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EVALUATION OF *CALLISTEMON CITRINUS*, PUNICA GRANATUM AND PUMPKIN AGAINST MOLLUSCICIDAL AND FREE LARVAL STAGES OF *SCHISTOSOMA MANSONI*

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ABSTRACT

The molluscicidal activity of methanolic extract of *Callistemon citrinus* leaves, *punica granatum* and Pumpkin has a promising toxic effect against *Biomphalaria alexandrina* snail. The present study showed that the concentration LC_{50} and LC_{90} for *Callistemon citrinus*, *punica granatum* and Pumpkin methylene chloride extracts has moderate effect on *S. mansoni miracidia* after 30 minutes of exposure. All *S. mansoni cercariae* exposed to LC_{90} of the *Callistemon citrinus* were dead after 30 minutes, while the methanol extracts killed 100 % *S. Mansoni* miracidia after 30 minutes of exposure. All *S. mansoni cercariae* exposed to LC_{90} of the *Callistemon citrinus* were dead after 30 minutes, Pumpkin (methanol extract) was less toxic to cercariae with 40% percentage of mortality after 60 min. As regards to the direct effect of methanol extract of *Callistemon citrinus, punica granatum* and Pumpkin on *S. mansoni* worm *in vitro*, data revealed a marked lethal effect after 30 min. for *Callistemon citrinus* at 30 µg/ml while complete death of worm after 60 min at 30 µg/ml resulted with Pumpkin extract, for punica *granatum* methanol extract yielded 100% dead worms *in vitro* after 90 min at 30 µg/ml. In conclusion, the methanol extract of *C. citrinus* leaves, *punica granatum* and Pumpkin seeds may be used as a promising molluscicidal and as an alternative for schistosoma treatment not only reduce drug resistance but also reduce its side effects and the cost of the treatment especially in developing countries.

Keywords: *Biomphalaria alexandrina, Callistemon citrinus*, *Punica granatum* and Pumpkin seeds molluscicide, *Schistosoma mansoni*

INTRODUCTION

Schistosomiasis is a group of chronic parasitic diseases afflicting at least 240 million people in over 70 countries (Larson *et al*., 2014). Recent World Health Organization estimates indicate that 200 million people are infected worldwide, with over 600 million people at risk of infection (Ferguson, 2012). Almost, no country of the African continent is safe from the parasite, as about 85% of the infected populations worldwide are Africans (WHO, 2010; Nyakaana *et al*., 2013).

It is a snail-borne trematode infection of humans, domestic and wild animals in different parts of Asia, Africa, the Middle East, South America and the Carribbean (Bakry & Mohamed, 2011). The importance of the disease lies in the fact that it affects not only the overall health status and fitness of the infected people, but also the human productivity and national economy **(**El-Garem *et al.,* 1994). *Biomphalaria alexandrina* snails are the intermediate host of *Schistosoma monsoni* (Standley *et al*., 2012) with widespread distribution all over Egypt (Bakry *et al*., 2011 and El-Sheikh *et al*., 2012).

The incidence of schistosomiasis is increasing as a result of the construction of dams and the introduction of irrigation schemes which inadvertently provide ideal breeding sites for the snail intermediate hosts of the parasite. So, it is generally agreed that control of these snails is an essential part of the fight against schistosomiasis (Mohamed *et al*., 2012). Different approaches have been used these snails either chemically (WHO, 1967), biologically (Madsen, 1983) or physically (Fagitta & Egami, 1984).

World Health Organization recommended further investigations on plant molluscicides .Most of the plants screened against schistosomiasis cercariae and miracidia were generally effective at levels less than that of their molluscicidal ones. It was recorded that no *S. mansoni* worms were detected from mice exposed to cercariae previously treated for one hour with 100 ppm of the plant *Anagallis. arvensis* dry powder *(Mahmoud and Gawish,2005)*. *Solanumnigrum*, also, has a suppressive effect on the infectivity of *S. mansoni* cercariae to albino mice *(Helmy,* 2007*)*. *S. mansoni* miracidia and cercariae were killed by 100 ppm dry powder of *Calendula micrantha* within 2 and 24 h of exposure, respectively *(El-emam, 1986).*

During molluscicidal operations in water bodies, some schistosomiasis transmission sites receive sublethal concentrations from the molluscicides under application. Therefore, the present study evaluates the attenuation effect of sublethal

concentrations of methanol extract from some plant species on the infectivity of *S. mansoni* cercariae to albino mice.

Plant extracts with molluscicidal and cercicidal properties may provide cheap, locally produced, biodegradable and effective control agents in rural areas of developing countries where schistosomiasis is endemic(Brachenbury,1998).

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MATERIAL AND METHODS

Biological material

The snail material used was adult *Biomphalaria alexandrina* snails, 8-10 mm in diameter, collected from the irrigation canals near Cairo and accommodated under laboratory conditions for several weeks before being used. During this period, they were examined several times for natural parasitic infection and healthy snails only were used. The snails were fed on boiled lettuce leaves and blue green algae.

The parasite material was *Schistosoma mansoni* eggs, cercariae and worms, obtained from the Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute, Egypt.

The schistosome eggs were extracted from the intestines of hamsters infected seven weeks earlier. They were cleaned and hatched to get the miracidia in small amounts of dechlorinated tap water.

Collection of plants:

Plant *Callistemon citrinus*

The "red bottle brush plant" *Callistemon citrinus*, a member of the Myrtaceae family, was collected from Zoo garden, Giza, Egypt in May 2014 -2015. Authentication was carried out by Agriculture Engineer, Terese Labib. The plant was dried in shade then in an oven at 50^{o}C; finely powdered by Soaking with 95% methanol (0.5 kg / liter) for seven days at room temperature according to Shoeb*et al*., (1984) & (1993), Singh & Agarwal (1987) and Salomi *et al*., (1991). Then the solvent was filtered and distilled under vacuum and the crude extract

residues were stored in clean dry dark vessel till for the bioassay tests.

Pomegranate *(punica granatum)* and **Pumpkin seeds** used in the present study were purchased from Harraz Company for agriculture seeds and medicinal plants, Cairo, Egypt. They extracted with methanol at ambient temperature by percolation. Extracts were filtered and methanol was evaporated to dryness pressure and totally freed from water by freeze – drying and stored under freezing at -20°C until use.

