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Hematological Evaluation of some Aqueous Plant Extracts on *Clarias gariepinus*

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Abstract: This study deals with evaluation of the hematological effect of natural medicinal plants, *Allium sativum* and *Thymus vulgaris* on parasites of African Catfish, *Clarias gariepinus* as an effective solution for improving fish aquaculture and fish health. A total of 200 specimens, *Clarias gariepinus* were divided into four groups, control (G1), 0.1 g/L of garlic water extract (G2), 0.1 g/L of thyme water extract thyme (G3) and 0.05 g/L of garlic water extract mixed with 0.05 g/L of thyme water extract (G4) groups. Each fish group was placed in 250 liters of water from River Nile for 4 weeks. Results concluded that beneficial supplementation of *T. vulgaris* improves growth rate of common catfish and improved blood parameters (eg, hemoglobin count, RBC count, WBC count). The present results displayed positive effects of garlic and thyme supplemented on healthy status as natural source for fish aquaculture.

keywords: Garlic, Thyme, *Clarias gariepinus*, Hematological, Blood picture.

1.Introduction

As the world suffering from the continuous increase in human population with subsequent increase in their nutritional demands, fish are expected to offer a great hope in dissolving the problem of animal protein deficiency (17).

The African catfish, *Clarias gariepinus* is an important farmed fish in tropical and subtropical regions. *C. gariepinus* is a fish known for its rapid growth rate and resistance to adverse conditions such as handling, temperature fluctuations, low oxygen, deteriorating water quality, and high stocking densities (4).

In general, plant extracts containing abundant proteins, carbohydrates, vitamins, lipids, and minerals can play an important part in enhancing growth performance and modifying physiological function of fishes. Medicinal plants can modulate the innate immune response. Plants may directly initiate activation of the innate defense mechanisms acting on receptors and triggering intracellular gene activation that may result in the production of antimicrobial and parasitic molecules. Disease outbreaks in commercial aquaculture may be prevented by the enhancement of innate and adaptive immunity through the application of plants extracts (15).

Garlic (*Allium sativum*), commonly known as garlic, is a member of the family Alliaceae, and it has a history of dietary and medicinal applications for curing various diseases. The medicinal effects of garlic are based on the organo-sulfur compounds, particularly allicin, which has potential antibacterial, antiprotozoal, antifungal, antiviral, antitumorigenic, antimutagenic, antioxidant, and detoxification properties (12).

Thyme (*Thymus vulgaris* L.) locally known as "Zaatar" is an herbaceous perennial plant belonging to the Lamiaceae family. *T. vulgaris* (garden thyme) is a small shrubby plant with a strong, spicy taste, and odor. This plant is indigenous to the Mediterranean region of Europe and is extensively cultured in the United States. Thyme has a strong antimicrobial, antiparasitic and antioxidant activity due to its very high contents of thymol, p-cymene, carvacrol, eugenol, and 4-allylphenol (5). Thymol, a major component of thyme essential oils, has been widely studied for its antimicrobial properties (3).

The aim of this study is to evaluate the effect of natural medicinal plants, *Allium sativum* and *Thymus vulgaris* on hematology (Hb content, PCV value, RBC and WBC count) of *C.*

gariepinus as an effective solution for improving fish aquaculture.

2. Materials and methods

2.1. Sampling of the African fish, *Clarias gariepinus*

A total of 200 specimens of the African catfish, *Clarias gariepinus* Cuvier and Valenciennes, 1840 were collected from the River Nile in El-Mansoura City (El-Dakahlia Governorate, 120 Km north to Cairo, Lower Egypt, coordinates: 31° 7' 30" N, 31° 25' 49" E).

The fishes were placed in the laboratory in a plastic tank aquarium supplied with 250 liters of water from the River Nile in order to be similar to its natural environment as possible and was equipped with an air pump and an electric filter.

2.2. Sampling of experimental plants and preparation of extracts

Fresh lobes of garlic (*Alium sativum*) and fresh plant leaves of thyme (*Thymus vulgaries*) were collected. Plant parts were cleaned, cut into small pieces, minced and weighed. The aqueous extract (juice) was prepared by dissolve dried 100 g of plant extract in 1 L in water.

