Sara A Mansour[1,2], Maha FM Soliman[1]*, Fatma AA El Deeb[2] and Nahla S El-Shenawy[1]

[1]Zoology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt

[2]Environmental Research Department, Theodor Bilharz Research Institute, Giza, Egypt

*Corresponding author: Maha FM Soliman, Zoology Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt, E-mail: maha_soliman@hotmail.com

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Research Article

Factors Affecting Lethality of Bisphenol a on *Biomphalaria alexandrina* Snails

Abstract

The present study was undertaken to investigate the influence of some environmental factors including temperature, water vegetation, bed mud and pH on LC_{50}, LC_{90} and LT_{50} of bisphenol A (BPA) on the snail host of *Schistosoma mansoni, Biomphalaria alexandrina.* Effects of exposure to the sublethal concentrations of BPA on some biological aspects of the snails and on the cercarial output form *S. mansoni* infected snails were studied. Results showed that temperature, water vegetation, bed mud and pH markedly affected the lethality of BPA. The biological parameters of *B. alexandrina* including survival rate, egg hatchability and egg laying capacity were greatly affected by exposure to BPA and the response was dose dependent. Regarding the possible effect of BPA on transmission of schistosomiasis, results showed that exposure to different concentration of BPA for 7 days before miracidial infection caused the death of all the snails before reaching the patent period while, no cercarial output was recorded from snails exposed to BPA for 24 hrs till their death. In conclusion, our results showed that the environmental characteristics may alter the biological impacts of BPA and the exposure of snail to BPA may affect the transmission of schistosomiasis.

Introduction

Studying the environmental conditions related to the occurrence of snail intermediate host can provide valuable information on its breeding sites and consequently on the model of schistosomiasis transmission [1]. The anthropogenic ecological transformations have a considerable impact on altering many of the environmental and social conditions necessary for schistosomiasis transmission [2].Human activities resulting in chemical contamination of the environment have increased the potential stresses on molluscs in exposed habitats [3].

Bisphenol a (BPA) is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic (e.g., water bottles), epoxy resins (e.g., inside coating in metallic food cans) and as a non–polymer additive to other plastics [4,5]. BPA is a pseudo-persistent chemical, which despite its short half-life is ubiquitous in the environment because of continuous release [6]. Post-consumer releases are primarily via effluent discharge from municipal wastewater treatment plants, leaching from landfills, combustion of domestic waste, and the natural breakdown of plastics in the environment [7,8].

It has been suggested that some invertebrates appear to be quite sensitive to BPA, and effects have been documented at environmentally relevant concentrations [6]. Both midge *Chironomus riparius* larvae and the marine copepod *Tigriopus japonicus* showed developmental inhibition at very low concentrations of BPA [9,10]. However, higher exposure caused premature larval metamorphosis and settlement in the marine polychaete worm *Capitella capitata* [11].

In the freshwater snail *Marisa cornuarietis*, exposure levels >1.0 mg L^{-1} were found to result in super-feminization (additional female organs, enlarged sex glands, oviduct deformities, and increased fecundity), oviduct rupture, and mortality [12]. In the mollusk *Mytilus edulis*, spawning induction, as well as oocyte and ovarian follicle damage, was observed following BPA exposure for 3 weeks at 50.0 mg L^{-1} [13]. The effect of BPA appears to vary considerably among related taxa, and it appears that some invertebrates may be hypersensitive to BPA exposure (freshwater molluscs and insect larvae, and marine copepods in particular).

The present study was undertaken to investigate the influence of some environmental factors including temperature, water vegetation, bed mud and pH on the effect of bisphenol A on the fresh water snails, *B. alexandrina* and consequently the transmission of schistosomiasis. The possible effect of BPA on the *Schistosoma* cercarial output form the snails was also studied.

Materials and Methods

Maintenance and rearing of *B. alexandrina* snails

B. alexandrina snails were collected from fresh water canals in Warrak El-Arab village, Giza Governorate. The collected snails were maintained in glass tank filled with dechlorinated tap water under constant temperature (25 ± 2 ºC) with diurnal alteration and fed on dried lettuce leaves for at least three weeks before being used for the following experiments. Maintenance and rearing of the snails were done according to [14].