A-Experimental Materials:

1. Snails:

Biomphalaria alexandrina snails used in the present study were collected from the River Nile and irrigation schemes at Giza Governorate (from some canals in Abu-Rawash (30 km from Cairo), Giza). The collected snails were transferred and maintained at Malacology laboratory, Theodor Bilharz Research Institute (TBRI), where, they washed thoroughly with dechlorinated tap water, maintained in plastic aquaria (16 x 23 x 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails / L) and covered with glass plates. They were maintained in air conditioned room at 24°C and fluorescent light was reflected 30 cm over them during day time. Oven dried lettuce leaves and blue green algae (*Nostocmuscorum*) were used for feeding. Lettuce leaves were given daily and its amount was adjusted as possible to the number and size of the snails and the algae were added weekly. *N.muscorum*algae were originally obtained from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI) and was then cultivated in Medical Malacology Laboratory according to Liang *et al.* (1987). Water in the aquaria was changed weekly. Snails were examined twice weekly for natural trematode infection for one month before being used in bioassay tests. The eggs of these snails are maintained till hatching and growing up, and then were used in the experimental tests (Zidan*et al*., 2000 and El-Fiki& Mohamed, 1978).

For collection of egg masses, small pieces of polyethylene sheets were introduced into the aquaria (Pellegrino &Goncalves, 1965).They were collected daily with their attached egg masses and kept in small jars till hatching.

2. Cercariae and miracidia:

Schistosoma mansoni ova and cercariae used in this study were obtained from SBSC, Theodor Bilharz Research Institute (TBRI), where, ova were taken from previously infected mice and cercariae from infected snails. The ova were allowed to hatch in small amount of dechlorinated water (24°C) for about 15 minutes under a

direct light. Then, the hatched miracidia were used in the experimental tests.

Molluscicidal activity

1. **On mature snails**

Biomphalaria alexandrina snails used in this study were obtained from the laboratory-bred stock in Theodor Bilharz Research Institute (TBRI). The snails (8-10 mm in size) were then transferred to glass aquaria (50×30×30 cm), 10 snails/liter of dechlorinated tap water (pH 7.0-7.5 and 25-27^{o}C) to avoid crowding. Boiled then oven dried lettuce leaves were used for feeding snails and commercial fish food (tetramin D_{5420}Melle, Germany) was added once a week to the aquaria. The water in the aquaria was changed weekly, while dead snails were removed daily **(Mossalem, 2003).**A stock solution of 1000 ppm was prepared from *Callistemon citrinus* powder on the basis of w/v using de-chlorinated water (pH 7.0 to 7.5). (World Health Organization1965). Three replicates were used, each of ten snails (6 to 8 mm/L, for each concentration. Exposure and recovery periods were 24 h each; at 25 ± 2°C. For each test, 3 replicates of control snails were maintained under the same experimental conditions in de-chlorinated water. After exposure, the snails were thoroughly washed and transferred to fresh water for another 24 hrs for recovery. The effectiveness of *Callistemon citrinus* has been expressed as LC50 and LC90 (Litchfield and Wilcoxon, 1949). The sublethal concentrations were calculated through a computer program .the statistics program SPSS package version 7.5 was used for calculation.

2. Free living stages of *Schistosoma mansoni*:

- **Miracidicidal tests**

Miracidia of *Schistosoma mansoni* were obtained from Schistosome Biological Supply Programm unit (SBSP) at Theodor Bilharz Research Institute (TBRI).

Ten ml of water containing about 100 freshly hatched miracidia were put in small graduated Petri dishes. They were mixed with equal volume (10 ml) of various concentrations of *Callistemon citrinus* , *pomegranate (punica granatum)* and Pumpkin seeds to get 20 ppm, 15ppm, 10 ppm, 8ppm, 6 ppm 4 ppm, 2ppm, about equal numbers of miracidia in 20 ml of dechlorinated water were used as control. Two replicates were run in each case. Microscopical observation of the viability of both organisms, as indicated by motility, was performed after 15 minutes exposure. Miracidia were considered dead when motion ceased completely and the dead organisms were counted. Then after all miracidia were killed by adding a drop of Bouins fluid to each Petri dish, and the total numbers of organisms were determined and

the mortality rate was computed in each case.

- **Cercaricdal tests**

Cercariae of *Schistosoma mansoni* were obtained from Schistosome Biological Supply Program unit (SBSP) at Theodor Bilharz Research Institute (TBRI).

Ten ml of water containing about 100 freshly shed cercariae were put in small graduated Petri dishes. They were mixed with equal volume (10 ml) of various concentrations of

Callistemon citrinus, to get 10 ppm, 5ppm, 4 ppm, 3ppm,2 ppm,1ppm and 0.5 ppm about equal numbers of cercariae in 20 ml of dechlorinated water were used as control. Two replicates were run in each case.

pomegranate (punica granatum) to get 10 ppm, 5ppm, 4 ppm 3 ppm and 2ppm, about equal numbers of cercariae in 20 ml of dechlorinated water were used as control. Two replicates were run in each case. And **Pumpkin seeds** to get 50 ppm, 40ppm, 30 ppm, 25ppm, 20 ppm 15 ppm, 10ppm, 5ppm, 4 ppm and 3 ppm about equal numbers of cercariae in 20 ml of dechlorinated water were used as control. Two replicates were run in each case. Microscopical observation of the viability of both organisms, as indicated by motility, was performed after 15 minutes exposure. Cercariae were considered dead when motion ceased completely and the dead organisms were counted. Then after all cercariae were killed by adding a drop of Bouins fluid to each Petri dish, and the total numbers of organisms were determined and the mortality rate was computed in each case.

Statistical analysis

The data are presented as mean ± standard deviation of mean (Mean ± SD). The mean values of each group were calculated from the mean values of individual mice. The mean groups were compared by analysis of variance **(Snedecor** and **Cochran, 1981).** The comparison between two groups was carried out using student's "t" test. The data were considered significant if P values were equal to or less than 0.05.

All statistical analysis was performed with the aid of the SPSS computer program (version 7.5 Windows).

RESULTS

Plant extracts with molluscicidal and cercicidal properties may provide cheap, locally produced, biodegradable and effective control agents in rural areas of developing countries where schistosomiasis is endemic (Brachenbury,1998). Attenuation of *S. mansoni* cercariae with a molluscicide was previously achieved in vitro (Perrett*et al*, 1994).

