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Effect of Dietary Natural Phytobiotics Mixture on Growth, Body Composition, Immune, and Antioxidant-Related Gene Expression of Nile Tilapia (*Oreochromis niloticus*) Fries

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Abstract

THIS study investigated the effects of a natural phytobiotics mixture (Syrena Boost®) on the growth, feed utilization, body composition, water quality, genetic regulation of growth performance, immune system, and antioxidant pathways in Nile tilapia (*Oreochromis niloticus*) fries. The Phytobiotics mixture contains *Quillaja Saponaria*, Star anise oil, and capsaicin. Fries (N=6,000 average weight 0.01 ± 0.012g) were randomly assigned to 12 aquatic compartments (500 fries per compartment). The fries were divided into four sets: Control set (CS) receiving a basal diet and three sets S1, S2, and S3, the basal diet was fortified with 0.1 g kg^{-1}, 0.2 g kg^{-1}, and 0.4 g kg^{-1} Syrena Boost, respectively, for a period of 21 days. This study demonstrated significant improvements ($p < 0.05$) in fish growth performance as well as body composition. In addition, dietary supplementation of Syrena Boost induced up-regulation of insulin-like growth factor (IGF-1) and growth hormone (GH), antioxidant enzymes (CAT, GPX), and immune cytokines (TNF-V, IL-1) genes that can impact the growth, antioxidant capacity and immune system of fries respectively. The total ammonia nitrogen and unionized ammonia (NH3) were significantly reduced by Syrena Boost compared to CS. Consequently, dietary supplementation of Syrena Boost improves Nile tilapia fries' growth, feed utilization, body composition, and water quality. Moreover, it enhances growth, antioxidant, and immune gene expression. The best dosage was found to be 0.2 g kg^{-1} Syrena Boost (S2).

Keywords: *Quillaja Saponaria*, Star anise oil, Capsaicin, Fries, Gene expression.

Introduction

The aquaculture industry has emerged as a primary global source of high-quality animal protein [1]. Nile tilapia (*Oreochromis niloticus*) is a prominent aquaculture species valued for its adaptability to various diets, tolerance of high-density culture, rapid growth rate, and resilience to adverse environmental conditions [2, 3]. To increase production, farmers often intensify aquaculture practices [4]. However, this intensification can lead to various environmental challenges, including increased disease susceptibility, weakened immune systems, and high mortality rates [5]. To address these challenges, dietary supplementation with phytobiotic feed additives has gained significant attention. These additives offer a range of benefits, including antimicrobial, antioxidant, and antiparasitic properties, as well as stimulating bile secretion, digestive enzyme activity, growth, and appetite [6-8].

Capsaicin (CAP), an alkaloid, possesses antibacterial, anti-inflammatory, gut-stimulating, and antioxidant qualities [9]. CAP acts as an anti-inflammatory to protect the gastrointestinal tract from sepsis and systemic inflammation while

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simultaneously increasing the antioxidant enzymes' action in the gastrointestinal mucosa [10]. Changes in the quantity and composition of the intestinal microbiota have been connected to dietary consumption of CAP [11]. Studies on CAP suggested that it may have a major impact on chili peppers and physiologically active substances.

Quillaja saponin (QS), specifically quillaic acid, possesses robust biological activity [12]. Derived from plants, bacteria, and some lower marine organisms, QS is known for its antimicrobial and antibiotic properties [13]. Widely used in aquaculture, QS has proven effective as a natural growth promoter when added to aquafeeds [14, 15]. It contributes to reduced fish mortality, a strengthened immune system, and protection against oxidative stress [16]. According to [17], QS have ability to improve water quality and enhance fish performance.

Illicium verum, commonly known as star anise, is a traditional spice with medicinal uses. Its extracts are abundant in bioactive compounds like quercetin, trans-anisole, eugenol, and glycosides, which have demonstrated anti-inflammatory, antiviral, and antibacterial effects [18]. The chemical composition of star anise is multifaceted, with star anise oil (SAO) and shikimic acid being the most significant and extensively researched components [19]. SAO, a volatile oil derived from star anise fruit, possesses antimicrobial, antioxidant, and anti-inflammatory properties. Moreover, SAO is employed in food flavoring [20].

The ability of Nile tilapia fries to grow and thrive is heavily dependent on the nutritional composition of their feed. Therefore, the primary purpose of this study is to investigate the effects of Syrena Boost®, a natural phytobiotics mixture consisting of *Quillaja Saponaria*, Star anise oil, and capsaicin, on the Nile tilapia (*Oreochromis niloticus*) fries growth performance, feed utilization, body composition, water quality, and expression of genes related to growth, immunity, and antioxidant capacity.

Material and Methods

Ethical approval

The procedures and conduct of the current investigations were certified by the IAACUR Committee, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt (Serial approval number: IAACUC-KSU-56-2023).

