

Evaluation Of The Efficiency Of Aqueous Extract Of Mentha Piperita Leaves On Menacanthus Stramineus In Vitro

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Abstract

Background: Lice is a widespread external parasite that causes inconvenience to infected birds. This is due to its direct feeding on feathers and skin. Its bites are severe and painful, in addition to its saliva being stimulating and irritating. In the event of a severe infestation, it causes scratches and wounds, especially in cold weather. Body lice, *Menacanthus stramineus*, are a reservoir and transmitter of pathogens, Given the resistance shown by lice to insecticides and the role played by plant extracts in treating many diseases, we decided to conduct this study to test the effectiveness of the aqueous extract of mint leaves on biting lice in vitro.

Materials and Methods: Lice samples were collected from the Indian blue peacock present in some breeding stations in Babil Governorate, and after confirming their diagnosis, equal numbers of lice (8 lice) were placed in each dish, in the amount of four dishes, After preparing the aqueous extract of *Mentha piperita* leaves, three concentrations were prepared. The first plate was treated with a concentration of 5 mg/ml, the second plate was treated with a concentration of 10 mg/ml, the third plate was treated with a concentration of 15 mg/ml, while the fourth plate was left without any addition as a control group. The dishes were monitored during Three different times (10, 20, 30) minutes In order to determine the optimal time for the effectiveness of this substance, with its different concentrations, against lice infesting peacocks, live lice were counted if they showed any form of repetitive movement, either spontaneously or in response to touch with a soft brush.

Results: The results of the examination of 39 samples of peacocks showed that 11 birds were infected with one type of biting lice represented by the genus *Menacanthus stramineus* at a rate of 28.2%. The results of the current study, which included testing the effectiveness of the cold aqueous extract of mint leaves *Mentha piperita* at three different concentrations and for three times against biting lice outside the body of the living organism In Vitro, indicated an increase in the percentage of lice death with increasing concentration and duration of exposure to the substance, as the percentage of death in concentrations of 5, 10 and 15 mg/ml after 30 minutes of treatment reached 75%, 95%, 100% respectively, which represents the highest percentage of lice death compared to the percentage of Lice treated with the same concentrations and shorter periods of time, while the lowest mortality rate was in the group treated after 10 minutes, reaching 55%, 69.5%, 85% respectively. Through these results, the time period (30 minutes) was determined as the best time period for the effectiveness of this substance at its different concentrations. The results of the statistical analysis showed the presence of significant statistical differences at the probability level ($P < 0.05$) between the different groups compared to the control group.

Keyword: Lice; *Pavo cristatus*; *Mentha piperita*; *Menacanthus stramineus*; Iraq.

Date of Submission: 15-11-2024

Date of Acceptance: 25-11-2024

I. Introduction

Lice are common parasites in wild and domestic birds and are especially prevalent among domestic birds Ouarti et al., 2020), and are characterized by being small insects ranging in length from 3-2 mm, generally white to brown in color, wingless, very active, with six legs, a flat body from top to bottom, and mouth parts adapted for chewing (Pickworth and Morishita, 2007), Lice usually feed on feather barbs and skin scales, causing skin irritation and feather damage to infected chickens (Kaufman et al., 2006), and may feed on sweat and mucous secretions, and some species of biting lice feed on eggs and nymphs of the same species (Ford, 2004) and on dried blood collected on the skin at the site of irritation caused by lice (Whiteman and Parker, 2004).

Mentha piperita belonging to the family labia is an important plant, widely spread throughout the world. It is used in folk medicine due to its wide range of biological and pharmaceutical effectiveness. It is known as a tonic for the body's immunity and treats many diseases of the digestive system. It works as a sedative for the central nervous system and a topical analgesic for headaches. It is an antioxidant and antispasmodic for respiratory muscles. It has the ability to lower high blood pressure, and is an antibacterial and antifungal in addition to various therapeutic uses and an insect repellent (Khanna et al., 2014; Ruepert, et al., 2011; Keifer, et al., 2007) As its leaves contain many active ingredients such as menthol and volatile oils such as menthone ,menthyl acetate, menthofuran and cineol. In addition to the presence of small amounts of some other components such as limonene, pulegone, caryophyllene and pinene (Eccles, 1994; Leung, 1980) Given the resistance shown by lice to insecticides and the role played by plant extracts in treating many diseases, we decided to conduct this study to test the effectiveness of the aqueous extract of mint leaves on biting lice outside the living body.

