Laboratory Evaluation of the Effect of Egyptian Native Plants Against Some Parasitic Vectors

Mısır’dağı Yerli Bitkilerin Bazı Parazitik Vektörlere Karşı Olan Etkisinin Laboratuvara Değerlendirilmesi

Eman Taher¹, Narmeen Mahmoud¹, Maha Mahmoud²

¹Clinic of Parasitology, Research Institute of Ophthalmology, Giza, Egypt
²Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt

ABSTRACT

Objective: Four plant extracts possessing molluscicidal and insecticidal efficacy were evaluated under laboratory conditions versus Biomphalaria alexandrina, Lymnea cailliaudi snails, their egg masses and Culex pipiens larvae. These extracts included Grape seed, Eucalyptus, Pomegranate, Verbesina alcoholic extracts, as well as Eucalyptus oil.

Methods: Different mortalities in the exposed vectors were recorded due to the four plant extracts using different concentrations and exposure time.

Results: Total snail mortality LC100 was (100 ppm/12-24h) for Grape seed, (200 ppm/18-24h) for Eucalyptus, (100 ppm/12-18h) for Pomegranate, (100-200 ppm/24h) for Verbesina alcoholic extracts and (100-200 ppm/12h) for Eucalyptus oil. However, only Eucalyptus, Verbesina alcoholic extracts and Eucalyptus oil revealed snail ovicidal effects. LC100 was (100-200 ppm/24h), (100-200 ppm/24h) & (100-200 ppm/12-48h) respectively. Moreover, the same plant extracts were able to induce total Culex pipiens larvicidal mortality, LC100 was (200 ppm/48h). However, Grape seed and Pomegranate alcoholic extracts did not induce either snail ovicidal or Culex pipiens larvicidal total mortalities. Activities of the studied plant extracts were considered using reference molluscicidal (Copper sulfate) and insecticidal (Temephos) substances.

Conclusion: Egyptian native plants continue to provide a wealth of potential sources for biologically active agents that may have a promising role in the production of safe, biodegradable eco-friendly and natural molluscicidal and insecticidal agents.

Key Words: Molluscicides, insecticides, grape seed, eucalyptus, pomegranate, Verbesina, Biomphalaria alexandrina, Lymnea cailliaudi, Culex pipiens

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ÖZET

Amaç: Mollosidal ve insektisidal etki gösteren dört bitki ekstratı laboratuvar koşulları altında Biomphalaria alexandrina, Lymnea cailliaudi salyangozlardan, bunların yumurtaları ve Culex pipiens’in larvalarına karşı olan etkilerini açığa vurmak için değerlendirilmiştir. Bu ekstraktlar, üzüm tohumu, ökaliptus, nar ve Verbesina’nın alkollü ekstratları ve ökaliptus yağdır

Yöntemler: Farklı konsantrasyonlar ve farklı sürelerde bu bitki ekstratlarına maruz bırakılan vektörlerde farklı ölüm oranları elde edilmiştir. Özellikle bitki ekstratları ve référence mollosikal ve insektisidal uygulamalar (Copper sulfate ve Temephos) nüanslandığı düşünülmiştir.

Bulgular: Salyangozlar için toàn âm lượng LC100 (100 ppm/12-24 saat) üzüm tohumu için (100 ppm/12-18 saat), ökaliptus için (200 ppm/18-24 saat), nar için (100 ppm/12-18 saat), Verbesina için (100-200 ppm/24 saat) ve ökaliptus yağ için (100-200 ppm/12 saat) olarak belirlenmiştir. Ancak sadece ökaliptus (LC100=100-200 ppm/24 saat) Verbesina LC100=100-200 ppm/24 saat) ve ökaliptus yağ (LC100=100-200 ppm/12 saat) salyangoz yumurtalarının (%98) ölü olarak belirlenmiştir. Bundan başka bazı bitki ekstratları C. pipiens larvalarına %99 oranında etki göstermiştir. Bu, bitki ekstratları ve référence üretech uygulamaların etkisini belirlemek için hazırlıkta.
INTRODUCTION

Phytomedicine has been used to treat parasitism. Moreover, many modern commercial medicines are derived from plants. However, scientific evidence on the anti-parasitic efficacy of most plant products are limited (1). The use of natural products of plant origin (botanical derivatives) is a recent alternative approach for mosquito and snail control. In spite their toxicity to pests and snails, they are readily biodegradable and usually lack toxicity to higher animals’ which means that they are eco-friend-ly (2).

