



Effect of lantana camara as feed additive on the growth performance and health status of Oreochromis niloticus

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Abstract

Sixty juvenile Nile tilapia (*Oreochromis niloticus*) ranging from 30 – 40 g were divided randomly into 3 groups: control, group (A) and group (B) (in duplicate) with 10 fish in each group. The control group was fed basal diet. The second group (A) was given feed with addition of 0.5% Lantana camara extract. The third group (B) was given feed with addition of 2% Lantana camara extract. After 60 days, groups fed with diet mixed with different concentration of Lantana camara extract showed significant improvement in growth performance, hematological and biochemical parameters compared to the control group. At the end of the experiment fish were challenged with *Streptococcus* species, groups fed on diet mixed with different concentration of Lantana camara extract showed decrease in mortality rate compared to the control group. In conclusion, adding 2% Lantana camara to fish diet promote growth, improve fish health and reduce mortality in association with challenge with *Streptococcus* sp.

Keywords: lantana camara, *Oreochromis niloticus*, *Streptococcus* species

Introduction

Aquaculture is one of the most important industries which play a major role in developing countries (Manoj and Vasudevan, 2009). Aquaculture industry in recent years shows fast growth, with an average yearly growth rate elevated by 6.1% from 36.8 million tonnes in 2002 to 66.6 million tonnes in 2012 (FAO, 2014). This development in aquaculture was accompanied by enhancement of the intensive culture system against emerging diseases (Harikrishnan et al., 2011). Use of chemotherapeutic agents like antibiotics in treatment of diseases may lead to development of antibiotic resistant bacteria, environmental pollution and the accumulation of toxic residues in fish (Cañada et al., 2009). The antibiotics may reduce the larval growth, inhibit defense mechanisms of the fish larvae and produce other undesirable side effects (Citarasu, 2009). Since immemorial time, medicinal plants have been used for the treatment of widespread infectious diseases (Rios and Recio, 2005). Medicinal plants are considered effective alternative agents to treat the bacterial diseases that affect fish

culture and mitigate many of side effects that are associated with synthetic antimicrobials (Madhuri et al., 2012). Medicinal plants are considered cheaper source for treatment with greater accuracy than chemotherapeutic agents without causing toxicity (Ahmed et al., 2009). Lantana camara is a plant that belongs to the family Verbenaceae, is the most widespread species of genus Lantana and it is very popular as ornamental plant (Ghizalberti, 2000). Lantana camara is one of the most important medicinal plants of the world because the major phytochemical constituents as terpenoids, steroids, and alkaloids (Ganjewala et al., 2009). Lantana camara essential oil containing β -caryophyllene, bornyl acetate, geranyl acetate, terpinyl acetate and limonene extremely inhibited the growth of many bacteria and fungi (Deena and Thoppil, 2000). Thus, the aim of this study to investigate the effects of two concentration of methanolic extracts of L. camara on growth performance and health status of *Oreochromis niloticus* challenged with *Streptococcus* sp.

Materials and methods

Collection of plant samples: The fresh leaves of *Lantana camara* were collected in January, 2016, from El Orman Garden, Giza, Egypt.

Preparation of Plant Extracts: Preparation of methanolic extract of *Lantana camara* leaves was done according to the method of **Lilybeth et al. (2013)**.

Experimental design: Sixty juvenile *Oreochromis niloticus* ranging from 30-40g were adapted for two weeks prior to the experiment. Fish were fed on the control pelleted diet twice daily. Fishes were divided randomly into 3 groups: control, group (A) and group (B) (in duplicate) with 10 fish in each group and kept in glass aquarium. Each aquarium was supplied with air pump and electrical filter. Fish were fed at a rate of 3% of their body weight twice daily. Fish were weighted every two weeks and the amount of feed was adjusted according to the weight.

Feed preparation: The first group was given feed without any addition of *L. camara* extract and served as the control group. The second group (A) was given feed supplemented with 0.5% *L. camara* extract according to **Ramkumar and Perumal (2014)**. The third group (B) was fed on diet supplemented with 2% *L. camara* extract.

Fish performance: Fish performance was evaluated by calculating body weight gain, average growth rate, feed efficiency ratio and specific growth rate according to **Ballestrazzi et al. (1994)**

Collection of blood samples: EDTA anticoagulated blood samples were collected from the caudal vessels of individually caught fish according to method described by **Black (2000)** for estimation of

Results

Result of growth parameter: Growth parameter of *O. niloticus* after feeding on diet mixed with different concentration of methanolic extract of *Lantana camara* leaves for 60 days showed

hematological parameter and differential leukocytic count. Some blood samples collected without EDTA, then centrifuged at (6000) r.p.m for (10) min for separation of serum to determine ALT, AST and total protein.

Determination of hematological parameters: Hemoglobin concentration determined with Drabkin solution according to **Stoskopf (1993)**. Red and white blood cell count determined according to **Kanaeu (1985)**. The packed cell volume is determined according the methods of **Sarder et al. (2001)**. Estimation of differential leukocytic count according to the method of **Blaxhall and Daisley (1973)**.