Determination of LC_{50}, LC_{90} and LT_{50} of bisphenol A against *B. alexandrina* snails

Bisphenol A (4,4´–isopropylidinedi-phenol) was purchased from Sigma Aldrich Company, Germany. Stock solution of BPA (100 mg L^{-1}) was prepared according to the method of [15]. A series of

concentrations (1.0, 3.0, 5.0, 7.0, 9.0, 11.0 and 13.0 mg L^{-1}) were used for determination the LC_{50} and LC_{90} values of BPA. Three replicates of adult snails (10 snails/replicate/L capacity tank) with 9.0 - 11.0 mm shell diameter were used for each concentration after three weeks of maintenance. Snails were exposed for 24 hrs to BPA concentrations then transferred to dechlorinated tap water for another 24 hrs for recovery. Mortality rate was determined after recovery period [16]. A set of control snails was prepared using dechlorinated water was run parallel to tested concentrations. Computation of LC_{50} and LC_{90} values and slope function were determined utilizing the statistical program SPSS (2001) for windows.

The LT_{50} was carried out by exposing separate groups of snails to the previous concentrations of BPA for 24, 48, 72 and 96 hrs, followed by 24 hrs of recovery for each exposure period.

Studying the effect of certain environmental factors on LC_{50} and LC_{90} values of BPA

This experiment was designed to evaluate the effect of temperature, water vegetation, river- bed mud and pH on LC_{50} values of BPA. Three replicates of adult snails (10 snails / replica) were used for each experiment. The control group of snails was maintained at the same experimental condition in de-chlorinated tap water. Exposure and recovery periods for all experimental tests were 24 hrs. Mortality rates were determined in each experiment after recovery period in clean de-chlorinated water for 24 hrs [17].

Temperature: snails were exposed to LC_{50} and LC_{90} (9.7 and 12.3 mg L^{-1}, respectively) of BPA at 18, 24 and 30 °C.

Water vegetation: water plants namely *Lemna gibba* (Family: *Lemnaceae)* and *Ceratophyllum demersum* (Family: Ceratophyllaceae) were used. Snails were exposed to LC_{50} and LC_{90} with / without *L. Gibba* or *C. demersum* in different amount (1.0, 2.0 and 4.0 g L^{-1}).

River-Bed mud particles: snails were deposed to different amounts of very fine mud (5.0 and 10.0 g L^{-1}) with LC_{50} and LC_{90} of BPA. A set of control snails was prepared using de-chlorinated water and mud at the same amount. Tanks were provided with gentle air stream to maintain continuous and thoroughly mixing the mud [18].

pH: LC_{50} and LC_{90} of BPA were prepared in standard reference water solutions previously adjusted with NaOH or HCl at pH values of 9.0, 7.0 and 4.0. Three replicates were performed for each pH value and BPA concentration. Control snails were prepared in de-chlorinated water having the same experimental pH values [17]. The pH measurements were made only once in this experiment, before adding BPA.

Effect of sub-lethal concentrations of BPA on some biological parameters of the snail

The semi-static system was used with renewing the treatment solution twice a week to study the effect of sublethal concentrations (0.01, 0.05 and 0.1 of LC_{50}-24 hrs) of BPA on some biological parameters of *B. alexandrina* for 28 days. Half-life of BPA from manufacturing effluent is 2.5:4.0 days [19].

Survival rate and shell diameter: Juvenile snails (4.0 ± 0.5 mm) were used in this experiment. For each concentration, a group of 30 juvenile snails (3 replicates) was exposed in glass jar of 1000 mL capacity. Another group of snails was maintained in dechlorinated tap water as control. Snails were fed daily with dried lettuce leaves. The survival rate was recorded weekly [20]. The shell diameter was measured weekly using a caliper. The shell diameter of juvenile snails was calculated as the difference between size of snails of each week and the size of snails in the previous week divided by the number of lived snails in this week according to the method of [21].

Hatchability rate of the eggs: The aged egg masses of one and six days were collected from nylon sheets placed on the water surface in the aquaria contained *B. alexandrina* snails that were supplied with dried lettuce leaves and tetramine (fish food) for being able of oviposition. Three replicates of each egg masses age were used separately. Each egg-age was exposed to the sublethal concentration of BPA. A group of each egg-age was maintained in dechlorinated water as control. Then, all treatment and control egg masses were transferred to clean dechlorinated water to recover for 24 hrs at 25.0 ±1.0 °C [22].

