Original Article

Biocidal effect of *Nigella sativa, Pelargonium graveolens* and *Azadirachta indica* extracts on *Bulinus truncatus* snail and aquatic stages of *Schistosoma haematobium*

Heba A Aminou[1], Hanan S Mossalem[2], Yosra Alam-Eldin[1]

Departments of Medical Parasitology, Faculty of Medicine, Ain-Shams University, Cairo[1], and Medical Malacology, Theodor Bilharz Research Institute, Giza[2], Egypt

ABSTRACT

Background: Schistosomiasis is a public health problem of social and economic importance in the developing world. Schistosomiasis *haematobium* is still endemic in many foci along the Nile valley, especially in El Fayoum and Beni-Suef governorates. Chemotherapy and snail eradication are the most important control measures with few studies that dealt with its snail intermediate host, *Bulinus truncatus*. Because of drug resistance and recurrence of exposure to infection, snail control becomes a better method for control, keeping in mind that chemical molluscicides may have toxic effects on non-target organisms.
Objectives: The aim of the study is to search for biologically derived molluscicides such as plant extracts, that are less expensive, available, biodegradable, non-toxic and easily applicable than synthetic ones.
Material and Methods: The effect of oil extracts of three natural plants; *Nigella sativa*, *Pelargonium graveolens* and *Azadirachta indica* were investigated as molluscicidal agents against *B. truncatus* snail. Histopathological examination of the soft tissue of the snail was performed after its removal from the shell and its staining with hematoxylin and eosin (HE). Effect of the three plants on miracidia and cercariae of *S. haematobium* was also evaluated.
Results: The potent molluscicidal effect was demonstrated by disturbance of normal histology and presence of vacuolated tissues evident by microscopical examination of the dead snails after HE staining. A schistosomicidal effect was also recorded against aquatic stages of the parasite, demonstrated by reduction in the movement of the miracidia, followed by their sinking down together with the cercariae. *N. sativa* showed the most potent molluscicidal, miracidicidal, as well as cercaricidal activities, followed by *P. graveolens*, while *A. indica* had the least effect.
Conclusion: These findings recommend the use of the studied plant extracts as safe and effective agents in the control of *S. haematobium* in Egypt.

Keywords: *Azadirachta indica*, cercaria, miracidia, *Nigella sativa*, *Pelargonium graveolens*, *S. haematobium*.

Received: 17 August, 2020, **Accepted:** 13 October, 2020.

Corresponding Author: Heba A Aminou, **Tel.:** +20 1001550924, **E-mail:** hebaaminou74@hotmail.com

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 13, No. 3, December, 2020.**

INTRODUCTION

Schistosomiasis has been recognized for centuries as a public health burden in many countries across the world[1]. It is considered one of the major communicable diseases and is second to malaria with socio-economic and health importance in many developing countries[2]. In Egypt, the disease is a major health problem because it affects millions of young farmers and their families, negatively affecting their productivity[3]. Haggag *et al*.[4] determined the prevalence of schistosomiasis *haematobium* among schoolchildren in five governorates in Upper Egypt. The overall prevalence was 1.3% (varying from 0% to 13.4%). The national schistosomiasis control program (NSCP) followed a new elimination strategy using praziquantel (PZQ) and targeting all transmission areas in order to eradicate schistosomiasis *haematobium* in Egypt. Moreover, to complete the strategy of effective control, the program recommended that interruption of disease transmission is mandatory[4].

Chemotherapy, good hygiene, and snail eradication are the most important control measures. However, chemotherapy does not eliminate the disease entirely. The risk of recurrence of infection and drug resistance are still major problems. An essential method to control schistosomiasis *haematobium* is to combat *B. truncatus*, the snail intermediate host[5] in Egypt. The use of molluscicides to control this snail is still one of the most promising means in the battle against schistosomiasis[6].