II. Material And Methods

Collecting lice sample

Lice samples were collected from the Indian blue peacock found in some breeding stations in Babylon Governorate, then the lice samples were placed in tubes containing 70% ethyl alcohol with drops of glycerin until the time of permanent loading, and the lice samples were diagnosed based on Soulsby (1982). After diagnosing the lice, other samples of lice of the same genus and species were brought in alcohol-free tubes for the purpose of knowing the effect of the aqueous extract of mint leaves on those samples

Preparing the cold aqueous extract of *Mentha piperita* leaves

The mint leaves were obtained from the local markets in the city of Diwaniyah. The leaves were left for a week at a temperature of 25°C for the purpose of drying them. The leaves were ground after drying using an electric grinder and the powder was stored in tightly sealed glass bottles at a temperature of 4°C until used. Mix 50 grams of mint leaf powder with 500 ml of distilled water and place the mixture on a magnetic stirrer for 24 hours at laboratory temperature. Then use a centrifuge at 3000 rpm for 15 minutes to separate the filtrate from the precipitate. Concentrate the filtrate using a rotary evaporator, then place the filtrate in Petri dishes and leave it at laboratory temperature until it dries. Then store it in the refrigerator (Al-Ani, 1998).

The effect of aqueous extract of *Mentha piperita* leaves on lice *Menacanthus stramineus*

The effect of aqueous extract of *Mentha piperita* leaves was tested using the method of Sioutas et al.(2023), where isolated lice were placed inside plastic dishes and the edges of the dishes were covered with Vaseline to prevent lice from escaping. Before starting the test, the dishes containing the isolated lice were placed inside the refrigerator (20°C) for 15 minutes to limit the movement of lice and make them easier to handle. After that, filter paper (5.5 cm in diameter) was placed at the bottom of plastic Petri dishes (6 cm in diameter) and each paper was sprayed once with either 5 mg/ml or 10 mg/ml or 15 mg/ml of the above-mentioned extract, while the fourth dish was left as a control group. After that, the lice were separated into 4 groups consisting of 8 adults using a dissecting microscope and a soft brush and placed inside Petri dishes. After that, they were sprayed Lice were treated twice using the extract, and the edges of the dishes were covered with Vaseline to prevent lice from escaping. Before closing the lid of each dish, a total of 3 replicates were performed simultaneously for each concentration. After that, the samples were examined to calculate the number of lice that were still alive and the number of deaths under the same microscope used to identify them during different times, in order to determine the optimal time for the effectiveness of this substance at its different concentrations against lice parasitizing the peacock. Live lice were counted if they showed any form of repetitive movement, either spontaneously or in response to touching with a soft brush.

Statistical analysis

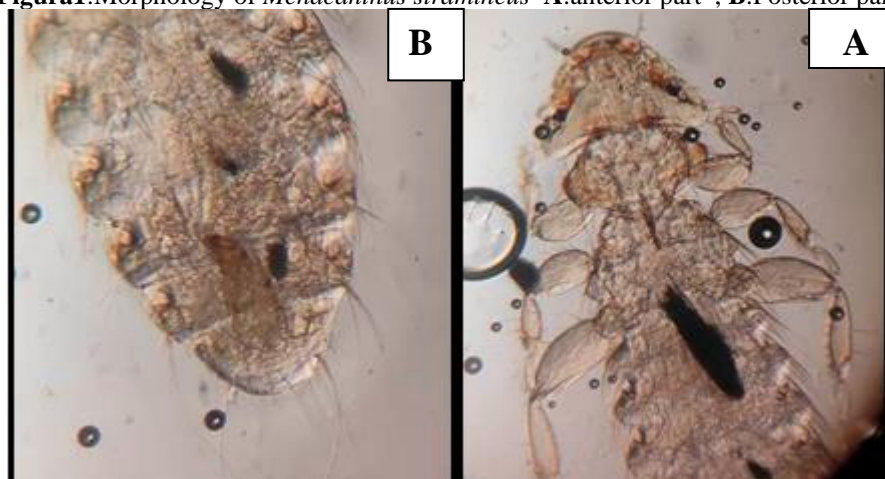
The Chi-square test X² was used to analyze the research results statistically, based on Al-Rawi (2000)

III. Result

During this study, one type of biting lice *Menacanthus stramineus* was isolated in the blue peacock found in some breeding stations in Babylon Governorate in the chest area and under the wings as shown in Figure (1) and the infection rate reached 28.2%. This species prefers to settle in areas that are not dense in feathers and is characterized by its activity and ability to move quickly. The head appears triangular and the front is round and prominent. The antennae consist of 4 segments. The fourth segment appears swollen while the third segment appears small in size. The chest has a triangular shape and a triangular piece appears from its side surrounded by thorns. The abdomen is oval in shape and each abdominal segment is characterized by the

presence of a group of short hairs and three long hairs. The abdominal side of each segment contains 4 groups of short hairs and 3 transverse rows of hairs ,the abdomen also has paired plates.

