Effect of extracts of two aquatic plants *Typha domingensis* (Pers), and *Lemna minor* (Lamarc) on Ecophysiology of common carp *Cyprinus carpio* (Linnaeus)

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Abstract

This study aimed at investigating the impacts of aquatic extracts of two macrophytes *Typha domingensis* (Pers) and *Lemna minor*(Lamarc) on the Ecophysiology of the common carp *Cyprinus carpio* (Linnaeus) reared in waste water. A total of eighty fish were divided into four groups, each group consists of ten fish. Four treatment were used with two replicates of they were made as follow; waste water, clean water (control), waste water- extract of *T. domingensis* and waste water- extract of *L. minor*. Healthy freshwater common carp weighing about 35.75 ± 0.60g were obtained from the aquaculture station of Marine Science Centre /University of Basrah. Physicochemical properties of waters used were recorded and controlled. The diet given to waste water–aquatic plant extract groups was mixed for one night. Blood smears were taken. White blood cells/1 mm^3 were counted by using Neubaur Improved Haemocytometer and light compound microscope. The results indicated that the number of white blood cells of common carp for the control sample was more than that of waste water. While the numbers of white blood cells of the fish in separately treated waste water with *T. domingensis* and *L. minor* extracts were close to the number of the control sample. Blood smears of fish reared in clean water (control sample), waste water- *T. domingensis* extract and waste water- *L. inor* extract seemed quite normal, where as the smear of the blood of fish reared in waste water showed abnormalities in the blood such as disruption of cell walls and deformation of the shape of red blood cell.

Introduction

Fish are not only a major ecosystem component, making it important to study their physiological response mechanism when confronted with environmental stress (Wu *et al*., 2002). Any environmental disturbance can be considered as a potential source of stress as it promotes a number of responses in the fish to deal with the physiological changes triggered by exterior challenges (Martinez-Alvares *et al*., 2002). Nowadays, new biochemical research is focusing on the use of whole-plant-derived dietary supplements,

phytochemicals and provitamins that assist in maintaining good health and combating disease, referred to as functional foods or nutraceuticals (Hoareau and Dasilva, 1999). In several industrialized societies, plant-derived prescription drugs constitute an element in the maintenance of health (UNESCO,1996). The prophylactic and therapeutic effects of plant food extracts are increasingly being recognized as promoter's potential health in both man and livestock (Bizimenyera *et al.,* 2007). Vinodhini, (2010) mentioned that plants need scientific validation and exploration before used as phytotherapeutic agents in control of acute and chronic disorders, when he studied two medicinal plants, *Nelumbo nucifera* and *Aegle marmelos,* which were previously recognized for their remarkable therapeutic effect in the management of various diseases and in the ayurvedha and siddha system of medicine and were selected as herbal drugs. Vinodhini, (2010 evaluated the efficacy and detoxification effect of *Nelumbo nucifera*nd, *Aegle marmelos* in an aquatic system exposed to 5 ppm combined heavy metals, using common carp as an experimental model In the current study the extracts*. T. domingensis* and *L.minor* were used to evaluate their effects on Ecophysiology of common carp.

Material and methods

The extracts of two aquatic plants *T. domingensis* and *L.minor* were prepared as they were described by Heinrich *et al* ,(2004),by taking 50 g of aquatic plants and putting it in 200 ml of distilled water, it was heated till boiling and was filtered ,then the filtrate as the extract was taken. These two extracts were thoroughly mixed

with the artificial diet of common carp fish for one night. Common carp weighing about 35.75 ± 0.60 g were obtained from the aquaculture station of the Marine Science Centre / University of Basrah. Fish were individually checked for external necrosis,
infection and parasites. Those fish which did not show any pathological signs (i.e the very healthy ones) were kept for the study. The feeding frequency(twice a day), and body weight of the fish and physicochemical properties of waters used for rearing fish in control, waste water and separately treated waste water with two aquatic plant extracts (*T. domingensis* and *L. minor* extracts) treatment groups were controlled according to Vinodhini and Narayanan (2009b). Total of eighty fish were distributed among four replicated treatment groups (waste water, with separately treated waste water extracts of *T. domingensis* and *L. minor*, control). Ten fish for each treatment were used.

