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Effect of some herbal extracts on the health status of cultured Oreochromis niloticus.

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ABSTRACT

The present study was conducted to evaluate the effect of (onion leaves-barley seeds-rice straw) extract on growth, hematological and some biochemical parameters of tilapia fish (*oreochromis niloticus)*. The experiment was carried on seven fish groups (10fish/group with triplicate) for 8 weeks. Seven experimental diet were formulated at 25% crude protein representing the following levels {control (0), Onion (0.5) ,Onion (1), Barley (0.5) ,Barley (1), Rice (0.5) and Rice (1) g/kg diet} .210 fish (wt. 30gm±5) were fed twice daily 3% of body weight. At the end of experiment there was significant increase ($P \leq 0.05$) in weight gain in groups fed onion and barley extracts (0.5-1) g/kg diet. After 8 weeks, RBCs, HB, WBCs, glucose, uric acid and creatinin were measured. No changes in RBCs count and HB level in all treated groups while there was significant increase in WBCs count in onion and barley groups. The blood glucose level showed significant decrease in onion groups than control one. The analysis of AST, ALT, and ALP showed no significant changes in all treated groups. The results of this study demonstrated that onion and barley extracts could improve growth and health of cultured *oreochromis niloticus.*

Keywords: Plant extract, tilapia, growth, biochemical parameter.

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INTRODUCTION

Aquaculture is considered one of the most effective solutions to cover the gap in food consumption where tilapia fish represents one of the main principal sources of the national income (1). Due to increasing demands for more proteins, fish aquaculture has increased worldwide (2). Responding to the increased demand for fish, many fish species are now being cultured at intensive levels specially tilapia spp. leading to spread of infectious diseases (3).

Using antibiotics as feed additive or therapeutics lead to appearance of resistant bacterial strain and accumulation of residues in fish and environment may reach to human (4).On the other hand, vaccination has some disadvantage as difficulty application, more labor required, high cost and impossible to develop pro-active prophylactic strategies in new diseases emerging from time to time (5).

So the application of plant products as plant extract is more useful as they are safe and eco-friendly, directly taken by humans as food or medicine. Also they are gaining importance in aquaculture as its advantages are available in plenty, cheap, their action is effective, they act as the substitution for feed, fertilizer in aquaculture, no adverse effect on natural ecosystem, act as a growth promoter, immune stimulant ,anti-bacterial substrate, anti-fungal agents and anti-parasitic agents (6).

Herbs and medicinal plants are promising to be important sources of therapeutics in fish culture since these products provide a cheaper source for treatment and greater accuracy without causing toxicity (7). In general, plants have a variety of functions due to the presence of various active compounds like alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids and essential oils (9).

Onion *(Allium cepa)* has medicinal uses as they are high in flavonoids which are concentrated on the outer layer of the flesh (10). Onion has growth promoting effect on *Clarias gariepinus* as it increases the villi length and width (area of absorption) so enhance absorption and digestion (10) and has prebiotic activity due to their high soluble fiber content (11). Also onion has hypolipidemia and hypocholesterolemia in blood serum so decrease stress on fish, in addition, it increase immunity in Olive flounder juvenile by increasing lysozyme activity (12). Barley (*Hordeum vulgare*) contains antioxidant compounds (13) which act as anti-inflammatory, anti-carcinogenic and anti-microbial activities (14). Barley straw is used in aquaculture for cleaning wastes from water by inhibiting the growth of certain types of algae in laboratory studies and field trials (15). Barley bran when used in fish diet increases final body weight, weight gain and specific growth rate (16).

Rice straw (*Oryza sativa*) is one of the abundant lingo-cellulosic waste materials in the world. It usually causes the most environmental problem since it exists with enormous quantities so burning in fields is a common option for disposal but it causes serious air pollution. Therefor new economical methods for rice straw disposal and utilization must be developed (17). It is of low cost and its biodegradable favor the growth of bacterial biofilm and prephyton which used as feed for fish (18). Rice straw extract can be used in fish pond to decrease the turbidity (19).