Determination of biochemical parameters: Total Protein was determined by spectrophotometrically according to the method of **Gornal et al. (1949)**. Albumin was determined according to the method of **Doumas et al. (1971)**. Determination of glucose according to the method of **Trinder P. (1969)**. Alanine Aminotransferase (ALT) and Aspartate Transaminases (AST) was determined according to the method of **Reitman and Frankel (1957)**.

Experimental challenge: At the end of experiment the fish groups were challenged with streptococcus sp. strain kindly supplied by the Department of Fish Diseases and Management, Cairo university, Giza, Egypt. Each fish were intrapretonealy injected with 0.5 ml streptococcus sp. (8×10^8 CFU/ml) according to the method of **Yilmaz et al. (2012)**.

Statistical analysis: Results for each measured parameter were expressed as the mean \pm standard deviation (SD). Data were analyzed using one-way ANOVA and differences were considered statistically significant when $p < 0.05$.

significant increase in weight gain, average growth rate and feed efficiency ratio than control. Feed conversion ratio was improved in group (B) compare to group (A) and control. These result are illustrated in table (1)

Table (1). Growth performance of *O. niloticus* fed with different concentration of *Lantana camara* mixed diet.

parameters	Control group mean ±SD	Group (A) 0.5% mean ±SD	Group (B) 2%mean ±SD
Initial weight (g/fish)	26.79±6.96 ^a	32.14±8.71 ^a	28.93±7.64 ^a
Final weight (g/fish)	41.79±8.21 ^a	54.29±19.99 ^b	58.57±14.73 ^b
weight gain(g/fish)	15±2.77 ^a	22.14±12.04 ^b	29.64±7.96 ^c
Average growth rate (g/d)	0.25±0.04 ^a	0.37±0.20 ^b	0.49±0.13 ^c
Specific growth rate(SGR,%BW/day)	0.77±0.18 ^a	0.82±0.28 ^a	1.18±0.16 ^b
Feed conversion ratio (FCR)	4.70±0.98 ^a	4.47±3.26 ^a	2.58±0.85 ^b
Feed efficiency ratio (FER) (g/g)	0.22±0.04 ^a	0.32±0.17 ^b	0.42±0.11 ^c

The values represented as mean of ten samples ±SD . Mean values bearing same superscript among the groups are not statistically significant, (P<0.05)

Result of hematological parameter:
Hematological parameters of blood in *O. niloticus* showed significant difference in number of RBC, WBC, HB and PCV in

group (A) and group (B) compared to the control. The observed changes in the number of lymphocyte and neutrophil are shown in table (2).

Table (2). Hematological parameters of blood in *O. niloticus* fed with different concentration of *Lantana camara* mixed diet.

parameters	Control group mean ±SD	Group (A) 0.5% mean ±SD	Group (B) 2%mean ±SD
HB (g/dL)	5.99± 0.45 ^a	6.20±0.78 ^a	6.62±0.24 ^b
RBCs×10 ⁶	1.13±0.12 ^a	1.87±0.32 ^b	1.77±0.21 ^b
WBCs×10 ³	51.67±4.04 ^a	62.67±17.21 ^{ab}	76.33±9.29 ^b
PCV (%)	18.67±2.89 ^a	29.33±1.53 ^b	30.33±4.93 ^b
Lymphocyte%	49.13±30.95 ^a	59.46±17.06 ^a	67.91±12.94 ^a
Neutrophil %	15.33±1.11 ^a	12.37±5.40 ^a	13.17±5.99 ^a

The values represented are the mean of three samples ±SD. Mean values bearing different superscript among the groups are statistically significant, (P < 0.05).

Results of biochemical parameters:
Biochemical parameters of blood in *O. niloticus* fed with different concentration of *Lantana camara* mixed diet showed that the protein, globulin and albumin level was significantly increased in group (A) and

group (B) compared to the control. There are no significant change in glucose level and liver enzymes (ALT and AST) between groups fed on *Lantana camara* and the control group. These result are illustrated in table (3).

Table (3). Biochemical parameters of blood in *O. niloticus* fed with different concentration of *Lantana camara* mixed diet .

parameters	Control group mean ±SD	Group (A) 0.5% mean ±SD	Group (B) 2%mean ±SD
Total protein (g/dL)	2.23± 0.12 ^a	4.24± 0.47 ^b	4.37± 0.37 ^b
Albumin (g/dL)	0.92± 0.04 ^a	1.59± 0.13 ^b	1.82± 0.08 ^c
Globulin (g/dL)	1.31± 0.17 ^a	2.38± 0.81 ^b	2.66± 0.35 ^b
Glucose (mg/dL)	46.08± 7.23 ^a	57.77± 7.44 ^a	59.63± 9.05 ^a
ALT (U/L)	60.00± 2.00 ^a	65.00± 3.00 ^a	69.33± 9.81 ^a
AST (U/L)	64.33±9.87 ^a	67.67±2.89 ^a	62.67±10.41 ^a

The values represented are the mean of three samples ±SD. Mean values bearing different superscript among the groups are statistically significant, (P < 0.05).

