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***Research Article***

# DETECTION OF *CHILODONELLA* AS THE PREDOMINANT SKIN PARASITE INFECTING *POECILIA RETICULATE* (GUPPY) IN IRAN, 2014 AND A BIOLOGICAL CONTROL APPROACH FOR INFECTION IN LABORATORY SCALE

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## ABSTRACT
The aim of present study was detection of predominant parasites in guppy fish and control of infection by the natural product, bee propolis. Infecting parasites were isolated from 75 signed guppy aquarium fish (*Poecilia reticulate*) and identified according to morphological characteristics. To analyze the effect of ethanol extract of bee propolis on treatment of skin infection, propolis was mixed in ethanol 96% and after homogenizing and distillation; it was dissolved in dimethylsulphoxide (DMSO). The concentrations of 0.1 g $L^{-1}$ and 0.2 g $L^{-1}$ were treated in a statistical test, on two groups; each with 10 *Poecilia reticulates* infected by the predominant parasite. Three groups of healthy *Poecilia reticulates* (each with 10 poecilia reticulates) were also considered as the control group to analyze the effects of extract and the parasites on the survival of the mentioned fish. The results were recorded according to daily numbers of the parasites in water and the death rates of the fish after the 8-day treatment period, as compared to the control groups. The predominant parasite was identified as *Chilodonella* based on morphological characteristics. The concentration of 0.2 g $L^{-1}$ of the extract was more effective on reducing the number of parasites and survival of the fish, than the extract with the concentration of 0.1 g $L^{-1}$ ($p<0.05$), such that it reduced 97% of the parasites and 90% of the fish death in the treatment group as compared to the diseased control group after the 8-day treatment. Moreover, the extract had no effect regarding the fish death in the control group. The results for the first time proposed the extract of bee propolis for treatment of parasite infection of *Poecilia reticulate*, as a certain preventive and therapeutic method.

***Keywords:*** *Poecilia Reticulate, Chilodonella, Biological Control, Bee Propolis*

## INTRODUCTION
Due to their beautiful appearance, small size, and easy keeping, reproduction and growth; aquarium fish had prominent development in the past few decades. Increasing the inclination of people in growing ornamental fish, the attention to treating of aquarium fish infections has also been developed. Despite of the hygienic control and preventive actions for diseases control, the emergence of different infections is possible in ornamental fish (Craig *et al.*, 1996).
The apparent fit body and health of the fish is quite important in their selection. The living environment of fish (water), feeding, respiration, excretion of wastes, the presence of other fish and creatures are also important. When this environment is fertile with regards to feedings, balanced for its chemical and physical materials, and healthy with regards to pathogenic factors, it leads to better growing and fish health, as well as achieving the production aims. Sometimes water causes diseases in the fish. About one thousand parasites could live on the surface or inside the body of fish. There are different factors for the parasitic diseases, including water quality, water temperature, unsuitable feeding, and stress (Ellender and Weyl, 2014).
One of the damaging parasites to the skin and bronchia, causing death of the fish in all seasons, especially in cold seasons is *chilodonella*. This parasite belonging to *chilodonide* species, is the agent of lethal chilodonelose disease among aquarium fish. In stress conditions, high compaction of fish and unsuitable feeding, *Chilodonella* causes high rate of fish loss. This parasite feeds from the secretions and wastes from damaged skin and bronchia. *Chilodonella* has a worldwide growth among the fish (Lom and Dykova, 1992; Fan *et al.*, 2014).

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Traditional medicine and treatment by herbs are historical globally. Propolis that is also known as bee adhesive is a natural product derived from herbal resins accumulated by bees.
This material which is transported to the hives by bees is supplemented with other products such as wax and used in hive walls. Structurally, propolis is made of different components such as aromatic acids and esters, flavonoides and terpenoids.
Propolis is an anti-microbial and anti-inflammatory material as well as its effectiveness on self-immune diseases and controlling cancerous tumors (Marcucci, 1995).
The aim of this research was detection of predominant parasites infecting *Poecilia reticulate* and using the antimicrobial property of propolis for survival of fish and controlling the infections by the isolated predominant parasite.

## MATERIALS AND METHODS

### *Sampling*

The number of 75 signed guppy aquarium fish were collected randomly from Esfahan province by the end of summer and mid-autumn, 2014. The fish kept in separated aquariums in laboratory for the following experiments. Samples were taken from the fish skins by pressure and scratching. Identification of the parasite was done by microscopic morphology and comparisons with identification keys and resources (Lom and Dykova, 1992; Spring *et al.,* 2013).

