Efficiency of the water and alcohol extracts of *Melia azedarach* fruits for treating the common carp *Cyprinus carpio* infected with some monogenean parasites

Zahra M Al-Turaihi and AJ Al-Rudainy

**Abstract**

A total of 235 specimens of *Cyprinus carpio* was examined to detect the external monogenean parasite infested the skin, fins and gills, represented five species (*Gyrodactylus elegans, Dactylogyrus extensus, D. achamerowi, D. minutus* and *D. vastator*), treated with different concentrations of water and alcohol extracts of *Melia azedarach* fruits, treated treatments were: T1, control (without treating included infected and non-infected fish): T2, 1%; T3, 2%; T4, 3%; T5, 4% and T6, 5% by applying dip method in a water bath for 24h. The results showed that the higher the concentration of the both extracts, the greater the mortality ratio of the five parasites achieved 100% in T6 (5%). Blood parameters of infected fishes before and after treatment with water and alcohol extracts of *M. azedarach* fruits were changed, the results of water extract showed no significant differences in red blood cell (RBC) counts between T6 (5%) compared to the other treatments, and the values of white blood cell (WBC) were decreased with increasing the concentrations of water extract, decreased from 36.79×10^6 in T2(1%) to 29.24×10^6 in T6 (5%). Packed cell volume (PCV) and hemoglobin (Hb) showed no significant differences (p>0.05) between treatments, also between T6 and non-infected control treatment. The values of RBCs count have increase with the increasing of alcohol extract concentrations ranging between 0.90×10^6 in T2(1%) to 1.27×10^6 in T6 (5%). No significant differences (p>0.05) between the five experimental treatments, increased significant (p≤0.05) when compared with infected control treatment. The mean values of WBCs have decreased with increasing the concentrations of alcoholic extract, which decreased from 37.49 in T2(1%) to 27.04×10^6 in T6 (5%), also significant differences (p≤0.05) between T2 compared to T5 and T6, while no significant occurs among T2, T3 and T4. The mean values of PCV and Hb for treatments ranged between 21.33 – 25.50% and 7.06 – 8.47/dl in T2 and T6 respectively. Results of statistical analysis of PCV and Hb showed no significant differences (p>0.05) between all treatments, also between T6 and non-infected control treatment.

**Keywords:** *Cyprinus carpio*, parasite, *Melia azedarach*, treatment

1. **Introduction**

Fish consider are important food sources because their meat contains high protein ratio, vitamins, fats and salts [1]. Cyprinid fish is one of the largest and most important treatments of fish in the world, they dominate freshwater with 2000 species [2]. Parasites live in a normal condition with their fish hosts in their natural healthy environments, however if some harmful changes such as bad water quality and weak physiological condition of fish, resulted the equilibrium between fish hosts and their parasites may be disturbed, and outbreaks of species of parasites may occur [3]. Parasites infections of fishes are caused clinical or subclinical diseases that might lead to economic losses which reduced the production and increased the cost of treatment [4]. Monogenean trematodes a class of flattened and leaf-like animals that complete their direct life cycles in a single host, Class Monogenea (Phylum Platyhelminthes) includes small ectoparasites living on the skin, fins and gills of fishes, it represents the largest treatment of fish parasites with direct life cycles [3]. Monogeneans are important fish pathogens, particularly for carp fingerlings under extensive fish culture practice and their direct life cycles and fish crowding is good conditions for their easy spread among fishes [3]. The high spread of fish farms, cages and hatcheries in Iraq led to the spread of parasitic diseases, therefore, necessary find search for an effective treatment to eliminate parasites, because of significance parasites is related directly to the importance of the fish that it may be affected, it produces economic losses as increase mortality fish, excessive farm intake via.
treatment expenses and growth rate reduction. The present study aimed for treatment of some external parasites of the studied fish by using plant extract of *Melia azedarach*. *M. azedarach* L. is a small to medium sized deciduous tree, it ranges between 5 to 15m in tall and 30 to 60 cm in diameter it is native in Pakistan, India, Indochina, Southeast Asia and Australia, it is widespread and naturalized in most of the tropics and subtropical countries [5], it cultivated in Iraq, Saudi Arabia, Syria, Egypt and Libya [6]. The chemical composition of *M. azedarach* is highly complex, its main chemical composition is a blend of 3 to 4 related main compounds and over 20 others present in smaller amounts, these compounds are mainly triterpenes with the most effective being the limonoids abundant in its oil, at least nine limonoids are effective in inhibiting insect growth, especially some of the most deadly varieties found in human health and agriculture worldwide [7]. Since ancient times it has been millions of Asians use the tree for medicinal purposes, where this tree has earned a good reputation as a remedy against a different diseases [8]. Various preparations of *M. azedarach* are used for the treatment of several diseases [9, 10]. The powder of dried fruits of this plant was shown to be effective in diabetes [11].