2.3. Grouping of experimental fish

The collected fish were divided into four groups, in which each group is applied for one month in a separate tank as the following:

A- Control group (G1); in which the fish was bred in the tank without adding any additional factors for the investigation period.

B- *Allium sativum* group (G2); in this group amount of 0.25 L of prepared garlic juice forming 0.1 g/L in the tank water of *A. sativum* was added to the tank twice weekly for four weeks

C- *Thymus vulgaris* group (G3); in this group amount of 0.25 L of prepared thyme juice forming 0.1 g/L in the tank water of *T. vulgaris* was added to the tank twice weekly for four weeks

D- *Allium sativum* & *Thymus vulgaris* group (G4); in this group amount of 0.125 L of prepared garlic juice forming a concentration of 0.05 g/L in the tank water of the aqueous extract of with *A. sativum* mixed with a same concentration of *T. vulgaris* (0.05 g/L) were added to the tank twice weekly for four weeks

2.4. Haematological analysis and blood collection

Blood analysis of fish serves in diagnostic purposes; in addition this main purpose, it is also to analyze the effect of plant extracts on fish.

Blood samples were collected via caudal vein puncture as described by (8).

The parameters of blood picture including red blood cells count (RBCs), Haemoglobin (Hb), Hematocrit value (Ht), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cells Count (WBCs) were analyzed using Celtal Alpha MEK-6400 hematology analyzer.

2.5. Statistical data analysis

One-way ANOVA test using SPSS (version 25 for Windows) was used to detect differences between hematology during the experiment in different groups. If significant differences were found, multi-range comparisons (least significant difference) were selected from PostHoc tests (Tukey HSD) on the same statistical package to detect differences between infestation levels for different parasites in each group. The cluster analysis program (6) is used to determine the similarity index of hematogical analyses between different groups.

3. Results and Discussion

3.1. Complete blood picture (CBC)

Complete blood picture and enzyme analyses were determined in all groups at the end of investigation period on specimens of *C. gariepinus* in all investigated groups.

3.1.1. Hemoglobin (HGB)

Mean Hemoglobin (HGB) values are determined in specimens of the four groups in which the highest hemoglobin value was recorded in group 3 (12.43 ± 1.72 g/dl), followed by group 1 (11.20 ± 3.55 g/dl) and group 4 (9.87 ± 3.72 g/dl) then finally, the lowest value of hemoglobin was recorded in group 2 (4.00 ± 0.28 g/dl) as shown in Table (1).

Table (1): Blood picture analysis of *Clarias gariepinus* in the four studied groups.

Blood Parameter	Group 1	Group 2	Group 3	Group 4
HGB (g/dl)	11.20 ± 3.55	4.00 ± 0.28	12.43 ± 1.72	9.87 ± 3.72
RBC (x100^{3}cells/µl)	1.88 ± 0.60	0.72 ± 0.03	2.55 ± 0.37	2.05 ± 0.84
HCT (%)	29.76 ± 10.82	10.89 ± 0.53	37.51 ± 5.18	26.40 ±13.45
MCV (fl)	156.67 ± 7.02	153.50 ± 4.95	147.33 ± 8.62	127.00 ±24.25
MCH (pg)	59.60 ± 0.72	55.35 ± 1.77	48.80 ± 1.65	49.03 ± 4.53
MCHC (g\dl)	38.10 ± 1.66	36.25 ± 0.21	33.23 ± 2.90	39.23 ± 5.78
WBC (x10^3/mm^3)	79.45 ± 14.26	69.99 ± 3.46	85.27 ± 6.20	66.93 ±26.94
PLT (10³/µl)	29.33 ± 7.51	30.50 ± 2.12	101.00 ± 26.96	88.00 ±27.51

3.1.2. Red blood cells count (RBCs)

Mean red blood cells count (RBCs) values are detected in specimens of the four groups. The highest RBCs value was 2.55 ± 0.37 (x100^3 cells/µl) in G3, followed by 2.05 ± 0.84 (x100^3 cells/µl) in G4 and 1.88 ± 0.60 (x100^3 cells/µl) in G1 then finally, the lowest value of RBCs was 0.72 ± 0.03 (x100^3 cells/µl) that recorded in G2 (Table 1).