The present results revealed that survival rates of juvenile *B. alexandrina* snails exposed to the tested compounds were concentration dependent. The present data showed that the methanol extracts of *Callistemon citrinus punica granatum* and Pumpkin induced a considerable molluscicidal activity. Methanolic extract of *Callistemon citrinus* have $LC_{90}$of (18.32 µg/ml) after 24 hr& (36.14µg/ml) after 5 days incubation exposure period respectively. The methanolic extract of *punica granatum* yielded LC90 of (751.6214µg/ml) after 24 hrs& (444.43 µg/ml) after 5 day incubation exposure. Pumpkin methanolic extract also have molluscicidal activity against *Biomphalaria alexandrina* snails, LC90 was (727.62 µg/ml) after 24 hrs. But after 5 days LC90 was (419.53 µg/ml).The present results may be due to metabolic disorders, loss of muscular coordination which leads to snail's death Table (1, 2, 3).

Table (1): Molluscicidal activity of *Callistemon citrinus* against *Biomphalaria alexandrina*s nails after 5 days at 25 ^{o}C±2^{o}C

Groups	Conc. ppm	Number. of snails(number 30) dead *Biomphalariaalexandrina*snails after incubation for (5days)				
		1 day	2 days	3days	4 days	5 days
Control	0	0	0	0	3	5
*Callistemon citrinus*methanol extract	5	3	5	9	10	15
	10	5	10	15	19	23
	15	12	22	25	30	30
	20	15	23	28	30	30
	30	18	25	29	30	30
	35	20	26	30		
	40	25	27	30		
Lc50 (µg/ml)		10.15	16.60	20.59	23.41	25.16
Lc90(µg/ml)		18.32	29.26	33.38	37.47	36.14

Table (2): Molluscicidal activity of Pumpkin against *Biomphalaria alexandrina* snails after 5 days at 25 ^{o}C± 2^{o}C

Groups	Conc. ppm	Number. of snails(number 30) dead *Biomphalariaalexandrina* snails after incubation for (5days)				
		1 day	2 days	3days	4 days	5 days
Control	0	0	0	0	1	3
Pumpkin methanol extract	25	0	0	0	2	5
	50	0	0	1	4	7
	100	0	0	5	6	9
	150	0	2	7	10	14
	200	0	3	9	15	18
	300	1	5	11	19	22
	400	3	7	15	23	25
Lc50 (µg/ml)		561.06	515.72	360.59	242.59	188.41
Lc90(µg/ml)		727.62	775.30	623.74	457.75	419.53

Table (3): Molluscicidal activity of *pomegranate* against *Biomphalaria alexandrina* snails after 5 days at 25 ºC± 2ºC.

Groups	Conc. ppm	Number. of snails(number 30) dead *Biomphalaria alexandrina* snailsafter incubation for (5days)				
		1 day	2 days	3days	4 days	5 days
Control	0	0	0	1	3	5
pomegranate methanol extract	25	0	0	3	5	6
	50	1	2	5	7	9
	100	3	7	8	11	14
	150	5	9	12	14	17
	200	7	11	15	16	18
	300	9	14	17	19	20
	400	10	17	20	22	25
Lc50 (µg/ml)		444.44	315.52	257.21	214.87	198.26
Lc90(µg/ml)		751.62	571.35	531.07	507.87	444.43

Table (4): Effect of different concentration of *Callistemon citrinus*, *pomegranate (punica granatum)* and Pumpkin seeds (pepitas) methanol extract on activity of *S. mansoni* miracidia

Groups	Number of dead Miracidia and % after incubation for (min)							
	15min		30 min		45min		60 min	
Control	0		0		0		0	
	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)
Callistemon citrinus	7.61	14.39	4.61	10.21	1.54	7.15	0	0
pomegranate (punicagranatum)	5.38	12.71	3.4	8.39	0.19	4.09	0	0
Pumpkin seeds (pepitas)	6.44	9.70	4.81	8.05	3.22	6.46	1.61	3.83

Table (5): Effect of LC10 of Callistemon *citrinus*, *pomegranate* and Pumpkin concentration on cercariae output of *Biomphalaria alexandrina* infection with *Schistosoma mansoni.*

Items	Control group	Exposed group (*Callistemon citrinus)*	Exposed group (*pomegranate)*	Exposed group (Pumpkin)
Exposed snails	100	100	100	100
%of infection rate.	46%	6.4%	14%	21%
Total mean number of shedding cercariae / infected snail	237.15±91.2	21.3±6.3	37±11.2	45±19.7

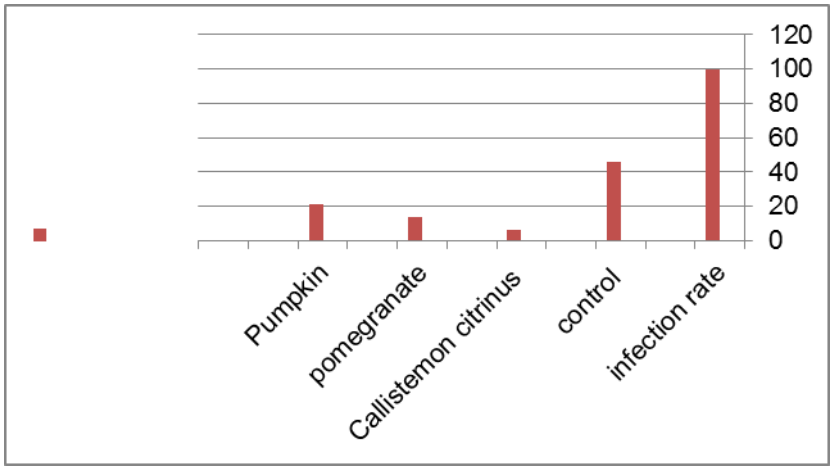

Fig. (1): Effect of LC10 of *Callistemon citrinuspom egranate* and Pumpkinon infection rate, *Biomphalaria alexandrina* infection with *Schistosoma mansoni.*