2. Materials and Methods

2.1 Fish and aquariums

The study was conducted at the laboratory of Animal and Fish Resources Center. A total of 235 specimens of Common carp *C. carpio* were obtained in December/2016, ranging between 14- 24 cm in total length and 81-250 g in body weight. Parasites have been classified according to [12]. Using aquariums with dimensions of 60× 60 × 30 cm filled with tap water, supplied with oxygen for treatment of infected fish by using a water and alcohol extract of fruit *Melia azedarach*, other aquariums with the same specification used as recovery aquariums which fish are placed after treatment.

2.2 Chemical compounds detection of the plant

The study detecting the presence of chemical compounds in the plant *M. azedarach* including Alkaloids, Glycosides, Flavonoids, Tannins, Saponins, Resins, Coumarins and measuring the pH, the following tests were conducted in the biochemical laboratories in College of Veterinary Medicine, University of Baghdad.

2.3 Preparation of plant extracts

2.3.1 Water extract

Two hundreds gram of grinded dry weight of fruit extract of *M. azedarach* was prepared by using the water, which prepared 200g dry weight after grinding with the electric grinder according to [13]. The sample was placed in glass flask capacity 2000ml containing 1400ml of distilled water, blended with an electric mixer for three hours, the solution filtered through Whatman filter paper (No.1) using a Buchner funnel connected with vacuum filter, the filtered was concentrated by rotary evaporator at 40 °C to get the exact size, then completed the size to 1000ml of distilled water, stored the alcohol extract in refrigerator until use.

2.3.2 Alcoholic Extract

Two hundred grams of grinded dry weight of fruit extract of *M. azedarach* was prepared, 1000ml ethyl alcohol (95%) was added to the 200g. grinded fruit, blended with an electric mixer for an hour, the solution was filtered through Whatman filter paper (No.1) using a Buchner funnel connected with vacuum filter, the filtered was concentrated by rotary evaporator at 40 °C to get the exact size, then completed the size to 1000ml of distilled water, stored the alcohol extract in refrigerator until use [13].

2.4 Experimental design

According to [14] the extract was added to the aquarium for a limited time to determine the period needed for treatment until healing.

2.4.1 The antiparasitic efficacy of the experimental water and alcoholic extract of *M. azedarach* against monogenetic parasite *in vivo* within low concentration in 24 h.

Fish were examined by microscope to identify present parasites. To evaluate the efficiency of water and alcoholic extracts of *M. azedarach*, a series of concentrations for five treatments as well as the control (two duplicate) as described previously (Fig. 1).

![Fig 1: Experimental design illustrate the concentrations for fish treatment](image)

Preparation of each concentration was placed in aquarium provided with artificial ventilation and temperature regulator. The chemo-physical parameters of the water were measured during the experimental period as follows: Temperature ranged between 20 - 21 °C, Dissolved O2 ranged between 9-10 mg / l, pH ranged between 6.7 - 7.3, Salinity 0.1 g /l. Calculated the antiparasitic efficacy of each treatment according to the following formula modified from [15] as following: Mortality ratio = Number of parasites killed / Total No. of parasites × 100

2.5 Hematological Parameters

Blood was collected in the end of the experiment from the caudal vessels using a sterile disposable plastic syringe 3 ml, transferred immediately to a test tube containing (EDTA) (Ethylene di amine tetraacetic acid) for studying hematological parameters, including the red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and haemoglobin concentration (Hb) and these parameters were determined as described by [16].