Several methods have been used to eradicate snail hosts. One of these methods is by using chemical molluscicides, that may have toxic side effects on the non-target organisms. Therefore, a top priority is to search for biologically derived molluscicides to replace the synthetic ones[7]. Some plants' extracts were reported as molluscicides against *Biomphalaria alexandrina*. The use of dry powders of *Solanum nigrum* and *Dizygotheca kerchoveana* continuously for

 DOI: 10.21608/puj.2020.39631.1085

4 w suppressed the egg laying capacity of *B. alexandirna* snails due to degeneration of their hermaphrodite gland cells and evacuation in gametogenic stages[8]. This report also recorded that exposure to LC_{25} of *Panicum repens* dry powder resulted in significant reduction of *B. alexandrina* infection rates with *S. mansoni* miracidia. Such molluscicides were reportedly cheap, available, safe, and successfully used with simple techniques.

Nigella sativa (Family: Ranunculacea), commonly known as black seeds, is an annual herbaceous plant growing in Mediterranean countries and it is one of the native plants that are widely distributed in Egypt[9]. It has been traditionally used in Arabian countries, Indian subcontinent and Europe for medicinal purposes as a natural remedy for a number of illnesses and conditions, including asthma, hypertension, diabetes, bronchitis, headache, eczema and influenza[10].

The genus *Pelargonium* (Geraniaceae), consisting of approximately 280 plant species, originate mostly from South Africa, but also Australia, New Zealand, and the Far East[11]. Extracts from *Pelargonium* species are used as a traditional medicine for treating dysentery, fever, respiratory infections, liver ailments, and wounds. From many medicinal plants indigenous to South Africa, *Pelargonium sidoides* was reported as the most traditionally used plant for primary health care[12]. *Pelargonium* species possess chemically based defenses effective against insects and pathogens[13]. Geranium oil is also an effective insect repellent[14].

Azadirachta indica, commonly known as neem, is an evergreen tree of the tropics and sub-tropics native to the Indian subcontinent with great importance in agriculture as well as in the pharmaceutical industry. This ancient medicinal tree, often called the "wonder tree", is regarded as a chemical factory of diverse and complex compounds that are extremely difficult to imitate by chemical synthesis[15]. In addition to its insecticidal property, the plant is also known for its antimicrobial, antimalarial, antiviral, anti-inflammatory, antioxidant, molluscicidal and anti-filarial properties[16].

In the present work, we investigated the effect of oil extracts of three natural plants; *N. sativa*, *P. graveolens* and *A. indica* as molluscicidal agents against *B. truncatus* snail, the intermediate host of *S. haematobium*. Moreover, evaluation of their effect on miracidia and cercariae of *S. haematobium* was performed.

MATERIAL AND METHODS

This observational analytical study was performed in Schistosome Biological Supply Centre, Theodor Bilharz Research Institute (TBRI), Giza, Egypt, during the period from May till July 2019.

Plants: Oil extracts of *N. sativa*, *P. graveolens* and *A. indica* were purchased from the Agricultural and Biological Research division, National Research Centre, Gizah, Egypt. They were coded NsO, PgO and AiO, respectively. Oil was dissolved in dimethylsulfoxide (DMSO) and then diluted in incubation medium (fresh dechlorinated water). One liter of solution was prepared with a concentration of 50 ppm of each oil extract in each solution.

Effects on the snails: *B. truncatus* snails, were obtained from a laboratory bred colony in SBSC, in TBRI. Adult *B. truncatus* snails, 2-3 mm shell height were used in the study. The snails were fed on boiled lettuce leaves, blue green algae, and fish food[17]. The efficacy of plant extracts against the adult snails was primarily determined according to the standard method of WHO recommendations[18]. To a sample of 100 ml from each of the previously prepared solutions, 10 snails were added. For negative control, two groups of snails were maintained in fresh dechlorinated water under the same experimental conditions. The currently conventional molluscicide, niclosamide, was used similarly as positive control starting with a concentration of 10 ppm. The snails were maintained in the solution for 24 h at 25°C ± 2°C. After exposure, the snails were thoroughly washed and transferred to fresh water for another 24 h for recovery. At the end of recovery period, the snails were examined for viability, and the dead snails were counted and recorded to calculate the mortality rate[19]. Oil extracts were then retested by the same method using descending concentrations for LC_{50} and LC_{90} determination.

Snails histopathological examination: Snail samples were randomly selected from the experimental groups. The soft parts of snails were dissected out of the shells after gentle crushing between two glass slides and the shell fragments were removed using pointed forceps under the dissecting microscope. The tissue was fixed in 10% neutral buffered formalin solution, washed, dehydrated, cleared and paraffin embedded. Serial sections cut at 5 µ thickness, were hydrated, HE stained[20], then microscopically examined and photographed.