### *Preparation of Bee Propolis Extract*

Propolis was prepared from bee keepers of Najafabad town in Esfahan, Iran. The amount of 30 g of propolis was divided into smaller pieces and resolved in 1200 ml of ethanol 96%. After 3 days mixing in 150 rpm and keeping for 14 days in 24°C in darkness, the extract was separated from the alcohol by distillation. The achieved extract was soluble in dimethyl sulfoxide and ethanol 96%.

### *Effect of Extract on the Parasite*

The concentrations of 0.1 g $L^{-1}$ and 0.2 g $L^{-1}$ of the extract were prepared. Then 4 different treatments were tested as follows:
Treatment I: Healthy control group including 10 healthy guppies, which 2 ml of propolis extract with the concentration of 0.2 g $L^{-1}$ was daily added to their water during their growth. The effect of propolis on the health of the fish was observed in this treatment.
Treatment II: The diseased control group including 10 diseased guppies, which 375 isolated parasitic cells was added to each milliliter of their water during their growth.
Treatment III: The treated sample groups included two groups of 10 guppies exposed to parasites in water and 2 ml of the extract with 0.1 g $L^{-1}$ concentration. The extract was added daily to their water.
Treatment V: The treated sample groups included two groups of 10 guppies exposed to parasites in water and 2 ml of the extracts with 0.2 g $L^{-1}$ concentration.
The extract was added daily to their water.
The results were analyzed after 8 days according to the mortality rates and daily counting of parasites in the water by using neobar slide in the treated groups and comparing them with the control groups. SPSS software was used for the analyses, and Wilcoxon method (binary comparison) was used to compare the inter-grouping frequencies.
According to hypothesis, the significance of the difference between the number of treated alive fish and the control group and also between the number of under-treatment parasites and the control group were considered.

## RESULTS AND DISCUSSION

### *Results*

*Identified Parasites*

Table 1 indicates the rate of skin infection from each parasite among 75 signed guppy aquarium fish. As shown, C*hilodonella* has the most frequency (41.4%) among the parasites in the sample population. Also figure 1 shows the microscopic morphology of the isolated predominant parasite (C*hilodonella*).

**Table 1: The rate of infection caused by different parasites in sample population**

| Identified parasite | Rate of infection in sample population (%) |
|---|---|
| *Chilodonella* | 41.4 |
| *Dactylogyrus* | 16.8 |
| *Gyrodactylus* | 14.2 |
| *Ichthyobodo* | 27.6 |

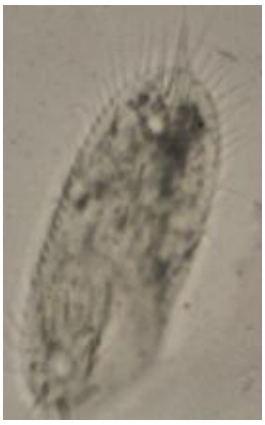

**Figure 1: Morphology of *Chilodonella* isolated from guppy. The parasite appears leaf shaped abdominal ciliated with a granular inside. Also the pharyngeal basket is seen that appears as a clear bubble on the front side of the organism**

***Effect of Ethanol Extract of Propolis on the Predominant Parasite***

*Comparison of groups in pairs:* The results of paired comparisons approve the significant difference between two groups, for all the studied groups. Tables 2 shows the results according to fish death and table 3 shows the results according to the number of alive parasites in each ml of water. Every two groups in all comparisons confirmed the significant differences.

**Table 2: Paired comparison of treatments regarding number of alive fish after 8 days**

| Group | | Wilcoxon value | Z-value | Significance level |
|---|---|---|---|---|
| Diseased control group compared to: | A: Treatment by the concentration of 0.1 g $L^{-1}$ | 3 | -2.00 | 0.046 |
| | B: Treatment by the concentration of 0.2 g $L^{-1}$ | 3 | -3.16 | 0.002 |
| | C: Healthy control group | 3 | -3.31 | 0.001 |
| healthy control group compared to: | A: Diseased control group | 3 | -3.31 | 0.001 |
| | B: Treatment by the concentration of 0.1 g $L^{-1}$ | 6 | -3.46 | 0.001 |
| | C: Treatment by the concentration of 0.2 g $L^{-1}$ | 45 | -4.24 | 0.000 |
| Treatment by the concentration of 0.1 g $L^{-1}$ compared to: | A: Diseased control group | 3 | -2.00 | 0.046 |
| | B: Treatment by the concentration of 0.2 g $L^{-1}$ | 6 | -3.31 | 0.001 |
| | C: Healthy control group | 6 | -3.46 | 0.001 |
| Treatment by the concentration of 0.2 g $L^{-1}$ compared to: | A: Diseased control group | 3 | -3.16 | 0.002 |
| | B: Treatment by the concentration of 0.1 g $L^{-1}$ | 6 | -3.31 | 0.001 |
| | C: Healthy control group | 45 | -4.24 | 0.000 |