2.6 Statistical Analysis

One way analysis of variance (ANOVA) was used to determine the significant differences between the variables. The differences of means were analyses at probability value 0.05. A probability level equal or less than *P* <0.05 were considered significantly different.
4. Results

4.1 External monogenean parasitic infection
A total of 235 specimen of Cyprinus carpio were examined for detection the external monogenean parasite in the skin, fins and gills, represented five species of monogenetic (Gyrodactylus elegans, Dactylogyrus extensus, D. achamerowi, D. minutus and D. vastator).

4.2 Chemical detection of active compounds in M. azedarach

According to [20], the chemical composition of M. azedarach is varies notably between its wild and cultivated state, that given the variability in the chemical composition depending on the environment and management. The results of general chemical detection of active compounds of M. azedarach were with positive results, the emergence of yellow-orange sediment indicates the presence of Alkaloids, the appearance of a red precipitate means presence of Glycosides, when mixing equal sizes of solution (A) and solution (B) and the emergence of the yellow color indicates the presence of Flavonoids, as the appearance of bluish green color indicates the presence of Tannins, appearance of a dense foam thickness of 1 cm indicates the presence of Saponins, the appearance of turbidity indicated presence of Resins, the filter paper moistened with a diluted solution of sodium hydroxide and leaves in boiling water bath for ten minutes, The filter paper exposed to the source of UV rays, appearance of yellow color indicates presence of Coumarins, measuring pH was 4.5.

4.3 Treatment of infected fish

4.3.1 Treatment with water extract of M. azedarach at different concentrations in 24 h

General experimental conditions for fish: Total length ranged between 18-24 cm., Total weight ranged between 82 - 189 g. Number of fish: two in each aquarium, The frequency of experiment: twice.

The results showed that the fish was in normal behavior and have not been affected when transported in treatments aquarium including different concentrations, the mortality ratio of parasites was 21.66, 43.33, 63.33, 81.66 and 100% in T1, T2, T3, T4 and T5 respectively after 24 h. The higher the concentration of the extract, the greater the mortality ratio of the parasites, so the results of statistical analysis of mortality ratio in parasites showed significant increased (p< 0.05) between T5 compared to the other treatments.

4.3.2 Treatment with alcoholic extract of M. azedarach at different concentrations in 24 h

General experimental conditions for fish: Total length ranged between 20-24 cm. Total weight ranged between 126 - 235 g. Number of fish: two in each aquarium, The frequency of experiment: twice.

Fish became normal after treatment. The mortality ratio of parasites was 14.28, 24.28, 41.42, 71.42 and 100% in T2, T3, T4, T5 and T6 respectively after 24 h. The higher the concentration of the extract, the greater the mortality ratio of the parasites, with significant increased (p< 0.05) between T6 compared to the other treatments.