The waste water was obtained from a close river that was containing domestic sewage water. Glass aquaria were used, each aquarium contained 40 L. of treatment water. The fish diet was composed of; 20% fish meal, 28% Soya bean meal,25%Yellow corn flour, 25% Wheat bran,2 % Vitamins and minerals, protein ratio was30% ,feeding ratio was 5% of fish Body weight. The same diet was given to control and waste water treatments, while for treatment of waste water-plant extract, the plant extracts were mixed with diet for over one night before they were introduced to fish. At the end of the experiment that lasted for ten days, blood-test samples through heart puncture were taken and put in Monovet units with anticoagulant, EDTA (Arnaudov *et al.,* 2009). Blood smears for all studied groups were taken. White blood cells were counted by using Neubaur Improved Haemocytometer and light microscope (Dacie and Lewis, 1975).

Results

The number of white blood cells per 1 mm^3 of the fish from waste water, fresh water (control), waste water plus *T. domingensis* extract and waste water plus *L.minor* extract were counted (Table 1). The highest number was 127000 white blood cells / 1 mm^3, was found in the control fish, while the least one was found in the waste water fish. (29000 white blood cells/1 mm^3), common carp fish from waste water plus *T. domingensis* extract and waste water plus *L.minor* extract showed numbers of WBC close to that of the control (125000,112500 white blood cells/1 mm^3, respectively).

Table (1) The numbers of blood cells in1 mm^3 of blood of common carp (*Cyprinus carpio*) after treatments.

Number of white blood cells per 1 mm^3 in:			
Waste water	Freshwater (control)	Waste water- *T. domingensis* extract	Waste water- *L.minor* extract
29000 ±1400	127000 ±1200	125000 ±950	112500 ±1080

Table2. shows the survival rate % of common carp at the end of the experiment were twenty fish (100 %) in each aquarium (the control and waste water separately treated with each of the two aquatic plant extracts) , except the fish of a waste water (untreated with aquatic plant extracts) all the fish died except two fish (10 %) were very weak .

Table (2) Explained as survival rate percentage of common carp at end of the experiment.

Waste water	Freshwater (control)	Waste water - *T. domingensis* extract	Waste water -*L. minor* extract
10 %	100 %	100 %	100 %

The smears of blood cells of common carp fish that were reared in fresh water, waste water and waste water separately treated with extracts of *T. domingensis* and *L. minor* extracts were shown in Figures 1, 2.3 and 4.

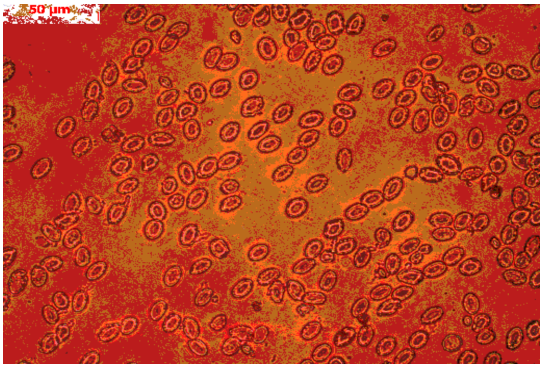

Figure (1) Illustrates the blood smear of common carp cultivated in fresh water treatment 1(control)

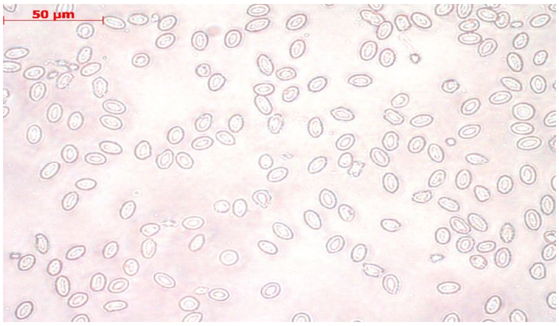

Figure (2) Blood smear of common carp fish cultivated in waste water plus *T. domingensis* extract (treatment 2).