Therefore, the demand for replacement of natural products has been rising with a focus on plant products as growth promoter and alternative to antibiotics. In this regard, several plants extracts such as: Onion leaves, barley seeds and rice straw were tested in this study to evaluate their effect on growth, physiological and blood parameters of tilapia fish.

MATERIAL AND METHOD

Fish:

A total of 210 apparently healthy *Oreochromis niloticus* with an average body weight 30±5 g, were collected alive from private fish farm at Kafr Elshikh governorate. Out of 10 apparently healthy fish were selected randomly in each tank. Fish were kept for 2 weeks for acclimation and supplied with de-chlorinated water. Continuous aeration was maintained in fully prepared glass tank 40L by using electric air pumps**.**

The water quality criteria for all groups were within the permissible limits (temperature was adjusted to 24.5±0.1°C, PH 6.8±0.2, O_2 5.5mg/l) (20).

Feed:

Fish fed for 8 weeks from (April to May).

a. **Diet:**

Commercial fish diet 25% crude protein, 4.5 % fiber and 5% fat as well as vitamins and minerals.

b. **Plant extract:**

- Plants (onion leaves, barley seeds, rice straw) purchased from global market**.**
- Preparation of plant extracts.

Plant samples will dry at room temperature and ground to a fine powder, using a mortar and pestle. The extraction will process using (methanol) solvent. The extracted material filtered through Whatman No. 1 filter paper and the final extract was evaporated by rotary evaporator (21).

Feed preparation:

Standard commercial fish diet was mixed with extracts (onion leaves, barley seed, and rice straw) and 6 experimental rations were prepared.

Growth parameters determination:

a. Body weights: weights were estimated biweekly throughout the experimental period.
b. Body weight gain: Final fish weight (g) – Initial fish weight (g) according to Annet (22).

Clinicopathological examinations:

a. Haemgram:

- **Determination of hemoglobin concentration (Hb %):**

Hemoglobin concentration was determined by acid hematin method according to Coles (23).

- **Erythrocytes and leukocytes count:**

Blood cells were counted using a haemocytometer according to Kanaeu (24).

- **Differential leukocytic count :**

Thin blood smears were prepared and stained with Giemsa stain for 30 minutes. One hundred leukocytes were identified and the percentage values of different white cells were calculated as measured by Jain (25).

Biochemical analysis:

- **Liver enzymes:**

Serum ALT, AST and ALP activities were estimated calorimetrically using Vitro Scient kits as described by Reitman (26).

- Uric acid was estimated by kits of spectrum according to Schultz (27)
- Creatinin was estimated according to Rock *et al.* (28)

- Glucose was measured by kits of spectrum comp. according to Borges *et al.* (29)

Statistical analysis:

Data were presented as means ± standard error (SE) and the significance of differences was evaluated using analysis of variance (ANOVA) test SPSS 14, (2006).

RESULTS

- **Growth performance:**

The result of growth performance of different fish groups demonstrated in table (1-2) indicated significant increase in body weight and weight gain in all treated groups than control except rice (1, 0.5g) groups during the 8weeks of the experimental period.

Table(1): Weight (g) of different fish groups during the 8weeks of the experimental period.

Groups	2nd week	4th week	6th week	8th week
O 1g	37.35±1.75 ^{B}	49.75±0.45^{CD}	63.15±0.95^{E}	75.9±0.60^{B}
O 0.5g	37.23±0.32 ^{B}	47.13±0.27^{CD}	55.93±3.29^{CD}	74.46±1.47^{B}
B 1g	37.10±0.92 ^{B}	45.00±1.83^{BD}	50.90±0.36^{BD}	71.20±1.55 ^{B}
B 0.5g	36.63±0.92 ^{B}	42.23±1.55 ^{B}	49.66±1.78^{B}	60.10±4.30 ^{C}
R 1g	33.03±0.32^{A}	37.03±1.15^{A}	43.30±2.40^{A}	49.00±4.10^{A}
R 0.5g	33.77±0.49^{A}	36.40±0.55^{A}	39.92±0.43^{A}	43.55±0.58^{A}
Cont.	33.16±0.54^{A}	36.26±0.66^{A}	40.70±0.32^{A}	44.33±0.44^{A}

O=onion, B= Barley, R= rice, Cont. = control
Initial weight =30±5 gm.
Data represented as means ± SE; n= 10
Columns with different letters are significantly difference at p ≤0.05.