Results of the challenge test:Results of the challenge test showed that mortality rate was 20% with all concentration of *Lantana*

camara . The mortality rate in control group was 40%. These result are illustrated in table (4).

Table (4). Results of challenge with streptococcus sp. injected by intraperitoneal route and mortality rate %

challenged fish groups	No. of challenged fish	No. of dead fish	Mortality%
Control group	10	4	40%
Group A (0.5%)	10	2	20%
Group B (2%)	10	2	20%

Discussion

In this work the effect of methanolic extract of *L. camara* leaves on the growth performance and health status of *O. niloticus* has been investigated. The results showed significant increase in the final weight, weight gain and specific growth rate. Concerning the feed conversion ratio it was significantly decreased in group fed on 2% *L. camara*. These results supported the result of **Srinivasan et al. (2015)**, who reported significant increase in the final weight, weight gain, SGR and decrease in feed conversion ratio in *Catla catla* fed with different level of *Limonia acidissima* fruit. The improvement in growth performance could be attributed to the presence of flavonoids, tannin and saponin compound. The hematological parameters showed increase in RBC, WBC, PCV and HB in groups fed on different concentration of *L. camara* extract. These result supported the results of **Ramkumar and Perumal (2014)**, who found significant increase in RBC, WBC, PCV and HB in *Labeo rohita* fed on methanolic extract of *L. camara* mixed with diet. Increase in HB denoted an increase in oxygen transportation leading to improvement in immunity of fish. The increase in PCV and HB indicated that there are increase in hemopoiesis mechanisms and osmoregulation. Lymphocyte and neutrophil did not show significant different in groups fed on *L.camara* and control. These results supported the findings of **Nahak et al. (2014)**, who found no significance in lymphocyte and neutrophil count in *Clarias batrachus* fed on aqueous leaf extract of *Ocimum basilicum* mixed with diet for two

Conclusion

Methanolic extract of *Lantana camara* showed a significant improvement in the growth performance at concentration 2%.

weeks. Serum biochemical parameters showed increase in total protein and albumin levels. These results supported the findings of **Ramkumar and Perumal (2014)**, who found significant increase in protein and albumin in *Labeo rohita* fed on methanolic extract of *L. camara* mixed with diet. There were also an increase in globulin level. These results supported the findings of **Nahak et al. (2014)**, who found significant increase in globulin level in *Clarias batrachus* fed on aqueous leaf extract of *Ocimum basilicum* mixed with diet. The increase in protein and globulin contents, indicated strong innate immunity. There were no significant different in glucose level, ALT and AST values in groups fed on *L. camara* and control group. These results are more or less similar to those of **Bulfon et al. (2016)**, who found no significant difference in glucose level, ALT and AST value in *Oncorhynchus mykiss* fed on *Panax ginseng* root ethanolic extract mixed with diet. The result of glucose level in groups fed on *L. camara* indicate that fish were not stressed. The result of ALT and AST in groups fed on *L. camara* indicate that there are no toxic effect for *L. camara*. Results of the challenge test showed decrease in mortality rate compared to the control. These results supported the findings of **Yilmaz et al. (2012)**, who found decrease in mortality rate in *Oreochromis mossambicus* fed on different herbs thyme, rosemary and fenugreek mixed with diet after challenge with *Streptococcus* sp.. This due to the phytochemicals like tannins, alkaloids and flavonoids present in *L. camara* and other herbs that have anti-microbial activity. Both concentration of *L. camara* improved the hematological and biochemical parameter and decrease mortality rate after challenge with *streptococcus* sp..

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الملخص العربي

تأثير اللانتانا المقفوسة كمكملات غذائية علي معدل النمو والحالة الصحية للبلطي النيلي

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60 بلطي نيلي يتراوح حجمه من 30 - 40 جم تم تقسيمه بشكل عشوائي في 3 مجموعات: مجموعة ضابطة، مجموعة (أ) و مجموعة (ب) (من نسختين) مع 10 سمكات في كل مجموعة. المجموعة الضابطة أعطت علف بدون إضافة مستخلص نبات اللانتانا. المجموعة الثانية (أ) أعطت علف بإضافة 0.5% مستخلص نبات اللانتانا. المجموعة الثالثة (ب) أعطت علف بإضافة 2% مستخلص نبات اللانتانا. بعد 60 يوما المجموعات التي تغذت علي تركيزات مختلفة من مستخلص نبات اللانتانا أظهرت تحسنا كبيرا في اداء النمو واختبارات الدم ومصل الدم. بعد ذلك تم عدوة السمك بالمكورة العقدية، المجموعات التي تغذت علي تركيزات مختلفة من مستخلص نبات اللانتانا أظهرت تراجع في معدل الوفيات مقارنة بالمجموعة الضابطة. ونستنتج من هذه الدراسة أن إضافة 2% من مستخلص نبات اللانتانا في علف السمك عزز النمو، حسن صحة الاسماك وخفض معدل الوفيات.