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Effect of the bioactive compound "thymoquinone" extracted from *Nigella sativa* and loaded with chitosan nanoparticles (TQ/ChNPs) on free larval stages of *Schistosoma mansoni* and their infectivity to *Biomphalaria alexandrina* snails

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ABSTRACT

Biomphalaria alexandrina snail is the intermediate host of *Schistosoma mansoni* in Egypt. This study was designed to evaluate the molluscicidal, cercaricidal and miracidicidal activities of thymoquinone bioactive compound from *Nigella sativa* (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs)**.** The results proved that thymoquinone bioactive compound from *Nigella sativa* (TQ) and thymoquinone chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) against *B. alexandrina* showed no molluscicidal activity was recorded against *B. alexandrina* snails. On the other hand, the exposure of *S. mansoni* infected snails to TQ/ChNPs and TQ caused a considerable reduction in the infection percentages and cercarial production/infected treated snail. The average of snail infection in treated groups reached to 30% in comparison with 90.4% in control group. The results also, showed that thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) has a promising effect on free larval stages of *S. mansoni* (miracidia and cercariae). Cercaricidal and miracidicidal activity of TQ/ChNPs was higher than that of TQ.

INTRODUCTION

Schistosomiasis is one of the 17 neglected tropical diseases listed by the World Health Organization. WHO reported that in 2013, 261 million people requiring preventive chemotherapy for schistosomiasis, 92% of them are living in sub-Saharan Africa, and in 2010 schistosomiasis mortality could be as high as 280 000 per year in Africa alone (**Savioli *et al.*, 2017).** Control of schistosomiasis involves prevention of new infection by interruption of the life cycle of parasites, chemotherapy, snail control, sanitation and health education. Different approaches have been followed for controlling the snail intermediate hosts by molluscicides **(WHO, 1967)**, biological agents **(Madsen, 1983)** and physical practices **(Fagitta and Egami, 1984).** Many

authors study the impact of molluscicides on miracidia and cercariae to interrupt schistosomiasis life cycle **(El-Nahas and El-Deeb, 2002; Abdel Raouf 2007).**

The crude oil of *Nigella sativa* (black-seed oil) is one of the promising alternative drugs of plant origin that have an antischistosomal effects **(Ahamed and Mostafa, 2003; Mohamed *et al.*, 2005)**. Numerous active components have been isolated from *N. sativa* seeds and its oil including thymoquinone (TQ), thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellimine-N-oxide **(Das *et al.*, 2012; Muhammad, 2016)** and the most bioactive component of *N. sativa* is (TQ) (**Naz, 2011**).

Nanoparticles or colloidal carriers have been extensively investigated in biomedical and biotechnological areas. Nanotechnology employs curative agents at the nanoscale level to develop nanomedicines. The field of biomedicine comprising nanobiotechnology, drug delivery, biosensors, and tissue engineering has been powered by nanoparticles (**Mirza, *et al.*, 2014).**

Therefore, in the present study the activity of thymoquinone (from *Nigella sativa*) loaded with chitosan nanoparticles (TQ/ChNPs) on free larval stages of *S. mansoni* and their infectivity to *B. alexandrina* snails were determined.

MATERIALS AND METHODS

Preparation of extracts

N. sativa seeds were collected from local markets in Giza, Egypt. Seeds were ground using an electric mill and the fine powders were stored in dried containers for the extraction process. Extraction with different solvents (hexane, methanol and water) was done (**Iqbal *et al.*, 2018**). Briefly, for every 1L of the each solvent, 250 g of the seeds powder was used for extraction. After extraction for 3 consecutive days, each extract was dried using rotatory evaporator.

- Gas chromatography-mass spectrometry (GC-MS) analysis:

Extracts were injected to GC-MS technique. The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) equipped with a polar Agilent HP-5ms (5%-phenyl methylpolysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 Elm film thickness). The carrier gas was helium with the linear velocity of 1 ml/min. The injector and detector temperatures were 200 ºC and 250 ºC, respectively. Injection mode, split; split ratio 1: 10, volume injected 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250 ºC, and acquisition mass range 50–600 (**Morsi *et al.*, 2020**). The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY libraries as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

- Preparation of thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) **Ohya, *et al*. (1994)***:*

Materials: Chitosan (degree of deacetylation of (93%)), Sodium Tripolyphosphate (TPP), Phosphate buffer saline (PBS), Acetic acid and thymoquinone

Procedure:

1- Chitosan was dissolved in acetic aqueous solution at various concentrations (1, 2, 3 mg/mL).