Figura1:Morphology of *Menacanthus stramineus* A:anterior part , B:Posterior part



The results of the current study, shown in Table (1), indicated significant differences between the control group and the treatments. It was noted that the concentrations (10, 15) mg/ml of *Mentha piperita* leaves extract used to study the effectiveness of this extract against lice led to inhibition and paralysis of lice movement and then their death in different time periods, all of which were shorter than in the case of the control group. It was found that the higher the concentration, the shorter the time period required to paralyze the lice movement and the period of their death. It was found that the effect of the extract concentrations 5,10 15mg/ml after 30 minutes of treatment was 75%, 95%, 100% respectively, which represents the highest percentage of lice death, while the lowest percentage of death was in the treated group after 10 minutes, reaching 55%,69.5%,85% respectively. The percentage of death increased directly with time, as the percentage of death after 20 minutes reached 68.5%. 81.5%, 90% respectively. The results of the statistical analysis showed that there were significant statistical differences at the probability level ($P<0.05$) between the different groups compared to the control group.

Table 1 : The effect of different concentrations aqueous extract of *Mentha piperita* leaves on *Menacanthus stramineus* in Vitro

Concentration	10	20	30
5	55	68.5	75
10	69.5	81	95
15	85	90	100
Control groups	0	0	0

IV. Discussion

During this study, one type of external parasites was isolated, represented by the biting louse of the species *Menacanthus stramineus*, Soulsby (1982) provided an accurate description of this species that matched the description of the diagnostic models in this study. This species is considered one of the most widespread types of lice, as it was isolated by Al-Jubouri (2010) in Diwaniyah at a rate of 66%, and Al-Shabani (2013) recorded it in Diwaniyah at a rate of 26%. Al-Saeed and Al-Badran (2014) were able to isolate it in Dohuk at a rate of 24%, and Al-Shabani (2015) isolated it from turkeys at a rate of 2%. It was also recorded by Al-Ardi (2019) from resident and migratory birds in Al-Dalmaj Marsh at a rate of 19.75% and 37.01%, respectively. This species of louse is also distinguished by its length, which may reach to 3 mm and the presence of feathers on the dorsal plates of the middle and posterior thorax. The reason for the high incidence of this species is attributed to the environment suitable for the life of chicken body lice, as it is found in scattered places on the body, unlike other types, as well as its speed of movement, which enables it to escape the behavior of the bird cleaning its feathers, while Jeffer et al (2005) attributed the reason for the high incidence of this type to the shortness of its life cycle and the large number of eggs laid by the female, which ranges from 50 to 300 eggs.

The current study indicated that the concentrations (5, 10, 15) mg/ml of mint leaf extract used to study the effectiveness of this extract against lice led to inhibition and paralysis of lice movement and then their death in different time periods. It was noted that the higher the concentration, the shorter the time period required to paralyze the lice movement and the period of their death. It was found that the extract at a concentration of 15 mg/ml led to inhibition and paralysis of lice movement and their death after 30 minutes. This may be due to the

fact that this extract contains many active substances such as menthol and volatile oils such as menthol, menthyl acetate, menthofuran and cineol.

In addition to the presence of small amounts of some other components such as limonene, pulegone, caryophyllene and pinene. pinene (Eccles, 1994; Leung, 1980) which came into direct contact with the insect's body, leading to its death in a short period of time, or due to the insect's nervous and digestive systems being affected by touching this extract, or these materials entering through the breathing holes, leading to its suffocation (Halawa, 1998), or it may be explained by the ability of this extract to cause physical disability by adhering to its external structure and limbs, represented mainly by the feet and claws, which severely hindered its movement. Among the factors that helped in this adhesion is the presence of abundant hair on the body of the louse, especially in the abdominal area, which may lead to the blockage of the air breathing holes in that area, which causes suffocation. This study is consistent with what Al-Rahimy & AL-Essa, 2019, reached in their study of the effect of mint extract on *Culex molestus mosquitoes*, as they noted that the aqueous extract of mint caused morphological deformities in mosquito larvae and pupae in addition to the adult insect.

V. Conclusion

Laboratory treatment of lice with the aqueous extract of *Mentha piperita* leaves showed an increase in the rate of lice death by increasing the concentration and duration of exposure to the extract. The time period of 30 minutes is the best time period for the effectiveness of the aqueous extract at its various concentrations. It was also concluded that treatment at a concentration of 15 mg/ml per exposure period 30 is the optimal time and concentration for the effectiveness of mint leaf extract against lice

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