Egg masses were examined daily during the experimental period under a stereomicroscope and the newly hatched snails were recorded. At the end of experiment, the percentage of hatchability was calculated by dividing the number of newly hatched snails by total number of eggs at the beginning of experiment [23].

Egg-laying capacity: Thirty adult snails (9.0-11.0 mm) were used into three replicates for each experimental concentration. Untreated normal snails were maintained under the same experimental conditions and at 25.0 ± 2.0 °C. Daily dried lettuce leaves food was added. White foams were putted to aquaria for egg deposition. The egg masses laid by exposed and control snails were collected and counted weekly.

Dead snails were removed from aquaria and the number of lived snails at the end of each week was recorded. Snail's egg clutches deposited on the foam and the wall of the aquaria were gently collected weekly and their egg content was counted using a dissecting microscope. The surviving snails in each aquarium counted also weekly [24]. The terms used to express the tested parameters, (L_x) that refers to the survivorship or the ratio of surviving snails in each week, (M_x) represents the egg laying capacity or mean number of eggs/ snail/week. $(L_x M_x)$ refers to the reproductive rate in each week while $(\Sigma L_x M_x)$ is the total reproductive rate at the end of the experiment.

Experimental infection of *B. alexandrina* snails with *Schistosoma mansoni*

Schistosoma mansoni ova were obtained from Schistosomiasis Biological Supply Center (SPSC), Theodor Bilharzia Research Institute (TBRI), and Giza, Egypt. They were left in clean dechlorinated water for hatching under a desk lamp then fresh hatch miracidia were used in bio-assay and infection tests. Two replicates of adult *B. alexandrina* snails (10snails/replicate in 1Lglass container) were exposed to sublethal concentrations of BPA either for 24 hrs or for 7 days before miracidial exposure. Snails were exposed to miracidia individually (10 fresh hatched miracidia/snail) for 24 h under ceiling illumination. After that, snails were transferred to clean de-chlorinated water (26 ± 2°C) and daily fed with oven dried lettuce leaves throughout the

pre-patent and patent periods [25]. A control group of two replicates was exposed to miracidia concurrently with the experimental snails and treated similarly. Dead snails were removed daily and surviving snails were individually examined once weekly for cercarial shedding.

Statistical analysis

The hatchability percent and survival rate were analyzed by Chi-square values of contingency tables [26]. The data of growth rate was statistically analyzed for the significance difference between control and treated groups by using T-test and values were expressed as means ± S.E.

Results

After 34 hrs of exposure, there was no mortality recorded in the 0.0 to 5.0 mg L^{-1} concentrations. Mortality was 15.0 % for 7.0 mg L^{-1} and increased to be 35.0 % and 60 % for 9.0 and 11.0 mg L^{-1}, respectively (Table 1). Mortality was recorded 100 % when 13.0 mg L^{-1} concentration was applied. The calculated lethal concentrations LC_{50} 24 hrs and LC_{90} 24 hrs were 9.9 and 12.8 mg L^{-1}, respectively.

The toxicity of BPA against *B. alexandrina* was time and dose-dependent. There was a significant correlation between all LC_{50} values and exposure periods (Table 1). The LC_{50} values decreased from 9.9 mg L^{-1} at 24 hrs to 1.5 mg L^{-1} at 96 hrs as well as the LC_{90} values decreased from 12.8 to 4.2 mg L^{-1} at 24 and 96 hrs, respectively.

Time required reaching the values of LT_{50} and LT_{90} was reversely proportional to concentrations. The LT_{50} values were 69.4, 49.8, 32.9 and 19.3 hrs for the concentrations 5.0, 7.0, 9.0, 11 and 13, respectively.

LT_{90} values decreased from 94.2 to 45.9 hrs by increasing the exposure concentration to the snails from 5.0 to11.0 mg L^{-1}, respectively.

Data in Table 2 showed that the percentage of dead snails increased by increasing temperature. BPA cause no mortality observation for LC_{50} value at 18 °C while, 13 % of snails died when LC_{90} was applied. Mortality rate was much higher at 24 °C than that 18 °C. The mortality percentages were 50 % and 80 % in snails that exposed to LC_{50} and LC_{90} values of BPA at 24 °C, respectively. However, the temperature of 30 °C greatly increased the snails' mortality to be 80% and 100% at concentrations of LC_{50} and LC_{90} of BPA (Table 2).

