_XM_X			15.96			18.10			9.44			7.14
% of Reduction						-13.40			40.85			55.26

$\sum L_xM_x$: reproductive rate, L_x: survival rate, M_x: fecundity (number of eggs / snail/ week).

Table 3: Survivorship and fecundity of adult *Biomphalaria alexandrina* snails after exposure to sublethal concentrations of high phosphorus fertilizer (N:P:K, 5:40:5).

Weeks	Control			$^1/_{10}$ LC_{50} (160 ppm)			$^1/_4$ LC_{50} (400 ppm)			$^1/_2$ LC_{50} (800 ppm)		
	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X
1	1.00	----	----	1.00	---	---	1.00	---	---	1.00	---	---
2	1.00	0.75	0.75	1.00	---	---	1.00	---	---	1.00	---	---
3	1.00	2.85	2.85	1.00	0.9	0.9	0.96	0.63	0.60	1.00	---	---
4	1.00	0.35	0.35	0.96	---	---	0.96	---	---	0.86	---	---
5	1.00	1.52	1.52	0.93	0.53	0.49	0.93	---	---	0.73	---	---
6	1.00	3.88	3.88	0.86	0.84	0.72	0.90	0.28	0.25	0.66	---	---
7	1.00	3.41	3.41	0.73	4.44	3.24	0.86	1.25	1.25	0.63	---	---
8	1.00	1.70	1.70	0.70	5.62	3.93	0.76	0.90	0.68	0.50	---	---
9	0.96	0.31	0.29	0.70	3.25	2.27	0.76	---	---	0.46	---	---
10	0.96	0.37	0.35	0.66	2.8	1.84	0.63	---	---	0.46	---	---
11	0.93	0.06	0.062	0.63	---	---	0.56	---	---	0.46	---	---
12	0.80	1.00	0.80	0.50	---	---	0.50	---	---			
ΣM_X		16.2			18.39			3.06			0.00	
ΣL_XM_X			15.96			13.40			2.78			0.00
% of Reduction						16.04			82.58			100.0

$\sum L_xM_x$: reproductive rate, L_x: survival rate, M_x: fecundity (number of eggs / snail/ week).

Table 4: Survivorship and fecundity of adult *Biomphalaria alexandrina* snails after exposure to sublethal concentrations of high nitrogen fertilizers (N:P:K, 35:5:5).

Weeks	Control			$^1/_{10}$ LC_{50} (950 ppm)			$^1/_4$ LC_{50} (2375 ppm)			$^1/_2$ LC_{50} (4750 ppm)		
	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X	L_X	M_X	L_XM_X
1	1.00	----	----	1.00	---	---	1.00	---	---	1.00	---	---
2	1.00	0.75	0.75	1.00	---	---	0.86	---	---	0.70	---	---
3	1.00	2.85	2.85	1.00	0.75	0.75	0.86	---	---	0.70	---	---
4	1.00	0.35	0.35	0.96	---	---	0.76	---	---	0.66	---	---
5	1.00	1.52	1.52	0.63	---	---	0.50	---	---	0.60	---	---
6	1.00	3.88	3.88	0.60	0.40	0.24	0.50	---	---	0.60	---	---
7	1.00	3.41	3.41	0.60	---	---				0.56	---	---
8	1.00	1.70	1.70	0.60	1.60	0.96				0.56	---	---
9	0.96	0.31	0.29	0.50	---	---				0.53	---	---
10	0.96	0.37	0.35	0.50	---	---				0.50	---	---
11	0.93	0.06	0.062	0.50	---	---						
12	0.80	1.00	0.80	0.50	---	---						
ΣM_X		16.2			2.75			0.00			0.00	
ΣL_XM_X			15.96			1.95			0.00			0.00

% of Reduction						87.78			100.0			100.0

$\sum L_xM_x$: reproductive rate, L_x: survival rate, M_x: fecundity (number of eggs / snail/ week).

Table 5: Electrophoretic separation of tissue soluble protein from digestive gland of *B. alexandrina* snails exposed to sublethal concentrations of each composite fertilizer type (N:P:K) after 4 weeks of exposure period.

