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Comparing the Pesticide Effect of Copper Oxide Nanoparticles Synthesized by Green Chemistry and Plant Extracts on *Anopheles Stephensi* Mosquitoes

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Abstract

Objective: According to the importance of Anopheles mosquitoes, in the transmission of malaria and control of disease transmitter is one of the important strategies for the prevention of diseases. This study aimed to produce plant insecticides using blue extracts of oleander plants, tobacco, ferula, and Achilles Mille folium essential oils and copper oxide nanoparticles synthesized by pomegranate peel extract on the Anopheles Stephens mosquitoes to solve the problems by chemical insecticides.

Material and Methods: In this laboratory research, the effect of blue extracts with concentrations (0.66, 1.3, and 2.6) microliters in a milliliter of leaves and stems of oleander, tobacco, ferula, and Achilles Mille folium essential oil on the Anopheles mosquito (Anopheles. Stephens: Bandar Abbas strain) was studied. To optimize these extracts, antibacterial properties of thyme were used to prevent mold growth. In addition, the insecticidal effect of copper oxide nanoparticles synthesized from pomegranate peel extract was considered. To identify nanoparticles synthesized by spectroscopic analysis Vis-UV, scanning electron microscopy (SEM) and X-ray energy diffraction (EDX) was used.

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Copper Oxide Nanoparticles, Anopheles Stephens mosquito, SEM, Oleander, Tobacco, Pomegranate

Results: The results showed that copper oxide nanoparticles of pomegranate extract with antibacterial properties were obtained with LC50 = 0.70. It had a good insecticidal effect on Anopheles Stephens mosquito specimens. But oleander plants with LC50 less than copper Nan oxide showed more lethality.
Conclusion: But since fennel is a poisonous plant and is harmful to non-target organisms and humans, it cannot be a good alternative to plant insecticides. In contrast, copper oxide nanostructures do not harm humans or non-target organisms.

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Introduction

Mosquitoes can transmit the disease more than any other group of arthropods, affecting millions of people worldwide through mosquito-transmissible diseases [1]. Anopheles mosquitoes are the only biological transmitter of malaria. Malaria is an acute or chronic infectious disease of the blood. It's caused by the plasmodium parasite, which in turn infects humans and host insects, and has caused many social and economic losses [2]. Epidemiological studies show that more than 1-1.5 million deaths occur yearly in the world; most of them are children and pregnant women. The disease is prevalent in approximately 100 countries and approximately 2400 million people are at risk. Also, 60% of the eastern Mediterranean region is at risk of malaria. Iran is located in the temperate region of the northern and eastern Mediterranean and with various climates in the endemic area, there is a global map of malaria in the world [3]. Control of mosquitoes is mainly affected by the use of common insecticides [4], but these cases have their special problems, including destructive effects on the environment and the encouragement of pesticide resistance in some of the mosquitoes and adverse health effects on humans such as stomach-ache, vertigo, headache, squint, nausea, eye problems, and skin [5-7].

The approach of more efficient and attractive mosquito control programs is to target the larval stage in their reproduction grounds with larvae [8]. These efforts to develop new materials as mosquito larvae are necessary [9]. In recent years, the use of metal nanoparticles has found many applications in new technology. Copper oxide (CuO) is one of the most important semiconductor metal oxides that can be used as insecticides and pesticide [10]. Many microorganisms, such as Escherichia coli bacteria, Staphylococcus aureus, Pseudomonas aeruginosa, and non-toxic to animal cells [11-13]. Copper oxide nanoparticles are synthesized through various techniques and methods such as sonochemical, sol-gel [14], hydrothermal, and many other chemical methods [15]. Such methods are very expensive and in the process of preparing nanoparticles, harmful, toxic, and very dangerous chemicals are used, which leads to environmental problems [16]. Therefore, Nano chemists are looking for environment-friendly and compatible methods for the preparation of metal nanoparticles. Recently, the green synthesis of oxide nanoparticles from plant extracts has received much attention from researchers [17]. Method of Green synthesis is being developed and compatible with the environment. In this method, the extract is used as a reducing agent and coating for nanoparticles. Al-Mali et al. (2010) reported that the blue extract of dried shade leaves of Euphorbia hirta was used for the synthesis of silver nanoparticles and their antibacterial activity. Various studies have shown the effects of silver, gold, chitosan, and metal oxide nanoparticles on various parasites, including Giardia, Leishmania, Malaria, Toxoplasma, and insect larvae, which have a lethal or growth inhibitory effect. According to the importance of Anopheles mosquitoes in the transmission of malaria and control of vectors as an important strategy for the prevention of this disease, this study aimed to produce plant insecticides using aqueous extracts of oleander, tobacco, yarrow essential oil Anopheles stephensi was performed on mosquitoes to solve problems caused by chemical insecticides.