Table (2):Weight gain (g) of different fish group during the 8weeks of the experimental period.

Groups	2nd week	4th week	6th week	8th week
O 1g	7.35±1.75 ^{B}	19.75±0.45^{B}	33.15±0.95^{B}	45.90±0.6^{B}
O 0.5g	7.23±0.32^{B}	17.13±0.27^{B}	25.93±3.29^{B}	44.46±1.47^{B}
B 1g	7.10±0.92 ^{B}	15.00±1.83^{B}	20.90±0.36^{B}	41.20±1.55^{B}
B 0.5g	6.60±0.92 ^{B}	12.23±1.55^{B}	19.66±1.78^{B}	30.10±4.30^{B}
R 1g	3.03±0.32^{A}	7.03±1.15^{A}	13.30±2.40^{A}	19.00±4.2^{A}
R 0.5g	3.77±0.49^{A}	6.40±0.55^{A}	9.92±0.43^{A}	13.55±0.58^{A}
Cont.	3.16±0.54^{A}	6.26±0.66^{A}	10.70±0.32^{A}	14.33±0.44^{A}

O=onion, B= Barley, R= rice, Cont. = control
Initial weight =30±5 gm.
Data represented as means ± SE; n= 10
Columns with different letters are significantly difference at p ≤0.05.

Hematological studies:

- **The results of RBCs count, Hb and WBCs count:**

- The results of RBCs count and HB represented in table (3) showed non-significant difference in RBCs count and non-significant difference in HB level in all groups compared to control one.
- The results of WBCs count and differential leukocytes count of different fish groups were represented in Table (4). The results showed significant increase in WBCs count and lymphocyte % in all treated groups except rice (1-0.5gm) groups compared to control while there was no significant increase in heterophiles% and monocytes% in all treated groups compared to control one.

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Table (3): RBC$_S$ count ×10^6 and Hb (g/dl) in different fish groups.

Groups	R.BC$_S$×10^6	Hemoglobin(g/dl)
O 1g	3.05±0.05	12.20±0.18
O 0.5g	2.80±0.20	11.57±0.09
B 1g	2.90±0.05	11.39±.047
B 0.5g	2.73±0.24	11.50±0.25
R 1g	2.76±0.12	11.39±0.55
R 0.5g	2.75±0.13	10.61±0.41
Control	2.70±0.11	10.35±0.04

O=onion, B= Barley, R= rice, Cont. = control
Data represented as means ± SE; n= 3

Table (4): WBCs count and differential leukocytes count of different treated *O. niloticus* groups.

Groups	WBC$_S$×10^3	Lymphocytes%	Heterophiles%	Monocytes %
O 1	53.00±1.02^{CD}	80±1.24^{CD}	7.0±0.58	2.00±0.56
O 0.5	51.33±1.85^{CD}	78±1.32^{CD}	7.5±1.23	2.50±1.23
B 1g	48.33±0.88^{BD}	75±1.24^{BD}	6.6±1.56	2.00±1.74
B 0.5	44.00±2.64^{B}	72±0.89^{B}	8.4±1.35	1.50±1.38
R 1	35.66±4.70^{A}	71±1.21^{A}	7.2±2.01	1.40±1.76
R 0.5	31.10±0.68^{A}	71±0.97^{A}	6.5±1.02	1.50±1.45
Cont.	30.63±0.68^{A}	70±1.02^{A}	6.7±1.64	1.04±0.57

O=onion, B= Barley, R= rice, Cont. = control
Data represented as means ± SE; n= 3
Columns with different letters are significantly difference at p.value ≤0.05

Biochemical analysis of fish serum:

- **Serum transferases:**

The results of serum transferases (ALT-AST) of all fish groups were represented in Table (5) .There were no significant differences in ALT and AST level in all treated groups compared to control one.