2- Drop wise 5 mL of the chitosan solution was added to 2mL of TPP solution under magnetic stirring (1000 rpm, 1 hour) at room temperature.

3- The opalescent suspension was formed under the same above mentioned conditions.

4- The nanoparticles were separated by centrifugation at 20,000 g and 14°C for 30 minutes.

5- The freeze-dried nanoparticles were stored at 5 ± 3°C.

6- The weights of freeze-dried nanoparticles were measured.

7- Thymoquinone -loaded nanoparticles were formed by the addition of chitosan solution to TPP solution containing 100 mg/mL concentrations of thymoquinone and chitosan concentrations (1, 2, 3 mg/mL)

8- Thymoquinone -loaded nanoparticles were separated from aqueous suspension by centrifugation at 20,000 g and 14°C for 30 minutes.

9- The supernatant was collected and protein content (free) in supernatant was determined by the Bradford protein assay spectrophotometric method at 595 nm.

10- The encapsulation efficiency (AE) and loading capacity (LC) of nanoparticles were calculated as follows:

$\%AE = [(A-B)/A] \times 100$

$\%LC = [(A-B)/C] \times 100$

Where A is the total amount of thymoquinone, B is the free amount of thymoquinone and C is the weight of nanoparticles.

Experimental animals:

Snails:

Adult *B. alexandrina* snails were collected from irrigation canals in Giza Governorate and transferred to Medical Malacology Laboratory, (TBRI). The collected snails were thoroughly washed and maintained in dechlorinated water at temperature of 25 ± 2ºC in plastic aquaria (10 snails /1 Liter) fed on dried powder of lettuce leaves for 4 weeks before used in the toxicity tests. The snails were examined weekly individually for their natural infection. Clean (negative) adult *B. alexandrina* (Shell diameter: 8-10 mm) were supplied by foam sheets for egg deposition, the water of each aquarium was changed weekly. egg masses were collected and transferred to another separate aquaria where they were hatched and reared **(Souzal, 2002).** Hatched snails were fed on lettuce leaves until used in infection experiments.

Toxicity to snails:

A stock solution of 1000 ppm from each of thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) was prepared. A series of concentrations from each compound (100, 200& 300 ppm) was performed. For each concentration 30 adult *B. alexandrina* snails (8-10 mm) were used, 10 snails/1000 ml of the experimental suspension as 3 replicates. The result was calculated as average of the replicates, another 3 replicates also were prepared as control group. The exposure period was 24 hrs. The snails were removed from the plant extracts solutions, washed with dechlorinated water and kept in dechlorinated water for another 24hrs as recovery period for all groups. Death of the snails was determined according to **Nolan *et al.* (1953)** and **Jove (1956)** by immersing the snails in a small amount of 15-20% sodium hydroxide solution in a Petri dish.

Infection of *B. alexandrina* snails with *S. mansoni* miracidia:

Snail's infection:

S. mansoni ova used were isolated from livers of mice treated with thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) post 4 weeks of infection with cercariae. Mouse was orally treated with 200 mg/kg from each compound, 3 times/week for 4 successive weeks. By the 8^{th} week mice livers were dissected out and used in this experiment. The ova were allowed to hatch then, the fresh hatched miracidia were used in snail infection. The mass infection technique was followed by exposing four groups of *B. alexandrina* each of 10 snails (4 – 6 mm) in glass container, 10 miracidia/snail. Group 1: served as the positive control (Infected with *S. mansoni* miracidia isolated from untreated mice) (24 snails), Group 2: Infected with *S. mansoni* miracidia isolated from treated mice with thymoquinone (TQ), (24 snails), Group 3: Infected with *S. mansoni* miracidia isolated from treated mice with thymoquinone loaded with chitosan nanoparticle (TQ/ChNP) (24 snails), Group 4: Infected with *S. mansoni* miracidia isolated from treated mice with chitosan (24 snails)

Snails were placed under direct illumination source for three hours, then washed and maintained in dechlorinated water at 25 ± 2ºC for 21 days The water of each group was changed weekly.

Examination of snails for *S. mansoni* cercarial shedding:

After 21 days of snails` exposure to miracidia, survived ones were individually examined by stereomicroscop for cercarial shedding. Shedding snails (positive) were counted and the day of first shedding in each group was recorded. The experiment continued for negative snails (non-shedding snails) and examination was repeated weekly for cercarial shedding. This examination was carried out once weekly and the following parameters were recorded: infection rate, duration of cercarial shedding and the number of cercariae/snail.