Culex pipiens mosquito, Biomphalaria alexandrina and Lymnea cailliaudi snails are major pests of medical importance having different roles in transmitting many diseases (3).

Mosquitoes can transmit dreadful and fatal diseases to more than 700 million people, such as malaria, dengue, yellow fever, and filariasis (4). In various areas of the world, mosquitoes have been found to become resistant to several of the biological and conventional chemical insecticides used for their control (5).

Schistosomiasis is an important disease in Egypt and other tropical countries (6). It is considered as the world's most wide-spread parasitic disease (7). More than 207 million people are infected worldwide (8). A sure way to tackle the problem of diseases transmitted through snails as intermediate hosts is to destroy the carrier snails and remove an essential link in the life-cycle by using molluscsid (9). This type of control appears feasible and cost effective especially in poor countries (10). However, synthetic organic molluscsidcs are toxic to non-target animals and may have long term effects on the aquatic environment (9).

In this respect, the present work is an attempt to evaluate molluscsidal and or insecticidal properties of Grape seed extract, Pomegranate, Verbesina, Eucalyptus alcoholic extracts and Eucalyptus oil for their effect on B. alexandrina (vector of Schistosoma mansoni), L. cailliaudi (vector of Fasciola gigantica) snails and their egg masses as well as on larvae of Culex pipiens.

METHODS

Plant material

Grape seeds: the fruit of Vitis vinifera, Eucalyptus, Pomegranate the fruit of Punica granatum L., and Verbesina alternifolia, were collected from different areas from Egypt, during 2010. Plants were washed with tap water and dried at room temperature. The air-dried powdered parts were exhaustively extracted with 90% ethanol by maceration. The total alcoholic extract was combined and evaporated under reduced pressure by vacuum distillation at a temperature not exceeding 40°C to yield a semi-solid residue. Essential oil extract was obtained by hydrodistillation for 2 hours using a Clevenger-type apparatus using the methods of Cetin and Yanikoglu (11). Investigation of the prepared oils was carried out on an Agilent (USA) GC-MS system. The extracts and oil were concentrated and stored in dark glass tubes under refrigeration at (4°C) until evaluation (12).

Tested Snails & their Egg Masses

Biomphalaria alexandrina and Lymnea cailliaudi snails (5-6 mm) were collected from irrigation canals in Giza Governorate. Snails were identified according to Christensen and Frandson (13). They were screened for natural infection with any trematodes. Uninfected snails that didn’t harbor any trematoda parthenaen were maintained in the laboratory conditions in fresh de-chlori-nated tap water fed with fresh lettuce leaves with daily cleaning for at least 7 days before using in the experiment. Snails were prevented from crawling out of the solution by means of a fine mesh placed above the water surface.

Snail aquaria were supported by cellophane sheet for egg de-po-sition. Freshly laid, 3-5 days old B. alexandrina and Lymnea cailliaudi egg masses were collected. Number of sound active embryos and their stage of development were identified micro-scopically per each snail egg mass.

Tested Mosquito

The vector mosquito, Culex pipiens, was used as test organism. Mosquito eggs were obtained from The Medical Research Institute of Insects. Eggs were soaked in de-chlorinated and fil-tered tap water to develop into first instar larvae. The larvae reared after this in the same aquarium till development to the third larval stage.

Molluscsidal and Larvical bioassay

The bioassay was done according to WHO (14) guidelines with slight modifications. Trematoda free; medium sized; active B. alexandrina, L. cailliaudi snails and their egg masses as well as late third instar larvae of C. pipiens; 30 in number for each group; were picked up from the aquaria and allocated in three 250 mL jars for screening the efficacy of the tested plant extracts.