Table 2 showed that the effect of different densities of *L.gibba* and *C. demersum* on the mortality of *B. alexandrina* exposed to LC_{50} and LC_{90} of BPA. For both plant, the LC_{50} of BPA that mixed with different amount of plant densities (1.0, 2.0 and 4.0 g L^{-1}) had more toxicity and caused more mortality than applied LC_{50} value without plant. On the other hand, results showed that both plants have no effect on the LC_{90} values at 1.0 and 2.0 g of both densities. However, the mortality was slightly increased at LC_{90} value of BPA with 4.0 g of *C. demersum* when compared with amount of *L. gibba* in which all the snails died.

Mud particles influenced the BPA toxicity against *B. alexandrina* snails as shown in Table 3. The values of LC_{50} and LC_{90} of BPA against snails were decreased when mixed with 5.0 g L^{-1} of mud to reach to 30% and 66.66%, respectively. The percentage of the snails died when exposed to LC_{50} of BPA (9.7 mg L^{-1}) mixed with 10.0 g L^{-1} of mud was 70 %. The same pattern was recorded for LC_{90} of BPA that mixed with 10.0 g L^{-1} mud, the percentage of dead animals was 76.6 % (Table 2).

Table 1: Percentage of dead *B. alexandrina* snails exposed to different concentrations of BPA at different time.

Concentrations (mg L^{-1})	Time of exposure (hrs)			
	24	48	72	96
Control	0.0	0.0	0.0	0.0
5.0	0.0	20	45	95
7.0	15	45	80	100
9.0	35	75	95	100
11	60	90	100	100
13	95	100	100	100
Values presented the mortality rate (%)				

Table 2: Effect of water temperature, water vegetation, bed mud and pH on the mortality of snails using LC_{50} and LC_{90} of BPA.

Concentrations (mg L^{-1})	Temperature degrees (°C)			*L. gibba* (g)			*C. demersum* (g)			Amount of mud		pH of water		
	18	24	30	1.0	2.0	4.0	1.0	2.0	4.0	Low (5 g L^{-1})	High (10 g L^{-1})	Alkaline (9.0)	Neutral (7.0)	Acidic (4.0)
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LC_{50} (9.7)	0.0	50	80	85	85	95	60	85	85	30	70	43.3	50	23.3
LC_{90} (12.3)	13	80	100	90	90	100	90	90	93.3	66.6	76.6	76.6	90	66.7
Data presented the percentage of dead snails at each treatment														

Table 3: Survival rate and shell diameter of juvenile *B. alexandrina* after 4 weeks of exposure to sub-lethal concentrations of BPA.

Age (weeks)	Control snails		0.01 LC_{50} (0.097 mg L^{-1})		0.05 LC_{50} (0.485 mg L^{-1})		0.1 LC_{50} (0.97 mg L^{-1})	
	%Lx	Growth (mm)	Lx (%)	Growth (mm)	%Lx	Growth (mm)	Lx (%)	Growth (mm)
0	1.00	4.2 ± 0.1	1.00	4.2 ± 0.2	1.00	4.2 ± 0.1	1.00	4.2 ± 0.1
1	1.00	4.5 ± 0.1	1.00	4.4 ± 0.1	0.80	4.4 ± 0.1	1.00	4.3 ± 0.1
2	1.00	4.6 ± 0.1	0.95	4.6 ± 0.1	0.80	4.7 ± 0.1	1.00	4.3 ± 0.1
3	1.00	4.7 ± 0.1	0.85	4.8 ± 0.1	0.75	4.7 ± 0.1	0.55	4.4 ± 0.1
4	1.00	4.9 ± 0.1	0.80	5.0 ± 0.1	0.70	4.8 ± 0.1	0.55	4.5 ± 0.1
Data was presented as mean ± S.E								

Toxicity of BPA against *B. alexandrina* differed by variation in the pH values of water (Table 2). In alkaline medium at pH 9.0, the percentage of dead snails was 43.3%. However, the percentage of dead snails sharply decreased in to reach to 23.3 % at acidic medium (pH 4.0). The LC_{90} of BPA against snails also decreased to record 76.6 and 66.66 % of died snails in alkaline and acidic media, respectively (Table 2).

Regarding the effect of BPA toxicity on juvenile snails, the results in Table 3 indicated that the survival rate (L_x) of treated snails with 0.01- and 0.05- LC_{50} for 24 hrs (0.097 and 0.485 mg L^{-1}, respectively) showed a gradual decrease during the experiments. On the contrary, the L_x value of juveniles exposed to 0.97 mg L^{-1} was markedly decreased at the third week that was significant at as compared to 100 % Lx in control group. Comparing the mean shell diameter of control group with that of treated ones, results did not show any significant differences (Table 3).

