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Evaluation of the efficiency of chitosan nanoparticles in the viability of chewing lice isolated from *Numida meleagris* in vitro

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Abstract

Chewing lice are a widespread external parasite that shows resistance to many of the chemical pesticides used. Therefore, the study aimed to research and investigate the effectiveness of chitosan nanoparticles on the species of lice that infect guinea fowl *(Numida meleagris)* in vitro.

During this study, five species of lice were recorded: *Menacanthus stramineus* , *M. pallidullus* , *M. cornutus* , *Cuclotogaster heterographus* and *Bonomiella columbae*. , with a total infection rate of 80%, as it was isolated from 36 samples of guinea fowl, and no species was recorded in only 9 samples.

The results of treating lice with the three concentrations of 40, 60, and 80 mg/ml of chitosan particles at different time periods (5,10,15,30,60) minutes after treatment showed that the concentration of 80 mg/ml is the most efficient in killing lice at times 15, 30. 60 minutes, as the rate of lice death reached 100% compared to the two concentrations of 40,6 0 mg/ml. This study is considered a new study and for the first time in Iraq, whether at the level of isolating lice samples from guinea fowl or in terms of evaluating the efficiency of chitosan nanoparticles against bird lice.

Keywords: Chitosan ,Nanoparticles, *Numida meleagris*, Iraq**.**

Introduction

Bird breeding in the world is witnessing great and rapid development, accompanied by development and diversity in methods of controlling diseases that affect them, as their meat has become a staple food in many countries of the world, but despite this, their breeding still suffers from serious problems that cause major economic losses as well as Some diseases are linked to public health, and the fluctuations of the seasons, the difference in temperature between night and day, the age of the bird, the quality of food, diseases, and predation are among the main problems facing bird farming (1,2). Deaths also occur frequently as a result of viral, bacterial and fungal diseases, as well as external and internal parasitic infections, they all create a major obstacle to the progress of these livestock (3).

External parasites are treated with anti-parasitic chemical treatments, but over time, resistance to these drugs has arisen due to overuse, while recent years have witnessed tremendous development in the field of nanomedicine to combat parasites, as some nanoparticles have shown promising results in treating various types of parasitic infections (4), where scientists have proven that nanoparticles (NPs) can be used to treat bacterial, viral, fungal, and parasitic diseases (5).

Chitosan is a polysaccharide polymer derived from chitin and produced by removing the acetate portion of it. It is extracted from the shells of crustaceans such as crabs as well as from the cell walls of fungi. It is a biodegradable and biocompatible polymer that is considered safe for human food use and is approved for wound dressing. It has been approved by the US Food and Drug Administration (FDA) for tissue engineering and drug delivery (6) .Recent research indicates its use as an ectoparasiticide as well as a treatment for a group of parasitic protozoa, as(7) indicated in his experiment on *Rhipicephalus* ticks, which included three different concentrations of chitosan nanoparticles, the ability of these particles to reduce the mass of female tick eggs, noting in At the same time, it was possible to use it as an acaricide in livestock, and in another study, Attia et al., (8) indicated that spraying chitosan nanoparticles on pigeons infected with the fly *Pseudolynchia canariensis* in cages for 15 minutes gave a promising result as an anti-parasitic agent as well as healing the wound resulting from the bite insects. Therefore, the current study aimed to evaluate the effectiveness of chitosan nanoparticles against lice in vitro.

Material and Methods

Examining birds and collecting lice samples

For the purpose of isolating lice samples, 45 guinea fowl *Numida meleagris* were examined, the feathers of the *Numida meleagris* included in the study are examined with the naked eye and with the help of a hand lens. The method of Abu al-Hab (9) is used to examine the samples obtained. After that, the lice samples are transferred to %70 ethyl alcohol to preserve them, after which they are placed in a cold potassium hydroxide KOH solution at a concentration of 10% for 24 days. An hour for the purpose of clarification, then washed with distilled water and placed in Xylole for 1-2 minutes, then placed on a glass slide using Canada Balsam, and the slide cover is placed on it and left until it dries to perform the diagnosis and classification process. Lice samples were diagnosed based on a set of diagnostic characteristics, such as the shape of the head, the number of antennal brains, and the number of hairs on the abdomen, which are mentioned in taxonomic keys prepared by several researchers (10-12).

2. Preparation of chitosan nanoparticles

Chitosan nanoparticles were purchased in the form of ready-made oxide from one of the Chinese companies(Nanjing High Technology Nano material) in powder form, with a purity of 99.8% and a size of 10-30nm.The nanocomposite of the chitosan compounds was prepared in the form of a stock solution based on (13) , 2g were dissolved in 100 ml of water, the solution was sterilized with an autoclave, then the solution was mixed using an ultrasonic homogenizer for 15 minutes, then three concentrations of 40,60, 80 mg/ml were prepared and stored in the refrigerator until used in the experiment.