Initially, 2 mL of each plant distillate was dissolved in 100 mL distilled water containing 0.3% Tween 80 to make 1% stock solu-tion. Immersion technique was adopted according to WHO (14). Efficacy of each tested plant was evaluated using series of upgraded concentrations (400, 200, 100, 50, 25 and 12.5 p.p.m) each for (3, 6, 12, 24, 36, and 48) hours exposure time. Each experiment set contained three replicates. Control group was run simultaneously for each plant extract with the used solvent material in tap de-chlorinated water. All experiments were con-ducted simultaneously in the laboratory at room temperature (26±2°C).

At the end of the exposure time, tested solution was removed; snails, egg masses and larvae were washed for five times using
Molluscicidal and larvicidal activities of four plant extracts included Grape seed, Eucalyptus, Pomegranate, as well as Verbesina, were tested at upgraded concentrations ranging from (12.5) to (400) p.p.m detecting LC$_{50}$ and LC$_{100}$ against Biomphalaria alexandrina; Lymnea cailliaudi snails and their egg masses as well as Culex pipiens larvae. These extracts included Grape seed extract, Pomegranate alcoholic extract, Verbesina alcoholic extract, Eucalyptus alcoholic extract as well as, Eucalyptus oil. Copper sulphate and Temephos were included as reference molluscicidal and insecticidal substances (17, 18).

The tested plant extracts and oil proved to have molluscicidal activity but some of them had snail ovicidal as well as larvicidal activity versus C. pipiens larvae. Generally, there was a direct relationship between the efficacy and increasing the concentration and exposure time.

Results of the present study cleared that Grape seed extract was able to kill 50% of the exposed snails (LC$_{50}$) at a concentration of (100 ppm/6-12 h). These concentrations didn’t show killing ability versus snail egg masses. In the same time total mortalities of the exposed snails (LC$_{100}$) were achieved using the same previous concentration after increasing the exposure time to 12-24h.

This extract showed weak effect versus C. pipiens larvae, mortality didn’t increase over 50% after increasing the concentration more than (200 ppm for 48h).

Eucalyptus alcoholic extract induced 50% mortalities in the exposed snails and its egg masses at (100 ppm for 12-24h). Total mortality of L. cailliaudi snails and their egg masses was achieved at concentration of (100 ppm) and at (200 ppm) for B. alexandrina snails and their egg masses after 24h exposure time.

C. pipiens larvae showed more resistance, as they were able to tolerate higher concentrations of this material more than snails as LC$_{50}$ & LC$_{100}$ of this material reached up to (200 ppm after 24-48h) exposure time respectively.

The present study recorded rapid effect by Pomegranate alcoholic extract versus L. cailliaudi and B. alexandrina snails as 50% mortality was noticed at (100 ppm) after 3-6 hours exposure. On the contrary 50% mortalities (LC$_{50}$) for L. cailliaudi and B. alexandrina egg masses was recorded at (100-200 ppm) after 24h exposure. However, it was 200 ppm for C. pipiens larvae after 48h exposure. In the same time Pomegranate alcoholic extract couldn’t induce total mortalities neither for both snails’ egg masses nor for C. pipiens larvae.

Total mortalities (LC$_{100}$) of L. cailliaudi and its egg masses was revealed after using 100 ppm after 24h exposure of Verbesina alcoholic extract, higher concentration (200 ppm) for the same exposure time was required to get complete mortality for B. alexandrina snail and their egg masses. While 200 ppm after 48h was required for achieving total mortality of C. pipiens larvae.

The present study determined 100 ppm/12h as LC$_{100}$ for Eucalyptus oil versus L. cailliaudi snails and its egg masses. While 200 ppm was required for the LC$_{100}$ versus B. alexandrina, after 12h. The concentration of (200 ppm/48h) was considered as LC$_{100}$ of the oil versus B. alexandrina egg masses as well as C. pipiens.