Effect on infection of B. alexandrina during exposure to S. mansoni miracidia:

Four groups each of 24 snails (4-6 mm) were prepared as the previous experiment. *S. mansoni* ova collected from untreated mice were used in this expermint. *S. mansoni* ova were obtained from Medical Malacology Laboratory, TBRI.

Toxicity to the free larval stages of *S. mansoni*:

The miracidicidal and cercaricidal activities of thymoquinone, chitosan, thymoquinone loaded with chitosan nanoparticles were studied on *S. mansoni* miracidia and crecariae. *S. mansoni* ova and cercariae were obtained from Medical Malacology Laboratory, (TBRI). The concentrations used were 100, 200 and 300 ppm from each compound. For 10 ml of each concentration about 100 miracidia or cercariae were introduced. Another 10 ml of dechlorinated tap water containing about 100 miracidia or cercariae was used as control. Microscopical examination of the miracidial or cercarial movement was carried out after different intervals of time either in control and chitosan solutions (½, 1, 1½, 2, 2½ and 3 hours), while (TQ) and (TQ/ChNPs) the time was (10, 20, 30 and 40 mins.). Stationary miracidia or cercariae was assumed to be dead and the total number of miracidia or cercaria was counted at the end of the experiment after adding Iodine solution **(El- Nahas, 1994).**

Statistical analysis:

Data are expressed as means ± SD. The results were computed statistically using the T-test analysis values of $p<0.05$ were considered statistically significant by using oneway analysis of variance (ANOVA). Also, using Microsoft office excel application and GraphPad InStat 3 Program **(Spiegel, 1981).**

RESULTS

1- **GC-MS analysis of different extractsof black seeds**

GC-MS analysis of hexane, methanol and water extracts were carried out and the results are presented in the Table (1). The identification of the components of each extract was performed by their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (%). These compounds are listed according to their retention times. The identified compounds in the extracts are 16 compounds by different concentration of each compound. The main constituents of *N. sativa* seeds extracts as detected by GC/MS were p-cymene (13.4% - 21.8%), 1, 8 cineol (9.8% - 15.7%), 9, 12-Octadecadienoic acid, methyl Ester (7.8 %- 10.71%), Hexadecanoic acid (11.02% - 29.32 %) and Thymoquinone (9.03 %- 29.03%).

Table (1): Results of GC-MS analysis of hexane, methanol and water extracts of **black seeds**

Peak no.	Components	MF	MW	t_R	Area % of hexane Ext.	Area % of MeOH Ext.	Area % of H_2O Ext.
1	α-Thujene	$C_{10}H_{16}$	136	7.02	2.9	4.8	6.5
2	α-Pinene	$C_{10}H_{16}$	136	8.35	1.99	3.1	2.1
3	Sabinene	$C_{10}H_{16}$	136	10.26	2.06	3.08	1.9
4	Dodecane	$C_{12}H_{26}$	170	10.59	2.04	1.03	0.9
5	Myrcene	$C_{10}H_{16}$	136	11.75	0.7	0.04	0.29
6	p-Cymene	$C_{10}H_{14}$	134	12.3	13.4	15.8	21.8
7	Nonanoic acid, 9-oxo-methyl ester	$C_{11}H_{22}O_3$	186	12.56	1.5	2.04	1.09
8	1,8 cineol	$C_{10}H_{18}O$	154	13.6	9.8	15.7	11.2
9	9- Octadecenoic acid	$C_{18}H_{34}O_2$	282	14.43	2.34	0.4	1.05
10	Hexadecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242	19.08	1.12	0.06	2.33
11	9,12-Octadecadienoic acid, methyl Ester	$C_{19}H_{34}O_2$	294	21.86	10.2	7.8	10.71
12	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	22.89	29.32	11.02	13.05
13	Linalool	$C_{10}H_{18}O$	154.	23.01	2.01	0.8	1.02
14	Thymoquinone	$C_{10}H_{12}O_2$	164	23.25	9.03	29.03	17.66
15	Carvacrol	$C_{10}H_{14}O$	150	24.9	3.02	0.99	.0.87
16	Longifolene	$C_{15}H_{24}$	204	25.6	2.09	0.25	3.08
Total of identified compounds%					93.52	95.85	94.68

2-Effect on the free larval stages of *Schistosoma mansoni:*

a- Miracidicidal activity: Data from **(Figs. 2, 4, 6)** revealed that thymoquinone (TQ) and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) were highly toxic to miracidia than chitosan. The mortality rate of miracidia reached 100% when exposed to 200 ppm (TQ), 300 ppm chitosan and 200 ppm thymoquinone loaded with chitosan nanoparticles

(TQ/ChNPs) after 10 mins,1 ½ hour and 10 mins., respectively compared to the mortality rate of control 28.7% after 2 ½ hours.

b- Cercaricidial activity: (Figs. 1, 3, 5) showed thymoquinone (TQ) and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) were highly toxic to cercariae than chitosan. The mortality rate of cercariae reached 100% when exposed to 300ppm (TQ), 200ppm chitosan and 300ppm thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) after 10 mins,1 ½ hour and 10 mins., respectively compared to the mortality rate of control 18.9% after 2 ½ hours.