3.Testing the efficiency of chitosan nanoparticle concentrations

To test the effectiveness of chitosan nanoparticles on lice in vitro, 10 Petri dishes were used, and an equal number of lice (8) were placed in each dish for three different times 30, 10, and 5 minutes, dishes 1-3 were treated with a concentration of 40 mg/ml. As for the dishes 4-6 were treated with a concentration of 60 mg/ml, while dishes 7-9 were treated with a concentration of 80 mg/ml, dish 10 were also left without any treatment as a control group.The dishes were observed during the above-mentioned times under a microscope for the purpose of determining the numbers of live and dead lice.

4.Statistical analysis

Completely Randomized Design (CRD) was used in a factorial experiment with two factors (concentrations and time periods) and three replications, and the results were tested according to Least Significance Differences (LSD) and at the probability level P<o.o5 .

Results

During the period between October 2023 and May 2023, 45 guinea fowl were collected, including female samples and male samples. Through laboratory examination, it was found that guinea fowl are infected with five species of lice: M*enacanthus stramineus* , *M. pallidullus* , *M. cornutus* , *Cuclotogaster heterographus* and *Bonomiella columbae*.(Fig.1). The total infection rate reached 80%, with 36 infected samples as in Table (1).

Table (4-1):Numbers and percentages of infection with ectoparasites in guinea fowl .

guinea fowl	Numbers	Percentages %

Number infection	36	80
Number non -infection	9	20
Total number	45	

The results of testing three different concentrations of chitosan nanoparticles in Table (2) showed an increase in the percentage of lice death with increasing concentration and duration of exposure to the chitosan nanoparticles, where the percentage of death in concentrations reached 40, 60, and 80 mg/ml after an hour of treatment 75%, 100%, and 100% respectively, The highest percentage of lice death compared to the percentage of death of lice treated with the same concentrations and for shorter periods of time, while the lowest percentage of death in the treated group was 5 minutes after treatment, as it reached 12.5%, 12.5%, and 50% respectively. The percentage of death increased directly with time, as the percentage of death reached after 10 minutes, reaching 12.5%, 37.5%, 62.5% respectively, followed by the death rate of lice after 15 minutes of treatment, where the death rate reached 50%, 62.5%,100% respectively, followed by the death rate of lice after 30 minutes, which reached 62.5%, 75%, 100% respectively.

Table.2:The effect of different concentrations of chitosan nanoparticles on lice mortality rates in vitro.

Concentration of chitosan nanoparticles mg/ml	**Percentage of lice death % / time period after treatment (minutes**				
	5 min	10 min	15 min	30 min	60 min
40	12.5	12.5	50	62.5	75
60	12.5	37.5	62.5	75	100
80	50	62.5	100	100	100
Control groups	0	0	0	0	0