Larvicidal (miracidicidal and cercaricidal) activity: Miracidia and cercariae of *S. haematobium* were also obtained from SBSC. Miracidia were obtained from hatching eggs in small amounts of dechlorinated tap water. While the cercariae were produced from experimentally infected *B. truncatus* snails at 25°C±2°C. Miracidia and cercariae were exposed for one hour to the LC_{50} of oil extracts of the three tested plants as determined on the exposed snails, according to Eissa *et al*.[17]. Indication of miracidial and cercarial death was determined by ceased motion for one minute, followed by sinking down, and the detachment of the cercarial tail[17].

Statistical analysis: The statistical program SPSS package version 20 was used for calculation. Linear regression analysis was applied to determine the relationship between mortality (of snail or parasites) and concentrations of plant extracts. Probit regression graphing was used to determine LC_{50} and LC_{100}.

RESULTS

Molluscicidal activity: The molluscicidal activity of oil extracts of *N. sativa*, *P. graveolens* and *A. indica* on *B. truncatus* snails after 24h of exposure indicated that *N. sativa* was the most effective plant on the snails (LC_{50} 5.8 ppm, LC_{100} 10 ppm), followed by *P. graveolens* (LC_{50} 15 ppm, LC_{100} 28 ppm) and the least activity was for *A. indica* (LC_{50} 28 ppm, LC_{100} 47 ppm) (Table 1).

Table 1. Molluscicidal activity of oil extracts of *N. sativa*, *P. graveolens* and *A. indica* on *B. truncatus* snails after 24 h of exposure under laboratory conditions.

	LC_{50} (ppm)	LC_{100} (ppm)
N. sativa	5.8	10.0
P. graveolens	15.0	28.0
A. indica	28.0	47.0
Niclosamide	0.2	0.6

LC: Lethal concentration, **ppm:** Part per million.

Concerning histopathological findings, control snails showed normal female and male hermaphrodite glands. Primary gametogenesis was registered with 1st gametocyte and 2nd gametocyte, and spermatogonia stages (Figure 1a). Figure 1b showed digestive gland with normal complete connective tissues (CT) and regular cell membrane. Head foot tissues were normal with thick and cutaneous layers of connective tissue (Figure 1c). After 24 h exposure to *N. sativa* oil extract, hermaphrodite glands of *B. truncatus* showed absence of developmental stages of gametogenesis and spermatogenesis with vacuolated tissues (VT) (Figure 2a); cell layers of digestive glands were disturbed with vacuolated connective tissues (Figure 2b); while head foot region showed laceration of cutaneous layers and vacuolated tissues (Figure 2c). With exposure to *P. graveolens*, hermaphrodite glands showed partial disturbance in development (Figure 3a), digestive glands showed slight vacuolated tissues (Figure 3b), and gradual distortion in shape of cell layer in the head foot region (Figure 3c). After exposure to *A. indica*, the hermaphrodite glands showed regular contents (Figure 4a); digestive glands showed slight degree of vacuolation of cytoplasm (Figure 4b); head foot region showed slight disturbance in shape of layers (Figure 4c).

Miracidicidal and cercaricidal activities: *N. sativa*, *P. graveolens* and *A. indica* showed variable degrees of biocidal effect on aquatic stages of *S. haematobium*. *N. sativa* was the most effective (lethal dose; LC_{100} that lead to complete death of miracidia was 5 ppm, while that of cercariae was 7 ppm), while *A. indica* had the weakest killing power on both miracidia and cercariae (LC_{100} that caused complete death of miracidia was 18 ppm, while that of cercariae was 26 ppm) (Table 2). This is demonstrated by reduction in the movement of the miracidia, followed by their sinking down together with the cercariae to the bottom of the container with detachment of the cercarial tail, indicating death of both organisms.

Table 2. Effect of oil extracts of *N. sativa*, *P. graveolens* and *A. indica* on *S. haematobium* miracidia and cercariae after 1h exposure.

