

Effect of *Artemisia herba-alba* water extract in treating the common carp *Cyprinus carpio* infected with the anchor worm *Lernaea cyprinacea*

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Abstract:

Seven concentrations (35%, 40%, 45%, 50%, 55%, 60% and 65%) of water extracts of the white wormwood *Artemisia herba-alba* were experimentally tested to kill the anchor worm *Lernaea cyprinacea* infecting the common carp *Cyprinus carpio* which were obtained from Al-Furat Fish Farm, Babylon province, Iraq. The dip method for five minutes was applied to treat the infected fishes. The extract had caused varying degrees of effects on treated fish behaviour (irritation, increasing number of opercular movements, sudden and speedy swimming movements, hitting the inside walls of the treatment tank, trying to jump out of the tank and upside down inversion of some specimens). A gradual kill of the parasites started with the concentration of 40% when 38% of the parasites were killed. The concentration of 65% had achieved a total killing of the parasites. These treated fishes were returned to special recovery tanks and watched for two to seven minutes until getting their normal state, depending on the concentration of the extract.

Keywords: *Cyprinus carpio*, *Lernaea cyprinacea*, *Artemisia herba-alba*, Dip method.

تأثير المستخلص المائي لنبات الشيح *Artemisia herba-alba* في معالجة أسماك الكارب الإعتيادي *Cyprinus carpio* المصابة بالدودة الكلابية *Lernaea cyprinacea*

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الخلاصة:

تم تجريبياً إختبار سبعة تراكيز (35%، 40%، 45%، 50%، 55%، 60% و 65%) من المستخلص المائي لنبات الشيح لقتل الدودة الكلابية المتطفلة على أسماك الكارب الإعتيادي المأخوذة من مزرعة أسماك الفرات في محافظة بابل، العراق. تم استخدام طريقة التغطيس لمدة خمس دقائق لمعالجة الأسماك المصابة. تسبب المستخلص بدرجات مختلفة التأثير في سلوك الأسماك المعالجة (تهيج، زيادة عدد حركات الغطاء الغلصمي، حركات سباحة مفاجئة وسريعة، اصطدام بالجدران الداخلية للحوض، محاولة القفز خارج الحوض وإنقلاب بعض النماذج رأساً على عقب). بدأ القتل التدريجي للطفيليات عند التركيز 40% حيث تم قتل 38% من الطفيليات. حقق تركيز 65% القتل الكلي للطفيليات. تمت إعادة الأسماك المعالجة إلى أحواض نقاهة خاصة وتمت مراقبتها لحين إستعادتها لوضعها الطبيعي لمدة تتراوح بين دقيقتين إلى سبع دقائق، اعتماداً على تركيز المستخلص.

Introduction

The anchor worm *Lernaea cyprinacea* Linnaeus, 1758 belongs to the family Lernaeidae, order Cyclopoida, class Hexanauplia, subphylum Crustacea of the phylum Arthropoda [1]. The females of this crustacean parasite infect 34 fish species in Japan [2]. A total of 23 fish species were

listed as hosts for this parasite in Argentina [3]. They also infect a minimum of 79 different native and exotic fish species in North America [4]. *L. cyprinacea* is also known to infect some amphibians [2], especially frog tadpoles [5, 6]. It has been introduced to many countries by translocation of cyprinids [7, 8].

The anchor worms are freshwater fish parasites, causative of lernaosis, can cause intense inflammation, leading to secondary bacterial and fungal infections. The secondary infections sometimes worsen and kill the infected fishes. Larger numbers of parasites on gills can interfere with respiration, causing death [9]. It causes damage to scales, skin, and underlying muscle tissue. Severe lesions including ulcers, haemorrhages and fibrous nodules were found on the body surface of parasitized fishes [10]. *L. cyprinacea* attaches itself to all external parts of the host body and also inside the mouth, in the gill chambers, occasionally on the gill filaments or even in the eyes of fishes [11]. In Iraq, *L. cyprinacea* was firstly identified in Al-Zaafaranyah Fish Culture Station, south of Baghdad [12]. A recent study revealed its occurrence from a total of 31 native and exotic fish species from different inland water bodies as well as from fish aquaria, fish cages and different fish farms in different parts of Iraq [13].

Some works were done in Iraq on the control of *L. cyprinacea* by using different treatment agents such as quicklime [14], sumcidin [15, 16], dipterex and other organophosphorus compounds [12, 17, 18, 19], formalin [12], potassium permanganate [20] as well as some plant extracts [21, 22, 23, 24].