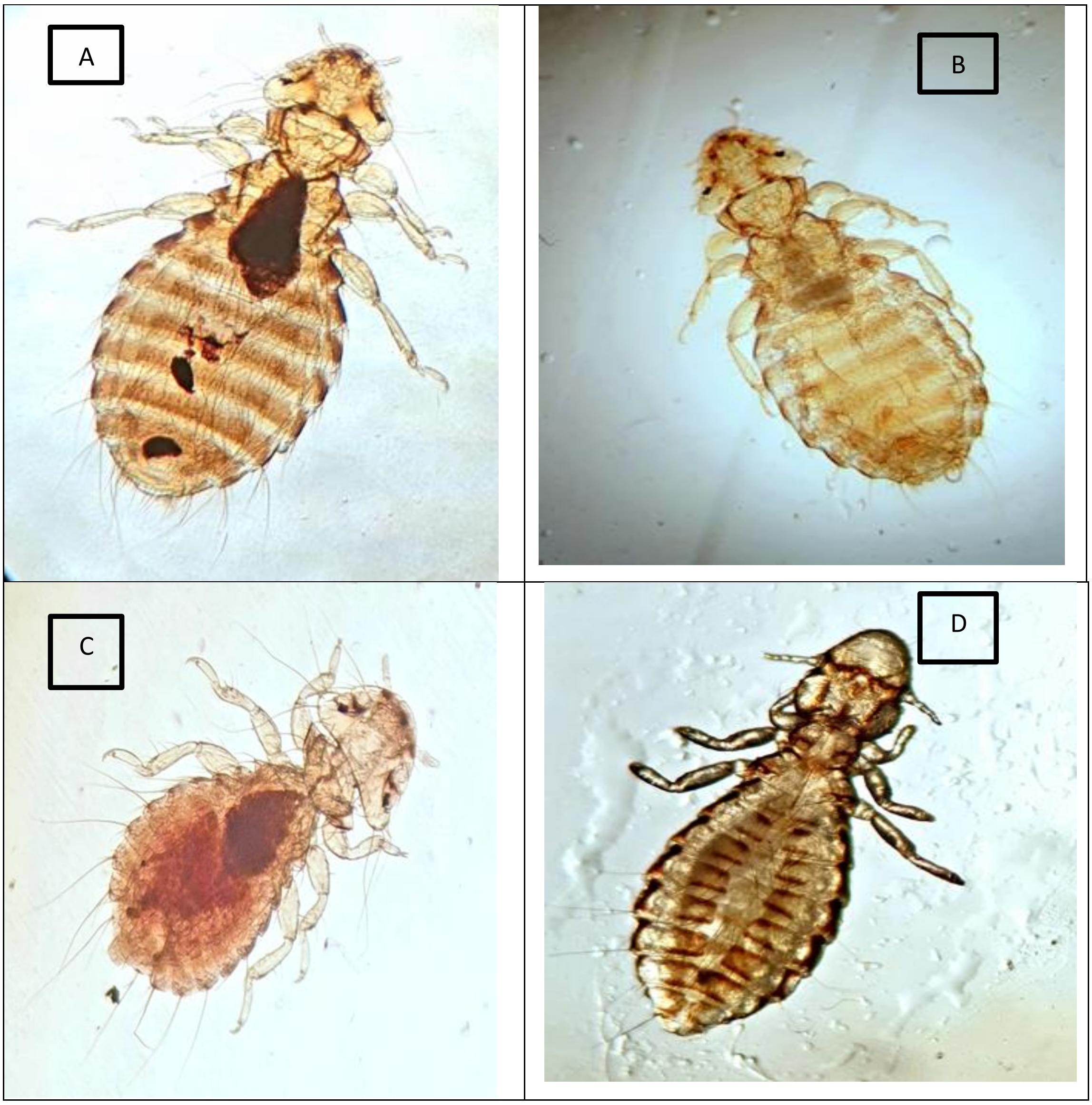
A
B
C
D

Fig.1:A:*Menacanthus stramineus*♀ **, B:***M. pallidullus*♀ **, C:***M. cornutus* **,D:** *Cuclotogaster heterographus* ♂ **, E:** *Bonomiella columbae*♀**.**

Discussion

Lice is considered a harmful parasite for birds, as it leads to a lack of production and general weakness. In addition, it reduces the birds' resistance to diseases and makes them vulnerable to secondary infections and may lead to the death of the infected birds. Lice disturbs the infected birds as a result of their direct feeding on the feathers and skin. In addition to that Its stings are severe and painful, in addition to its saliva being stimulating and irritating, and in the case of severe infection, it leads to scratches and wounds(14).

The results of the current study indicated that the rate of infection of guinea fowl with lice reached 80%, which is less than percentage recorded by (15) in his study of different species of guinea fowl ,the reason for the difference in the recorded percentages (higher and lower) may be due to the difference in the number of birds examined, study areas, and climatic conditions, which may play a major role in the increase or decrease in the infection rate. This study is the first study in Iraq, where these parasites were isolated from other birds, such as domestic chickens and other birds, and they were not recorded in guinea fowl.

The results of the current study indicated that the death rate of lice increased proportionally with increasing concentration and duration of exposure to chitosan nanoparticles. Three increasing concentrations were used in this study 40, 60, and 80 mg/ml, in addition to lice treated with 0.9% physiological saline solution, representing the control group. Calculating the percentage of lice death after five times, 5,10,15,30,60 minutes, for each of the concentrations mentioned above, where the percentage of death of lice treated with concentrations of 40, 60, and 40 mg/ml after an hour of treatment was 75%, 100%. 100% respectively, which represents the highest percentage of death of lice treated with the same concentrations and shorter periods of time, while the lowest percentage of death of lice was 5 minutes after treatment, as it was 12.5%, 12.5%, and 50% respectively, and these are very low percentages when compared with the percentages of death Lice after 10, 15 and 30 minutes.

Nanocomposites in general, including chitosan, are biocompatible materials with an anti-parasitic effect in the veterinary field (16), as some nanoparticles have shown promising results in treating various types of parasitic infections (4) and the reason may be due to In this regard, the ability of chitosan nanocomposites with a size of 20-30 nanometers enables them to penetrate the skin of arthropods or the wall of any microbial cell, and this in turn increases the permeability of the skin and rupture of the insect (17,18). This study indicates that chitosan nanoparticles kill lice directly, and that applying them at a concentration of 80 mg/ml led to a 100% kill rate in 15, 30, 60 times, this will reduce the effects of parasitic lice on birds in general.

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