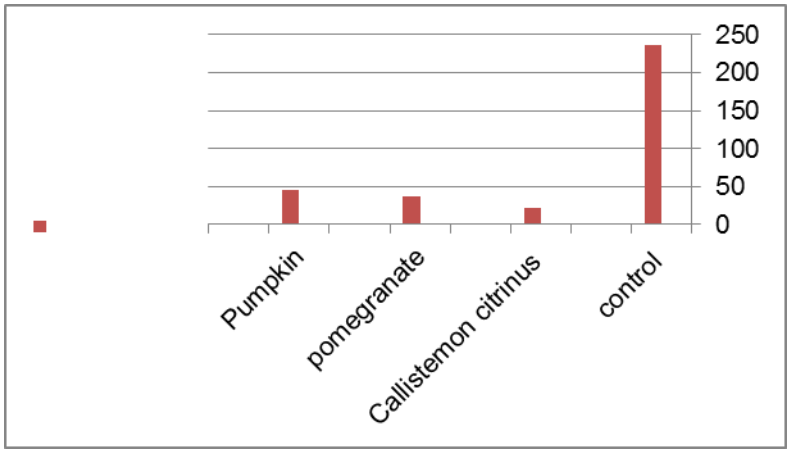

Fig (2).Effect of LC10 of *Callistemon citrinus pomegranate* and Pumpkinon,Total mean number of shedding cercariae / infected snail *Biomphalaria alexandrina* infection with *Schistosoma mansoni*

As regards the cercarialautput of infected *B. alexandrina* snails exposed to the different methanolic plant extracts, *Callistemon citrinus*LC10.reduced the infection rate of *B. alexandrina* snails to 6-4% in addition to decrease in the number of shedded cercariae to reach21.3±6.3comared to 237.15±9.2 cercariae emitted from infected untreated control snails Table (4,5), Fig (1,2). Regarding the miracidicidal and cercaricidal activities, the present study showed that the concentrati on LC_{50} and LC_{90} for *Callistemon citrinus*, *punicagranatum* and Pumpkin methanol extracts killed 100 % of the treated *S. mansoni* miracidia after 30 minutes of exposure. Besides, all *S. Mansoni*c ercariae exposed to LC_{90} of the *Callistemon citrinus* experimental plants were dead after 30 minutes Table (5).On the other hand, Pumpkin (ethanol extract) was less toxic to cercariaea percentage of mortality 40% after 60 min. exposure to LC_{50} (20.41 µg/ml) & LC90 (41.74 µg/ml) resulted in80µg/ml after 60 min of incubation compared to other 2 plants, which were used in this test. This difference may be due to the difference in plant species Table (6,7).

Table (6): Effect of different concentration of *Callistemon citrinus*, *pomegranate (punica granatum)* and Pumpkin seeds (pepitas) methanol extract on activity of *S. mansoni* cercariae

Groups	Number of dead cercariae and % after incubation for (min)							
	15min		30 min		45min		60 min	
Control	0		0		0		0	
	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml	Lc90 (µg/ml)
Callistemon citrinus	2.79	4.72	2.00	3.76	1.36		0.96	1.79
pomegranate (punicagranatum)	4.61	5.34	4.28	5.11	3.91	5.02	3.22	4.47
Pumpkin seeds (pepitas)	37.46	55.35	33.26	58.76	24.31	46.44	20.41	41.74

Table (7): Effect of different concentrations on *Callistemon citrinus pomegranate* and Pumpkin, *methanol* extract on *Schistosoma mansoni* worm *in vitro*

Groups	Number of dead adult worms and % after incubation for (min)									
	30min		60 min		90 min		120 min		24h	
Control	0		0		0		0		0	
Control (PZQ)	0		8.3		41.66		83.3		100	
	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)	Lc50 (µg/ml)	Lc90 (µg/ml)
Callistemon citrinus	6.83	21.44	2.29	14.84	1.86	9.51	0.88	7.72	0.44	3.09
pomegranate (punicagranatum)	19.63	35.0	13.28	33.47	6.05	25.15	2.63	13.77	0.18	4.76
Pumpkin seeds (pepitas)	13.67	26.41	10.89	22.58	6.61	20.31	1.25	13.18	1.01	5.84

DISCUSSION

Schistosomiasis control focuses on reducing disease through periodic, large-scale population treatment with praziquantel; a more comprehensive approach including potable water, adequate sanitation and snail control would also reduce transmission (WHO, 2016). Planorbid snails of the genus *Biomphalaria* are major intermediate hosts for the digenetic parasite *Schistosoma mansoni* (Vallejo *et al.,* 2014). Hence, control of the snail intermediate hosts of schistosomiasis by molluscicides is the best available method for effective and quick reduction of the disease transmission (Mohamed *et al.*, 2012).

In Egypt, where schistosomiasis represents a major national health problem, screening of local plants for molluscicidal, cercaricidal and schistisomicidal activity has received increasing attention (Shoeb& Diwan, 1984; Shoeb *et al.*, 1985; 1990; 1991; El-Emam*et al.*, 1990; Tantawy & El-Deeb, 2001; Kamal, 2005; EL-Komy, 2006 and EL-Naggar, 2007, Gawish, 2008).

Awareness is growing that the burden of schistosomiasis is largely underestimated and requires revision, as it might actually rank close to that of malaria, It is encouraging that signification progress in the control of schistosomiasis has been achieved over the last several years in Brazil, China and Egypt (Bergquist, 2002 and WHO, 2002).

The present results revealed that survival rates of juvenile *B. alexandrina* snails exposed to the tested compounds were concentration dependent. The data of this study, showed that the methanol extracts of *Callistemon citrinus, punica granatum* and Pumpkin induced a considerable molluscicidal activity. Methanolic extract of *Callistemon citrinus* have LC_{90} of (18.32 µg/ml) after 24 hr exposure and (36.14µg/ml) after 5 days incubation

period respectively. The methanolic extract of *punica granatum* yielded LC90 of (751.6214µg/ml) after 24 hrs& (444.43 µg/ml) after 5 day incubation period. Pumpkin methanolic extract also have molluscicidal activity against *Biomphalaria alexandrina* snails, LC90 was (727.62 µg/ml) after 24 hrs, but after 5 days LC90 was (419.53 µg/ml).The present results may be due to metabolic disorders of the snails, loss of muscular coordination which might lead to snail's death.