4.5 Hematological parameters of experimental fish

Tab.1 showed the mean of hematological values of control treatment and treated fish by water extract. Mean RBCs counts in infected control and non-infected treatment reached 1.35 and 0.78x10^6 respectively. The results of statistical analysis showed a significant decreased (p< 0.05) of infected control treatment compared with non-infected treatment. Tab.(1) pointed out that the values of RBCs count increase with the increasing of water extract concentration ranging between 1.05 in T2 to 1.25x10^6 in T6. The results of statistical analysis showed no significant differences (p>0.05) among T3, T4, T5 and T6 compared with non-infected treatment, while T2, T3, T4, T5 and T6 showed an increasing significant (p< 0.05) when compared with infected control treatment. Tab.(1) illustrate that the mean values of WBCs decreased with increasing the concentrations of water extract, which decreased from 36.79 in T2 to 24 ×10^3 in T6. Results of statistical analysis showed a significant differences (p< 0.05) between T2 and each of T5 and T6, while no significant occurs among T2, T3 and T4. The mean values of PCV and Hb for treatments ranged between 21.33 – 24.50% and 7.06 – 8.12 g/dl in T2 and T6 respectively. Results of statistical analysis of PCV and Hb showed no significant differences (p>0.05) between treatments, also between T6 and non-infected control treatment (Tab.1).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RBC x10^6</th>
<th>WBC x10^3</th>
<th>PCV %</th>
<th>Hb g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Control (Without treated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infected Fish</td>
<td>1.35 ± 0.06 a</td>
<td>21.55 ± 0.47 c</td>
<td>28.00 ± 0.70 A</td>
<td>9.01 ± 0.20 a</td>
</tr>
<tr>
<td>Infected fish</td>
<td>0.78 ± 0.09 c</td>
<td>37.86 ± 0.59 a</td>
<td>20.66 ± 0.66 B</td>
<td>6.83 ± 0.23 b</td>
</tr>
<tr>
<td>T2 (1%)</td>
<td>1.05 ± 0.02 bc</td>
<td>36.79 ± 0.23 a</td>
<td>21.33 ± 0.66 B</td>
<td>7.06 ± 0.23 ab</td>
</tr>
<tr>
<td>T3 (2%)</td>
<td>1.18 ± 0.03 ab</td>
<td>35.28 ± 0.33 a</td>
<td>22.00 ± 0.40 B</td>
<td>7.3 ± 0.40 ab</td>
</tr>
<tr>
<td>T4 (3%)</td>
<td>1.21 ± 0.03 ab</td>
<td>33.16 ± 0.30 ab</td>
<td>22.66 ± 0.66 B</td>
<td>7.53 ± 0.23 ab</td>
</tr>
<tr>
<td>T5 (4%)</td>
<td>1.23 ± 0.02 ab</td>
<td>30.31± 0.40 b</td>
<td>23.33 ± 0.66 B</td>
<td>7.76 ± 0.23 ab</td>
</tr>
<tr>
<td>T6 (5%)</td>
<td>1.25 ± 0.02 ab</td>
<td>29.24 ± 0.61 b</td>
<td>24.50 ± 0.95 Ab</td>
<td>8.12 ± 0.30 ab</td>
</tr>
</tbody>
</table>

Different letters represent significant variations at (p≤0.05). The mean values of RBCs counts in infected control treatment and non-infected treatment reached 0.09 and 1.43x10^6 respectively (Tab.2). The results of statistical analysis showed a significant decreased (p ≤ 0.05) of infected control treatment compared with non-infected treatment. Results showed that the values of RBCs count increase with the increasing of alcoholic extract concentrations ranging between 0.90 and in T2 to 1.27x10^6 in T6. The results of statistical analysis showed no significant differences (p > 0.05) among the fifth experimental treatments (T2, T3, T4, T5 and T6), but it's showed an increasing significant (p ≤ 0.05) when compared with infected control treatment. The results of Tab.2 illustrated that the mean values of WBCs counts in infected control treatment and non-infected treatment obtained 39.17 and 20.33 x10^3 respectively. The results of statistical analysis showed significant increased (p ≤ 0.05) of infected control treatment compared with non-infected treatment. The current results showed that the mean values of WBCs decreased with increasing the concentrations of alcohol extract, which
decreased from 37.49 in T2 to 27.04 × 10³ in T6. Results of statistical analysis showed a significant difference (p<0.05) between T2 compared to T3 and T6, while no significant occurs among T2, T3, and T4. The mean values of PCV and Hb in non-infected and infected control treatment reached 28.5% and 9.15 g/dl and 20.50%, 6.8 g/dl respectively. The results of statistical analysis showed a significant decreased (p ≤ 0.05) in PCV and Hb of infected control treatment compared with non-infected treatment, the mean values of PCV and Hb for treatments ranged between 21.33 – 25.50% and 7.06 – 8.47 g/dl in T2 and T4 respectively. Results of statistical analysis of PCV and Hb showed no significant differences (p>0.05) between all treatments, also between T3 and non-infected control treatment (Tab.2).