Bands	Marker		control		balanced fertilizer (N:P:K, 20:20:20)					
					$^1/_{10}$ LC_{50} (50.57 ppm)		¼ LC_{50} (126.4 ppm)		½ LC_{50} (252.8 ppm)	
	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%
1			239.5	18.0						
2					236.5	22.5				
3										
4							219.9	8.3		
5									216.6	28.4
6	195.7	13.4								
7										
8					172.8	7.6				
9			159.2	10.1						
10					135.9	14.5				
11										
12			122.9	6.78						
13	107.1	4.4								
14			94.87	25.1						
15							91.90	43.7	91.90	37.5
16					88.98	30.8				
17										
18										
19										
20										
21										
22	59.2	7.1								
23							57.29	16.4		
24									53.7	16.6
25	41.2	14.3								
26	27.5	9.7								
27	20.5	0.39	20.5	22.5			20.81	16.4		
28	15.1	21.4								
29			8.27	8.9	8.68	16.0	8.68	9.8	8.68	10.8
30	6.4	28.9								
31			5.22	8.4	5.73	8.4	5.73	4.6	5.73	6.4
Number of bands	8		7		6		6		5	
Similarity index					0.30		0.50		0.33	

high phosphorus fertilizer (N:P:K, 5:40:5)						high nitrogen fertilizer (N:P:K, 35:5:5)					
$^1/_{10}$ LC_{50} (160 ppm)		¼ LC_{50} (400 ppm)		½ LC_{50} (800 ppm)		$^1/_{10}$ LC_{50} (950 ppm)		¼ LC_{50} (2375 ppm)		½ LC_{50} (4750 ppm)	
M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%
223.2	57.9										
		219.9	18.7					219.9	9.4	219.9	12.0
				189.8	13.1						
										129.4	13.5
88.98	42.0	88.98	22.3	88.98	27.7	88.98	88.7			88.98	6.7
								86.11	26.5		

		41.2	5.7	41.2	6.5						
----		8.89	49.6	9.29	48.2	----		9.28	57.9	9.49	62.7
						6.06	11.2	6.23	6.0	6.06	4.9
----		5.89	3.4	5.73	4.2	---					
2		5		5		2		4		5	
0.0		0.33		0.33		0.0		0.18		0.16	

M.W = molecular weight

Table 6: Electrophoretic separation from hemolymph protein of *B. alexandrina* snails exposed to sublethal concentrations of each composite fertilizer type (N:P:K) after 4 weeks of exposure period.

Bands	Marker		control		balanced fertilizer (N:P:K, 20:20:20)					
					$^1/_{10}$ LC_{50} (50.57 ppm)		¼ LC_{50} (126.4 ppm)		½ LC_{50} (252.8 ppm)	
	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%
1			269.87	4.7						
2							266.88	2.1		
3					263.9	2.1				
4									259.42	9.9
5										
6										
7			237.02	4.6						
8					228.06	8.2				
9	195.7	13.4								
10										
11										
12										
13			179.29	0.89					179.29	0.56
14					176.14	0.38				
15							171.43	10.2		
16	107.1	4.4	107.1	8.9						
17					98.17	9.4				
18									90.92	11.6
19							87.45	10.2		
20										
21										
22										
23			70.80	5.09						
24					65.46	6.6				
25	59.2	7.1								
26			4813	8.5						
27					45.53	7.16				
28	41.2	14.3							42.88	74.1
29	27.5	9.7								
30	20.5	0.39								
31	15.1	21.4								
32	6.4	28.9	7.69	62.0	7.51	65.5	6.95	77.2	6.77	0.28
33			4.31	5.04	4.24	0.32	4.09	0.0	4.24	3.4
No. of bands	8		8		8		5		6	
Similarity index					0.25		0.30		0.42	

M.W = molecular weight.

high phosphorus fertilizer (N:P:K, 5:40:5)						high nitrogen fertilizer (N:P:K, 35:5:5)					
$^1/_{10}$ LC_{50} (160 ppm)		¼ LC_{50} (400 ppm)		½ LC_{50} (800 ppm)		$^1/_{10}$ LC_{50} (950 ppm)		¼ LC_{50} (2375 ppm)		½ LC_{50} (4750 ppm)	
M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%	M.W. (kDa)	%
		250.46	10.6								

241.5	4.5										
										184.02	11.4
						182.44	10.6	182.44	10.9		
		179.29	0.29								
177.71	6.1			176.14	11.4						
		86.31	14.6								
										84.08	49.0
								82.98	42.2		
						79.76	12.0				
----		6.67	74.3	----		6.95	72.9	7.32	39.4	7.32	39.0
4.02	7.6	4.02	0.0	4.09	88.5	3.79	4.3	3.72	7.3	4.09	0.51
3		5		2		4		4		4	
0.18		0.46		0.2		0.33		0.33		0.33	

Discussion:

Owing to the extensive use of various agricultural fertilizers for growth crops, those ultimately reach the water canals and drainage system. So they may destroy the snail population or contaminate their normal environmental habitat Coura [6]. The present results showed that the (LC_{50}) values were 505.7, 1600 and 9500 ppm for balanced, high phosphorus and high nitrogen fertilizers, respectively. These results were in parallel with the pervious study of Ragab and Shoukry [21] who found that LC_{50} were 470, 1900 and 2200 ppm for ammonium nitrate, potassium sulphate and urea, respectively against *B. alexandrina* snails. Recently, Flefel [15] tested the toxicity of urea ($CO(NH_2)_2$) and composite fertilizers (N:P:K, 22:5:0) against adult *B. alexandrina* snails, the results of LC_{50} and LC_{90} values were (9636 and 15937 ppm) for urea and (2754 and 4092 ppm) for composite fertilizer, respectively.