	Miracidicidal effect (LC_{100} ppm)	Cercaricidal effect (LC_{100} ppm)
N. sativa	5.0	7.0
P. graveolens	10.0	15.0
A. indica	18.0	26.0

LC: Lethal concentration, **ppm:** Part per million.

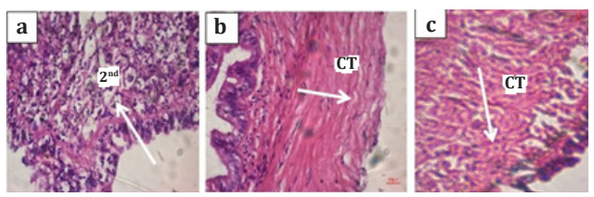

Fig. 1. Histological section of normal *B. truncatus* snail. Primary gametogenesis was registered with 1st gametocyte and **2nd** gametocyte, and spermatogonia stages (1a). Figure 1b showed digestive gland with normal complete connective tissues **(CT)** and regular cell membrane. Head foot tissues were normal with thick and cutaneous layers of connective tissue (1c) (X400).

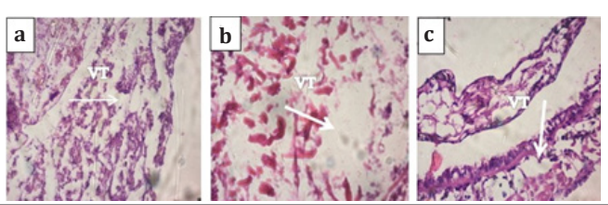

Fig. 2. Histological section of *B. truncatus* snail after 24h exposure to *N. sativa* oil extract; hermaphrodite glands showed absence of developmental stages of gametogenesis and spermatogenesis with vacuolation of tissues **(VT)** (a); cell layers of digestive glands were disturbed with vacuolated connective tissues (b); while head foot region showed laceration of cutaneous layers and vacuolated tissues (c) (X400).

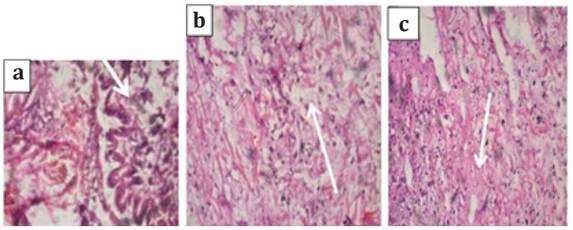

Fig. 3. Histological section of *B. truncatus* snail after 24h exposure to *P. graveolens* oil extract; hermaphrodite glands showed partial disturbance in development (a), digestive glands showed slightly vacuolated tissues (b), and gradual distortion in shape of cell layer in the head foot region (c) (X400).

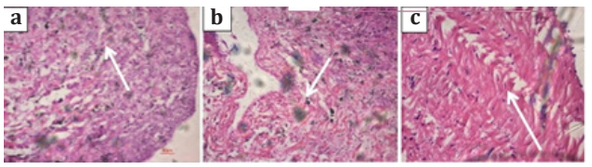

Fig. 4. Histological section of *B. truncatus* snail after 24h exposure to *P. graveolens* oil extract; hermaphrodite glands showed partial disturbance in development (a), digestive glands showed slightly vacuolated tissues (b), and gradual distortion in shape of cell layer in the head foot region (c) (X400).

DISCUSSION

Bulinus truncatus snails, the intermediate host of *Schistosoma haematobium* in Egypt, had colonized the River Nile from the Nile Delta throughout the country resulting in an increase in schistosomiasis transmission[21]. Use of molluscicides in snail control showed a significant effect in reducing both incidence and prevalence of schistosomiasis[5]. However, the negative impact of synthetic molluscicides on the environment and their high cost necessitated search for an alternative approach of using plant extracts for the control of schistosomiasis[22].

In the present work, the molluscicidal effect of oil extracts of three natural plants; *N. sativa*, *P. graveolens* and *A. indica* was studied against *B. truncatus* snail. Essential oil of *N. sativa* was proved to have many therapeutic effects. It was found to exert an anti-*T. gondii* and anti-malarial effect as it decreased the parasitaemia and increased the survival rate of mice infected with *Plasmodium berghei*[23]. It was also found to prevent liver damage induced by *S. mansoni* infection in mice by reducing inflammation and modulating the immune response[24]. Moreover, alcoholic extract of *N. sativa* was found to be as effective as metronidazole in the cure of giardiasis[25].

