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

Mısır'daki Yerli Bitkilerin Bazı Parazitik Vektörlere Karşı Olan Etkisinin Laboratuvarda Değerlendirilmesi

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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 LC_{100} 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. LC_{100} 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, LC_{100} 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.

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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* salyangoz türleri, bunların yumurtaları ve *Culex pipiens*'in larvalarına karşı olan etkinlikleri açısından değerlendirilmiştir. Bu ekstraktlar, üzüm tohumu, ökaliptus, nar ve *Verbesina*'nın alkol ekstraktları ve ökaliptus yağıdır

Yöntemler: Farklı konsantrasyonlar ve farklı sürelerde bu bitki ekstraktlarına maruz bırakılan vektörlerde farklı ölüm oranları elde edilmiştir.

Bulgular: Salyangozlardaki toplam ölüm oranları (LC_{100}) üzüm tohumu için 100 ppm/12-24saat, ökaliptus için 200 ppm/18-24saat, nar için 100 ppm/12-18saat, Verbesina için 100-200 ppm/24saat ve ökaliptüs yağı için 100-200 ppm/12saat olarak belirlenmiştir. Ancak sadece ökaliptus (LC_{100}=100-200 ppm/24saat) ve Verbesina LC_{100}=100-200 ppm/24saat) alkol ekstraktları ile ökaliptus yağı (LC_{100}=100-200 ppm/12-48saat) salyangoz yumurtaları üzerine öldürücü etki göstermiştir. Bundan başka bazı bitki ekstraktları *C. pipiens* larvaları üzerine olan öldürücü etkiyi indüklemiştir LC_{100}=200 ppm/48saat). Bununla birlikte, üzüm tohumu ve nar alkol ekstratkları ne salyangoz yumurtaları üzerine ne de *C. pipi-*

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ens larvaları üzerine etkili olmamıştır. Çalışılan bitki ekstraktlarının aktiviteleri referans mollusid (bakır sülfat) ve insektisit (Temefos) maddelere benzer olarak değerlendirilmiştir.
Sonuç: Mısır'daki yerli bitkilerin biyolojik olarak aktif vektörler için zengin potansiyel kaynaklar olduğu, güvenli, biyolojik olarak yıkılabilir, çevreye dost ve doğal mollusid ve insektisisidal ajanlar üretilebileceği görülmüştür. *(Turkiye Parazitol Derg 2012; 36: 160-5)*

Anahtar Sözcükler: *Mollusid*, insektisit, üzüm tahomu, ökaliptus, nar, *Verbesina, Biomphalaria alexandrina, Lymnea cailliaudi, Culex pipiens*

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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-friendly (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 widespread 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 lifecycle by using molluscicide (9). This type of control appears feasible and cost effective especially in poor countries (10). However, synthetic organic molluscicides 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 molluscicidal 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 *Vistis 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 parthenata were maintained in the laboratory conditions in fresh de-chlorinated 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 deposition. 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 microscopically 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 filtered tap water to develop into first instar larvae. The larvae reared after this in the same aquarium till development to the third larval stage.

Moulliscicidal and Larvicidal 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 solution. 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 conducted 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

de-chlorinated tap water, kept in a new aquarium for 24 hours post exposure. Mortality was checked using crushing technique (5% sodium hydroxide solution) as described by WHO (15). Dose of plant extract in part per million (p.p.m) that killed 50% of the exposed stages (LC_{50}) and the dose that killed all of the exposed stages (LC_{100}); were calculated according to Kovendan et al. (16) using the following equation.

$$\text{Percentage mortality} = \frac{\text{Number of dead snails (larvae)}}{\text{Number of exposed snails (larvae)}} \times 100$$

RESULTS

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 moulliscicidal and insecticidal substances (17, 18).

The tested plant extracts and oil proved to have moulliscicidal 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. piepiens* 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 *Verbisina* 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 200ppm 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 moulliscicidal activity of the studied plant extracts was higher than the moulliscicdal 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 mo squitoes' 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

Table 1. Molluscicidal &insecticidal effect of plant extracts

No.	Tested Material	LC_{50}					LC_{100}				
		B. alexandrina		*L. cailliaudi*		*C. pipiens*	*B. alexandrina*		*L. cailliaudi*		*C.pipiens*
		snails	Egg masses	snail	Egg masses	Larvae	snails	Egg masses	snails	Egg masses	Larvae
1.	*Grape Seed* Extract	100ppm/ 12h	-ve	100ppm/ 6h	-ve	200ppm/ 48h	100ppm/ 24h	-ve	100ppm/ 12h	-ve	-ve
2.	*Eucalyptus Alcoholic* Extract	100ppm 12h	100ppm 24h	100ppm 12h	100ppm 12h	200ppm/ 24h	200ppm/ 18h	200ppm/ 24h	100ppm 24h	100ppm 24h	200ppm/ 48h
3.	*Pomegranate Alcoholic* Extract	100ppm/ 6h	200ppm/ 24h	100ppm/ 3h	100ppm/ 24h	200ppm/ 48h	100ppm/ 18h	-ve	100ppm/ 12h	-ve	-ve
4.	*Verbesina Alcoholic* Extract	200ppm/ 12h	-ve	100ppm/ 12h	100ppm/ 24h	200ppm/ 12h	200ppm/ 24h	200ppm/ 24h	100ppm/ 24h	100ppm/ 24h	200ppm/ 48h
5.	*Eucalyptus oil*	100ppm/ 12h	200ppm/ 24h	50ppm/ 24h	50ppm/ 12h	200ppm/ 24h	200ppm/ 12h	200ppm/ 48h	100ppm/ 12h	100ppm/ 12h	200ppm/ 48h
6.	Reference Molluiscicide (Cu Sulphate)	10ppm/ 12h	10ppm/ 8h	5ppm/ 6h	10ppm/ 6h	-ve	20ppm/ 24h	20ppm/ 24h	10ppm/ 24h	10ppm/ 24h	-ve
7	Reference Insecticide	-	-	-	-	0.5ppm/ 24h	-	-	-	-	0.5 ppm/ 48h

* In the same time no mortalities was recorded in control non exposed snails in water or in the used alcoholic solvent in each case

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 molluscicidal 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 molluscicidal effect could be accepted as it possesses a wide array of pharmacological and biochemical actions (32). This includes anti-inflammatory, anti-carcinogenic 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, anti-bacterial/antimicrobial and anthelmintic (34). Moreover, it was reported that *Pomegranate* extract gave very promising results as an anti-*trichomoniasis virginalis* (35). *Pomegranate* contains high levels of phytochemicals including polyphenols, sugars, fatty acids, aromatic compounds, amino acids, tocopherols, 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).

Molluscicides 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 acetylcholinestrase 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.

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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