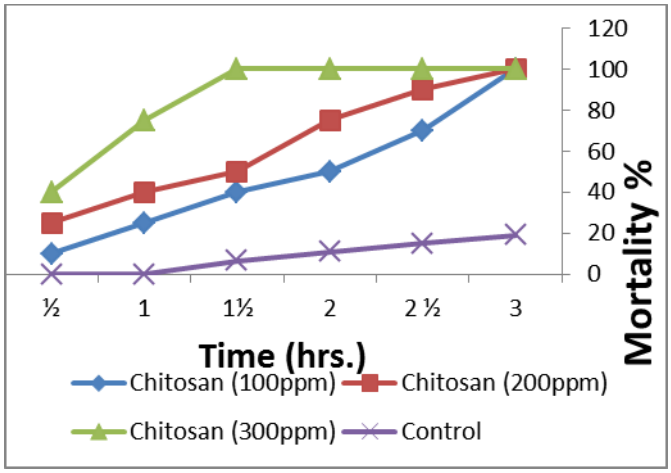

Fig. (1): Cercaricidal activity of chitosan against *S. mansoni* cercariae post different exposure times

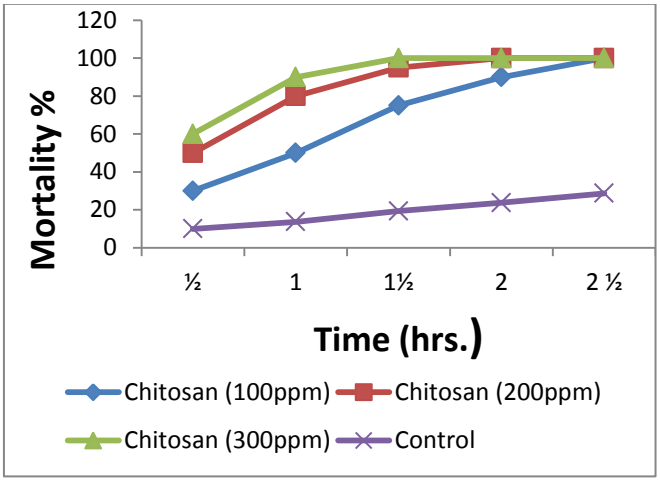

Fig.(2):Miracidicidal activity of chitosan against *S. mansoni* miracidia post different exposure times

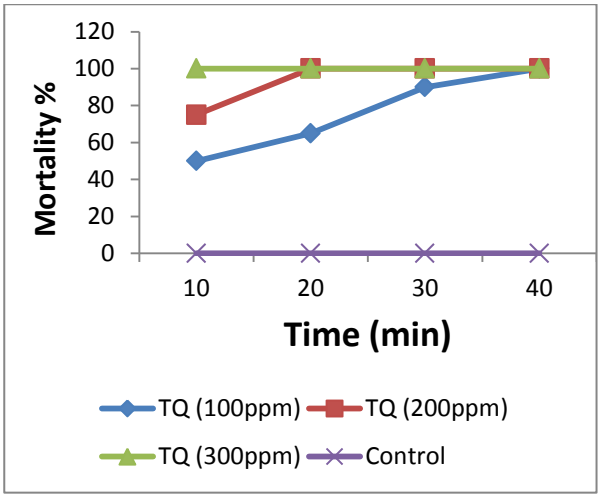

Fig. (3): Cercaricidal activity of (TQ) against *S. mansoni* cercariae post different exposure times