It is noteworthy that no study could be traced on the molluscicidal activity of *N. sativa* extracts on the snail intermediate hosts of *S. haematobium*. In our present work, oil extract of *N. sativa* showed a lethal effect on *B. truncatus* snail. LC_{50} was 5.8 ppm, while LC_{100} was 10 ppm. The histopathological findings showed severe distortion in the structure of the snail with absence of developmental stages, disturbance of digestive glands with excessively vacuolated tissues (Figure 2).

Similar histopathological findings were obtained by Yousef and EI-Kassas[26] who observed numerous vacuoles in the digestive and excretory cells of *B. alexandrina* and *B. truncatus* snails when exposed to three Egyptian wild plant-extracts; *Euphorbia splendens* (Euphorbiaceae), *Ziziphus spina-Christi* (Rhamnaceae) and *Ambrosia maritima* (Asteraceae).

Our present study is the first report, to the best of our knowledge, to show evidence that *N. sativa* has a potent molluscicidal activity. Its powerful effect on *B. truncatus* snail makes it a new candidate in molluscicides family. Since miracidia, after infecting snails, form sporocysts, with the production of thousands of cercariae, that are the infective stage to human, therefore, killing the miracidium represents an effective way to control schistosomiasis. We showed that the *N. sativa* oil had a good biocidal effect against both miracidia (with LC_{90} of 5 ppm) and cercariae (with LC_{90} of 7 ppm) of *S. haematobium*. Miracidia proved to be more sensitive than cercariae to *N. sativa* lethal effect. This is supported by other studies in which miracidia of *Schistosoma* were more liable than cercariae to the biocidal effect of other plant extracts as berries of *Phytolacca decandra*[27] and latex of *Euphorbia milli*[28].

In 2005, Mohamed *et al*.[29] tested *N. sativa* seeds *in vitro* against *S. mansoni* miracidia, cercariae, and adult worms. Results showed its strong effects against all stages of the parasite and also indicated an inhibitory effect on egg-laying of adult female worms. The effect of crushed seeds was also studied on some antioxidant enzymes that protect adult worms against host oxidant killing. Disturbance of such enzymes, using *N. sativa* seeds, could in turn render the parasite exposed to damage by the host, thus suggesting its role in the anti-schistosomal potency of the used drug.

In another study, conducted by Abo-Zeid and Shohayeb[30], it was found that oil extract of *N. sativa* exerted a lethal effect on both miracidia and cercariae of S. *mansoni*. Miracidia were more sensitive than cercariae. At 0.39 ppm and 50 ppm, *N. sativa* oil killed 100% of the miracidia after 25 and 0.5 min and cercariae after 90 and 5 min respectively. Therefore, the lethal effect of *N. sativa* oil extract shown in this study on aquatic stages of *S. haematobium*, recommends its future use, as potent cercaricidal and miracicidal agent.

In the present work, the molluscicidal activity of *P. graveolens* was also investigated, where it showed a toxic effect against *B. truncatus* snail. LC_{50} was 28 ppm, while LC_{100} was 15 ppm. Histopathological alterations included partial disturbance of hermaphrodite glands, with general distortion and vacuolation of snail tissues (Figure 3). In 2012, El-Tantawy *et al*.[31] demonstrated high toxic effect of *P. graveolens* on *Lymnaea caillaudi* and *B. alexandrina* snails. Also in another study, it was found to have potent molluscicidal activity against *B. alexandrina* snail, confirmed by the decreased protein content and the modulation in the activities of vital enzymes in the snail hemolymph and tissues, which indicated damage to the snail tissue and disturbance of the physiological activities required for parasite development and cercarial production[32].

