



المكافحة البيولوجية للعلق النيلي في المياه باستخدام بعض

المستخلصات النباتية (التباكو ، الرقف) كبديل لكبريتات النحاس

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Biological control of Nile leech in water using some plant extracts

(*Nicotiana tabacum*, *Cyclamen persicum*) as an alternative to

copper sulphate.

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الملخص:

يشارك نبات التبغ *Nicotiana tabacum* ونبات الرقف *Cyclamen persicum* في احتوائهما على مجموعة من المواد المنفرة والقاتلة للعلق النيلي وهي القلويدات Alkaloids صممت هذه التجربة لتقييم مدى إمكانية استخدام جذور نبات الرقف *cyclimn persicium* والتي تتركز فيها القلويدات واوراق نبات التبغ ومقارنتها بإمكانية استخدامها كبديل لكبريتات النحاس المسرطنة، تم تجميع العلق النيلي *Limnatis nilotica* من الحقل وتم تعريضه للمستخلص المائي البارد من كلا النباتين مع تثبيت زمن التعرض للعلق 30min بدء تأثير نبات الرقف عند 90 g/1000ml وتمثلت الاعراض بالشلل وارتخاء محاجم العلق النيلي وعدم قدرتها على الالتصاق وكانت الجرعة القاتلة النصفية 250g/1000ml مع متوسط وقت النفوق 18.41 ± 23.18 دقيقة بينما كانت الجرعة القاتلة 400gm/1000ml مع متوسط وقت النفوق 6.53 ± 19.2 اما بالنسبة لنبات التبغ كانت الجرعة الفعالة 50g/1000ml وكانت الجرعة النصفية القاتلة 80g/1000ml مع متوسط وقت النفوق 3.13 ± 8.4 دقيقة أما الجرعة القاتلة كانت 50g/1000ml مع متوسط وقت النفوق 15.87 ± 14.2 بينما كانت الجرعة الفعالة لكبريتات النحاس 600g/1000ml والجرعة النصفية القاتلة 250 g/1000ml مع متوسط وقت النفوق 2.93 ± 4.67 والجرعة القاتلة LD 600gm/1000ml متوسط وقت النفوق 5.78 ± 27.63 وبالتالي أظهرت فعالية استخدام نبات الرقف ونبات التبغ كبديل لكبريتات النحاس حيث يمكن استخدامها كمنتج دوائي قوي وفعال لمكافحة العلق النيلي.

الكلمات المفتاحية: القلويدات، نبات بخور مريم ، (نبات الرقف)، نكوتينا توباكم، نبات التبغ

Abstract:

The *Nicotiana tabacum*, and *Cyclamen persicum* share a group of substances that are repulsive and fatal to Nile leeches *Limnatis nilotica*, which are alkaloids. This

experiment was designed to evaluate the possibility of using the roots of the *Cyclamen persicum* plant, in which alkaloids are concentrated, and the leaves of the *Nicotiana tabacum* plant, and to compare them to the possibility of using them as an alternative to carcinogenic copper sulphate. Nile leeches collected from the field were exposed to cold water extract from both plants, with the exposure time set at 30 min. The effective dose of the *Cyclamen persicum* cold water extract began at 90g/1000ml, and the symptoms were paralysis and the relaxation of the leech suckers. The LD₅₀ was 250g/1000ml, with an average death time of 23.18±18.41 min, while the lethal dose was 400gm/1000ml, with an average death time of 19.2±6.53min. As for the tobacco plant The effective dose was 50g/1000ml and the lethal dose was 80g/1000ml with a mean time to death of 8.4±3.13 min, while the effective dose for copper sulphate was 100g/1000ml LD₅₀. 372 g/1000ml with an average mortality time of 4.67±2.93 and the (LD) of 600gm/1000ml with an average mortality time of 27.63±5.78.

Thus, the effectiveness of using the aqueous cold extracts of *Nicotiana tabacum* and *Cyclamen persicum* as an alternative to copper sulphate has been shown, as they can be used as a powerful and effective medicinal product to control the Nile leeches at the water surfaces

Keywords: *Limnatis nilotica*. Alkaloids. cold water extract. *Cyclamen persicum*. *Nicotiana tabacum*.

