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Study of the effect of *Nerium oleander* extract in the destruction of *Argulus foliaceus* and *Lernaea cyprinacea* in ornamental fish

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ABSTRACT

The effect of the extract of the *Nerium oleander* was studied on ornamental fish in vivo and in vitro, Using different concentrations and different periods. The results of the current study showed that the destruction of *Argulus foliaceus* in 50 g / 5 ml and 0.25 g / 5 ml of water after 62 and 135 minutes respectively when using the extract directly on the lice (in vitro), *Lernaea cyprinacea* was tested in the fish. The results of the study showed that *Lernaea cyprinacea* was destruction at a concentration of 50 mg/L after 15 minutes but did not affect lice, The results of the study showed that fish were cured from lice after one month of treatment at 33.33 mg/L for 72 consecutive hours. When fish were placed at 50/1500 g/L of the extract, they were treated from lice but caused the fish to die after 15 minutes after placing in a pure water bath.

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INTRODUCTION

Ornamental fish, Like other animals, are exposed to diseases which are classified as viral, bacterial, fungal and parasitic diseases (AL-Darwesh *et al.*, 2014). Most of the health problems involve external diseases (Notash, 2012), as well as reducing the nutritional and physical value of fish, which may cause mass deaths, especially in fish farms (Hoffman, 1998). *Argulus foliaceus* is a common parasite that affects freshwater fish.

Lernaea, known as "anchor worms," is a crustacean that can infect and cause disease and death in many freshwater fish species, particularly wild species that are caught and reared, the most common in the summer months and more common in stagnation or slow movement of water bodies. There are approximately 110 species of *Lernaea cyprinacea*, one of the most common species around the world especially in common carp and golden fish; however, it can infect other species of fish and cause the death of catfish in ponds in the state Arkansas (Hossain *et al.*, 2018).

Nerium oleander is an important medicinal plant in folk medicine and all parts of the plant are toxic and contain a variety of heart glycosides that include neriin, oleandrin, cardenolides, gentiobiosyl and odoroside. The plant also contains secondary metabolites such as alkaloids, flavonoids and steroids that have pharmaceutical applications. It also plays a role in important pharmacological activities such as antimicrobial, anthelmintic, anti-inflammatory, anti-oxidant, anti-fungal, anti-cancer and anti-HIV active (Farooqui and Tyagi, 2018).

Many plant species are not consumed by a large number of insects due to toxic or repellent secondary substances contained in these plants. And the use of plants as a means to combat fish parasites without side effects on the environment can be an alternative way to overcome the negative effects of spraying pesticides against pests, and the importance of this plant in the treatment of parasites and

other causes came the idea of this research, which focused on the following isolate the parasite *Argulus foliaceus* from infected fish skin.

Study of the effect of alcohol extract of *Nerium oleander* plants in the treatment of fish infected with *Argulus foliaceus* and *Lernaea cyprinacea* invitro and in vivo.

MATERIALS AND METHODS

Preparation of the alcoholic extract

The leaves were collected from the public nurseries and then washed to remove the suspended soil. The leaves were dried in the shade and then grinded well and placed in the extractor. They took 60g of leaves and 600ml of absolute ethyl alcohol. When the extraction process was complete, the solution was placed in the oven at 40 ° C for drying. Then save the extract stored in the refrigerator until use.

Preparation of experimental animals

During the months of November and December, the fish were placed in two glass basins with dimensions of 30 x 50 cm and were provided with artificial ventilation and artificial feed.

A convalescence basin has been prepared to transport the fish to it after the experiment. Examination of the skin and gals of the fish with the naked eye to observe the lice. Four experiments were conducted as follows:

The first experiment was conducted to determine the lethal dose of the extract by placing the non-infected fish in a fish basin containing a 66.66 mg/L extract.

Second experiment: *Lernaea cyprinacea* and *Argulus foliaceus* were infested in a water bath containing 50 mg/L extract for 15 minutes and placed in a pure water bath. The experiment was repeated for 3 days

Experiment III: Fish infected with *Argulus foliaceus* was immersed in a water bath containing the extract at a concentration of 50 mg/L and 33.33 mg/L for 3 consecutive days.

Experiment IV: Fish infected with *Argulus foliaceus* was immersed in a water bath containing the extract at a concentration of 50 g/L and then calculated the duration of the lice destruction.

The fifth experiment: isolate the lice from the skin of the fish using a soft brush. The samples were transferred to a petri dish containing the extract of the alcohol of the plant concentration of 0.50 mg/5 ml and 0.25 mg/5 ml and then calculated the duration of the destruction of lice in the laboratory.

The behavior of the fish during the treatment period was observed, such as tail movement, fish breathing, and sluggisness when placed in different concentrations of the experiment.

RESULTS AND DISCUSSION

The results showed that the death of healthy fish was 96 hours after exposure to the extract (Table 1). This is not consistent with, noting that the LC_{50} for alcoholic extract of the leaves of the plant on the predatory fish was 24 hours later. This is due to the different species of fish used.

The study also investigated the effectiveness of the extract of the *Nerium oleander* plant in the treatment of fish infected with *Lernaea cyprinacea* and *Argulus foliaceus.*

The results of the present study showed that the plant extract is effective in the destruction of the worm *Lernaea cyprinacea* and treatment of fish infected with worm within 15 minutes of exposing the infected fish to the extract where the worm was separated from the body of the fish but not effective in the loss of lice over the period of 3 days as shown in Table 2.

The results of the present study also showed the effect of the extract in treatment of long-term fish when it was placed in the extract at a concentration of 33.33mg/l for parasite destruction after one month of treatment, while not treated when placed in concentration 50mg/l for 3 consecutive days, resulting in death of fish as shown in Table 3.