Al-Sayed *et al*.[33] explained the molluscicidal effect of *Pelargonium* species. They stated that these species are a rich source of tannins, flavonoid derivatives[34], coumarins[35] and phenolic acids[36]. Phenolic compounds with their strong killing action against *B. glabrata* and *B. alexandrina* snails, are suitable for snail control especially as their plants are widely distributed. Moreover, phenolic compounds are less toxic than saponins for other non-target organisms[33]. All these findings show that *Pelargonium* can be used as an effective molluscicidal agent against snail intermediate host of *Schistosoma* in Egypt and hence, control schistosomiasis. Our study confirmed the potency of the oil extract of *P. graveolens* by recording a schistosomicidal effect against miracidia (LC_{90} at 10 ppm) and cercariae (LC_{90} at 15 ppm) of *S. haematobium*. Similar results were obtained by Al-Sayed *et al*.[33], who demonstrated a strong miracidicidal activity of LC_{90} at 15.9 ppm and cercaricidal activity of LC_{90} at 55.9 ppm of the phenolic-rich plant, *P. graveolens*.

Concerning *A. indica*, the oil extract of this plant showed the least molluscicidal activity against *B. truncatus* where LC_{50} was 47 ppm, and LC_{100} was 28 ppm. Histopathological findings showed slight distortion of snail tissue with few vacuolations (Figure 4). However, this effect is still in the range of the WHO standard for toxicity, which requires an LC_{50} of less than 100 ppm for plant molluscicides[37]. Moreover, its miracidicidal (LC_{100} at 18 ppm) and cercaricidal (LC_{100} at 26 ppm) activities were the lowest among the three plant extracts evaluated in this study. In 1986, Ayoub and Yankov[38] observed that water extract (100 ppm) of neem bark is active and toxic against *B. pefifferi* and *B. truncatus*. Singh and Agarwal[39] showed that molluscicidal activity of the standard molluscicide, niclosamide (24 h; LC_{50}, 11.8 mg/l) is only 1.4 times higher than neem oil (24 h; LC_{50}, 17.35 mg/l). Moreover, among the neem-based pesticides, nimbecidine is more toxic against *Indoplanorbis exustus* and *Lymnea acuminata* snails, possibly due to the presence of more limonoids in neem oil (used in the preparation of nimbecidine). However, the toxic effect of azadirachtin neem oil is short-lived. Its maximum effect is observed only within 24 h, due either to the fact that pure azadirachtin is not stable in water after 24 h or it is metabolised by the snail within 24 h. However, this time-dependent effect can be stable up to 96 h if used along with other chemical components of the neem[40].

In a study conducted by Singh and Singh[41], they found that a combination of *A. indica* oil with *L. inermis* seed powder and *Cedrus deodara* oil was more toxic for *L. acuminata* snail than their individual components. Following this declaration, Singh and Singh[42] reported the effect of sublethal treatment (20% and 60% of LC_{50} /24 h) of some plant-derived molluscicides, including *A. indica* oil, on the reproduction of snail *L. acuminata*. They observed that the combination of plant derived molluscicide and their active molluscicidal components caused a significant reduction in fecundity, hatchability, and survival of young snails. Withdrawal of snails to fresh water after the above treatment caused a significant recovery in the fecundity of *L. acuminata*. All these findings, in addition to ours, suggest that neem oil can be used as a good molluscicide that is less expensive and less hazardous to the environment than synthetic products.

Conclusion: Aiming to develop new and safe candidates with molluscidal and schistosomicidal activities, and hence promotion of schistosomiasis control in Egypt, the present work showed that oil extracts of *N. sativa*, *P. graveolens* and *A. indica* can be considered as potent mollusccidal agents against *B. truncatus* snail as well as effective agents against miracidia and cercariae of *S. haematobium*. Further studies are recommended.

Authors contribution: Aminou HA designed the plan of work, performed the practical part, analyzed the data, wrote, and revised the manuscript. Mossalem HS performed the histopathological study of snails. Alam-Eldin Y shared in designing the plan of work, analyzing the data, writing, and revising the manuscript. The manuscript has been read and approved by all named authors. We further confirm that the order of authors listed has been approved by all of us.

Conflicts of interest: We confirm the absence of known conflicts of interest associated with this publication.
Financial support: There was no financial support for this work that could have influenced its outcome.

REFERENCES

1. Fenwick A, Jourdan P. Schistosomiasis elimination by 2020 or 2030. Int J Parasitol 2016; 46(7): 385-388.
2. Bergquist NR, Colley DG. Schistosomiasis vaccines, research to development. Parasitol Today 1998; 14: 99-104.
3. El-Baz MA, Morsy M, EL-Bandary M, Motaweae SM. Clinical and parasitological studies on the efficacy of

Mirazid in treatment of scistosomiasis *haematobium* in Tatoon, Esta center, El-Fayoum Governorate. J Egypt Soc Parasitol 2003; 33: 761–776.