1. INTRODUCTION

Pollution of surface and floating waters and the need to access new sources of water are the biggest problems in developing countries, on which international studies focus. As the population grows and water sources decrease, there is an urgent need for clean water sources. •Monika Hinteregger (2009) Poor water quality and pollution kill 1.7 million people worldwide every year. Death rate due to contaminated water: 3.1 million deaths worldwide (UN-Water 2021.) According to statistics issued by the World Health Organization, 75% of human diseases are due to lack of access to safe water for drinking and sanitary purposes (swimming, bathing, etc. (UN-Water 2021.) Water sources are contaminated with various chemical contaminants such as heavy metals, germs, bacteria and parasites. Leeches are classified as parasitic aquatic pollutants, and there are 650 species of aquatic and terrestrial leeches. Among them are the Nile leeches, *Limnatis nilotic*, which is from the Hirudinea family. Most of this family is characterized by being

predatory and blood-sucking aquatic leeches and is considered a parasite that transmits many diseases, whether to humans or various animals. It also causes the disease Hirudiniasis, and it is called the horse leech. This species lives in the fresh water of springs, lakes and swamps, and it is found in the countries surrounding the Mediterranean basin. (Ichrak Khaled* & Issam Saidi 2024). Nile leeches can cause economic losses to livestock as a result of the leeches infesting them in the respiratory passages of them and of various animals when they drink water contaminated with indigo leeches, which causes their death. (BENAMER and NEGM-ELDIN, 2013) They also work as carriers of some infectious and dangerous diseases for animals and humans (Bahmani M., et.al. 2006). There are many medical reports indicating that Nile leeches are found in all natural orifices of the human body lined with mucous membranes, such as the nose, throat, pharynx, esophagus, urethra, vagina, and anus (Bahmani, BANIHABIB, & AHANGARAN, 2015) Livestock farmers resort to several methods to get rid of this problem. Either they close the water source contaminated with leeches and look for a second source of water, or they continue to treat the infected animals with expensive medications. There are several ways to disinfect water sources. Water can be disinfected from indigo leeches with compounds and chemicals that have a carcinogenic effect on humans and a negative impact on the environment, such as hydrated copper sulphate and other chemicals (BAHMANI, BANIHABIB, & AHANGARAN, 2015). Which is difficult to obtain by livestock breeders, so they resort to folk medicine using various medicinal plants or their active ingredients. Among the most important anthelmintic substances are plant compounds called alkaloids. The alkaloids in some plants have great value in treating some diseases as well as the pharmaceutical industry (Pirbalouti, 2009). There are several reports of human infections with leeches (Rasouli & Bahmani, 2013) and some reports of positive effects. medicinal plants on these parasites. (BAHMANI, BANIHABIB, & AHANGARAN, 2015) In this study, the possibility of using alcoholic extracts as a biological control that is not harmful to animals, humans or the environment was investigated for two plants: *Cyclamen persicum*, which is rich in alkaloids (Sharara, A et al, 2024). It is used in folk medicine as an anthelmintic and is considered a Libyan wild plant, and *Nicotiana tabacum* leaves, which are rich in nicotine. In controlling water sources polluted by Nile leeches and as an alternative to aqueous copper sulphate for disinfecting wastewater from Nile leeches. *Limnatis nilotica* (BAHMANI, BANIHABIB, & AHANGARAN, 2015) Modern scientific research in various fields promotes biological control as an alternative to the use of

chemicals that have side effects that harm the environment, animals, and humans. Leeches are considered one of the harmful parasitic pollutants for humans and animals around the world. Many scientific researches have addressed the use of Aqueous and alcoholic extracts of various plants as an alternative to harmful chemicals. Plants that contain alkaloids were chosen, as they are repellent and repulsive and also lethal to annelid worms, especially the Nile leech, *Limnatis nilotica*. In this research, the *Cyclamen persicum* plant, which is rich in these alkaloids and is considered one of the available Libyan wild plants, will be used and its effectiveness will be compared to copper sulphate and the *tabacum* *Nicotiana* plant, which is considered one of the natural plants approved for eliminating leeches in surfaces of water.

2 Methodology

2.1A *Limnatis nilotica* taxonomy (Savigny, 1822)

Name	<i>Limnatis nilotica</i>
Kingdom	Animalia
Phylum	Annelida
Class	Clitellata
Order	Arhynchobdellida
Family	Hirudinidae
Genus	<i>Limnatis</i>
Species	<i>L. nilotica</i>

2.1B *Nicotiana tabacum* taxonomy (L, 1753)

Name	<i>Nicotiana tabacum</i>
Kingdom	Plantae
Phylum	Spermatophytes
Class	Dicotyledon
Order	Solanales
Family	Solanaceae
Genus	<i>Nicotiana</i>
Species	<i>N. tabacum</i>

2.1C *Cyclamen persicum* taxonomy (L, 1753)

Name	<i>Cyclamen persicum</i>
Kingdom	Plantae

Phylum	Spermatophytes
Class	Dicotyledon
Order	Ericales
Family	Primulaceae
Genus	Cyclamen
Species	C. persicum