The present result showed that on using metabolic extract of *C.citrinus* there was a marked reduction in the survival rate of snails treated with sublethal concentrations of this plant compared to the other extracts and the control. These results were in agreement with many investigators (Sharif El Din, 2003; Tantawy *et al.,* 2004; Gawish *et al.,* 2004; El Sayed, 2006; and Abd El Rahman & Hassan, 2008) *C. citrinus*has several advantages as a plant molluscicide, it grows easily in wide area, its toxic parts (leaves) can be harvested without killing the plant and the plant material could be easily powdered, extracted and applied to water bodies by simple techniques Martin et al , (2006); Gawish , (2008). The study revealed that methanol extract of *C. citrinus* leaves may be presented as a promising molluscicide. The tested plant is mostly responsible for the defect of the internal defense system; hence the compatibility of *Biomphalaria alexandrina* snails to *Schistosoma mansoni* infection was decreased This reduction may be due to the fact that snails become unhealthy and change in physiological parameter as the result of continuous exposure to this plant extract. However, further investigations are necessary in order to explore the active constituents of this plant in an attempt to produce large quantities for comprehensive laboratory and semi-field bioassays.

This result agrees with the findings of Sharaf El-Din (2003), and Souza & Andrade (2006), Kamel *et al,* (2006, 2007).In addition, another study indicated that maintenance of snails in LC_{10} concentration of *C. citrinus* for one week resulted in significant reduction of the infection rate and cercarial production for treated snails versus control ones. This may be explained by the deterioration of physiological parameters of snails making them unsuitable for the parasite development (Gawish 2008). These results agree with other authors (Al Shakaway *et al*, 1996; Gawish and El Bardicy, 2004) who reported the toxic molluscicidal effectiveness of various plants against schistomiasis vector snails and their susceptibility to infection with schistosoma miracidia. El- Ansary *et al*.,

(2000) reported that *Ambrosia maritime* caused a remarkable decrease in mercurial shedding from *Biomphalaria* treated with the plant powder. Moreover Massoud *et al*., (2004) found that no *S.mansoni* cercariae were produced from *B.alexandrina* snails treated with LC_{20} of oleo resin from the plant *Commiphora molmol* (Myrrh), whereas, those exposed to LC_{10} showed a considerable reduction in the infection rate and cercarial production.

*C. citrinus*has several advantages as a plant molluscicide, it grows easily in wide area, its toxic parts (leaves) can be harvested without killing the plant and the plant material could be easily powdered, extracted and applied to water bodies of by simple techniques. The study of Gawish,(2008),concluded that methanol extract of *C. citrinus* leaves may be used as a promising molluscicide being mostly responsible for the defect of the internal defense system; hence the compatibility of *B. truncates* snails to *S. haematobium* infection was decreased. However, further investigations are necessary in order to explore the active constituents of this plant in an attempt to produce large quantities for comprehensive laboratory and semi-field bioassays.

The tested plant extracts proved positive molluscicidal activity against *B. alexandrina* snails. It may be suggested that the target tissues, for the tested extracts, were the hermaphrodite gland and the digestive tract.

Regarding the miracidicidal and cercaricidal activities, the present study showed that the concentration LC_{50} and LC_{90} for *Callistemon citrinus*, *punica granatum* and Pumpkin methanol extracts killed 100 % of the treated *S. mansoni* miracidia after 30 minutes of exposure. Besides, all *S. mansoni* cercariae exposed to LC_{90} of the *Callistemon citrinus and punica granatum* metabolic extracts were dead after 30 minutes.

The mechanisms of action of *Punica granatum* on snails is due to presence of tannins and phenolic substances were able to precipitate on the protein of cell membrane during its penetration according to Bakir, (1997). These compounds form hydrogen bounds with nitrogen free and multi hydroxyl-groups, causing inhibition of some enzymes which are very essential to the organism (Reed, 1995; Covington, 1997). Al-Mayah, (2002) reported high effect of aqueous extract of *Punica granatum* against *Fasciola gigantica* parasite and their larval stages such as miracidia and the redia which isolated from infected *Lymnaea auricularia*.

The extract of pomegranate rind could be considered as a candidate for control of *B. Arabica* snail. *P. granatum* extract also has

the advantage of being friendly to the environment as it is safe to fishes and animals Abo zaid*et al* (2013).

In India the extract of stem bark of pomegranate was tested against, the intermediate hosts of *Fasciola hepatica, Lymnae acuminata* snails (Kushwaha*et al* 2004). *P. granatum* bark extract had a molluscicidal activity and at the same time was not toxic to fishes (Kushwaha *et al* 2004).

The fruit rind extracts of *P. granatum* was lethal to snails of *B. Arabica* and their embryos at low concentrations. The LC50s of the fruit rind against snails and embryos of egg-mass after 24h were 47.80 and 65.66 ppm respectively and the LC90s were 100 and 150 ppm respectively. The ability of fruit peel extracts to kill embryos of egg-mass would further the ability of pomegranate to eradicate snails and control its population Abo zaid*et al* (2013).

Hmamouchi, *et al.* (2000), in Morocco, demonstrated the molluscicidal activity of alcoholic extracts of *Cit. colocynthis*, against *Bulinus truncatus* the intermediate host of *Schistosoma haematobium*. The extract killed 50% and 90 % of the snails at 70 ppm and 122 ppm respectively Hmamouchi, *et al*. (2000). The higher lethal concentration found in this study might be attributed to the difference in the type of snail used

Such molluscicidal activities of various plant species were observed by Tripathi and Singh, (2000) who used two plants; *Punica granatum* bark and *canna indica* root against *Lymnaea acuminate* snails and found that their molluscicidal effect was time and dose dependent. Tadros*et al*., (2008) found that the steroidal saponin-containing fraction from methanolic extract of the plant *Dracaena fragrans* had a considerable molluscicidal activity against *B*. *alexandrina* and *B. truncatus* snails.

On the other hand, Pumpkin (methanol extract) was less toxic to cercariae with a percentage of mortality 40% after 60 min. exposure to LC_{50} (20.41 µg/ml) & LC90 (41.74 µg/ml) resulted in 80 % mortality after 60 min of incubation compared to the other 2 plants, which were used in this test. This difference may be due to the difference in plant species.

As regards the cercarial output of infected *B. alexandrina* snails exposed to the different methanolic plant extracts, *Callistemon citrinus* LC10.reduced the infection rate of *B. alexandrina* snails to 6-4% in addition to decrease in the number of sheddedcercariae to reach21.3$\pm$6.3comared to 237.15$\pm$9.2 cercariae emitted from infected untreated control snails.