**Table 3: Paired comparison of treatments regarding the number of parasites after 8 days. Since no parasite has entered in healthy group sample, the healthy group eliminated and comparison was done between other groups**

| Group | | Wilcoxon value | Z-value | Significance level |
|---|---|---|---|---|
| Diseased control group compared to: | A: Treatment by the concentration of 0.1 g $L^{-1}$ | 4560 | -28.54 | 0.000 |
| | B: Treatment by the concentration of 0.2 g $L^{-1}$ | 55 | -27.01 | 0.000 |
| Treatment by the concentration of 0.1 g $L^{-1}$ compared to: | A: Diseased control group | 4560 | -28.54 | 0.000 |
| | B: Treatment by the concentration of 0.1 g $L^{-1}$ | 55 | -10.19 | 0.000 |
| Treatment by the concentration of 0.2 g $L^{-1}$ compared to: | A: Diseased control group | 55 | -2.7.01 | 0.000 |
| | B: Treatment by the concentration of 0.1 g $L^{-1}$ | 55 | -10.19 | 0.000 |

The number of living fish and the number of parasites are seen in figures 2 and 3, respectively. In the treatment group receiving 0.2 g $L^{-1}$ of the extract, 90% of the fish are alive that shows a considerable result as compared to 20% of diseased control group. Also the rates of parasites showed 75% and 97% decrease for the concentrations of 0.1 g $L^{-1}$ and 0.2 g $L^{-1}$ in 8 days, respectively, as compared to the diseased control group.

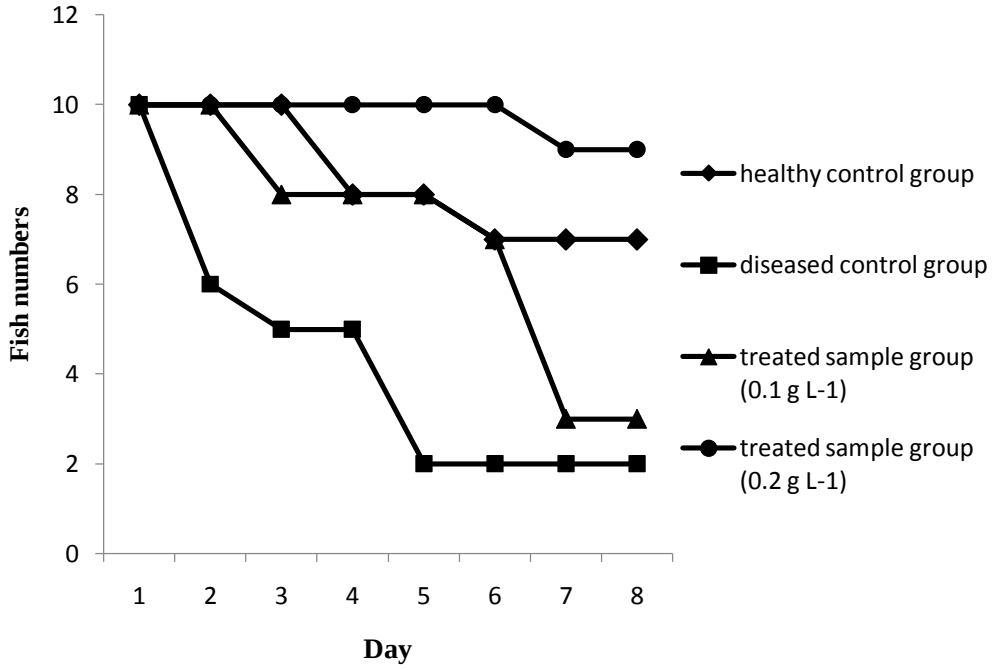


**Figure 2: Number of living fish in each day in treated and control groups. Lowest rate of mortality is observed in treatment group receiving the concentration of 0.2 g $L^{-1}$ propolis ethanol extract**

