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Research Article

Comparison of Disinfection Activities of Nicotine with Copper Sulphate in water Containing *Limnatis nilotica* [1]

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Abstract

In this study, we investigated the potential use of nicotine in controlling water polluted by leeches. The nicotine and copper sulphate LC50 values were also determined following 30 min exposure. The anti parasitic effect of nicotine was also compared with that of copper sulphate as positive control. The anti-leech effect of nicotine was evaluated against L. nilotica in which the number of dead and alive leeches in each utensil was counted for 30 min. The positive control group was copper sulphate and the negative control was distilled water. Our data showed that the LD_{50} value for nicotine was $6/10^3$ ppm with mean death time of 1.25±0.45 min while the LD50 value for copper sulphate was $637/10^2$ ppm with a mean death time of 12.00±3.69 min. Based on the obtained results nicotine is highly effective on leeches and might be used for disinfection purposes.

Keywords: *Disinfection assay, L. nilotica, Nicotine, Copper sulphate, LC_{50}*

Limnatis nilotica Bulunan Suda Dezenfeksiyon Amaçlı Olarak Nikotinin Bakır Sülfat İle Karşılaştırılması

Özet

Bu çalışmada, sülük ile kontamine suda nikotinin kirliliği kontrol altında tutmak amaçlı olarak potansiyel kullanımı araştırılmıştır. Nikotin ve bakır sülfatın LC50 değerleri 30 dakikalık maruz bırakmada hesaplanmıştır. Nikotinin anti-parazitik etkisi control olarak kullanılan bakır sülfat ile karşılaştırılmıştır. Nikotinin anti-parazitik etkisi L. nilotica'ya karşı 30 dakika süresince ölü ve canlı sülüklerin sayılması ile gerçekleştirildi. Pozitif kontrol olarak bakır sülfat ve negatif kontrol için ise distile su kullanıldı. Çalışmanın sonuçları nikotin için LD_{50} değerinin $6/10^3$ ppm, ortalama ölüm zamanının 1.25±0.45 dakika olduğunu, bu değerlerin bakır sülfat için ise sırasıyla $637/10^2$ ppm ve 12.00±3.69 dakika olduğunu gösterdi. Elde edilen sonuçlar nikotinin sülüklere karşı oldukça etkili olduğunu ve dezenfeksiyon maksatlı kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Dezenfeksiyon testi, L. nilotica, Nikotin, Bakır sülfat, LC_{50}*

INTRODUCTION

Contamination of superficial and supernatant water and the need for access to new sources are the biggest problems in developing countries, focused by international studies. With the growth of population and a decline in water supplies, clean water sources are more urgently needed [1]. Poor quality of water, environmental sanitation, and hygiene kill 1.7 million people worldwide annually. The mortality rate due to contaminated water is 3.1 million deaths in the world [2].

According to statistics released by the World Health Organization, 75 percent of diseases of human are due to the lack of access to safe water with hygienic (swimming, bathing, etc.) and drinking purposes [3]. Water sources are contaminated with various chemical pollutants such as heavy metals, germs, bacteria, and parasites. Leeches are parasitic elements of water contamination.

So far, 650 species of aquatic and terrestrial leeches have been identified. Leeches have been found in different parts of the human bodies, such as membranes, conjunctiva,

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nose, larynx, pharynx, esophagus, urethra, vagina, and anus [4]. Parasitic infestation with leeches happens through contaminated water supplies.

Disorders such as anemia, chest pain, coughing, difficulties in swallowing, breathing, fever, vomiting, bloody diarrhea, and vaginal bleeding complications occur with aquatic leeches [5].

There are several ways to disinfect water supplies. Disinfection of water suppliess contaminated with *Giardia lamblia* might be done by electricity flows [6], sunshine [7], gamma rays [8,9], hydrogen peroxide-silver complex and chlorine [10].

A group of medicinal plants or their active ingredients are used to improve or prevent human and animal diseases [11]. One type of plants by-products (nitrogenous) are alkaloids. These compounds are the largest group of plant secondary compounds. Traditional uses of alkaloids by human go back to over 3000 BC. Nowadays, the alkaloids from certain plants have a great value in the treatment of certain diseases as well as pharmaceutical industries [11]. There are several reports on human infections with leeches and some reports exist on the positive effects of medicinal plants on these parasites. Nicotine or 3-(1-Methyl-2-pyrrolidinyl) pyridine, (S)-(-)-Nicotine ($C_{10}H_{14}N_2$) with the following formula is an alkaloid of tobacco plant [12].