Table(5): AST,ALT and ALP of different fish groups.

Groups	ALT(U/L)	AST(U/L)	ALP (U/L)
O 1	10.03 ±0.42	25.2 ±0.05	0.29±0.06
O 0.5	9.61±035	24.96±0.17	0.30±0.04
B 1	9.36±0.42	25.30±0.12	0.25±0.07
B 0.5	9.93±0.30	24.73±0.12	0.22±0.04
R 1	9.97±0.09	24.26±0.18	0.24±0.03
R 0.5	9.87±0.10	24.10±0.11	0.28±0.03
CON.	10.30±.082	24.06±0.08	0.27±0.05

O=onion, B= Barley, R= rice, Cont. = control
Data represented as means ± SE; n= 3

- **Alkaline phosphatase**

The results of ALP represented in table (5) indicated no significant difference in all treated groups than control.

- **Glucose level:-**

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Glucose level of different fish groups represented in table (6) the results indicated significant decrease in glucose level in onion (0.5gm-1gm) than control.

Table (6) Glucose, creatinin and Uric acid of different fish groups.

Groups	Glucose (g/dl)	Creatinin (g/dl)	Uric acid(g/dl)
O 1g	49.87± 0.08 ^{B}	0.77 ± 0.09^{ED}	1.27 ± 0.09^{A}
O 0.5g	50.68± 0.18 ^{B}	0.78± 0.11^{E}	1.38 ± 0.06^{A}
B 1g	54.22± 0.12^{A}	0.83 ±0.07^{BC}	2.13 ±0.11^{B}
B 0.5g	54.29± 0.07^{A}	0.74± 0.012^{BD}	1.91 ±0.04^{B}
R 1g	54.73± 0.25^{A}	0.86 ± 0.07^{BC}	1.24 ±0.05^{A}
R 0.5g	55.45± 0.04^{A}	0.84 ± 0.02^{BC}	1.45 ±0.07^{A}
Cont.	54.42± 0.12^{A}	0.94 ± 0.03^{A}	1.35 ±0.07^{A}

O=onion, B= Barley, R= rice, Cont. = control
Data represented as means ± SE; n= 3
Columns with different letters are significantly difference at p.value ≤0.05

- **Creatinin in different fish group:-**

The results of creatinin represented in table (6) indicated significant decrease in Creatinin level in all treated groups than control groups.

- **Uric acid in different fish groups:-**

The results of uric acid represented in table (6) indicated significant increase in uric acid in barley (1, 0.5gm) groups than control group.

DISCUSSION

With the adverse effects of antibiotics people are now shifting to natural products. Plant extracts are safe and cheap and they do not lead to drug resistance (6). Herbal preparations are known to play an important role in disease control due to their active ingredients as phenolics, alkaloids, steroids, flavonoids, and essential oils which act as antimicrobial, antioxidant, growth promotion, anti-stress, tonic, appetite stimulation, and immune stimulation (30). The application of these herbal plants has been used in many laboratories and reported in some papers with narrow field practice (31).

Our study was aimed to discuss the potential of plant extracts as effective substitute for chemical treatments by natural products and as a growth promoter.

The present results declared a significant increase in body weight and weight gain in all treated groups than control except rice groups during the 8weeks of the experimental period.

In our study onion has the highest results in weight gain this may be due to the effect of onion extract on the intestinal villi as onion extract affect the villi length and width (area of absorption) in *C. gariepinus* so enhance absorption and digestion (10). Also onion has prebiotic activity due to their high soluble fiber content (32), specially inulin and fructo-oligosaccharides which stimulate the growth of specific microorganisms in the colon, as bifidobacteria and lactobacilli, with a general positive health effect (11). Additionally, a previous study revealed that the highest weight gain (WG %) and specific growth rate (SGR) was observed in beluga juvenile group fed with 1% onion ($P < 0.05$) than control group (33). However, unlike this study, dietary

inclusion of various 0, 0.5, 1, 2, 3, and 5% onion powder had no distinctive improvement on weight gain, specific growth rate and feed efficiency of the juvenile olive flounder (12).