It is worth to be mentioned that the molluscicidal activity of the studied plant extracts was higher than the molluscicidal activity of the reference material (copper sulfate) were its LC$_{50}$ was (5-10 ppm) after 6-12 h exposure time and its LC$_{100}$ reached up to (10-20 ppm) after 24 h. Moreover, the study recorded LC$_{50}$ and LC$_{100}$ of the reference insecticide (Temephos) as (0.5) after 24-48 h exposure time.

No mortalities were recorded in the control non exposed snails in water or in the used alcoholic solvent in each case. Results are presented in the Table 1.

**DISCUSSION**

Different snails and mosquitos’ species are of the most important vectors transmitting diseases all over the world; that constitute health problems and remaining as leading causes of mortality (19). Development of molluscicidal and insecticidal substances of botanical origin may serve as suitable alternatives to synthetic ones (17). Although several plants from different families have been reported for their molluscicidal and insecticidal activities, only few botanicals have moved from the laboratory to field use (20). In Egypt, screening of local plants for molluscicidal & mosquito larvicidal activities had received increasing attention (21). This was also previously reported by (22, 23).

Grape seed, Eucalyptus, Pomegranate, Verbesina alcoholic extracts, as well as Eucalyptus oil were evaluated in this study. The obtained results (LC$_{100}$) of the tested insecticidal activity of Eucalyptus was closely related to previous studies reported the larvicidal activity of Eucalyptus essential oil and others (24). It was also found that, essential oils of medicinal plants showed different mosquitocidal activities (25). Other studies also recorded that Eucalyptus alcoholic extract had molluscicidal activity (26). However, the present study revealed that Eucalyptus efficacy was less than that of Grape seed and Pomegranate alcoholic extracts. Moreover, C. pipiens larvae resisted its effect. Eucalyptus possesses insect and limited vermin control properties together with repellent properties. Eucalyptus alkaloids exhibited chemosterilant effect in addition to larvicidal and growth inhibition properties (27).

However, Verbesina induced 100% mortality after 48h with higher concentration than that was recorded by other studies (28). Medicinal uses of Verbesina alternifolia appeared to be limited and not widely documented although it was reported that it has an anti-inflammatory action. The major phyto-constituents are terpenoids, flavonoids and aromatic compounds (29). Poisoning
caused by Verbesina may be corresponded to high levels of nitrates and galegine (30).

The present study revealed that Grape seed and Pomegranate alcoholic extracts had potent molluscidal activity. They caused significant behavioral changes in B. alexandrina and L. cailliaudi with the most obvious sign of distress being muscular and spiral twisting of the body, followed by crawling on one another. The nature and rapid onset of these behavioral responses shows the extracts probably contains neurotoxins, which amongst other things, might be active at the neuromuscular system of the exposed animals. Some of these characters were previously described (31).

In the author’s opinion and in agreement with previous studies, Grape seed extract proved molluscidal effect could be accepted as it possesses a wide array of pharmacological and biochemical actions (32). This includes anti-inflammatory, anticarcinogenic activities. Moreover, it inhibits enzymes involved in the formation of histamine which might be attributed to its rich content of Proanthocyanidins.

Phytochemistry and pharmacological activities of Pomegranate (Punica granatum) were previously described (33). Medicinal properties are; antioxidant,anticancer, anti-inflammatory, antibacterial/antimicrobial and anthelmintic (34). Moreover, it was reported that Pomegranate extract gave very promising results as an anti-trichomoniiasis vulgaris (35). Pomegranate contains high levels of phytochemicals including polyphenols, sugars, fatty acids, aromatic compounds, amino acids, tocochromers, sterols, terpenoids and alkaloids (36).

Generally, mortality to the plant distillates increased as concentration and exposure time increased (37). This could be due to that uptake of active moiety is time dependant, leading to progressive increase of entrance of the volatile oil and its effect in the snail body (20). Molluscidic affect snails through inhibition of its respiratory enzymes either by direct contact or affecting the metabolic activities of snails (38). They act on different enzymes mainly those of respiration and carbohydrate metabolism. Inhibition of acetylcholinesterase enzyme, increasing its concentration at the synapses, leading to paralysis and eventually death (21). The adverse effect of these plant extracts can be accepted through such a mode of action. This was proved by appearance of L. cailliaudi as more sensitive than B. alexandrina snails.