4. Haggag AA, Rabiee A, Abd Elaziz KM, Gabrielli AF, Abdelhai R, Hashish A, *et al*. Elimination of schistosomiasis haematobia as a public health problem in five governorates in Upper Egypt. Acta Tropica 2018; 188: 9-15.
5. Singh SK, Yadav RP, Singh A. Molluscicides from some common medicinal plants of Eastern Uttar Pradesh, India. J Appl Toxicol 2010; 30(1): 1-7.
6. Perrett S, Whitfild PJ. Currently available molluscicides. Parasitol Today 1996; 12: 156-159.
7. Abd El-Ghany AM, Salama A, Abd El-Ghany NM, Gharieb RMA. New approach for controlling snail host of *Schistosoma mansoni*, *Biomphalaria alexandrina* with cyanobacterial strains-derived c-phycocyanin. Vector Borne Zoonotic Dis 2018; 18(9): 464-468.
8. Ibrahim AM, El-Emam MA, El-Dafrawy SM, Mossalem HS. Impact of certain plant species on *Schistosoma mansoni-Biomphalaria alexandrina* system. Proceeding 3rd International Conference Science 2004; 3: 390- 413.
9. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res 2003; 17(4): 299–305.
10. Harzallah HJ, Noumi E, Bekir K, Bakhrouf A, Mahjoub. Chemical composition, antibacterial and antifungal properties of Tunisian *Nigella sativa* fixed oil. Afr J Microbiol Res 2012; 6(22): 4675–4679.
11. Van der Walt JJA, Vorster PJ. *Pelargonium*s of Southern Africa, Natl Botanical Garden, Cape Town, South Africa 1988; 3: 54.
12. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol 2000; 88: 308–316.
13. Walters DS, Craig R, Mumma RO. Effects of mite resistance mechanisms of geraniums on mortality and behavior of foxglove aphid (*Acyrthosiphon solani*: Kaltenbach). J Chem Ecol 1990; 16: 877–886.
14. Kayser O, Kolodziej H. Antibacterial activity of extracts, constituents of *Pelargonium sidoides* and *Pelargonium reniforme*. Planta Med 1997; 63: 508–510.
15. Kharwar RN, Sharma VK, Mishra A, Kumar J, Singh DK, Verma SK, *et al*. Harnessing the phytotherapeutic treasure troves of the ancient medicinal plant *Azadirachta indica* (neem) and associated endophytic microorganisms. Planta Med 2020; 86(13-14): 906-940.
16. Mohammad AA. Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. Hindawi Publishing Corporation Evidence-Based Complementary and Alternative Medicine 2016, Article ID 7382506, 11 pages.
17. Eissa MM, El Bardicy S, Tadros M. Bioactivity of miltefosine against aquatic stages of *Schistosoma mansoni*, *Schistosoma haematobium* and their snail hosts, supported by scanning electron microscopy. Parasites and Vectors 2011; 4: 73-83.
18. World Health Organization. Molluscicide screening and evaluation. Bull World Health Organ 1965; 33:567-581.
19. El-Khayat HMM, Metwally KM, Abououf NA, El-Menyawy HM. Histopathological and ultrastructural studies on *Biomphalaria alexandrina* snails infected with *S. mansoni* miracidia and treated with plant extracts. Egypt J Hosp Med 2018; 71(3): 2792-804.
20. Bancroft JD, Stevens A. Theory and practice of histological techniques" 3rd ed. Edinburgh: Churchill Livingstone 1996: 766.
21. World Health Organization. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. Expert Committee World Health Organization Technical Report Series 2002.
22. Kiros G, Erko B, Giday M1, Mekonnen Y. Laboratory assessment of molluscicidal and cercariacidal effects of *Glinus lotoides* fruits. BMC Res Notes 2014; 7: 220.
23. Abdulelah H, Zainal-Abidin B. *In vivo* anti-malarial tests of *Nigella sativa* (Black Seed) different extracts. Am J Pharmacol Toxicol 2007; 2(2): 46–50.
24. Mahmoud MR, El-Abhar HS, Saleh S. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J Ethnopharmacol 2002; 79:1–11
25. Issa RM. Using *Nigella sativa* (Habbet El baraka) in treatment of some parasitic diseases. Egypt J Med Sci 2003; 24: 435–446.
26. Yousef AAH, EI-Kassas NB. Ultrastructure and histopathological effects of some plant extracts on digestive gland of *Biomphalaria alexandrina* and *Bulinus truncatus*. J Basic Appl Zool 2013; 66(2): 27-33.
27. Birrie H, Balcha F, Erko B, Bezuneh A, Gemeda N. Investigation into the cercariacidal and miracidiacidal properties of Endod (*Phytolacca dodecandra*) berries (Type 44). East Afr Med J 1998; 75: 311-314.
28. De-Carvalho RR, Maldonado AJr, Oliveira-Filho EC, Ribeiro AC, Paumgartten FJR, Rey L. Effects of *Euphorbia millilátex* on *Schistosoma mansoni* eggs, miracidia and cercariae. Mem Inst Oswaldo Cruz 1998; 93: 235-237.
29. Mohamed AM, Metwally NM, Mahmoud SS. Sativa seeds against *Schistosoma mansoni* different stages. Mem Inst Oswaldo Cruz 2005; 100(2): 205–211.
30. Abo-Zeid K, Shohayeb M. Evaluation of the biocidal activity of alkaloids, saponins and volatile oil extracted from *Nigella sativa* seeds against miracidia and cercariae of *Schistosoma mansoni*. Intern J Pharmac Sci Invent 2015; 4(1): 47-54.
31. El-Tantawy SAM, Mansour MFA, Fala HM. Molluscicidal potency of four plant extracts on three pulmonate snails (Gastropoda) in Egypt. Proc. 7th Int Con Biol Sci (Zool) 2012: 236 – 242.
32. Al-Sayed E, Abdel Hamid H, Hanaa M, Abu El Einin HM, Ali E, Fahmy Z. Antiprotozoal and molluscicidal activities of *Pelargonium graveolens*. Int J Pharm 2014a; 4(4): 209-214
33. Al-Sayed E, Abdel Hamid H, Abu El Einin HM. Molluscicidal and antischistosomal activities of methanol extracts and isolated compounds from *Eucalyptus globulus* and *Melaleuca styphelioides*. Pharm Biol 2014b; 52(6): 698-705.
34. El-Sherei MM, El-Sakhawy FS, Kaseem HA, Meselhy KM, Sleem AA. Bioactive phenolics from *Pelargonium*