3.1.3. Hematocrit (HCT)

Mean Hematocrit (HCT) values are observed in specimens of the four groups. The highest HCT value was (37.51± 5.18%) in group (3), followed by (29.76± 10.82%) in group (1) and (26.40± 13.45%) in group (4) then finally, the lowest value of RBCs was (10.89± 0.53%) that recorded in group 2 (Table 1).

3.1.4. Mean Corpuscular Volume (MCV)

Mean of Mean Corpuscular Volume (MCV) values are detected in specimens of the four groups. The highest MCV value was recorded in group 1 (156.67± 7.02 fl), followed by group 2 (153.50± 4.95fl) and G3 (147.33± 8.62 fl) then finally, the lowest value of MCV was in group 4 (127.00± 24.25 fl) that recorded in (Table 1).

3.1.5. Mean Corpuscular Hemoglobin (MCH)

Mean of Mean Corpuscular Hemoglobin (MCH**)** values are found in specimens of the four groups. The highest MCH value was 59.60± 0.72 pg in G1, followed by 55.35± 1.77 pg in G2 and 49.03± 4.53pg in G4 then finally, the lowest value of MCH was 48.80± 1.65 pg that recorded in G3 (Table 1).

3.1.6. Mean Corpuscular Haemoglobin Concentration (MCHC)

Mean of Mean Corpuscular Haemoglobin Concentration (MCHC) values are determined in specimens of the four groups in which the highest (MCHC) value was recorded in G4 (39.23± 5.78 g\dl), followed by G1 (38.10± 1.66g/dl) and group 2 (36.25± 0.21g/dl) then finally, the lowest value of (MCHC) was recorded in group 3 (33.23± 2.90 g/dl) as shown in Table (1).

3.1.7. White Blood Cells Count (WBCs)

Mean White Blood Cells Count (WBCs) values are detected in specimens of the four groups. The highest WBCs value was (85.27± 6.20 x10^3/mm^3) in G3, followed by (79.45± 14.26 x10^3/mm^3) in G1 and (69.99 ± 3.46 x10^3/mm^3) in G2 then finally, the lowest value of WBCs was (66.93 ± 26.94 x10^3/mm^3) that recorded in G4 (Table 1).

3.1.8. Platelets Count (PLT)

Mean Platelets Count (PLT) values are found in specimens of the four groups. The highest PLT value was 101.00 ± 26.96 10³/µl in G3, followed by 88.00 ± 27.51 10³/µl in G4 and 30.50 ± 2.12 10³/µl in G2 then finally, the lowest value of PLT was 29.33 ± 7.51 10³/µl that recorded in G1 (Table 1).

Statistical analysis (Independent samples One-way ANOVA on SPSS IBM package version 25) indicated that MCH and PLT showed a high significant difference between different groups ($p \leq 0.01$). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) MCH detected high significant differences between G1 with G3 and G4 ($p \leq 0.01$). Whereas, detected high significant differences between G1 with G3 ($p \leq 0.01$) and significant differences between G1 with G4 and G2 and G3 ($p \leq 0.05$).

On the other hand, statistical analysis indicated that HGB and RBCs showed a significant difference between different groups ($p \leq 0.05$). Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD)

HGB and RBCs detected significant differences between G2 and G3 only ($p \leq 0.05$).

Finally, statistical analysis indicated that HCT, MCV, MCHC and WBCs showed non-significant difference between different groups ($p > 0.05$).

3.2. Cluster analysis of blood picture analysis

The cluster analysis program analyzes the input data of blood picture analysis values of different fish groups then grouped them where, the high similarity index in blood picture analysis between group appears within the same group, while differential index blood picture analysis separates them in different groups (A, B, C and D).

The application of cluster analysis based on the similarity in blood picture analysis values of different fish groups (4 variables) for catfish, *C. gariepinus* led to the recognition of four groups (Fig. 1). Group A comprises one groups, G1, but Group B comprises one group, G2, while Group C comprises one group finally, G2 and Group D comprises one group, G3.

