Evaluation of molluscicidal activity and toxicity of some common medicinal plant and chemical synthetic agents against Biomphalaria alexandrina, the snail vector of Schistosoma mansoni infection

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Abstract- This study aims to evaluate and compare the toxicity and molluscicidal efficacy of three monoterpene oils (linalool, thymol, and eugenol) and artemether, plant derivative agents and atrazine, roundup and miltefosine, chemical synthetic agents against freshwater snails *Biomphalaria alexandrina*; intermediate host of *Schistosoma mansoni*. Following WHO guidelines, the sublethal (LC_0, LC_{10} and LC_{25}) and lethal (LC_{50} and LC_{90}) concentrations of tested materials were estimated after 24 hours exposure followed by another of recovery. The recorded results illustrated that all the tested agents had molluscicidal properties and a toxic effect against the examined snails. The most potent molluscicidal agent was atrazine expressed by LC_{50} of 1.31 ppm and LC_{90} of 4.07 ppm followed by roundup with LC_{50} 3.21 ppm and LC_{90} 12.61ppm, then miltefosine with LC_{50} 14.66 ppm and LC90 24.06 ppm, then artemether with LC_{50} 18.30 ppm and LC90 28.40 . Furthermore, the recorded results showed that the molluscicidal activity of the tested agents was arranged according to their lethal concentrations against *B. alexandrina* snails as the following: atrazine > roundup > miltefosine > artemether > thymol > eugenol > linalool. This study highlights plant derivative agents as safe natural molluscicides instead of harmful toxic chemical synthetic agents on non-target organisms and even man. It is recommended that more research studies should be carried out to assess the impact of linalool, thymol, eugenol and artemether in terms of biological, biochemical and molecular parameters of snails as well as their efficacy as schistosomicidal agents against *S. mansoni* worms.

Keywords – Plant molluscicides, Chemical molluscicides, *Schistosoma mansoni* control

I. INTRODUCTION

Fresh water *Biomphalaria alexandrina* snails have a great medical importance as vectors for *Schistosoma mansoni*. *S. mansoni* is a significant etiological agent of intestinal human schistosomiasis [1].Snail's control is represented one of the most effective method for schistosomiasis control and the best available mean for elimination a link in *Schistosoma* transmission cycle [2]. Generally, the use of molluscicides in snails` control has received particular attention due to the reduction of incidence and prevalence of schistosomiasis [3]. Economic and environmental concerns are increasingly linked to the use of molluscicides that are selectively effective, environmentally friendly, cheap and readily accessible in the affected areas (Bakry et al.,2017) [3].

The use of chemical synthetic molluscicides is hampered by their expensive cost, their toxicity for human and other non-target living organisms. Additionally, they may evoke harmful biological effects on the environment beyond those for which they were originally manufactured [2,3]. In view of these disadvantages, particular renewed interest has been directed to plant molluscicides that are inexpensive, safe, biodegradable, environmentally acceptable agents, readily available and simply applicable in the affected areas especially in the developing countries [4,5]. Molluscicidal activity has been evaluated for more than 1000 plant species [3]. Monoterpenes are a group of biogenic compounds that found in many different higher order plants and give them their unique odorous properties [6]. Monoterpenes have structures which derived from the coupling of two isoprenoid units that are made from isopentylpyrophosphatea. These compounds are usually aromatic oils which are frequently found in perfumes and other cosmetics and are often used as food additives and therapeutic medications (Abdelgaleil,, 2010) as well as they used as a natural pesticidal and molluscicidal agents [6, 7,8]. Furthermore, artemether is a methyl ether derivative of artemisinin that was extracted from the leaves of the Chinese wormwood plant (*Artemisia annua)* [4]. It possesses a broad spectrum of activities against all species of malaria [9]. Additionally, it has been shown that artemether was confirmed to have a molluscicidal efficacy against intermediate host of *S. mansoni* snails as the result of its bad effect on physiological and biological activity of treated snails [2, 4]. Also, artemether exhibited anti-schistosomal properties against the larval stages and adult worms of human schistosomal species [7, 10].