In addition the snails incubated in *punica granatum* and Pumpkin had an infection

rate of 14% and 21% respectively. The cercarial output from these snails one month later reached 37+11.2 and 45+ 19.7 cercariae for *punica granatum* methanolic extracts Pumpkin methanol extract respectively.

This reduction may be due to both the direct molluscicidal effect (table 5) of these plant extract on *B. alexandrina* snails in addition to the observed miracidicidal effect (table5) .thus resulting in marked effect on penetration ability of miracidia to infect the snails especially when using *Callistemon citrinus* methanol extract Fig (1,2) .

CONCLUSION

The use of molluscicides in the control of fresh water snails is now approaching a highly developed state and plants having molluscicidal properties were found to suppress the total number of snails.

It is recommended to study the efficacy of the purified extract of the plant in combination with as a prophylactic agent in schistosomiasis. Also, this purified extract could be given to evaluate re-infection in murine schistosomiasis.

It is concluded from the present results that the sublethal doses of methanol extract from plants species exhibited an acceptable and moderate antischistosomal activity against *S. mansoni*. In addition, plant species post receiving could have an important role in schistosomiasis control programs. This work light upon the importance of studying the plants of the promising plant's methanol extract, in addition to isolate and identifies the active constituents of such plant's extract for further antischistosomal evaluation.

Moreover the sub-lethal concentrations of the tested compounds have effects on the snails' growth. In addition, the damaged of snails treated with plants may effect to such snails. These effects will greatly reduce the population size of the snail intermediate host of *S. mansoni*.

Therefore, comprehensive studies are needed to define the proper technique for application of such tested agents in schistosomiasis control aiming to minimize water pollution and saving the non-target organisms in the treated water ecosystem.

REFERENCES

[1] Abdel Rahman, E. H. and Hassan, S.E. (2008): Molluscicidal saponins from leaves of *Hederacanariensis*. J. Egypt. Soc. Parasitol., 38(1): 293.

[2] Abozaid K H, El –wakil H , Ali E , Saad J and Shohayeb . (2013): Evaluation of the molluscicidal activity of *punica granatum*, *Calotropis procera, Solanum incanum* and *Citullus Colocynthis* against *Biomphalaria Arabica*.

World Applied Sciences Journal 26(7): 873-879.

[3] Al-Mayah, Z A (2002) .The effect of aqueous extract of *punica granatum* L. Pericarps in giant liver fluke *Fasciola gigantica* .Cobbold,1856.pp.102.

[4] Al-Sharkawy, I. M.; Mansour, M. A. and Tolba, G. A. (1996): Preliminary laboratory investigation of the molluscicidal activity of some wide distributed in Nile Delta. J. Egypt. Ger. Soc. Zool., 20(A): 245-266.

[5] Bakry, F. A. and Mohamed, R. T. (2011): Impact of *Euphorbia milii* latex on infectivity of *Schistosoma mansoni* larval stages to their hosts. Journal of Evolutionary Biology Research Vol. 3 (7), pp. 101-107.

[6] Bakry, F. A.; Hasheesh, W. S. and Hamdi, S. A. H. (2011): Biological, biochemical, and molecular parameters of Helisoma duryi snails exposed to the pesticides Malathion and Deltamethrin. Pesticide Biochemistry and Physiology 101: 86–92.

[7] Bergquist, N.R. (2002): Schistosomiasis: From risk assessment to control. *Trends parasitol.,* 18: 309-314.

[8] Bikir, M G (1997) the antibacterial and antifungal effects of pericarps of *Punica granatum* and some medical plants .M.Sc. Thesis, coll. Educ., Univ. Basrah:pp.125(in Arabic).

[9] Brachenbury T.D. (1998): Proposal standerdization of assessment protocols for plant molluscicides for use in self-help control programs, Am. Trop. Med. Parasitol. 92 (7) 803–816.

[10] El-Ansary, A. El-Bardicy, S., Soliman, M.S. and Zayed, N. (2000): Sublethal concentration of *Ambrosia maritima* (Damsissa) affecting compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* through disturbing the glycolytic pathway. *J. Egypt. Soc. Parasitol*. 30(3), 809

[11] El-Emam, M.A.; H.A. Shoeb; F.A. Ebid and L.A. Refai (1986):Snailcontrol by *Calendula micrantha officinalis*. *J. Egypt. Soc. Parasitol.,* 16 (2): 563-571.

[12] El-Emam. M.A.; S.M. El-Amin and W.S. Ahmed (1990): Mollucicidal properties of plants *Cestrum parqui* and *Headerac*

anariensis. *Egypt. J. Bilh.* 12: p.185.

[13] El-Fiki, S. A. and Mohamed, A. M. (1978): Effect of some herbicide on the toxicity of certain molluscicides against of *Biomphalaria alexandrina* snails. Egypt. J. Bilh., 5: 91-101.

[14] **Covington, A D. (1997**):Modern tanning chemistry. J.Chem.Soc .Rev., 26:73-146.

[15] El-Garem, A. A.; Kamel, L. M.; Mokhtar, S.; El- Behairi, N. M. and Abdel-Rahman, H. A. (1994): A clinical laboratory and social field study of schistosomiasis in an Egyptian village. *Egypt J. Comm. Med.*,12:29-32.

[16] EL-Komy, W. M. A. (2006): Evaluation of molluscicidal and schistosomicidal activity of some species of Egyptian *Artemisia* plant in *Schistosomiasis mansoni.* Ph.D.Thesis, Agricultural Science dep. Institute of Environmental studies and Research, Ain Shams Univ.

[17] EL-Naggar, D. M. (2007): Antibilharzial study of some extracts from Citharexylum quadrangular Jacq.Ph.D.Thesis, Pharmacognosy dep. Faculty of Pharmacy- Girls AL-Azhar Univ.

[18] El-Sayed, K.A., (2006): Effect of the plant *Cupressus macrocarpa* (Cupressacea) on some hematological and biochemical parameters of *Biomphalaria alexandrina* snails. J. Egypt.Soc. Parasitol., 36 (3), 911.

[19] El-Sheikh, Y. W. A. ; Eltamny, H. M.; Soliman, H. A.; Farag, A. A. and El Behary, M. H. H.(2012):Molluscicidal Activity of Eco-friendly natural compound (Rutin) Gained from Ethanolic Flowers Extract of *Calendula officinalis* on *B. alexandrina, B. truncatus* and *Lymanea* snails. New York Science Journal, 5(4):19- 27.