Table 4 showed the effect of BPA toxicity on hatchability rate of aged egg masses of one and six days old. There was slightly decrease in hatchability rate when 1-day-aged eggs were exposed to 0.097 and 0.485 mg L^{-1} of BPA for one day. However, the hatchability was decreased at 0.97 mg L^{-1} to reach to 72.4 % after one day of exposure. The continuous exposure the egg to BPA for a week, the hatchability rate of one- eggs and six-egg old showed gradual decrease with the three tested concentrations but eggs of six days old were less susceptible than one day old when exposed to 0.97 mg L^{-1} of BPA either for a day or a week.

L_x of adult snails was slightly decreased after exposure to 0.097 mg L^{-1} of BPA for two and three weeks as compared with control (Table 5). The Lx of snails exposed to 0.485 and 0.97 mg L^{-1} was decreased gradually until the 3rd week then had a very highly significant decrease by the 4th week. Comparing the fecundity (M_x) and reproductive rate (L_xM_x) of snails treated with 0.097 mg L^{-1}, data revealed that the two parameters were affected markedly by BPA exposure. The Mx and

LxMx values were higher than those of control after four weeks of exposure to low concentration of BPA. On the other hand, the Mx and LxMx were lower as compared to control group during the whole experimental period in the groups that exposed to 0.485 and 0.97 mg L^{-1}. There was a reduction in the total LxMx in snails reaching to 87.2 and 77.9 as the effect of 0.485 and 0.97 mg L^{-1}, respectively.

Mortality rates of the snails exposed to sub-lethal concentrations of BPA either for 24 hrs or for 7 days before miracidial exposure were shown in Figures 1,2. Results showed that exposure to different concentrations of BPA for 7 days before miracidial infection caused the death for the snails before reaching the patent period (cercarial output) as compared to the control group. Meanwhile, no cercarial output was recorded from snails exposed to BPA for 24 hrs till their death.

Discussion

Regarding the effect of environmental factors on the lethality values of BPA, results showed that temperature, water vegetation, bed mud and pH markedly affected the LC_{50} of BPA. A positive relationship was found between percentages of dead snails exposed to BPA and the temperature. This result was in agreement with those obtained by [17], who found that the molluscicidal activity of methanol extract of *Adenium obesum* plant against *Bulinus truncatus* snails was increased as the temperature increased.

Regarding the effect of different densities of *L. gibba* and *C. demersum* on the mortality of *B. alexandrina* exposed to LC_{50} and LC_{90}, results showed that the LC_{50} of BPA caused more mortality than applied LC_{50} value without plant. On the other hand, results showed that both plants have no effect on the LC_{90} values at 1.0 and 2.0 g of both densities. This result could be explained as aquatic plants can rapidly absorb BPA through their roots from water and metabolize it into several glycosidic compounds. The glycosylation of BPA by plants leads to estrogenicity of the parent compound. Two oxidative enzymes, peroxidase and polyphenol oxidase, are closely associated

Table 4: Effect of sub-lethal concentrations of BPA on the hatchability rates of *B. alexandrine'* eggs after two periods of exposure time.

Time of exposure	Age of eggs	Hatchability (%)			
		Control	Concentrations of BPA (mg L^{-1})		
			0.01LC$_{50}$ (0.097 mg L^{-1})	0.05LC$_{50}$ (0.485 mg L^{-1})	0.1LC$_{50}$ (0.97 mg L^{-1})
One day	One	100	99.9	94.7	72.4
	six	100	92.5	86.8	86.8
One week	One	100	90.0	81.8	56.5
	six	100	86.9	86.9	81.8

Table 5: Survivorship and fecundity of adult *B. alexandrina* after 4 weeks of exposure to sublethal concentrations of BPA.