The treatment of fish after a month of treatment when placed in the extract with a concentration of 33.33mg/l may be attributed to the effect of the plant extract on male and female parasites, which negatively affected parasite reproduction and non-production of eggs, In an experiment conducted by (Bagari *et al.*, 2015), On locusts, he fed the locust females and males to the leaves of the plant. The effect of leaves was found to reduce the physical activity of locusts males and female. There was no mating activity during the experimental period when feeding on leaves. Where pink stool was introduced by females, this can be caused by gastrointestinal disorders caused by their diet.

The anatomical study of the ovary in the desert locust showed toxic effects on females, resulting in a decrease in biological activity, reduced eating and inhibition of cellular reproduction and thus stopped ovarian growth.

The extract was applied to the skin of both animals (Farooqui and Tyagi, 2008) and human (Farooqui and Tyagi, 2009), Studies have

Table 1: The lethal dose in the healthy fish

Number of Fish	Concentration of the extract	Fish Death / Periods			
		24 hours	48 hours	72 hours	96 hours
10	66.66 mg/l	0	0	0	10

Table 2: The destruction of *Argulus foliaceus* and *Lernaea cyprinacea* and treatment of fish using water bath within 3 days

Number of Fish	parasite	Concentration of the extract	parasite destruction / time periods			
10	*Argulus foliaceus*	50 mg/l	Day 1 15 minutes	Day 2 15 minutes	Day 3 15 minutes	Fish death
10	*Lernaea cyprinacea*		0	0	0	0
			10	–	–	–

Table 3: The destruction of *Argulus foliaceus* on fish in vivo after 72 consecutive hours

Number of Fish	parasite	Concentration of the extract	parasite destruction /time periods				treatment
			24 hour	48 hour	72 hour	Fish death	
10	*Argulus foliaceus*	33.33mg/l	0	0	0	0	Parasite destruction after a month
10		50mg/l	–	–	–	10	–

indicated that this extract is safe, indicating that these compounds are not easily absorbed through the skin. This clearly indicates that the extracts of this plant can be safely used not only to control the pests but also can improve the immune system, so it has been used as a skincare product such as anti-ageing creams and cosmetics. Anti-mosquito control and other future pest control measures, it was also considered as safer environment for mosquito control and other future pest control measures.

It also has a role in important pharmacological activities as antimicrobial, anti-helmintic, anti-inflammatory, anti-oxidant, anti-fungal, anti-cancer and anti-HIV active, simultaneous research has found that the *Nerium oleander* plants properties for activating immunity are extremely positive. Research has confirmed that the *Nerium oleander* extracts showed activation of the immune system by stimulating the function and ability of specific subsets of monoclonal cells and specifically stimulates T. and B lymphocytes, i.e. both humoral and cellular immunity (Farooqui and Tyagi, 2018).

The results of the study also showed the effect of the extract in the destruction of lice when fish were placed in the extract at a concentration of 50g/1500ml and lice destruction after 75 minutes, but this led to the death of fish after 15 minutes of placing in the clean water bath as shown in Table 4.

The destruction of parasites due to the toxic compounds found in the leaves after being ingested, where they affect the metabolism of insects (Bagari *et al.*, 2015). All parts of the plant are toxic and contain a variety of heart glycosides including neriin, oleandrin, cardenolides, gentiobiosyl and odoroside. The plant also contains secondary metabolites such as flavonoids. Substances produced by plants, for example, alkaloids, are known to interfere with insect metabolism and reduce digestibility (Lebreton, 1982).

The poisonous effect of the plant may be attributed to its containment of heavy metals In an experiment conducted by on the content of the heavy metals, the results showed that the highest content in the leaves was mercury (16.27 mg), but the highest metal content was iron (0.689 mg) in the stems

Table 4: The destruction of the parasite Argulus foliaceus on fish in vivo using water bath within 24 hours

Number of Fish	parasite	Concentration of the extract	parasite destruction /time periods				tratment
			25 minutes	50 minutes	75 minutes	Fish death	
10	*Argulus foliaceus*	50g/1500ml	0	0	0	0	The fish died after 15 minutes after being placed in a pure water bath

Table 5: The loss of the Argulus foliaceus in vitro

No.of lice	parasite	Lice destruction / time periods				
		Concentratio	25 minutes	50 minutes	62 minutes	135 minutes
10	*Argulus*	0.5g/5ml	0	9	1	–
10	*foliaceus*	0.25g/5ml	0	0	2	8

and nickel Ni (8.177 mg) In flowers, The gravity of heavy metals comes from their bioaccumulation faster than their degradation through metabolism or output, The consumption of large amounts of them at high concentrations is harmful and even toxic and results in so-called heavy metal poisoning (Duffus, 2002).

The results of the study also showed the efficacy of the plant extract on fish lice *Argulus foliaceus* when treated in vitro by exposing the parasite to the extract using a concentration of 0.5g /5ml and 0.25g/5ml which resulted in the destruction of lice after 62, 135 minutes respectively As shown in Table 5.

Research has shown that the water extract of the plant is considered as a pesticide against eggs and insect larvae (Kumar *et al.*, 2012). The activity of this plant was also recorded against *Anopheles stephensi* as a pesticide of adults (Roni *et al.*, 2013). *Nerium oleander* was also used for crude hexane and the water extract of the flower as a pesticide of larvae for *Culex quinquefasciatus* (Raveen *et al.*, 2014).

CONCLUSION

Through the results of our current study, we deduce the efficiency of the extract of the plant in the destruction of some ornamental fish parasites and can be considered as a natural insecticide.

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