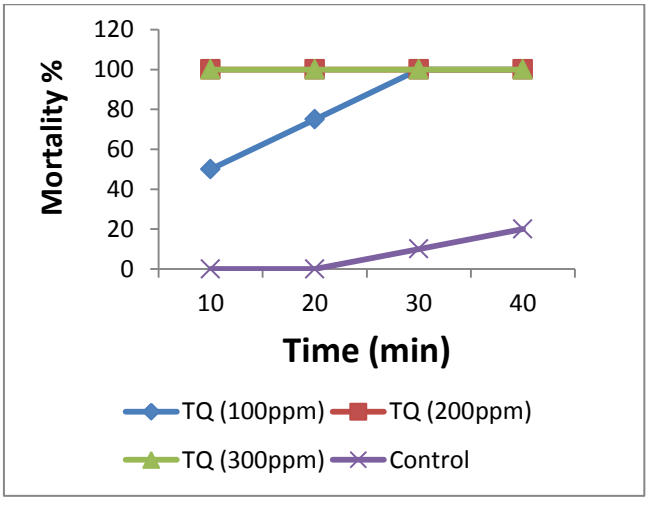

Fig. (4): Miracidicidal activity of (TQ) against *S. mansoni* miracidia post different exposure time

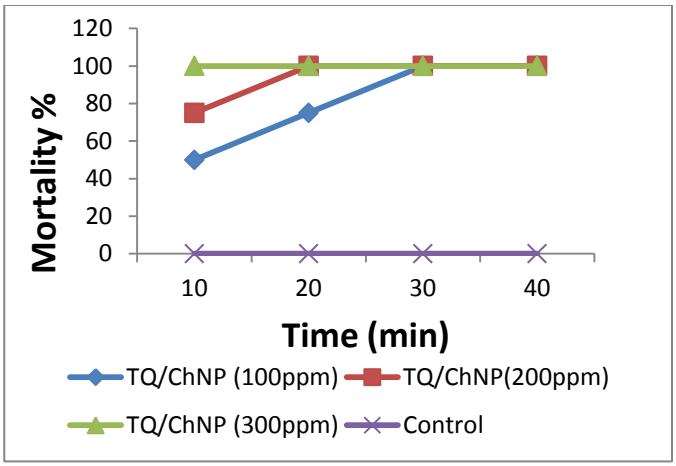

Fig. (5): Cercaricidal activity of (TQ/ChNPs) against *S. mansoni* cercariae post different exposure times

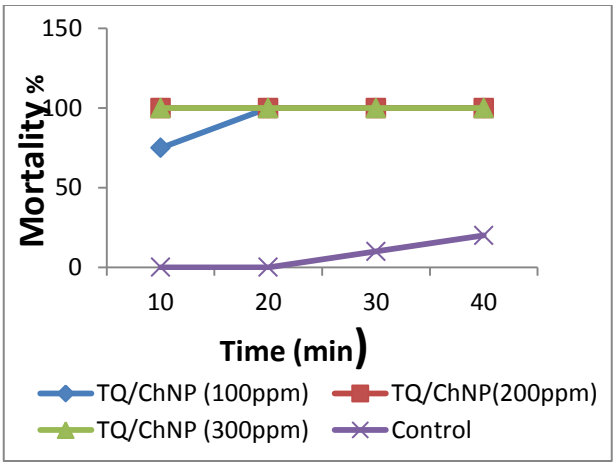

Fig. (6): Miracidicidal activity of (TQ/ChNPs) against *S. mansoni* miracidia post different exposure times

3- Molluscicidal activity against *B. alexandrina* snails:

It was observed from the current test that thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) were non toxic to *B. alexandrina*

4- Susceptibility of *B. alexandrina* snails to infection with *S. mansoni* miracidia from infected-mice treated with (TQ), (TQ/ChNP) and chitosan

Data in Fig (7) revealed that the infection rate of *B. alexandrina* snails infected with *S. mansoni* miracidia from infected mice treated with (TQ) and (TQ/ChNPs) were 20% and 9.52%, respectively while the infection rate of the control group was 90.4%. These rates were significantly lower ($P<0.05$) than that of the control group **(Fig. 7).** Meanwhile, reduction of infection rate of snail exposed to chitosan (77%) did not significantly different from that of control snails. The present data **also** indicated that *B. alexandrina* snails infected with *S. mansoni* miracidia from infected mice treated with (TQ) and (TQ/ChNPs) produced significantly low number of cercariae/snail **(**59.94 and 24.38 cercariae/snail, respectively) compared to 460 cercariae /infected control snail ($P<0.05$). Meanwhile, reduction of cercariae /infected snail exposed to chitosan (382 cercariae /infected) did not significantly different from that of control snails **(Fig. 8).**