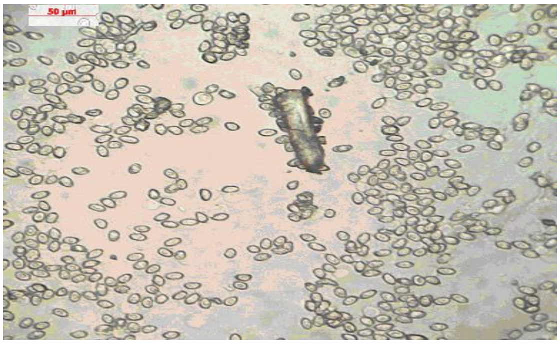

Figure (3) Blood smear of common carp fish cultivated in waste water plus *L. minor* extract (treatment 3).

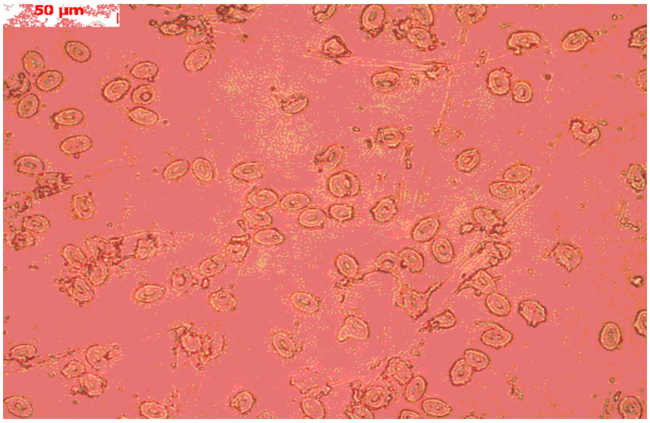

Figure (4) Blood smear of common carp fish cultivated in waste water (treatment 4).

Discussion

Table 1 shows the number of white blood cells Per 1 mm^3 for the common carp cultivated in four different treatments. The highest number 127000 of white blood cell was for common carp cultivated in fresh water treatment 1, while the less was for common carp cultivated in waste water treatment 2 (29000 white blood cells/mm^3), common carp cultivated in waste water plus *T. domingensis* extract and waste water plus *L. minor* extract showed numbers of WBC close to the that of the control treatment (fresh water treatment) and they were 125000, 112500 white blood cells/1 mm^3, respectively.

Recently in aquaculture, natural extracts were tested and used to control the infectious diseases in tilapia, besides that they are cheaper, safer, non-toxic, biodegradable, and biocompatible and have the highest antimicrobial activity (Pachanawan *et al.*, 2008). The herbal diets enhanced WBC, RBC and hemoglobin content compared to the control group (Mohamad and Abasali, 2010). Table 2 shows the surviving rate at the end of the experiment. 100% survival rate were recorded to fish in treatment 1, 2, and 3 while that for treatment 4(waste water treatment) 10% only.

Environmental stressors alter immuno competence (the white blood cells and immunity i fish) for instance in *Fundulus heteroclitus*, the evidence for immunosuppression was found following prolonged captivity, handling, hypophysectomy, radiation,

changes in temperature and salinity, and exposure to chemicals including environmental pollutants (Fries, 1986).
Figures 1, 2 and 3 the represented blood smears of common carp that were cultivated in fresh water (control), waste water plus *T. domingensis* extract and waste water plus *L.minor* extract. The blood of these fish seemed quite normal, whereas the smear of the blood of common carp fish reared in waste water treatment showed abnormalities in the blood such as disruption of cell walls and deformation of the shape of red blood cell. Any environmental disturbance can be regarded as a potential source of stress as it induces a number of responses in the fish to overcome the physiological changes caused by exterior challenges (Martinez *et al.,* 2002). These responses can be detected in fish in the form of changes in biomarkers, alteration in erythrocytes such as cell volume and enzyme activities (Vinodhini and Narayanan, 2008 b, 2009 c; Jee & Kang, 2005). Of all aquatic fauna, fish is the most susceptible to the effects of heavy metal toxicants (Nwaedozie, 1998; Agbozu *et al.,* 2007) Heavy metals are of prime importance among stress generators to fish (Vinodhini and Narayanan, 2009 a). Heavy metals cause differences in the physiological and chemical properties of fish blood (Hughes *et al.,* 1988). Zinc salts can precipitate the mucus on the gills of the fish causing their death from suffocation (Susan and Umminger, 1978; Alabaster and Llyod, 1982; Al-Kahem, 1993).