Pomegranate peel contains phenolic compounds, alginates, proanthocyanidins, complex polysaccharides, flavonoids, and a significant amount of trace elements, which

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have properties of anti-mutagenic, antioxidant, and antimicrobial. Common Achilles (*Achillea millefolium*) or thousand-leafed yarrow is a plant from the genus Achilles. The yarrow is a yearly herbaceous plant with straight stems and branches 25 to 60 cm high that grows in the Caspian Sea and most parts of Iran [10]. The parts used are its flowers and dried leaves. Yarrow contains blue oil, alkaloids, Achilles, tannins, small amounts of vitamin A, blood coagulation factors, a type of heterozygous, phytosterol, and other materials [10].

Alfalfa is a genus of poisonous and evergreen shrubs of the genus Gentianales and Apocynaceae, commonly planted in parks for ornamental purposes. All parts of oleander include leaves, flowers, grain, stems, bark, and roots. Its juice is toxic and contains cardiac glycosides. However, the seeds of the plant have the highest concentrations of these glycosides and are therefore more toxic, so eating a few of them can lead to death. After drying, the toxicity of oleander is maintained. This plant remains poisonous even after boiling. Also, steam and smoke from burning plant foliage contain cardiotoxic glycosides. Fenugreek cardiac glycosides are cardenolides and include (Oleandrin), (Neriin), (Digitoxigenin), (Nerianthin), (Rosagenin), and (Nerianthosidae). The most important cardiac glycoside of oleander probably is Oleandrin. Oleander, leaves have medicinal properties with the necessary precautions [18-21].

Tobacco or herbal tobacco is a broad-leaved plant that grew in North and South America and today is cultivated around the world. The name of tobacco is also given to the dried and cut leaves of this plant. Its dried leaves contain nicotine. The nicotine alkaloid is commonly recognized as a major component of tobacco, but nicotine alone isn't addictive. The reaction between beta-carbolines and nicotine seems to be the cause of most of the addictive properties of tobacco. The harmful effects of tobacco are from thousands of different compounds in its smoke, such as polycyclic aromatic hydrocarbons like benzopyrene, formaldehyde, cadmium, nickel, arsenic, tobacco-specific nitrosamines (TSNAs), phenols, and many others [2, 22, 23].

The highest amount of exploitation of the Badreh plant is done in Semnan, Khorasan, and Tehran provinces. Barijeh contains 95% of chemical and medicinal compounds that are removed from the stalk due to leaching and contains 9.5% of essential oil, 63.5% of resin, and 27% of gum. The essential oil is obtained by water vapor distillation and is light yellow with a pungent odor and contains 85% of terpenoid hydrocarbons such as alpha-phenine (7 to 21%), beta-pinene (45 to 65%), and delta 3-carne (2.5 to 16%). Its therapeutic properties and application include non-irritating, anticonvulsant, gastric pain-relieving, stomach tonic, and superficial wound healing. Industrial uses of Barijeh in preparing a special glue (diamond glue), cosmetics industry [26].

Materials and Methods

Methods

In this project, to make herbal insecticides to control Anopheles stephensi (sample in Bandar Abbas), tobacco, oleander, and ferula were extracted with the help of Soxhlet device. . To extract with the help of this device, first, the stalks and leaves of these plants were washed several times with distilled water to elimination contaminants and dust, so these plants were dried in the darkness for one to two weeks and Eventually their powder in size Twenty meshes were prepared and separately placed in the Soxhlet handle in different times. The average extraction time with this device was 5 hours. Also, yarrow essential oil was extracted by Clevenger and water distillation method and dehumidified by sodium sulfate and the duration of essential oil extraction was 3 hours.