Based on the mentioned background, the present work aims to assess the molluscicidal efficacy of three monoterpene oils (linalool, thymol and eugenol) and artemether, natural plant molluscicide products in comparison with the molluscicidal activity of two common pesticides; roundup (glyphosate) and atrazine and miltefosine (hexadecylphosphocholine), chemical synthetic molluscicides against *B. alexandrina* snails. Atrazine and roundup are used as herbicides in agricultural areas and in non-agricultural situations to combat weeds and grasses [11]. Whereas miltefosine is a membrane-active drug that was developed as oral medication to treat various parasite species such as different forms of leishmaniasis, *Trypanosoma*, *E. histolytica* and *Schistosoma*. Moreover, it used as anticancer agent [11, 12, 13].

II. MATERIALS AND METHODS

2.1 Experimental snails–

Uninfected adult *B. alexandrina* snails (8-10 mm in diameter) were brought from Schistosomiasis Biological Supply Centre (SBSC) at Medical Malacology Laboratory, Theodor Bilharz Research (Giza, Egypt). Snails were reared in dechlorinated water and fed daily with fresh lettuce leaves (0.015 g/day/snail) plus dried flakes (TetraMin, Hanover, Germany). Snails were maintained under standard laboratory conditions [14].

2.2. Experimental materials–

1- The monoterpene oils (linalool, thymol and eugenol) were purchased from Sigma Chemical Company, USA.
2-Artemether is an antimalarial agent was obtained in tablet form (Kunming Pharmaceutical Cooperation, PR China) with a documented purity of 99.6%.
3- Miltefosine (100 mg) was provided by (Sigma-Aldrich Chemie) and (GmbH, CA 58066-85-6, MW 407.57, Germany).
4- Roundup (glyphosate concentration 120 g/l in the form of glyphosateisopropylamine salt 162 g/l). Roundup pesticide was used in the liquid commercial form was supplied by Monsanto Company (St. Louis, MO, USA).
5- Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 97.8% purity) was purchased from Cluzeau Info Labo (Ste Foy La Grande, France).

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2.3. Estimation of molluscicidal activity of experimental materials:

The efficacy of the tested materials as molluscicidal agents against adult *B. alexandrina* snails was determined according to WHO (1965) [15]. A stock solution of 1000 ppm from each material (artemether, miltefosine, roundup and atrazine) was prepared according to its active ingredient (concentration/volume). For linalool, thymol and eugenol oils, stock solution (1:1) of each oil was prepared by measuring out 1 ml of each oil and emulsified with Tween-80 of about 0.003 ml or 3 drops from a needle tip [16]. The emulsified oil is then added up to 1liter of dechlorinated water to form stock solution (1000 ppm). A series of concentrations expressed in terms of part per million (ppm) were prepared from each stock solution that would permit the computation of experimental concentrations LC_{50} and LC_{90} [17]. The LC_0 was estimated as 1/10 of the LC_{50} value [14] (WHO, 1965). Mortality rates were recorded and SPSS was used to computer program under windows. This experiment was carried out by preparing three replicates of gradual concentrations from each stock solution. 30 snails / each replicate were used. The snails were exposed to the tested concentrations for 24 hours, then removed from the experimental concentration, washed with dechlorinated water and they kept in 1liter of dechlorinated tap water for next 24 hours for recovery (25 ± 2^oC).

2.4. *Statistical analysis*

Analysis of the obtained data statistically analyzed by using the Statistical Package for Social Science (SPSS version 15 package software). Each experiment was replicated three time and the recorded values were expressed as means ± S.E. The examined parameters of the treated and control snails were compared by student "t" test [18]. Statistically significant differences between groups were considered to have a p value of < 0.05.