denticulatum cultivated in Egypt. Egypt J Biomed Sci 2004; 15: 285-300.

35. Hauer H, Germer S, Elsasser J, Ritter R. Benzopyranones and their sulfate esters from *Pelargonium sidoides*. Planta Medica 2010; 76(4): 350-352.
36. Bauer H, Treutter D. Identification of *Pelargonium* genotypes by phenolic fingerprint, separation and identification of phenolic compounds in leaves. Gartenbauwissenschaft J 1990; 55(3): 113-118.
37. Marston A, Hostettmann K. Review article number 6: Plant molluscicides. Phytochemistry 1985; 24(4): 639-652.
38. Ayoub SMH, Yankov LK. The molluscicidal factor of tannin-bearing plants. Intern J Crude Drug Res 1986; 24: 16-18.
39. Singh DK, Agarwal RA. Correlation of the anticholinesterase and molluscicidal activity of the latex of *Euphorbia royleana* on *Lymnaea acuminata*. J Nat prod 1984; 47: 702-705.
40. Singh K, Singh A, Singh DK. Molluscicidal activity of neem (*Azadirachta indica*, A. Juss). J Ethnopharmacol 1996; 52: 35-40.
41. Singh A, Singh DK. Molluscicidal activity of *Lawsonia inermis* and its binary and tertiary combinations with other plant derived molluscicides. Indian J Exp Biol 2001; 39(3): 263-8.
42. Singh K1, Singh DK. Effect of different combinations of MGK-264 or piperonyl butoxide with plant-derived molluscicides on snail reproduction. Arch Environ Contam Toxicol 2000; 38(2): 182-90.