Preparation of copper oxide nanoparticles by pomegranate peel extract

To prepare the oxide nanoparticles, first, the fresh skin of the pomegranate fruit was washed well with distilled water and dried in an oven at 50 ° C for 6 hours. Then, 10 gm. of pomegranate peel powder with a size of 20 meshes was prepared for extraction by the Soxhlet machine. In this method, 2.5 g of copper acetate with a purity of 9.99% was added to 400 ml of distilled water twice and completely dispersed for 5 minutes with the help of a heater stirrer. (CH3COO) 2. H2O] was added. As soon as the first droplets were added, the color of the solution changed from blue to green and brown, indicating the formation of copper oxide nanoparticles.

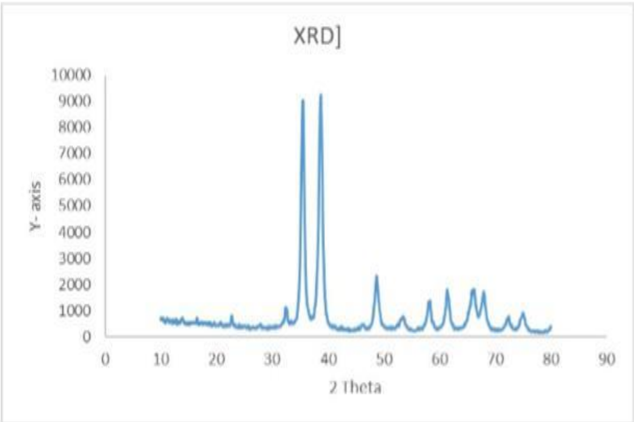

Figure 1. *XRD pattern of nanoparticles synthesized from pomegranate peel extract*

Characterization of Cuo NPs:

Scanning electron microscope SEM the size and morphology of the synthesized nanoparticles were examined using a 15 kV scanning electron microscope with a magnification of 10 x and a resolution of one nm.

EDX Energy- dispersive spectroscopy X-ray

X-ray energy diffraction spectroscopy (X, PERT Pro of Panalytical Company) along with SEM was used to investigate the presence of copper elements in SEM images. The 2θ peaks of 32.22, 36.50, 38.90, 56.56, 61.15, and 74.89 express the crystalline nature of copper oxide nanoparticles and confirm the crystal structure. (Figure 1).

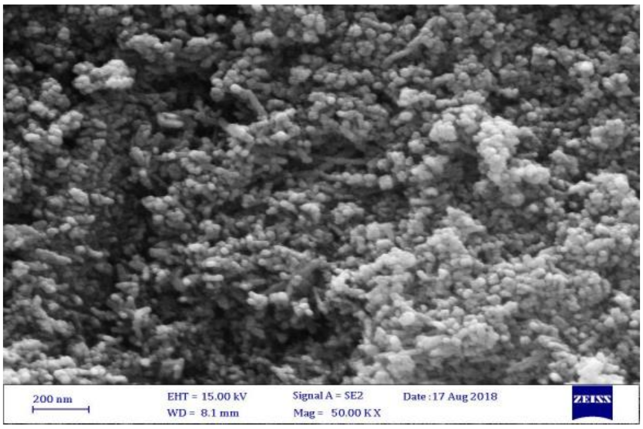

Figure 2. *SEM image of copper oxide nanoparticles synthesized*

Scanning electron microscope (SEM) after confirming the synthesis of nanoparticles using color change and also the absorption spectra of Vis-UV morphology of nanoparticles synthesized by scanning electron microscope) FESEM devise with German company ZEEISS model. According to images were synthesized copper nanoparticles with spherical morphology and the particle size was determined to be between 21 and 32 nm (Figure 2).

Preparation of toxic solutions

Each of the 250 ml compounds was prepared in 400 ml human beings with concentrations of 0.66-1.3 and 2.6 microliters per milliliter. Distilled water was used as a solvent. Anopheles stephensi, the main carrier of malaria in Bandar Abbas, was bred in the Medical Entomology and Carrier Control Laboratory of the College of Health and Public Health Research. To evaluate the toxicity of each of the extracts of fenugreek, tobacco, and oats, and yarrow essential oil and copper oxide nanoparticles, 20 samples of Anopheles mosquitoes were added to the contents of humans containing toxic solutions prepared in different concentrations. A human containing distilled water and 20 Anopheles mosquitoes were also prepared as controls. The experiments were performed at a temperature of 25 degrees Celsius and 50-60% humidity for 24 hours in the darkness and light hours. After 24 hours, the number of dead and living mosquitoes in the container was counted and the percentage of mortality or casualties was calculated.