III. EXPERIMENT AND RESULT

The molluscicidal property of natural tested materials against adult *B. alexandrina* snails after 24 h of exposure followed by another of recovery is presented in Table 1. The recorded data illustrated that artemether had the most toxic effect comparable with studied monoterpene oils, i.e. the least lethal concentration, LC_{50} (18.30ppm) and LC_{90} (28.40ppm) values were recorded for artemether with slope function value of 1.24. While, the highest LC_{50} and LC_{90} were 36.00ppm and 56 ppm, respectively with slope function value of 1.49 was recorded for linalool. Furthermore, it was noticed that the molluscicidal efficacy of natural studied materials had the following decreasing order according to their lethal and sublethal concentrations: artemether > thymol > eugenol > linalool.

Table -1 Experiment Result

Experimental material	LC_0 ppm	LC_{10} ppm	LC_{25} Ppm	LC_{50} ppm	LC_{90} ppm	Slope function
Linalool	4.00[a]	22.00[a]	28.00[a]	36.00[a]	56.00[a]	1.49[a]
Thymol	2.10[b]	9.45[b]	15.94[b]	21.90[b]	34.06[b]	1.65[b]
Eugenol	2.55[c]	12.05[c]	19.88[c]	26.77[c]	48.02[c]	2.05[c]
Artemether	1.83[b]	5.64[d]	10.50[d]	18.30[d]	28.40[d]	1.24[d]

Values means with the same letter for each parameters are not significantly different, otherwise they do ($P<0.05$).

Table 1 show the sublethal and lethal concentrations of natural experimental agents to illustrate their molluscicidal activity, where artemether has the lowest sublethal and lethal concentrations comparable with monoterpene agents.

These recoded results are in agreement with the results of Mossalem et al. (2013) [4] that cleared the efficacy of artemether as a toxic molluscicidal agent against *B. alexandrina* snails with LC_{50} of 21.06 ppm and LC_{90} of 32.69 ppm after 24 hours exposure. Similarly, after the same time period of exposure, Osman et al. (2019) [2] verified that LC_{50} and LC_{90} of artemether on *B. alexandrina* snails were 16.88 ppm and 27.97 ppm, respectively.They attributed the molluscicidal toxicity of artemether against snails due to immunological alterations that represented by negative effect on hemocytes, i.e. artemether evoked their degenerative changes and fragmentation. This distribution in snails' tissues induced by artemether that may deteriorate the biological and physiological activities of exposed snails. Several authors also reported that monoterpenes oils included eugenol, linalool and thymol had acute toxicity on schistosomiasis and fascioliasis vector snails [6, 19, 20]. Moreover, the results of El-Din (2006) [19] showed that thymol was the potent monoterpene oil against *B. alexandrina* snails at least LC_{50} (22 ppm) and LC_{90} (34 ppm) followed by eugenol (LC_{50} of 28 ppm and LC_{90} of 48 ppm) then linalool (LC_{50} of 34 ppm and LC_{90} of 56 ppm). Similarly, Hamdi et al. (2008) [6] showed that according to lethal concentrations (LC_{50} and LC_{90}), thymol was the most effective monoterpene followed by eugenol, then linalool against *Bulinus truncatus* (intermediate hosts of *S. haematobium*) and other aquatic snail species (*Planorbis planorbis*, *Helisoma dur*, *Physa acuta*, *Cleoparta bulimoides* and *Melanoides tuberculate*) that naturally associated with *B. truncatus*. Thymol exhibited high molluscicidal properties against *Schistosoma*, *Fasciola* snails and other harmful terrestrial snails [21,22] Monoterpene oils have molluscicidal activity as the result of their negative effect on the snails' physiology and biochemistry activities [6, 19, 23]. Additionally, it was proved that monoterpene oils (thymol, eugenol and linalool) evoked an inhibitory effect in the level of some enzymes such as transaminases activity in the haemolymph and tissues of exposed *Schistosoma* vector snails [6]. Also, the studies showed that monoterpene oils had other useful activities such as eugenol was larvicidal; antimicrobial antioxidant and anti-inflammatory against harmful snails [23]. Thymol had anti-inflammatory, fungicidal and bactericidal activities [24] , furthermore, it was often used as a rapidly decaying, toxic pesticide [25].