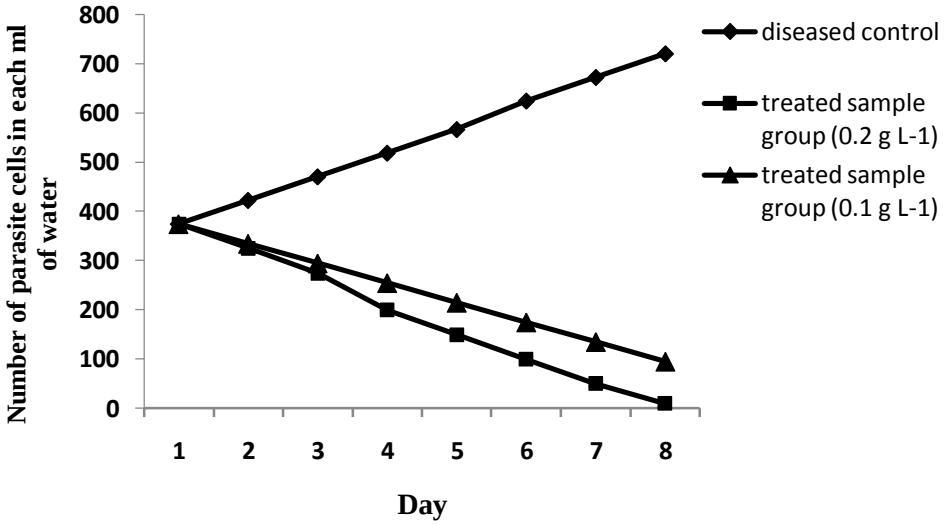


**Figure 3: Daily reduction of parasites in treated and control groups. Most reduction is observed in the concentration of 0.2 g $L^{-1}$ propolis ethanol extract**

***Discussion***

*Chilodonella* was isolated from guppies in Esfahan province, Iran by the end of summer and beginning of autumn, 2014. *Chilodonella* is a ciliated parasite which is important for fish in moderate waters and aquarium fishes. This parasite is elliptical with the length of 70 µm, covered by rows of cilia. *Chilodonella* shows continuous slipping moves on the covering affected cells. By removing its larynx organ, the parasite penetrates in host cell and sucks the internal substances. This parasite is mainly observed in low temperatures and grows by longitudinal divisions. The divisions are increased in 5-10°C (Lom and Dykova, 1992).

Many studies used herbal medicine to treat parasitic infections in the fish. For instance the effect of garlic and chamomile extracts were evaluated on *Ichthyophthirius multifiliis* parasites on guppy. The concentration of 0.1 g $L^{-1}$ of garlic extract and 0.4 g $L^{-1}$ of chamomile extract kill this parasite after 5 days (Sahandi *et al.*, 2012).

In the present study, a biological substance (propolis) was used for prevention and controlling parasitic infection caused by *Chilodonella* in guppies. Since this parasite exists on the skin of this fish, it was decided to use propolis in soluble form in water. Also the bathing method was used for the fish to be exposed more to the treating substance.

Notably propolis had not been used for the parasitic problems in fish before this research, but there are various studies in human beings. In a study, the anti-parasitic effect of propolis was tested on different types of *Leishmania*. The results indicated that the ethanol extract of red Brazilian propolis with the concentration of 0.6 g $L^{-1}$ reduced 84.5% of the parasite infecting activity after 3 days. This effect continued, increasing up to the concentration of 25 g $L^{-1}$. Higher concentrations could not be effective due to macrophage damages (Ayress *et al.*, 2007). It was shown in the present study that the concentration of 0.2 g $L^{-1}$ of propolis ethanol extract was the lowest concentration that could reduce 97% of *Chilodonella* cells after 8 days. Also, this concentration of propolis had no negative effects on the fish health in control group. The paired comparisons confirm the significant differences between two groups in all the comparing aspects.

Other studies have been shown that the characteristics of bee propolis depend extensively on the place of collection. For instance, in comparing Bulgarian and Brazilian propolis effects on different dermal leishmaniasis in human, it was indicated that although the ethanol extracts of propolis was effective on parasites, the Bulgarian one was more effective on all species (Machado *et al.*, 2007). The propolis taken from Esfahan region was used for the first time in this research for prevention and treating parasitic

infection of fish. The anti-parasitic activity of that extract could well be compared with propolis taken from other regions, such as the Brazilian type (Ayress *et al.*, 2007).

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