The calculation of the percentage of death in each sample at the specified concentration is given in the following equation

$$\text{(Mortality)}= \frac{\text{Total number of Anopheles mosquitoes in different concentrations of controles (total number of Anopheles mosquitoes lost)} \times 100}{\text{Total number of Anopheles mosquitoes in different concentrations}}$$

Results

According to Table 1, the highest mortality of Anopheles mosquitoes was related to oleander with a concentration of 2.6µl / ml, and the lowest mortality was related to ferula with a concentration of 0.66 µ l / ml and mortality was 3.75%.

The amount of LC50 is predictable according to the percentage of losses obtained at different concentrations (Table 2).

The mortality rate of oleander, tobacco, and ferula plants Shown in the following diagrams (Figure 3, 4, 5, 6 and 7).

Mor.	Con.
50	0.66
60	1.3
75	2.6

LC_{50}=0.600599

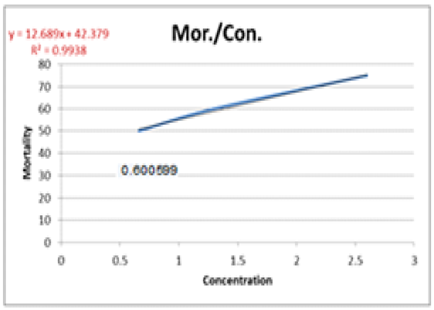

Figure 3. *The mortality rate of oleander toxin*

Mor.	Con
42.5	0.66
55	1.3
70	2.6

LC_{50}=0.6155124

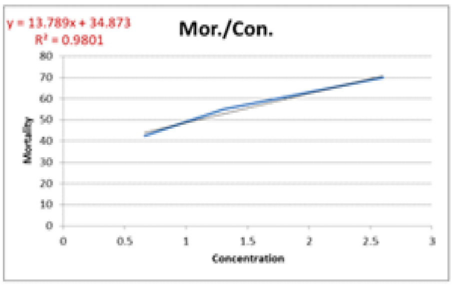

Figure 4. *Mortality percentage by copper oxide nanoparticles toxin concentration*