Table 2: Mean values (± SD) of hematological test for C. carpio treated with alcoholic extract of M. azedarach in low concentration in 24h.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>RBC × 10⁶</th>
<th>WBC × 10⁹</th>
<th>PCV %</th>
<th>Hb g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Without treated)</td>
<td>Non-infected fish</td>
<td>Infected fish</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1.43 ± 0.02 a</td>
<td>20.33 ± 1.1 e</td>
<td>28.85 ± 0.10 a</td>
<td>9.15 ± 0.05 a</td>
</tr>
<tr>
<td>T2 (1%)</td>
<td>0.90 ± 0.05 b</td>
<td>37.49 ± 0.37 ab</td>
<td>21.33 ± 0.06 bc</td>
<td>7.06 ± 0.23 ab</td>
</tr>
<tr>
<td>T3 (2%)</td>
<td>1.15 ± 0.02 B</td>
<td>35.34 ± 0.20 b</td>
<td>22.60 ± 0.06 bc</td>
<td>7.53 ± 0.23 ab</td>
</tr>
<tr>
<td>T4 (3%)</td>
<td>1.18 ± 0.03 b</td>
<td>33.46 ± 0.58 bc</td>
<td>23.50 ± 0.5 bc</td>
<td>7.82 ± 0.17 ab</td>
</tr>
<tr>
<td>T5 (4%)</td>
<td>1.22 ± 0.01 b</td>
<td>29.08 ± 0.39 cd</td>
<td>24.00 ± 0.81 bc</td>
<td>7.97 ± 0.26 ab</td>
</tr>
<tr>
<td>T6 (5%)</td>
<td>1.27 ± 0.01 b</td>
<td>27.04 ± 0.90 d</td>
<td>25.50 ± 0.95 ab</td>
<td>8.47 ± 0.30 ab</td>
</tr>
</tbody>
</table>

Different letters represent significant variations at (p≤0.05).

4. Discussion

The current results showed the observations on fish and the mortality ratio of parasites in each concentration of water and alcoholic extracts of M. azedarach, the extracts did not cause harm to the infected fish, but the mortality ratio of parasites is increases with increased concentration, this is due to the presence of active toxic compound such as Alkaloids, Saponins, Resins and Coumarins that are effective in killing parasites and insects [18]. The antiparasitic efficacy of Azadirachtin can be used as a potential agent for controlling Argulus sp. in infected Carassius auratus [19]. The present results of this study are agree with [20], who studied a combination of metobendazole at 0.4 mg/l and trichlorfon at 1.8 mg/l resulting 100% effective on both G. elegans and D. vastator. The minimum effective exposure time was 24h. Ekanem et al. [21] studied the effects of the crude methanolic extract of Mucuna pruriens leaves and the petroleum-ether extract of Carica papaya seeds against I. multifilis were investigated under in vivo and invitro conditions on Carassius auratus infected with parasites were immersed for 72h with M. pruriens extract, and for 96h with C. papaya extract, there was a 90% reduction in numbers of I. multifilis on fish after treatment of each plant extract at 200 mg / l compared to untreated controls. Ekanem et al. [22] showed a higher efficacy of methanol extract extracts Piper guineense against monogenean parasites G. elegans and D. extensus for infecting C. auratus which increased mortality ratio of parasite with increasing extract concentration. Wanga et al. [23] studied the anthelmintic activity of Bruea javanica fruits, the methanol extract from the fruits of B. javanica showed significant anthelmintic activity against D. intermedius in C. auratus, which was more effective than the positive control, after 48h exposure. The results of current study (Tab.1 and 2) showed the infected fish before treatment by using water and alcohol extracts, a significant decrease in number of RBCs, PCV and Hb compared to non-infected fish, due to the effect of parasite in the host body feeds on the blood of fish and bleeding in cases of severe injury, activities and various influences of parasite, while there was a significant increase in number of WBCs compared to non-infected, that is result defense reaction in host body against present parasite [24], after treated fish using water and alcohol extracts showed high mortality ratio of parasite, and the fish become healthy, particularly the blood parameters. The low values of both RBC and Hb does not mean occurrence of anemia in infected fish, this result agreed with [25] who examined C. carpio infected with monogenetic parasite D. vastator.

5. Conclusion

The results concluded that the higher the concentration of the water and alcohol extracts, the greater the mortality ratio of monogenean parasites infected Cyprinus carpio, followed by improvement in health status of infected fish.

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7. References