[20] Fagitta,S. and Egami, N. (1984): Effect of gamma irradiation on reproductive system of pond snail *Physaacuta.* Radist. Res., 98 (2): 362- 369

[21] Ferguson, J. (2012): Biology 112: Biology of Infectious Disease. Lecture 27: Schistosomiasis (Last modified Monday, February 06, 2012 12:09:36 PM).

[22] Gawish, F. A. (2008): Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails' reproduction and infection with

Schistosoma mansoni. New Egypt . J. Med., 39 (6): 103-10.

[23] Gawish F. and El-Bardicy S. (2004): Activity of *Solanumnigrum* and *Ambrosia maritima* plants on susceptibility of *Biomphalaria glabrata* snails and albino mice to Peurto Rican strain of *Schistosoma mansoni. J. Egypt. Ger.Soc. Zool*. (43D), 203.

[24] Gawish, F.; El-Bardicy, S.; Tadros, M.M.; Mahmoud, K. and Yousif, F. (2004): Biocidal activity of twenty eight plant species from two families, Myrtaceae and Agavaceae on *Biomphalaria alexandrina* snails and *Schistosoma mansoni* free larval stages. J. Egypt. Ger. Soc. Zool. (50D):89.

[25] Helmy, H.; Ahmed, H.A.; Ibrahim, H.; Kamal, L. and Ramzy, R.M. (2007): Attenuation of *S. mansoni* cercariae with molluscicide and cercaricide derived from the Egyptian weed, *Solanum nigrum* L. *Annls. Trop. Med. Parasitol*., 91(1): 941-950.

[26] Hmamouchi M, Lahlou M, Agoumi A.(2000) : Molluscicidal activity of some Moroccan medicinal plants. Fitoterapia.; 71(3): 308-14.

[27] Kamel, R.0. (2005): Evaluation of a new antischistosomal drug (Mirazid) on murine schistosomiasis mansoni. Ph. D. Zoology dep. Girls College, Ain shams Univ.

[28] Kamel, E. G.; Refaat, S. W.; El-Dafrawy, S. M.; Mohamed, A. H. and Mossalem, H. S. (2006): The effect of *Schistosoma mansoni* infection on *Biomphalaria alexandrina* haematocytes at ultra-structural level. Proceeding of the 4th International Conference of Biological Science (Zoology) p. 219.

[29] Kamel, E. G.; Refaat, S. W.; El-Dafrawy, S. M.; Mohamed, A. H. and Mossalem, H. S. (2007): Toxicological effect of certain plants and synthetic molluscicides on ultra-structural changes in haemocytes of *Biomphalaria alexandrina*. Egypt. J. Exper. Biol. (Zoology) 3:135.

[30] Kloos, H. and McCullough, F.S. (1987): Plants recognized molluscicidal activity in "Plant molluscicides" Moltedtion, John Wiley and Sons LTD, 44-108.

[31] Kushwaha RS, McGill Jr HC, Hausheer FH. (2004): Effect of Synthetic Truncated

Apolipoprotein C-I Peptide on Plasma Lipoprotein Cholesterol in Nonhuman Primates.J Biomed Biotechnol. (4):177-184.

[32] Larson, M. K.; Bender, R. C. and Bayne, C. J. (2014): Resistance of *Biomphalaria glabrata*13-16-R1 snails to *Schistosoma mansoni* PR1 is a function of haemocyte abundance and constitutive levels of specific transcripts in haemocytes. Int. J. Parasitol., 44(6): 343-353

[33] Litchfield, J. T. and WiLcoxon, F. (1949): A simplified method of evaluating Dose-effect experiments. J. Pharmacol. Exp. Therap., 96: 99- 113.

[34] Liang, Y. S.; Bruce, J. I. and Body, D. A. (1987): Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycle. Proc. 1st Sino-Amer. Symp., 1: 34- 48.

[35] Madsen, H. (1983): Distribution of *Helisomaduryi*, an introduced competitor of intermediate hosts of Schistosomasis, in an irrigation scheme in northern Tanzania. Acta. Trop., 40: 297-306

[36] Mahmoud S S., Gawish FA. (2005): Infection of *B. alexandrina* snails with *S.mansoni* miracidia from ova of mice infected with cercariae exposed to the plant *Anagallis arvensis*, Egyp. J. Sch. Infect. Endem. Dis. 27 85–93

[37] Martins-Souza, R.L, Pereira, C. A, Martins Filho, O.A, Coelho, P.M, Corrêa, A. J.r and Negrão-Corrêa, D. (2006) Differential lectin labeling of circulating hemocytes from*Biomphalaria glabrata* and *Biomphalaria tenagophila* resistant or susceptible to *Schistosoma mansoni* infection.*MemInst Oswaldo Cruz*. 101 Suppl., 1, 185.

[38] Massoud, A. Metwally, D. M., Khalifa, K. E. and Habib, F.S. (2004): Compatibility of *Biomphalaria alexandrina* snails to infection with *Schistosoma mansoni* after exposure to sublethal concentrations of Myrrh.*J. Egypt. Soc. Parasitol*. 34(3), 995.

[39] McCullough, F.S; P Gayral; J. Dunean and J. Christie (1980): Molluscicides in schistosomiasis control. *Bull. Wld. Hlth. Org.,* 58: 861.

[40] Mohamed, A. M.; El-Emam, M. A.; Osman, G. Y.; Abdel-Hamid, H. and Ali, R. E. M. (2012): Effect

of Basudin, Selecron and the phytoalkaloid Colchicine (pesticides) on biological and molecular parameters of *Biomphalaria alexandrina* snails Pesticide Biochemistry and Physiology 102, 68–78.

[41] Mossalem, H.S. (2003): Studies on the molluscicidal and miracidicidal effects of certain wild plants on *Schistosoma mansoni – Biomphalaria alexandrina* system. M. Sc. Thesis, Faculity of Science, Ain Shams University: 1-131the HPRT locus in patients with neurocysticercosis treated with praziquantel. *Mutat.Res.,* 305: 181-188.

[42] Nyakaanaa, S.; Stothardb, J. R.; Nalugwaa, A.; Websterc, B.; Langed, C.; Jørgensene, A.; Rollinsonb, D. and Kristensenf, T. K. (2013):*Bulinus globosus* (Planorbidae; Gastropoda) populations in the Lake Victoria basin and coastal Kenya show extreme nuclear genetic differentiation. ActaTropica., 128 (2): 226- 233.