Regarding the chemical synthetic agents, the data obtained in Table 2 clearly indicated that atrazine was the most potent synthetic material with the lowest sublethal and lethal concentrations against *B. alexandrina* snails followed by roundup then miltefosine. Stastically significant differences were recorded ($P < 0.001$). In comparison with the molluscicidal effect of the studied plant origin agents, the results detected that chemical synthetic agents were more toxic on *B. alexandrina* snails than the natural agents. The present study demonstrated that all the studied tested materials had a molluscicidal effect against intermediate host of *S. mansoni*, based on the standardized WHO method (1965) [15] , the median lethal concentration (LC_{50}) must not exceed 100 ppm for any molluscicidal material. The lowest LC_{50} was 1.31ppm for atrazine, whereas the highest value was 36.00 ppm for monoterpene oil linalool. According to the recorded values of sublethal and lethal concentrations in the present work, this order was noticed: atrazine > roundup > miltefosine > artemether > thymol > eugenol > linalool.

Table -2 Experiment Result

Experimental material	**LC_0 ppm**	**LC_{10} ppm**	**LC_{25} Ppm**	**LC_{50} ppm**	**LC_{90} ppm**	**Slope function**
Miltefosine	1.48^a	3.55^a	8.21^a	14.66^a	24.05^a	1.45^a
Roundup	0.64^b	0.88^b	2.03^b	3.21^b	12.61^b	2.40^b
Atrazine	0.21^c	0.35^c	3.19^c	1.31^c	4.07^c	2.21^c

Values means with the same letter for each parameters are not significantly different, otherwise they do ($P< 0.05$).

Table 2 show the sublethal and lethal concentrations of chemical synthetic experimental agents to illustrate their molluscicidal activity, where atrazine was most potent molluscicidal agent.

The recorded results agree with Barky *et al*. (2012) [11] who detected that atrazine was more efficient molluscicidal agent to freshwater snails than roundup. They reported a considerable killing effect of pesticides; roundup and atrazine to *B. alexandrina* snails with LC_{50} of 3.15 and 1.25 ppm, and LC 90 of 12.60 and 4.75 ppm, respectively. The authors determined also the sublethal dose LC_{10} (0.33 ppm for roundup and 0.84 ppm for atrazine). Whereas, the study of Omran and Salama (2013) [26] showed that the sublethal doses LC_{10} were 4.02 and 10.1 ppm for roundup and atrazine, respectively. Their recorded lethal doses LC_{50} values were 41.6 and 101.16 ppm, respectively on *B. alexandrina* after 24 hours of exposure followed by another of recovery. The toxicity of these herbicides against *Biomphalaria* snails is genotoxic leading to DNA damage which resulted from disturbance of antioxidant defensive system by increasing of free radical life span in exposed snails [27]. In addition to genotoxicity effect of roundup and atrazine, Bakry *et al*. (2012) [11]attributed their molluscicidal activity against *B. alexandrina* as the result of their adverse effects on metabolism and reproduction of snails. Furthermore, the results of Omran and Salama (2013) [26] proved that both of roundup and atrazine are considered endocrine disrupters due to their efficacy to alter the endocrine functions and cause cellular toxicity and histological alterations of *B. alexandrina* snails including deformation of sperms and oocytes. Regarding with molluscicidal effect of miltefosine against *B. alexandrina*, Eissa *et al* (2011) [11] proved that miltefosine is a potential promising molluscicidal compound against *S. mansoni* vector, *B. alexandrina* (LC_{50} of 7.5 ppm and LC_{90} of 9.9 ppm) and against *S. haematobium* vector, *B. truncates* snails (LC_{50} of 2.6 ppm and LC90 of 4.2 ppm). Meanwhile, the lethal concentrations of miltefosine against *B. alexandrina* were 13.80 ppm and 24.40 ppm for LC_{50} and LC_{90} as recorded by Osman *et al*. (2019) [2]. Miltefosine induced considerable morphological alterations of *B. alexandrina* such as extensive damage of the tentacles and the cilia at the foot as observed by scanning electron microscopy of exposed snails (Eissa *et al*., 2011a). Additionally, its toxicity as molluscicidal agent resulted from its negative effect on immunological aspects including fragmentation and degenerative of haemocytes [2].