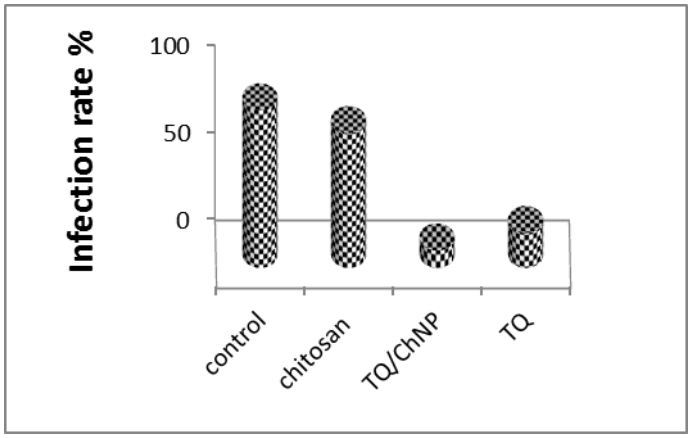

Fig. (7): Infection rate of infected *B. alexandrina* snails exposed to thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticle (TQ/ChNPs).

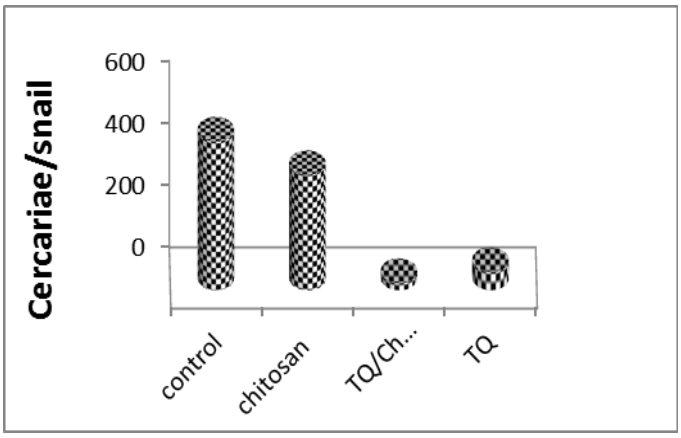

Fig. (8): Effect of thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticle (TQ/ChNPs) on cercarial production of *B. alexandrina* snails infected with *S. mansoni*

5- Effect on infectivity of *S. mansoni* miracidia to *B. alexandrina* snails

The present results in **(Fig. 9).** elucidated that snails infected with *S. mansoni* were significantly reduced to 40% and 30% when exposed to (TQ) and (TQ/ChNPs), respectively during exposure of *B. alexandrina* snails to *S. mansoni* miracidia compared to 90.4 % for control snails ($P<0.05$). Meanwhile, reduction of infection rate of snails exposed to chitosan (81%) did not significantly different from that of control snails. Also, *B. alexandrina* snails exposure to (TQ) and (TQ/ChNPs) during exposure to *S. mansoni* miracidia significantly reduced *S. mansoni* cercarial production/snail **(**66 and 48 respectively) compare to 460 cercariae /infected control snail ($P<0.05$). Meanwhile, reduction of cercariae /infected of snail exposed to chitosan did not significantly different from that of control snails **(Fig. 10).**

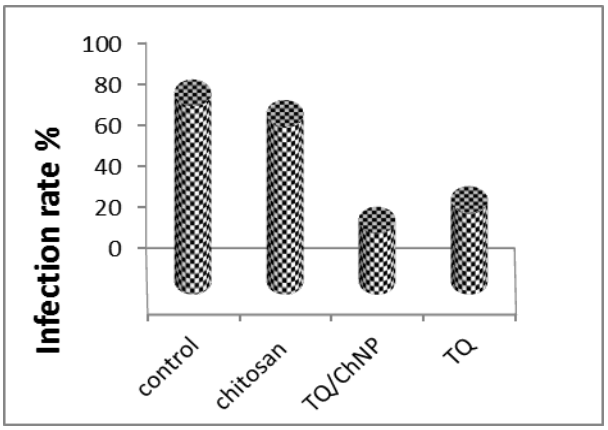

Fig. (9): Infection rate of infected *B. alexandrina* snails exposed to thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs).

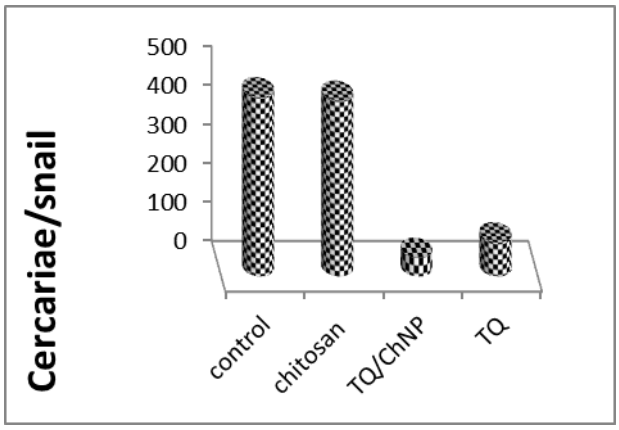

Fig. (10): Effect of thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticle (TQ/ChNP) on cercarial production of *B. alexandrina* snails infected with *S. mansoni*