This might be attributed to several factors; as L. cailliaudi has a wide aperture and large flappy body that needs more oxygen for the vital processes. As the majority of fresh water snails took their oxygen requirements throughout cutaneous pseudo bronchial and via pulmonary respiration. This is accompanied by high oxygen consumption level in comparison with B. alexandrina which has small snail body easily contracted inside snail with narrow aperture that protect its soft part of the body which is easily affected by the surrounding solution in comparison with the lower part of the body (snail foot) (21). Moreover, sensitivity of L. cailliaudi egg masses in comparison with that of B. alexandrina may be related to that L. cailliaudi egg is large in size with thin transparent ootheca in comparison with that of B. alexandrina which is small in size with thick ootheca (21).

It is worth to be mentioned that bioactivity of plant extracts and essential oils is affected by the plant species, cultivation conditions, plant storage, plant preparation and methods of extraction (39).

No behavioral symptoms or death occurred in control groups, indicating that no factors other than plant moieties were responsible for the altered behavior and mortality.

Table 1. Molluscidal &insecticidal effect of plant extracts

<table>
<thead>
<tr>
<th>No.</th>
<th>Tested Material</th>
<th>LC50</th>
<th></th>
<th>LC100</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. alexandrina</td>
<td>L. cailliaudi</td>
<td>C. pipiens</td>
<td>B. alexandrina</td>
</tr>
<tr>
<td></td>
<td></td>
<td>snails</td>
<td>Egg masses</td>
<td>snail</td>
<td>Egg masses</td>
</tr>
<tr>
<td>1.</td>
<td>Grape Seed Extract</td>
<td>100ppm/12h</td>
<td>-ve</td>
<td>100ppm/6h</td>
<td>-ve</td>
</tr>
<tr>
<td>2.</td>
<td>Eucalyptus Alcoholic Extract</td>
<td>100ppm/12h</td>
<td>100ppm/24h</td>
<td>100ppm/12h</td>
<td>100ppm/24h</td>
</tr>
<tr>
<td>3.</td>
<td>Pomegranate Alcoholic Extract</td>
<td>100ppm/6h</td>
<td>200ppm/24h</td>
<td>100ppm/3h</td>
<td>100ppm/24h</td>
</tr>
<tr>
<td>4.</td>
<td>Verbesina Alcoholic Extract</td>
<td>200ppm/12h</td>
<td>-ve</td>
<td>100ppm/12h</td>
<td>100ppm/24h</td>
</tr>
<tr>
<td>5.</td>
<td>Eucalyptus oil</td>
<td>100ppm/12h</td>
<td>200ppm/24h</td>
<td>50ppm/24h</td>
<td>50ppm/12h</td>
</tr>
<tr>
<td>6.</td>
<td>Reference Molluscicide (Cu Sulphate)</td>
<td>100ppm/12h</td>
<td>100ppm/8h</td>
<td>5ppm/6h</td>
<td>10ppm/6h</td>
</tr>
<tr>
<td>7.</td>
<td>Reference Insecticide</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* In the same time no mortalities was recorded in control non exposed snails in water or in the used alcoholic solvent in each case
The present study revealed that all of the tested plant extracts had molluscicidal effect as well as snail ovicidal and C. pipiens larvicidal effect of some of them with high LC$_{50}$ and LC$_{100}$ in comparison with reference molluscicide (Copper Sulphate) and insecticide (Temephos) substances. This might be explained as the identified characters of the examined plant extracts were evaluated (on the level of the present study) as crude non-selectively concentrated extracts while the used reference substances were more purified. So free using of commercially purified, extraction of botanical molluscicidal and insecticidal active component, is considered to be a new field of preparation of safe, rapidly biodegradable and eco-friendly alternative to chemical ones (22, 40).

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**Conflict of Interest**

No conflict of interest was declared by the authors.

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