2.2 Plant protocol preparation

2.2A cold aqueous extract of *Nicotiana tabacum*

The cold aqueous extract of *Nicotiana tabacum* is prepared according to the method (Harborne. 1973). wash the leaves sufficiently from impurities, then dry them sufficiently at room temperature and grind them with a regular blender. Then 100 grams of the dried powder of the *Nicotiana tabacum* plant are taken and placed in a 1500 ml beaker containing on 1000 ml of cold distilled water, the false magnetic material is removed for 15 minutes, then paper for 24 hours, then the solution is sprayed with Whattman2 type filter papers, and the filtrate is taken and placed in the centrifuge at a speed of 3000 rpm for ten minutes.

2.2.B cold aqueous extract of *Cyclamen persicum*

The cold aqueous extract of *Cyclamen persicum* is prepared according to the method (Harborne. 1973). In the *Cyclamen persicum* plant, the active substance is concentrated in the roots, of the plant wash the leaves sufficiently from impurities, then dry them sufficiently at room temperature and grind them with a regular blender. Then 400 grams of the dried powder of the *Cyclamen persicum* plant are taken and placed in a 1500 ml beaker containing 1000 ml of cold distilled water, Rotate the sample in the magnetic stirrer moved for 15 minutes, then leave for 24 hours, then the solution filterated with Whattman2 type filter papers, and the filtrate is taken and placed in the centrifuge at a speed of 3000 rpm for ten minutes. **2.3**

Analysis of

2.3 preparation of Copper sulfate

600 grams of copper sulphate are taken and placed in a 2000 ml conical flask, dissolved in distilled water, then rotated in a magnetic stirrer for 15 minutes and filtered using filter papers Whattman2

3 Results

A number of deaths were observed within a period of 30 minutes, and the dead leeches was enumerated,, in aqueous cold extracts of plants (aqueous extracts of *Nicotianac tabacum* and *Cyclamen persicum*) and copper sulphate, The affecting

dose of Nile leeches, which are diagnosed with leech paralysis and loosening of the suckers, was calculated in each of the three solutions, and also measure the LD50 and LD process Copper sulphate was the positive control and distilled water was the negative control as in Table No **1 and 2**

treatment	Effective dose gm/1000ml	LD50 gm/1000ml	LD gm/1000ml
DW	0	0	0
Copper sulphate	100	372	600
<i>Nicotiana tabacum</i>	50	80	100
<i>cyclimn persicum</i>	90	250	400

Table 1 shows the effective doses used to control Nile leeches

Compounds	LC50 (Mean±SD)	Mean±SD (min)	LD (Mean±SD)	Mean±SD (min)
cyclimn persicum	3.05±2.52	23.18±18.41	320±37.42	19.2±6.53
Nicotiana tabacum	8.4±3.13	8.4±3.13	176±78.64	14.2±15.87
Copper sulfate	4.66±2.93	4.67±2.93	265±93.67	27.63±5.78
DW	0	0	0	0±30

Table 2 shows the standard deviations of the solutions used

There was a significant difference between the treatment and control groups (P<0.05).

4 DISCUSSION

In this study, the potential use of aqueous cold extracts of plants (aqueous extracts of *Nicotiana tabacum* and *Cyclamen persicum*) and copper sulphate in controlling water polluted by *Limnatis nilotica* was investigated. aqueous cold extracts of plants (aqueous extracts of *Nicotiana tabacum* and *Cyclamen persicum*) and copper sulphate LC50 values were also determined following 30 min exposure. of *Nicotiana tabacum* and *Cyclamen persicum* showed an antileech activity The affecting dose of Nile leeches, which are diagnosed with leech paralysis and loosening of the suckers, was calculated for *Nicotiana tabacum* and *Cyclamen persicum*) and copper sulphate, it was 50-90 100gm/1000ml respectively. LD50 value of *Nicotiana tabacum* and *Cyclamen persicum*) and

copper sulphate is 80-250-372 gm/1000ml respectively at death time of 8.4 ± 3.13 min- 23.18 ± 18.41 min- 4.67 ± 2.93 min respectively

while the LD value for

Nicotianac tabacum and Cyclamen persicum) and copper sulphate was 100-400-600 g/1000 ml respectively with a mean death

time of 14.2 ± 15.87 min- 19.2 ± 6.53 min- 27.63 ± 5.78 min Based on the obtained results,

Nicotianac tabacum and Cyclamen persicum is highly effective on leeches and might be

used for disinfection purposes. And can use them as an alternative to copper sulfate. Which is considered a carcinogen and harmful to animals and the environment

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