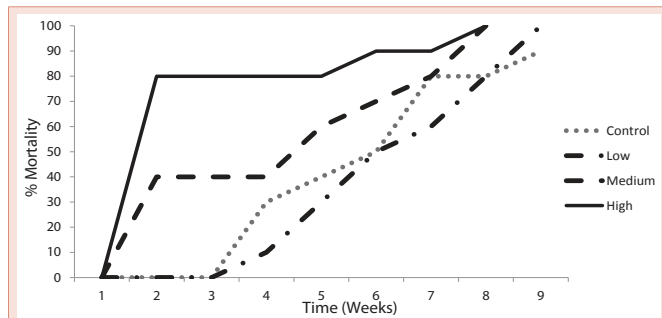| Time (weeks)	Control			0.01 LC$_{50}$ (0.097 mg L^{-1})			0.05 LC$_{50}$ (0.485 mg L^{-1})			0.1 LC$_{50}$ (0.97 mg L^{-1})		
	L_x	M_x	L_xM_x	L_x	M_x	L_xM_x	L_x	M_x	L_xM_x	L_x	M_x	L_xM_x
1	1.0	9.6	9.6	1.0	6.0	6.0	1.0	1.5	1.5	1.0	2.1	2.1
2	1.0	8.5	8.5	0.9	7.31	7.0	0.9	1.8	1.6	1.0	1.9	1.9
3	1.0	5.2	5.2	0.9	6.6	6.4	0.9	0	0	0.8	1.1	0.9
4	0.9	0.8	0.8	0.9	6.9	6.6	0.8	0	0	0.6	1.1	0.7
∑Mx	_	24.0		_	26.8		_	3.3		_	6.2	
∑LxMx	_		24.0	_		25.9	_		3.1	_		5.3
% of control	_			_	111.4	108.1	_	13.4	12.8	_	24.9	22.1
% of reduction	_			_			_		87.2	_		77.9

Figure 1: Effect of short exposure to different concentrations of BPA (24 hrs before miracidial infection) on the mortality rates of *B. alexandrina*. (Low= 0.01 LC_{50}; medium= 0.05 LC_{50}; High= 0.1 LC_{50}).
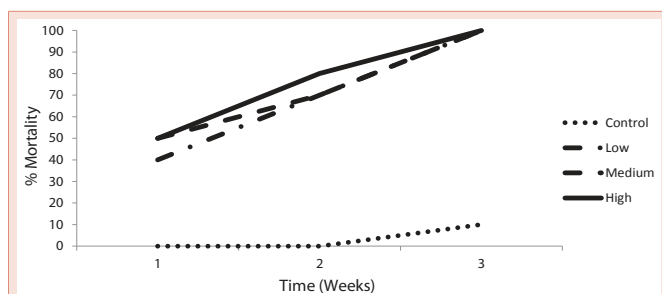

Figure 2: Effect of long exposure to different concentrations of BPA (7 hrs before miracidial infection) on the mortality rates of *B. alexandrina* (Low= 0.01 LC_{50}; medium= 0.05 LC_{50}; High= 0.1 LC_{50}).

with the BPA metabolism [27]. Reactive oxygen metabolites such as superoxide anion, hydroxyl radical, peroxyl radical, and hydrogen peroxide are cytotoxic agents because of their ability to induce oxidative stress [28]. BPA has been shown to induce oxidative stress in land and aquatic plants [29-31]. When the plants undergo decomposition; a large amount of nutrients (nitrogen, phosphorus) and organic material are released from the dying plants into the water causing death of snails.

Regarding the effect of different concentrations of bed mud on the values of LC_{50} and LC_{90} of BPA against the snails, LC_{50} and LC_{90} showed marked decrease in mortality of snails when mixed with 5.0 g L^{-1} of mud to reach to 30 % and 66.6 %, respectively. Increasing the concentration of mud to 10 mg L^{-1} increased the mortality of snails to be 70 % by using concentration of LC_{50} of PBA. This could be because the BPA had a moderate affinity for soil organic matter and is therefore unlikely to be mobile or bioavailable in soils than water column [32]. The half-life of BPA in soils has been estimated as 3 days [32], 7 days [33], and 37.5 days [34]. Increasing the concentration of mud caused increasing the toxicity of BPA on the snails. This could be explained as that much higher BPA concentrations in the sediments than in the upper water column [35]. It was noted a strong correlation between BPA levels near the base of the water column and those in the sediment [36].

The pH is an important factor in controlling the partitioning of BPA. Varying the pH of the water was achieved by adding 0.1 M HCl or 0.1 M NaOH. The effect of pH on mortility of snails was studied

at different pH. Results showed that the toxicity of BPA against *B. alexandrina* differed by variation of the pH values of water. The percentage of dead snails was 50% at pH 7 however, the number of dead snails decreased under acidic and alkaline conditions. The obtained results were in agreement with those recorded a decrease in the molluscicidal activity of *Agave filifera, Agave attenuate* and *Calendula micrantha* in acidic and alkaline pH level against *B. alexandrina* snails [37].