The white wormwood *Artemisia herba-alba* Asso belongs to the family Asteraceae, order Asteralis, class Magnoliopsida, phylum Tracheophyta of the kingdom Plantae [25]. It is a perennial shrub that grows commonly on the dry steppes of the Mediterranean regions in Northern Africa (Saharan Maghreb), Western Asia (Arabian Peninsula) and Southwestern Europe, and it is used in folk medicine for treatment of various diseases such as its use as antidiabetic, antihypertensive, antimicrobial and antispasmodic agents [26].

The present article is aimed to use the aqueous extract of *A. herba-alba* to treat the infected common carps *Cyprinus carpio* infected with *L. cyprinacea*, via dip method for five minutes, as plants are available and cheap. Using such extract is generally considered as harmless to the environment. Shortage of many drugs during the economic

blockade imposed against Iraq in 1990 encouraged Iraqi researchers to investigate the use of some plant extracts to control some pathogenic agents affecting human and animal life.

Materials and Methods

The common carps were fished from Al-Furat Fish Farm (previously known as Babylon Fish Farm) at Babylon province, mid Iraq. The infected fishes were transferred to the laboratory with containers filled with water from the farm. They were then stocked in porcelain tanks (110 x 40 x 36 cm) filled with non-chlorinated water with continuous aeration. The fishes were fed with food pellets, their faeces were daily removed and feeding was stopped one day before the start of the treatment trial. Recovery tanks (75 x 20 x 20 cm), filled with non-chlorinated water and supplied with aeration, were supplied to accommodate fishes directly after the end of each treatment trial.

Inflorescences of *Artemisia herba-alba* were bought from a local market, crushed at room temperature and stored until use. Details of the procedure of hot water extraction were according to procedures in phytochemical methods [27]. Seven different concentrations of the extract (35%, 40%, 45%, 50%, 55%, 60% and 65%) were prepared.

Water temperature, pH, dissolved oxygen and salinity of each treatment tank were recorded. The dip method for five minutes was applied. To determine the efficacy of each water extract, the anchor worms were inspected (for movement of the solutions in their bodies) before the treatment for five minutes in the treatment tanks and then after ten minutes of their transfer to the recovery tanks.

Results and Discussion

The followings are the main features of the treatment trials with the aqueous abstract of *A. herba-alba*: Fish total length ranged from 11-27 cm, water temperature $25 \pm 1^\circ \text{C}$, pH 8 ± 0.1 , dissolved Oxygen $8.6 \pm 0.2 \text{ mg/l}$ and water salinity 0.98 ppt.

Five fish specimens were used in each fish tank for each treatment trial. Three replicates were achieved for each trial. The results of the

treatments are given in Table 1. The use of ascending concentrations of *A. herba-alba* revealed that the increasing concentration of the solution had proportionally caused changes in fish behaviour. Such changes included irritation in fish swimming, increase in number of opercular movements, hitting

the insides of the tanks, trying to jump out of the tank and lately upside down inverse of some fish specimens. On the other hand, parasite killing was proportionally increased, with increasing concentration of the treatment agent, from 38% in case of 40% solution to 100% in case of 65% solution.

Table (1). Treatment of *C. carpio* infected with *L. cyprinacea* by using different concentrations of water extract of *A. herba-alba*.

Extract concentration (%)	Observations on both fishes and parasites
35	Fishes were normal during the first moments. Then, they seemed irritated with an increase in number of opercular movements. When transferred to the recovery tank, these fishes returned to their normal state after two minutes there. Parasites turned white in colour with slow movement of fluids inside their bodies. However, they stayed alive.
40	Firstly, fishes were normal, then they became irritated with quick and sudden swimming and showed an increase in opercular movements. When they transferred to the recovery tank, they returned to their normal state within two minutes. Parasite colour turned whitish and death of 38% of such parasites occurred.
45	Fishes were normal within the few moments, but then they became irritated and exerted an increase in opercular movements, sudden and speedy swimming movements and hitting themselves with inside walls of the tank. They became normal after three minutes of their transferring to the recovery tank. Parasites turned whitish in colour with a death of 61% of such parasites.
50	Fishes were normal within the few moments, but then they became irritated and exerted an increase in opercular movements, sudden and speedy swimming movements, hitting themselves with inside walls of the tank and trying to jump outside the tank. After five minutes of their transferring to the recovery tank, fishes became normal. Parasites turned whitish in colour with a death of 74% of such parasites.
55	Fishes were normal within the few moments, but then they became irritated and exerted an increase in opercular movements, sudden and speedy swimming movements, hitting themselves with inside walls of the tank and trying to jump outside the tank. Three fish specimens inverted upside down. After five minutes of their transferring to the recovery tank, fishes became normal. Parasites turned whitish in colour with a death of 84% of such parasites.
60	Fishes were normal within the few moments. Then they became irritated and exerted an increase in opercular movements, sudden and speedy swimming movements, hitting themselves with inside walls of the tank and trying to jump outside the tank. Three fish specimens inverted upside down. After seven minutes of their transferring to the recovery tank, fishes became normal. Parasites turned whitish in colour with a death of 97% of such parasites.
65	Fishes were normal within the few moments. Then they became irritated and exerted an increase in opercular movements, sudden and speedy swimming movements, hitting themselves with inside walls of the tank and trying to jump outside the tank. Three fish specimens inverted upside down. After seven minutes in the recovery tank, fishes became normal. Parasites turned whitish in colour and death of all such parasites occurred.