On the other hand in relation to barley, there was significant increase in body weight unlike study of (16) who reported that fish groups fed diet containing barley bran showed the highest body gain but the food conversion ratio was low.

Also the lowest results in weight gain was in fish groups fed on diet with rice straw extract this may be due to the high level of cellulose in rice straw as in (31) study who indicated that Nile tilapia are able to utilize dietary cellulose does not exceed 10% in their feed for optimum growth so as the rice straw extract contain upto 44% cellulose (35) tilapia fish can't digest and absorb the high level of cellulose.

Other result of introducing rice polish as a plant protein source for Nile tilapia and three diets were formulated (0, 10 and 20%) for a feeding trail of eight weeks to observe the growth performance and feed utilization revealed that it would be cost effective without any significant change in growth performance while the control diet revealed the highest body weight gain (36). This agrees with our results with rice straw extract as it didn't give any significant changes in growth performance.

Our study also indicated that there was no effect of onion, barley, rice extracts on the RBCs count than control in the Nile tilapia fish unlike (33) who concluded that as a result of feeding onion diet, RBCs (erythrocyte) count and Hct % increase in treated groups, exhibiting positive health effect on fish because of bioactive compounds in onion and (37) who showed that the administration of garlic (same family as onion) induced significant increases in all blood parameters (erythrocyte count, hemoglobin content, and hematocrit value) in tilapia fish.

The WBCs (leucocytes) act as the first lines of body defense and their numbers increase sharply when infections arise. The present result showed that there was an increase in WBCs count in onion and barley groups with no difference in rice groups than the control one and this agree with the previous work of (33) who indicated significant increase in WBCs count following feeding of onion diet that demonstrates the immunostimulatory effects and anti-infection properties of onion this may be due to the bioactive compounds polyphenols, flavonoids and quercetin found in onion prevented fish from infection by triggering immune system (38).

Serum biochemical parameters are useful indices for monitoring the health and physiological condition of aquatic vertebrates (39).

AST, ALT, ALP enzymes are used as indices of liver damage. Elevated levels may indicate degeneration, necrosis and destruction of the liver due to cellular damage. In addition, these enzymes may be used as indices to assess whether experimental materials and diet treatments create toxicity or not (40). This study indicated that there were no significant differences between control group and treated groups in ALT, AST level this may be due to flavonoids content in onion so it may be useful in prevention of diseases caused by oxidative stress and this agree with (33) who showed that onion has no adverse effects on liver function and have good nutritional status in addition to integrity of vascular system. Also this agree with study of (12) who indicated that there were no significant changes observed in AST and ALT of cultured juvenile olive flounder fed with diets enriched with onion powder. On the other hand (37) who showed that AST activity decreased significantly with increasing levels of *Allium sativum* in Nile tilapia. ALP results indicated no significant difference in all treated groups than control which agree with (34) who showed that ALT and ALP revealed a non-significant difference in all beluga fed diet containing onion compared to the control group.

Plasma or serum glucose level is often used as an indicator of non-specific stress (41). This study showed that fish fed on onion extract diet showed the lowest level of glucose than other extracts used (barley, rice) and the control one. The bioactive constituents from onion, such as quercetin, exert the anti-diabetic action by stimulating the production and secretion of insulin by pancreas, interfering with dietary glucose absorption, and favoring the insulin saving (42). Additionally Allicin and its derivative compounds are the main active substances responsible for the hypolipidemic and hypocholesterolemic effects of onion (43) .These results agree with the study of (44) who administered onion and garlic juices on *Clarias lazera* once a day for 5

days. Onion and garlic administration caused a temporary hypoglycaemia and a rise of the muscle glycogen levels and decrease the cholesterol level in *Clarias lazera* after 5 days of repeated dose of oral administration onion juice. Also (37) showed significant decreases of plasma glucose in Nile tilapia fish fed on diets containing 20, 30, and 40g *Allium sativum* / kg diet.

CONCLUSION

Methanol extract of onion leaves and barley straw improves the growth parameter and health condition in tilapia fish. Rice straw extract has no effect when added on fish diet.

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