It has been reported that the highest log Octanol-Water Partition Coefficient (Kow) occurred at approximately pH 8 [38]. The log Kow of BPA increased slightly at pH from 6 – 8 but decreased significantly at pH 10. A possible reason is the dissociation of BPA in the system, as pH 10 is within its pKa range (9.6 - 10.2) [39], in fact, BPA is a weak organic acid and can be deprotonated to exist in the system as an anionic form and/or neutral form of BPA. We can suggest that the acidic condition caused degradation to the BPA so, the toxicity of BPA decreased as the percentage of mortality of the snails decrease.

Regarding the effect of BPA toxicity on the survival rates of juvenile snails, results showed that the survival rate (L_x) of treated snails decreased during the experiments. The present findings can also be explained on the basis of storage depots as well as metabolic, physiological and biochemical hazardous effects of the accumulated doses. Our results are in accordance with those who found low concentrations of *Atriplex halimus* caused a sharp decline in the survival of *B. alexandrina* snail [40]. Moreover, it has been noticed that LC_{25} of both *Hedera canariensis* and *Pittosorum tobira varigatum* plants had more sharp effects than LC_0 and LC_{10} on the survival rates of both juvenile and adult *B. alexandrina* snails [41].

It has been noticed that plant cells have the ability to synthesize many phenolic compounds and some of these are conjugated by glucosylation and accumulate in the vacuole [42]. This could be explained why the percentage of dead snails increase in the present of aquatic plants (*L. gibba*) that more toxic than *C. demersum*.

Our findings reflected the important role of eggs age in determining the BPA toxicity against *B. alexandrina*. Hatchability rate showed a slight decrease upon exposure of 1-day-aged eggs to 0.097 and 0.485 mg $L^{-1}$of BPA for one day while it decreased to 72.4% upon exposure to 0.97 mg L^{-1} BPA. In addition, the continuous exposure to BPA fora week caused a decrease of the hatchability rate of one-eggs and six-egg old where the 6-day-aged eggs was less affected when exposed to 0.97 mg L^{-1} of BPA either for one day or one week as compared to-day-aged eggs. This may be due to thicker gelatinous egg coat in 1-day-aged eggs than those of 6-day-aged .Several authors recorded a similar harmful and remarkable reduction in hatchability of *B. alexandrina* eggs treated with different chemicals [34,44].

A significant increase in the mortality rates of snails exposed to sublethal concentrations of the tested material compared to the control group was observed in the present study. This finding agrees with those showed marked reduction in the survival rate of snails treated with sublethal concentrations of different plant species compared to the control [45,46]. It was also noted that adult snails are more tolerant than juvenile one. Similarly, it has been noticed that some plant derived molluscicides caused a significant reduction in the fecundity and survival of young snails than adult snails [47,48].

Regarding the effect of BPA toxicity on egg laying capacity of adult snails, the exposure to lowest concentration (0.01 LC$_{50}$) did not inhibit oogenesis or oviposition and recorded values showed an increase in the oviposition while increasing the concentration caused a complete disturbance in egg-laying capacity. Such reduction of snail's fecundity may arise as a result of the action of the tested agent upon the steroid hormones, the harmful effect on the male and female genital tract, or may arise from metabolic disorders as has been described [49]. These findings are in a harmony with those results [50] showed that LC$_0$, LC$_{50}$ and LC$_{10}$ of *Oreopanax guatemalensis* cause an elevation of egg production in *B. alexandrina* while LC$_{25}$ cause complete retardation.

Regarding the possible effect of BPA on transmission of schistosomiasis, results showed that exposure to different concentration of BPA for 7 days before miracidial infection caused the death of all the snails before reaching the patent period (cercarial output) while, no cercarial output was recorded from snails exposed to BPA for 24 hrs till their death. This may be explained by the deteriorations of physiological parameters of snails making them unsuitable for the parasite development [46]. These results were in accordance with those found a reduction in infection rate with different Biomolluscicides [50-52].

In conclusion, our results showed that the environmental characteristics may alter the biological impacts of BPA and the exposure of snail to BPA may affect the transmission of schistosomiasis.

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