DISCUSSION

Schistosomiasis is a parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma* and it is recognized as leading cause of significant mortality and morbidity worldwide (**Augusto and de Mello-Silva, 2018).** The control of their snail intermediate hosts is considered essential in controlling this parasite **(Kijprayoona *et al*., 2014).** Recently, nanomaterials have been proven to have molluscicidal activities either due to their toxic effect or/and due to its ability to reduce the snail fertility (**Ali *et al.*, 2012; Yang *et al.*, 2019**). Nowadays, several studies highlighting the potential of biogenic nanoparticles compared to their chemically made analogs (**Guilger *et al.*, 2017; Xiong *et al.*, 2018; Capeness *et al.*, 2019**). Of note, some of parasitological researches were conducted using chitosan nanoparticles (ChNPs) as carriers of drugs in treatment of parasitic diseases e.g. toxoplasmosis (**Etewa *et al.*, 2018**). Chitosan nanoparticles (CS NPs) as it are biodegradable and nontoxic **(Yien *et al.*, 2012**). Many studies used nanoparticles as vehicles to deliver drugs for the improvement of their therapeutic efficacy **(El-Temsahy *et al.*, 2016)**. In this study, chitosan was used as a carrier for thymoquinone (TQ) bioactive compound from *N. sativa* to improve its efficacy against *B. alexandrina* snails and *S. mansoni* miracidia and crcariae.

Firstly, the identification of the components of hexane, methanol and water extracts from *N. sativa* were performed by their retention time (RT), molecular formula (MF), molecular weight (MW), and concentration (%). The identified compounds in the extracts are 16 compounds by different concentrations of each compound. The main constituents of *N. sativa* seeds extracts as detected by GC/MS were p-cymene (13.4% - 21.8%), 1, 8 cineol (9.8% - 15.7%), 9, 12-Octadecadienoic acid, methyl Ester (7.8 %- 10.71%), Hexadecanoic acid (11.02% - 29.32 %) and Thymoquinone (9.03 %- 29.03%), the methanol extract has the highest % of Thymoquinone. These results are in agreement with previous studies reported by **Mahmoudvand *et al.*, 2014; Khalid and Shedeed, (2016); Kabir *et al.*, (2020).**

Also, the present study investigated the activities of thymoquinone (TQ) and thymoquinone chitosan and thymoquinone loaded with chitosan nanoparticle (TQ/ChNP) against *B. alexandrina.* The results showed that thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticle (TQ/ChNP) have no mollucicidal effect on *B. alexandrina*. However, certain studies explored the effect of NPs against *B. alexandrina* as ZnONPs which exhibited molluscicidal effects against *B. alexandrina* (**Fahmy *et al.*, 2014**). Also, iron nanoparticles caused significant mortality in *B. alexandrina* (**Khalil *et al.*, 2018**). These variations in the molluscicidal effects of the nanoparticles were due to the nature of structure materials and the size differences of the used nanoparticles (**Attia *et al.*, 2017).** The negative effect of chitosan on *B. alexandrina* snails may be because chitosan synthesized from snail shell **(Edokpayi *et al.*, 2015),** that primarily produced from chitin, which is widely distributed in nature, mainly as the structural component of the exoskeletons of arthropods

Another approach for the interruption of schistosomiasis life cycle is by killing the free larval stages of schistosomiasis (cercariae and miracidia). The present results evidenced that thymoquinone (TQ) thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) had a

promising cercaricidal and miracidicidal activities. These results are in harmony with **Mohamed *et al.* (2005)** who studied demonstrated that *N. sativa* crushed seeds possess strong schistosomicidal activity against *S. mansoni* miracidia and cercariae. Also, **Mansour *et al.* (2002**) revealed the miracidicidal and cercaricidal potency of the black seed at different extracts (petroleum ether, chloroform, ethanol, and water). **Abo-Zeid and Shohayeb (2015)** studied the lethal properties of *Nigella sativa* alkaloids, saponins and volatile oil in vitro against *Schistosoma mansoni* aquatic stages; miracidia and cercariae. The three bioactive constituents exerted a lethal effect on both miracidia and cercariae at concentrations below 1 ppm. Miracidia were more sensitive than cercariae to the lethal effect of three tested constituents. Also, **Tekwu *et al.*, (2017)** reported that the cercariacidal activity of *Nigella sativa* seeds and *Rauwolfia vomitoria* extracts were concentration dependent. From the present data chitosan nanoparticles had no cercaricidal and miracidicidal activities. Meanwhile, **Moustafa *et al.* (2018)** found that silver and gold nanoparticles caused significant increase in the mortality of *S. mansoni* cercariae and therefore, it could prevent or modulate the infectivity of cercariae in vivo.