Heavy metals such as cadmium, chromium, nickel and lead might alter the properties of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering the erythrocytes more fragile and permeable, which probably results in cell swelling deformation and damage (Witeska and Kosciuk, 2003). Physiological changes could be induced by xenobiotic that appear at the biochemical level, as in carbohydrate, protein metabolisms and in blood parameters (Barton and Iwama, 1991).

In aquaculture infectious diseases were controlled by chemotherapeutics and antibiotics. However it was found recently that the use of antibiotics and

chemotherapy created problems with drug resistance bacteria, toxicity and accumulation both in fish and environment. On the contrary natural products like plant extracts could have beneficial effects but cause no problems (Farag *et al.*, 1989; Citarasu *et al.,* 2002; Sadic and Ozcan, 2003). Some plants are rich sources of compounds like volatile oils, saponins, phenolic compounds, tannins,

alkaloids, polypeptides and polysaccharides, these are natural plant products that have various activities such as antistress, apipetizer, antimirobialand immunostimulants (Citarasu *et al.,* 2002, 2003).
Feeding the common carp with immunostimulants like chitin. increased the WBC count (Gopalakannan and Arul, 2006).

T. domingensis and L.minor extract immunostimulants that enable the common carp fish that were reared in polluted water plus *T.domingensis* extract and polluted water plus *L. minor* extract to survive, whereas the fish reared in polluted water alone most of them died.

Conclussion

T. domingensis and L. minor extracts could have immunostimulants that enable the common carp fish that were cultivated in waste water plus *T. domingensis* extract and waste water plus *L. minor* extract to survive and withstand the organic pollution where as the fish that were cultivated in waste water alone could not survive properly and only 10% survived

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تأثير مستخلصا النباتين المائيين البردي *Typha domingensis* (Pers) وعدس الماء (Lamarc) *Lemna minor* على بيئة وفسلجة الكارب الشائع *Cyprinus carpio* (*Linnaeus* 1958)

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الملخص

هدفت التجربة الى معرفة تاثير مستخلصين النباتين المائيين البردى *Typha domingensis*) وعدس الماء (*Lemna minor*) على مقاومة وبقاء اسماك الكارب الشائع *Cyprinus carpio*) عندما استزرعت فى الماء الملوث. استخدمت فى التجربة ثمانون سمكة وقسمت العينات الى اربعة مجاميع كل مجموعة بمكررين وهذه المجامبع تضم احواض الماء الملوث، الماء العذب (العينة الضابطة)) الماء الملوث المعامل مع مستخلص نبات البردى والماء الملوث المعامل مع مستخلص نبات عدس الماء، وضعت عشرة اسماك فى كل حوض من احواض التجربة. كانت الاسماك سليمة من الامراض وقد اخذت من احواض استزراع الاسماك التابعة لمركز علوم البحار، جامعة البصرة. سجلت الصفات الفيزيكيميائية لماء استزراع الاسماك وظلت تحت السيطرة. اعطيت مجاميع التجربة نفس الغذاء. ولكن مجموعتى الماء الملوث المعامل مع مستخلصى النباتين المائيين بقى الغذاء ممتزجا مع المستخلص ليلة كاملة قيس عدد خلايا الدم البيضاء فى المليمتر المكعب الواحد (باستخدام Haemocytometer والمجهر الضوئى المركب) واخذت مسحات دموية للاسماك المستزرعة فى احواض مجاميع الاسماك الاربعة المدروسة. كان عدد خلايا الدم البيضاء للاسماك فى حوضي الماء الملوث المعاملين مع مستخلصا نباتى البردى وعدس الماء قريبا جدا من عدد الخلايا الضابطة. ظهرت الخلايا في المسحات الدموية العائدة لاسماك المجموعة الضابط ة واسماك مجموعتى الماء الملوث المعامل مع مستخلصى نباتى البردى وعدس الماء بانها طبيعية وغير مشوهة ، بينما ظهرت الخلايا فى المسحة الدموية العائدة الى الاسماك المستزرعة فى حوض الماء الملوث بانها مشوهة الشكل وممزقة الجدران.