It is clear that the extract of *A. herba-alba* had exerted a noticeable effect on the anchor

worm. Such effect increased with the increase of its concentration (Figure 1).

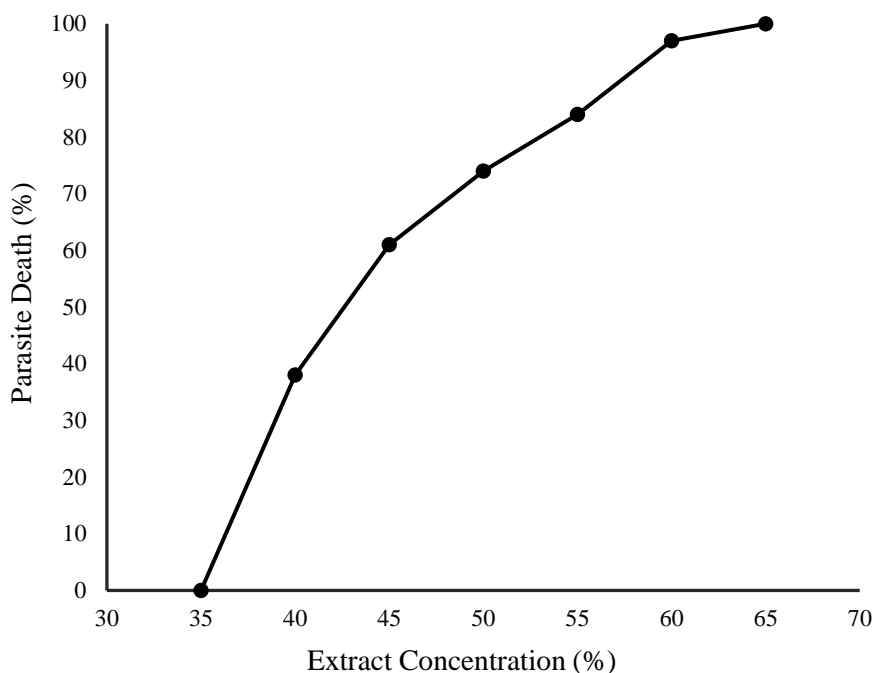


Figure (1). Mortality percentage of *L. cyprinacea* which exposed to different concentrations of water extract of *A. herba-alba*.

The concentration of 65% had achieved a total killing of the parasites. The efficacy of the aqueous extract of *A. herba-alba* is attributed to the presence of several compounds such as alkaloids, glycosides such as santonin, abselthin, thugon, artemisinin and almicinm soapin, tannins and other nonpolar water-soluble substances [28]. Available scientific literature published on *A. herba-alba* focused on the chemical constitutions which have been identified from this species, in addition to all of the reported biological effects, such as antioxidant, anti-venom, nematicidal, antibacterial, anthelmintic, *in vitro* antileishmanial activities as well as the pharmacology and toxicology which were demonstrated in an extensive review [29]. Phytochemical studies of *A. herba-alba* revealed the existence of many beneficial compounds such as herbalbin, cis-chryanthenyl acetate, flavonoids (hispidulin and cirsilincol), monoterpenes and sesquiterpene [26].

The extract of *A. herba-alba* was affective against some other parasites such as protoscolices of *Echinococcus granulosus in vitro* [30, 31], larvae and adults of *Toxocara*

cati in intestine of cats [32], *Hymenolepis nana* in mice [33], two inactive stages of the camel tick *Hyalomma dromedarii* [34], *in vitro* inhibition of egg hatching of the nematode *Haemonchus contortus* [35], against ascaridosis caused by *Heterakis gallinarum* in poultry industry [36] and against *Trypanosoma evansi* infection in rabbits [37]. Also, *A. herba-alba* exhibited promising antitumor efficacy with less toxic effects in case of mice bearing solid tumour [38].

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