The present study concerned to thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) during infectivity of *S. mansoni* miracidia to *B. alexandrina* snails, results showed that survival rate of *B. alexandrina* snails at first shedding of *S. mansoni* cercariae was reduced to 83.3% when exposed to (TQ) and (TQ/ChNPs) compared to 87.5 % for control snails ($P > 0.05$). While this parameter for snails exposed to chitosan during infection showed non-significant increase to 91.6% compared to control group. Such reduction of snail's survival may arise from metabolic disorders as results of the toxic effect of saponin compounds present in the two plant extracts in addition to the parasite-stress on the snail physiology to satisfy the needs for parasite proliferation and multiplication. This was confirmed by **Nabih *et al.* (1988)** who stated that rapid multiplication of the parasite requires a physiologically suitable intramolluscan environment with certain biochemical characteristics. This study findings is in a harmony with the results obtained by **Fathy *et al.* (2013)** who tested the low dose of methanol extract of *Oreopanax reticulatum , Azadirachta indica, Dizygotheca kerchoveana*, *Oreopanax reticulatum* and *Dizygotheca kerchoveana* plants on *B. alexandrina* snails and *S. mansoni* stages and recorded reduction in the snails survival rate, infection rate and number of shedding cercariae.Similarly, **Hasheesh *et al.* (2011)** found reduction in survival rates of *Bulinus truncatus* snails as well as in the infectivity of *Schistosoma haematobium* miracidia to the snail when used methanol extract of *Sesbania sesban* plant (LC_0, LC_{10} and LC_{25}).

In the same consequence, the present results confirmed reduction in the infection rates after exposure to thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs). This may be attriputed to the activity of certain compounds in thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) that have weakened the ability of the penetrated miracidia to proliferate and estabilished their developmental stages within different snail tissues. **Bakry *et al.* (2002)** observed reduction in the infection rate of *B. alexandrina* snails infected by *S. mansoni* miracidia and subjected to

LC_{25} methanol extracts of *Euphorbia lacteal*. These results also are in accordance with many investigations that used various chemical and plant molluscicides and revealed similar conclusions, **Mohamed *et al*., (2000)** examined *Abamectin,* **Tantawy *et al*., (2000)** examined *Solanium dubium*, **Bakry *et al*., (2001)** examined *Agava franzosin,* **Sharaf El-Din *et al*., (2001)** examined *Zygophyllum simplex* and **Bakry *et al*., (2004)** examined methanol extracts of *Oreopanax reticulatum* and *Furcraea selloea*. Also**, Shaldoum *et al.* (2016)** found that the infection rate of snails exposed to Cu2O NPs was significantly decreased.

Regarding to cercarial production, a significant decrease was observed of *B. alexandrina* snails exposed thymoquinone (TQ), chitosan and thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs)**,** this result is in agreement with **Bakry *et al*., (2017)** who found that effects of extracts of *Euphorbia pulcherima* and *Atriplex nummularia* caused a considerable reduction in the infectivity of *S. haematobium* miracidia to *Bulinus truncatus* snail. It caused a reduction in the number of cercariae per snail during the period of cercarial shedding and on infection rate. Also, **Ansari *et al*. (2000)** observed that the effect of *Artemisia maritima* caused a significant decrease in cercarial shedding and cercarial production in *B. alexandrina* the intermediate host of *S. mansoni*. **Sharaf El-Din *et al*. (2001)** treated *B. alexandrina* with sublethal concentrations of aqueous extraction of *Zygophyllum simplex* and obtained similar reduction in cercarial shedding and cercarial production.

CONCLUSION

In conclusion the thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) has a promising effect on free larval stages of *S. mansoni* (miracidia and cercariae). Cercaricidal and miracidicidal activity of TQ/ChNP was higher than that of TQ. While TQ/ChNPs**,** TQ and chitosan showed no molluscicidal activity against *B. alexandrina* snails. On the other hand, the exposure of *S. mansoni* infected snails to TQ/ChNPs and TQ caused considerable reduction in the infection percentages and Cercariae production of snail.

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