[43] Pellegrino, J. and Goncalves, M. G. R. (1965): A simple method for collecting egg clutches of *Biomphalaria glabrata (Australorbis glabratus}* and for rearing newly hatched snails. J. Parasitol., 51:10-14.

[44] Perrett, S.; Whitfild, P.J.; Bartlett, A. and Sanderson, L. (1994): Attenuation of *S.mansoni* cercariae with a molluscicide derived from *Mitteliathon ningii*. Parasitol., 109: 559.

[45] ReedJ D (1995): Nutritional toxicology of tannins and related polyphenols in forage legumes .J.Anim.Sci. 73: 1516-1528.

[46] Salomi, M.G.; Satish, C.N. and Pornikkar, K.R. (1991): Inhibitory effects of *Nigillasativa* and Saffrom (*Crows sativa*) on chemical carcinogenesis in mice. Nutrition and Cancer J., 16(1): 67-72.

[47] Sharaf El-Din, A. T. (2003): Effects of double infection with *Echinostoma liei* and *Schistosoma mansoni* on hemoglobin content and circulating haemocytes in the haemolymph of the host snail *Biomphalaria alexandrina*. Egypt. J. Schist. Infect. Endem . Dis. 25:41-52.

[48] Shoeb H.A. and B.H. Diwan (1984): Preliminary screening of some plant extracts for

molluscicidal activity. *Egypt J Pharm Sci.,* 25: 127.

[49] Shoeb, H.A.; M.A. El-Emam; S.M. El-Amin and N.S. Osman (1985): The molluscicidal properties of Euphorbiaceae *Euphorbia helioscopia soosiana*, 13: 63-68.

[50] Shoeb, H.A.; EL-Emam, M. A.; EL-Amin, S.M.; Refahy, L.A. and Khalifa, A.M. (1993): Preliminary screening of some plant as molluscicidal agents against *B. alexandrina* snails. Egypt. J. Appl. Sci., 8: 201.

[51] Shoeb. H.A; M.M. El-Sayed and A.M. Khalifa (1990): Some Egyptian plants with Mollucicidal activity. *Egypt. J. Bilh.,* 12: 35.

[52] Singh, S. (1998): Pharmacological and biotechnical effects of certain plant species against harmful snails. Ph.D. Thesis, Gorakhpour Univ., India

[53] Singh, D.K. and R.A. Agarwal (1987): Latex of *Euphorbia antisyphliticc*, a new potent molluscicide having anti acetylcholine estrase activity against the snail *Lymnaeaa cuminata*. *Sci. Environ.,* 61 (5): 211-216.

[54] Snedecor, G.W. and Cochran, W.C. (1981): One way classification analysis of variance. Ed. By Snedecor G.W. and Cochran,W.C. statistical methods. 6th(ed.). The IOWA state University Press, 258-296.

[55] Souza Sdos, S and Andrade, Z. A. (2006) .On the origin of the *Biomphalaria glabrata* hemocyt. *Mem. Inst. Oswaldo Cruz*. 101 Suppl 1, 213.

[56] Standley, C. J.; Vounatsou, P.; Gosoniu, L.; Jørgensen, A.; Adriko, M.; Lwambo, N. J. S.; Lange, C. N.; Kabatereine, N. B. and Stothard, J. R. (2012): The distribution of *Biomphalaria* (Gastropoda: Planorbidae) in Lake Victoria with ecological and spatial predictions using Bayesian modeling. Hydrobiologia 683, 249–264.

[57] Tadros, M. M.; Ghaly, N. S. and Moharib, M. N. (2008): Molluscicidal and schistosomicidal activities of a streriodalsaponin-contaning fraction from *Dracaena fragrans* (L.). J. Egypt. Soc. Parasitol., 38(2): 585-598.

[58] Tantawy, A.A. and F. El-Deep (2001): Biological and

biochemical studies on *Biomphalaria alexandrina* snails exposed to sublethal concentrations of Dodonaea viscose plant (Fam. Sapindaeae). *J. Agric. Sci. Mansoura Univ.,* 26 (10): 6479-6486.

[59] Tantawy A, Mostafa BB and Sharaf El-Din AT.(2004): Molluscicidal activity of *Synadenium grandii* (Euphorbiaceae) against *Biomphalaria alexandrina* and *Bulinus truncates* the intermediate host snails of schistosomiasis in Egypt and their infectivity with the parasite. *Egyptian J. Sci.*14: 183-196.

[60] Tripathi, S.M. and Sing, D.K. (2000): Molluscicidal activity of *Punica granatum bark* and *Canna indica root*. *Braz.J. Med Biol. Res*. 33, 1351.

[61] Vallejo, D.; Habib, M. R.; Delgado, N.; Vaasjo, L. O.; Croll, R. P. and Miller, M. W. (2014): Localization of Tyrosine Hydroxylase-Like Immuno reactivity in the Nervous Systems of Biomphalaria glabrata and Biomphalaria alexandrina, Intermediate Hosts for Schistosomiasis. J Comp Neurol.1; 522(11): 2532-52.

[62] World Health Organization (WHO, 1965): Expert committee on Bilharziasis choice of methods. 1- Snail control. WHO, No. 299: 5-56.

[63] World Health Organization (WHO, 1967): Reports on epidemiology and control of schistomiasis. Tech. Rep. Ser., 32: 2

[64] World Health Organization (2002): Prevenion and control of schistosemiasis and soil transmitted helminthiasis. Tech. Rep. Ser., 912:1-57. Report WHO. Expert Committee, World Health Organization, Geneva, Switzerland.

[65] World Health Organization (WHO, 2010): World health report on Schistosomiasis. Wikipedia, the Free Encyclopedia MHT

[66] World Health Organization (WHO, 2016): Schistosomiasis. http://www.who.int/mediacentre/factsheets/fs115/en

[67] Zidan, Z. H.; Mansour, A. A.; Abou El- Hassan, A. and El-Deeb, F. A. (2000): Factors influencing the molluscicidal activity of Niclosamide on *Biomphalaria*

alexandrina snails in laboratory. Arab Univ. J. Agri. Sci. Ain Shams Univ., 8(3): 863- 878.