Mor.	Con
27.5	0.66
40	1.3
45	2.6

LC_{50}=3.03265

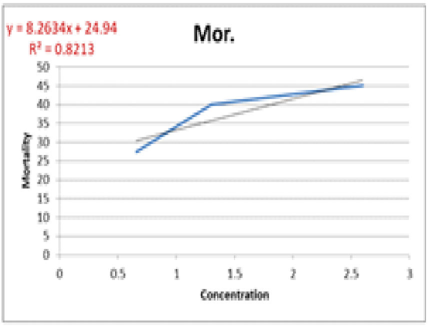

Figure 5. *The mortality rate of tobacco plant toxin concentration*

Mor.	Con.
20	0.66
35	1.3
40	2.6

LC_{50}=3.477973

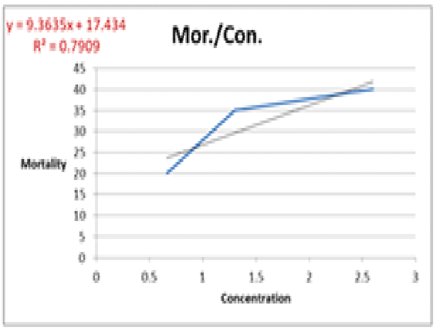

Figure 6. *Mortality percentage by the toxin of Achillea millefolium*

Mor.	Con.
3.75	0.66
15	1.3
25	2.6

LC_{50}=4.905006

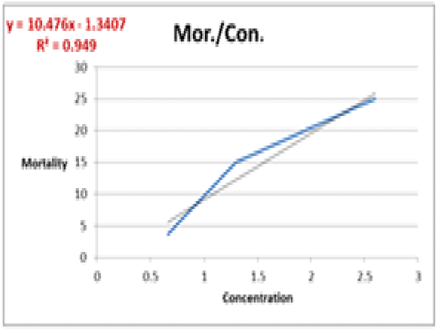

Figure 7. *Mortality percentage of ferula toxin concentration*

Discussion

Nanoscale compounds because of their potential as pest control agents have gained research interest in recent years (24). Among different ways of nano-synthesis using botanical extract to produce metal oxide nanoparticles as stabilized and, reduced agents is one of the most suitable ways due to their various advantages [25]. Green fabrication of nanoparticles compared to other methods is cheaper and quicker. The green synthesis also does not need high pressure and temperature. One of the most important advantages of green synthesis is that they do not require toxic compounds to produce nanoparticles [26]. Different studies show the promising results of using NPs as mosquitos controlling agents. CuO NPs proved their pesticide effect because of effective biocidal features [27, 28]. In table 1, we showed the mortality rate of the Anopheles. Stephensi. We found that among evaluated compounds, oleander extract remove 75% of the mosquitos and ferula showed the lowest level of mortality.

In this study, we tried to evaluate the potential mosquitocidal effect of CuO NPs and compare it to some plants extracts. The LC_{50} of the different compounds is shown in table 2.

Based on the results given in this study oleander had the best results but its toxic nature makes it less available as an applicable agent. Among toxic plants, extract ferula had the lowest level of efficacy (Table 1). Our results are in agreement with previous studies. *Selvan and his colleagues used* Green synthesized copper oxide nanoparticles against Aedes aegypti to evaluate its larvicidal activity and compared it with

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leaf extract of T. procumbens main extract. They showed that CuO NPs had a high level of larvicidal activity [29]. Copper nanoparticles produced by *Wrightia tinctoria* (*Wt*) R.Br extract also showed a good level of larvicidal activity. These particles LC_{50} and LC_{90} for their larvicidal activity against *A. aegypti* were 32.10 μg.mL^{-1} and 21.70 μg.mL^{-1}, respectively [30]. El-Saadony showed the potential of biosynthesized Cu-NPs against grain pest Tribolium castaneum. they showed that biosynthesized Cu-NPs were toxic with an LC50 value of 37 ppm after 5 days of treatment [31]. Malaikozhundan et al. examined the Bt-ZnO-NPs effect against pulse beetle (Callosobruchus maculatus). Bt-ZnO-NPs reduced the fecundity (eggs) laid) and hatchability of C. maculatus in a dose-dependent manner [32].

Based on our results the mortality rate of Anopheles stephensi from exposure to oleander was 75% from a concentration of 2.6μl/ml. compare to our results in the Lokesh study, the larvicidal activity of N. oleander against Culex larvae was 43 from the 30mg/ml after 24-hour treatment [33]. Another study conducted by el-akhal and his collogue showed promising results, the exhibited that Nerium oleander is lethal against Culex pipiens larvae [34]. In agreement with our study, another research showed that 0.1 mg/mL of oleander had 74% of mortality in 72 hours [35]. The molecular mechanism of nanoparticles against mosquito larval is under investigation. Some studies suggested that these particles penetrate the cellular space. After the penetration, the NPS destroy the essential cellular enzyme and organelles, which lead to a cellular loss of function [29, 36, 37].

Different types of green synthesized NPs evaluated for their larvicidal activities. AgNPs from Artemisia vulgaris leaf extracts exhibited a potential level of larvicidal activity. AgNPs could accumulate in the mosquito larvae midgut and create damage in the midgut, cortex area, and epithelial cells. In addition, green synthesized NPs produced from the fruit pulp of *Cassoa* fistula, Nelumbo nucifera, and *Solanum tuberosum showed a great deal of larvicidal activity [38].*

These studies are in complete agreement with our results. However, compare to oleander, green synthesized CuO NPs have shown the slightly same results. Green synthesized CuO NPs are much less toxic for humans and the environment, which makes them a suitable candidate for insecticide purposes.

Conclusion

Green synthesis of copper oxide nanoparticles was successfully performed in a very short time with a high dispersion rate and stability of copper oxide nanoparticles. Copper oxide nanoparticles with antibacterial properties were obtained with LC50 of about 0.70. However, oleander with LC50 was less lethal than copper monoxide.

But since fennel is a poisonous plant and is harmful to non-target organisms and humans, it cannot be a good alternative to plant insecticides. In contrast, copper oxide nanostructures do not harm humans or non-target organisms.

Data/Material availability

Data is available on request from the authors.

Ethical approval

There was no practical experiment on human or animals.

Funding/Support

None.

Conflict of interests

The authors have no conflicts of interest.

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