In the present study the potential use of nicotine in controlling water supplies polluted by leeches was investigated.

MATERIAL and METHODS

Taxonomy and Species of the Leeches

In this study 30 *L. nilotica* leeches were used. These species have morphological characteristics such as dark-green color surface with yellowish-orange rows and green spots on yellowish-orange dorsal surface [13].

Chemical Components

In this interventional screening study, nicotine (Merk, Germany) was prepared and then tested with copper sulphate (Sahand, Iran) ($CuSO_4$) as positive group.

Evaluation of the anti Annalida Activities

To investigate the effects of treatment, the method of Bahmani et al.[14] was used. The *L. nilotica* was placed in the plastic utensil containing water. Then, nicotine with compactness of $6/10^{+3}$ ppm was added to the utensil. The experiment was carried out in three replicates for each compound. The number of dead and alive leeches in each utensil was counted for 30 min. The positive control group was copper sulphate and the negative control was distilled water. The leeches were considered dead if they did not exhibit any internal or external movement when stimulated with a needle in the needle test [14].

Statistical Analysis

The differences between control and treatment groups were analyzed using one-way ANOVA statistical method by Sigma State 2.0 software.

RESULTS

During the 30 min of screening, the number of alive and dead leeches was enumerated. The results of the leech lethality trial are presented in *Table 1*. The LC_{50} values for nicotine as bioactive component less than the ones of positive (copper sulphate) or negative (distilled water) control groups.

There was a significant difference between the treatment and control groups ($P<0.05$). Normality failed with a median of 1, 11, and 30 for nicotine, $CuSO_4$, and water respectively.

Table 1. *Compounds, LC_{50} and effective dose for L. nilotica*
Tablo. *Maddeler, L. nilotica için LC_{50} ve etki dozu*

Compounds	LC_{50} (Mean±SD)	Dose (ppm)
Nicotine	1.25 ± 0.45^a	$6/10^{+3}$
$CuSO_4$	12.00 ± 3.69^b	$637/10^{+2}$
Distilled water	30 ± 0^c	10^{+2}

DISCUSSION

In this study, the potential use of nicotine in controlling water polluted by leech was investigated. The nicotine and copper sulphate LC_{50} values were also determined following 30 min exposure. Nicotine showed an anti-leech activity with LD_{50} value of $6/10^3$ ppm with a mean death time of 1.25±0.45 min while the LD_{50} value for copper sulphate was $637/10^2$ ppm with a mean death time of 12.00±3.69 min. Based on the obtained results, nicotine is highly effective on leeches and might be used for disinfection purposes. Several studies have investigated the efficacy of chemical and natural anti leech drugs. Considering the importance and frequency of contamination of surface water with leeches, study on the effects of different combinations of drugs is essential. Bahmani et al.[15] reported that garlic methanol extract (*Allium sativum* L.) had the anti immature *L. nilotica* effect. In another study Gholami-Ahangaran et al.[16] reported that *Vitis vinifera* L. and grape methanolic extracts, ivermectin, and niclosamide on *L. nilotica* had anti parasite activity against *L. nilotica*. Eftekhari et al.[17] investigated the anti *L. nilotica* effect of *A. sativum* L. extract and Levamisole on mature *L. nilotica*. Their results demonstrated that garlic methanol extract had a mean dead time of 144.55±57.217 min. In another study the disinfection effects (LC_{50s}) of *Nicotiana tabacum* extract, copper sulphate, and ammonium

chloride on *L. nilotica* were found to be $13/10^4$, $8/10^4$, and $370/10^4$ ppm, respectively. In previous studies, effective and positive effects of grapes, olives, ginger has been demonstrated to leech [18-21].

Nicotine is a highly toxic compound for some animals [12]. Due to the strong effects of nicotine in cleaning water supplies polluted with leech, it could be a natural compound to be used in the treatment of contaminated water supplies. The results of a study showed that LCD_{50} for nicotine was 1.25±0.45 min., which is a reasonable dead time and acceptable for clearing water supplies polluted by leeches. Another study showed that high doses of Harmal methanol extract had no effect on mortality of leeches, which are not consistent with the results of this study [14].

In the present study nicotine had a very good dead time against *L. nilotica*, therefore, it might be beneficial in controlling water supplies polluted with leeches. Although this compound is derived from a plant and the plants are usually safer than synthetic ones, its safety profile should be tested, in the same way as other compounds have been tested [22- 25].

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