Despite the efficacy of atrazine, roundup and miltefosine as the most potent molluscicidal agents comparable with plant natural molluscicidal agents linalool, thymol, eugenol and artemether against the examined snails, these synthetic materials may occasionally cause sublethal effects in various living organisms. So, the application of these synthetic products should be limited on their use as authorized molluscicides in the control program of snail vectors of schistosomiasis due to their biocidal effects on plants and animals, besides, their ecological toxicity, genotoxicity and carcinogenic impact on non-target organisms and even man, in addition to their expansive cost especially in developing countries [20]. Atrazine and roundup have the ability to accumulate in tissues of fish, phytoplankton and other aquatic invertebrates due to their physical, chemical properties and high water solubility as well as their extensive usage as grasses and weeds pesticides in agricultural applications and in non- agricultural situations [11]. Their bioaccumulation caused in turn harmful toxic effects for aquatic ecosystems through influencing on the reproduction of aquatic flora and fauna and interfering in the food chains of many species which in turn impacts on the community structure as a whole as documented by several researches [11, 28,29]. Roundup as a synthetic pesticide had an inhibitory effect on reproduction and development of arthropods, molluscan snails, fish, amphibians and plants by evoking biochemical, physiological and immunological alterations (Kielak et al., 2011). Similarly, the studies detected that atrazine induced serious environmental and human health risks and caused toxic effects to aquatic species [29]. It is an endocrine deactivate agent that deteriorates reproductive capacity of humans and animals [30]. Atrazine induced a decrease in oocyte meiotic maturation *in vitro* [31]. Furthermore, it altered the growth, behavior, reproduction and survival of algal, aquatic plants, invertebrates, zooplankton and fish. In addition to, it reduced cellular metabolism and deteriorate the antioxidant activity of aquatic organisms [29, 32]. As regards to toxic effect of miltefosine on non-target organisms, the results of Eissa et al. [12] demonstrated that the molluscicidal activity of miltefosine as a synthetic molluscicide agent had a broad biocide activity that might make it inappropriate for snail control. Additionally, the previous studies [33,34] showed that miltefosine is teratogenic agent and its action in treatment as anti-leishmanial drug was associated with severe side effects. Generally, human exposure to chemical synthetic pesticides or other synthetic agents poses a higher health risk than exposure to genetically modified foods [35]. So, the use of natural alternatives is represented a good solution that can reduce the side effects of impact of chemical synthetic molluscicides.

IV.CONCLUSION

The present study provides an information on the efficiency of artemether and monoterpene oils (linalool, thymol, and eugenol) as natural molluscicidal agents against *S. mansoni* snails. These tested agents were recommend as promising and safe natural molluscicide agents, besides they can be effectively implemented using simple techniques acceptable for developing countries. These were also mentioned as possible eco-friend molluscicides and alternatives to the currently available expensive and environmentally destructive molluscicides (atrazine, roundup and miltefosine). The obtained results could be useful to the National *S. mansoni* control programs and the public health service, therefore further research studies are warranted to evaluate the efficacy of artemether and linalool, thymol and eugenol on biological, biochemical and molecular parameters of snails as well as their effects on *S. mansoni* worms as schistosomicidal agents. Also, from the results of the present study, it showed that *B. alexandrina* snails may be used as a bioindicator of water pollution and pesticides toxicity in the surrounding water.

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