Also, data revealed that the survival rate of snails group that exposed to $^1/_{10}$ LC_{50} (50.57 ppm) of balanced fertilizer showed a gradual reduction reaching 80%. Although, this group did not lay eggs during the last four weeks of the exposure period, still their total reproduction rate higher than control group. However, this result agrees well with the effect of chelated copper on the same species of snails, where the exposing snails laid a number of eggs higher than control Ismail [16]. Moreover, the survival rate of snails groups that exposed to ¼ LC_{50} (126.4 ppm) and ½ LC_{50} (252.8 ppm) of balanced fertilizer, being 66% and 53%, respectively compared to 80% of control at the end of the experiment. Also, they had greatly impaired in their fecundity efficiency throughout the whole experimental weeks, the reduction in the total reproductive rate reaching 40.85 % and 55.26 %, respectively compared with control group. Also, the results supported by the findings of El-Sayed [13] who stated that ammonium choride at low concentrations greatly reduce the survival rate and fecundity of the same species of snails. On the other hand, the application of high phosphorus and high nitrogen fertilizers had more powerful effect on the treated snails with all sublethal concentrations and the declining of survivorship and egg laying capacity was directly proportional to the concentrations. These results are coinciding with findings on another species of snails by De La Cruz *et al.* [7] they declared that nitrogen addition in irrigated rice ecosystems cause a decrease in *Pomacea canaliculata* survival rate. Moreover, it was found a deleteriously suppressed by exposing snails to sublethal concentrations of high nitrogen fertilizer recording the reduction rate 87.78 % for snails exposed to $^1/_{10}$ LC_{50} and 100% for snails that are exposed to the rest of the concentrations (¼ LC_{50} and ½ LC_{50}). This great reduction means that snail's fertility was almost completely destroyed. These results support the findings of study by Ismail [16] who found that the egg production of the exposed snails to chelated zinc was completely inhibition 100 % during the exposure period.

As regards to SDS-PAGE patterns, results showed that many protein bands disappeared in treated snails and appeared in control and vice versa. These results were well corresponded to the findings of Bride *et al.*, Ragab,

Ismail, Bakry *et al.* and Abdel-Wareth [5, 20, 16, 2, 1] on the other effects of pesticides, oils, chemical fertilizers and fungal filtrate on the electrophoretic pattern of protein of the other snails species.

In the present work, the highest number of protein bands was observed in tissue and hemolymph of snail groups that subjected to all concentrations of balanced fertilizer in comparison to control, therefore, the maximum similarity indices values (0.50 and 0.42) were obtained when snails exposed to medium and high doses of balanced fertilizers in tissue and hemolymph, respectively. This result indicated that such concentrations had a slight effect on exposed snails and their protein fraction did not affect. Generally, the alterations in protein fractionation and metabolic processes could be due to a production of additional proteins, or diminishing ones by treated snails in additional to disturbance of polypeptide metabolism, Ragab [20].

On the contrary, the minimum number of protein bands was noticed in tissue and hemolymph of snails groups that subjected to high phosphorus and high nitrogen fertilizers compared to control protein fractions. Moreover, the low dose ($^1/_{10}$ LC_{50}) of both mentioned fertilizers types caused a powerful effect on tissue protein of exposed snail groups resulting in a marked dimensioned in protein bands reaching two bands only for each group. So that, there was no similarity between these two treated snail groups and untreated group. These results pointed to sever damaged in tissue protein patterns was occurred. These results are well agree with Ismail [16] who found that the low concentrations of (LC_{10}) of chelated zinc and chelated copper was similar in their effect on protein bands, and more effective than the higher concentrations (LC_{25} and LC_{50}) inducing change in protein pattern. Similar results was obtained by Bakry *et al.* [2] who found the less number of protein bands indicating that the pesticides were thought induce damage in DNA of treated snails. These results may be reflect to the toxic materials might affect the protein synthesis by decreasing the ATP synthesis and inhibition of RNA synthesis. Another interpretation, that protein inhibition may be result from the conversion of protein to amino acids, degradation of protein to release energy, or the direct effect of toxic substance on the amino acid transport of the cell Rawi *et al.* [23].

Conclusion:

So, we can conclude that, in spite of non specificity of the inorganic fertilizers for toxicity of snails, they like any chemical compounds may cause changes in the biological and physiological parameters when used for long-term.

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