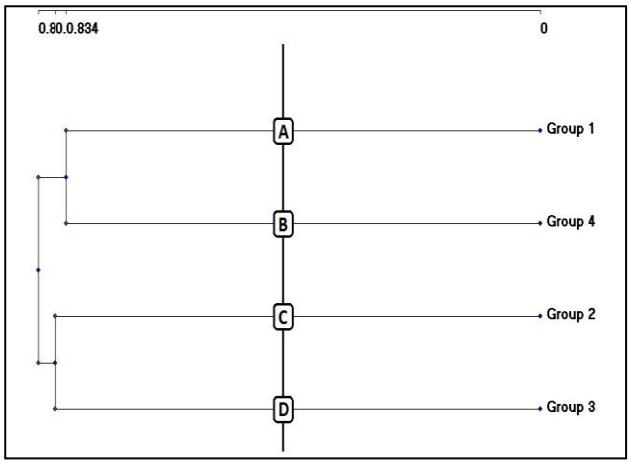

Fig. (1): Cluster analysis of different fish groups according to blood picture analysis values.

It is obvious that, each group of the four groups showed a significant difference in blood picture analysis values in which each of them appears in a separate group.

In order to investigate the *in vivo* effects of *Allium sativum* and *Thymus vulgaris* on internal helminth parasites of the clariid African catfish; *Clarias gariepinus* were investigated. This study was carried out on 4 groups, control groups (G1) and three other treated groups. G2 treated with *A. sativum*, G3 treated with *T. vulgaris* and G4 treated with a mixture of *A. sativum* and *T. vulgaris*.

The presence of parasites may cause some pathological effects on fish by delaying their growth, causing tissue disorder and even death. However, it can be assumed that parasitic infection of fish does not reduce its productivity, as shown in several studies (1).

Colorful parasites are associated with *C. gariepinus* in the wild and civilized terrain where they beget morbidity, mortality and profitable losses in monoculture practice in colorful corridor of the world (14; 2).

The application of herbal plant products as plant extract is more useful as they are safe and eco-friendly, directly taken by humans as food or medicine. It acts as a feed substitute, growth stimulator, immunostimulant, antimicrobial and fertilizer in aquaculture without any negative impact on the natural ecosystem (11).

Due to the high diversity of plant extract compounds, using them could lower treatment costs and be more environmentally benign as parasites are less likely to develop medication resistance (9).

Numerous studies have noted the advantages of plants like garlic (Allium sativum), which were successful in preventing fish illnesses and improving the cultured performance and immune response of farmed fish (7).

Alkaloids, saponins, reducing sugars, oils, steroids, and proteins were all present in moderate concentrations in the crude extract of the *A. sativum* bulb, whereas flavonoids and acidic compounds were only found in trace levels. An annual plant with a grassy appearance, *T. vulgaris* grows all over the planet. Due to its expectorant, antitussive, antibroncholytic, antispasmodic, antihelminthic, carminative, and diuretic qualities, it is frequently employed in traditional medicine. Because it contains more essential oils with a high concentration of Thymol, which has significant antitussive, spasmolytic, and expectorant properties, T. vulgaris is more frequently utilised in pharmaceutical dosage forms (16)

The outcomes demonstrated that garlic cloves and thymus leaves could improve particular immune responses. The outcome also

demonstrates that white blood cell counts in the treated groups were higher than those in the control group, indicating that the fish were able to develop an immunity to pathogens by ingesting thymus leaf and garlic extracts in water. Hematological parameters (RBC, hematocrits, haemoglobin, PLT, MCV, MCH, MCHC, RDW, PDW, lymphocytes, monocytes, and granulocytes) also was increased.

Statistical analysis (One-way ANOVA) indicated that complete blood picture analysis showed a significant difference between different groups. Regarding the Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected significant differences between different groups in some parameters (hemoglobin, RBC, MCH, LYM, MID, PLT, PCT).

The results of this investigation were consistent with those of (13), who found that *O. niloticus* fed various grades of *Allium sativum* and *Thymus vulgaris* saw a substantial ($p<0.05$) increase in erythrocyte count, haemoglobin content, and hematocrit value. (10)'s findings also demonstrated that *Allium sativum* supplementation boosted erythrocyte quantity, haemoglobin content, hematocrit value, leucocytes and thrombocytes in fish diets.

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