Dietary formulation, trial planning, and aquatic animal husbandry

This research was conducted at a private tilapia hatchery in Kafr Elsheikh Governorate, Egypt. The trial utilized four cement ponds (measuring: 6 m × 3 m × 1 m). A photoperiod of 16:8 hours light: dark was maintained for the fish, while 6,000 one-day-old Nile tilapia fries (*Oreochromis niloticus*) with an average weight of 0.01 ± 0.012 g were used for the study. Following acclimatization to the experimental system under standard conditions, the fries were randomly distributed into three replicates, with each compartment housing 500 fry. to establish four experimental sets: Set of Controls (CS) in which fish received the basal diet, S1: Fish were provided a basal diet fortified with 0.1 g kg^{-1} Syrena Boost (Delacon© Biotechnik GmbH, Austria), S2: Fish were provided a basal diet fortified with 0.2 g kg^{-1} Syrena Boost; and S3: Fish were provided a basal diet fortified with 0.4 g kg^{-1} Syrena Boost. this dose was determined according to [17]. The structure of the basal diet is presented in Table 1. Using gel to make mixing, the tested feed additive (Syrena Boost®) was added to the basal diet. Thirty percent of the fish's body weight was fed ten times a day and was then reduced to 26% then 22% according to the weight of fry every week. Over the course of 21 days, feeding times were distributed evenly over the 16-hour light period. Every day of the trial, mortality rates were recorded, and dead fries were removed from the experimental ponds [21]

Efficient fish growth and nutrient utilization

The fries were harvested at the end of the trial (21 days) using suitable net and the total biomass of each replicate was determined. The final weight of the randomly selected fish (50 fry/replicate) was determined. Selected fry (50/replicate) was measured for total length (L) on a measuring board. Feed consumption and growth performance were computed as follows: The formulas for body weight gain (BWG) = (W1 - W0), weight gain rate (WG%) = [(W1 - W0) / W0] × 100, specific growth rate (SGR%) = 100 × [(lnW1 - lnW0) / t], feed conversion ratio (FCR) = feed intake / BWG, condition factor (K) = 100 × (W1 / L^3), and survival rate (SR%) = (final number of fish / initial number of fish) × 100. Where W0 is the initial body weight, W1 is the final body weight and t- is the experimental period (days).

Basal diet and whole-fish chemical profile

The composition of the diet was evaluated prior to the experiment. Fish (15/replicate) were chosen at random from each aquatic compartment for whole fish chemical analysis at the end of the experiment. Cunnif's [22] Methods were used to determine the chemical composition of the diet and fish samples. To measure dry matter (DM), the samples were dried for 24 hours at 105°C in an oven (model type of oven, country). Measure crude protein done by using (Kjeltec auto analyzer, Model 1030, Tecator, Hoganas, Sweden). Using Soxhlet extraction with diethyl ether at 40–60°C, the lipid content was ascertained. The amount of ash was determined by burning samples for 12 hours at 550°C. Van's

method was used to calculate the fiber content of the fish and experimental diet [23].

Detection of Salmonella species

The red colonies with or without black centers on XLD agar (Biolife/Italia) were speculated as *salmonella* species and identified morphologically and biochemically according to Quinn et al., [21].

cDNA synthesis from isolated RNA followed by qRT-PCR

From each aquatic compartment, six fries were taken, and they were promptly flash-frozen in liquid nitrogen to be stored at -80°C. Following the manufacturer's instructions, RNA was extracted using the iTRAZOL RNA extraction kit (ITSI BIOSCIENCES, Cat #: A-0112). Using a NanoDrop UV-Vis spectrophotometer (Q5000/Quawell, USA), the quality and quantity of RNA were evaluated. Following the manufacturer's instructions, the SensiFASTTM cDNA synthesis kit (Bioline, UK) was used to create cDNA from the extracted RNA.

The primers used to amplify specific genes in Nile tilapia are listed in Table 2. These include the housekeeping gene β-actin, genes linked to growth look like insulin-like growth factor and growth hormone (IGF-1 and GH), immune-related pro-inflammatory cytokines look like tumour necrosis factor-alpha and interleukin-1 beta gene (TNF-α and IL-1β), and antioxidant enzyme activity look like catalase and glutathione peroxidase gene (CAT and GPX). A Stratagene MX300P instrument with SYBR Green chemistry (Agilent Technologies, USA) and TOP real™ preMIX SYBR Green qPCR master mix (Enzynomics, cat. RT 500) were used to conduct quantitative real-time PCR. The PCR was conducted under the following conditions: 45 cycles of 63°C for 60 seconds and 60°C for 60 seconds, followed by 95°C for 30 seconds. Normalized to β-actin, gene expression levels were computed using the 2-ΔΔCT method [29].

Water quality analysis

To ensure optimal water quality for Nile tilapia, temperature, dissolved oxygen, total ammonia nitrogen, and pH levels were monitored daily throughout the experiment. Using a portable photometer (Martini MI 405 MR), the amount of total ammonia nitrogen was measured. The pH, temperature, and dissolved oxygen in the aquatic compartments were measured using a pH meter (HACH PHC725-PH meter) and the dissolved oxygen and temperature were estimated using a Multiparameter probe meter (HI9829-03042-HANNA®insrruments), As well as a portable photometer (Martini MI 405 MR) was also used for total ammonia measurement [30]. The unionized ammonia was calculated from the values of total ammonia nitrogen, pH, and temperature of the same pond [31].

Statistical analysis

Residual analysis was done to make sure the data was normally distributed. To satisfy parametric test assumptions, percentage data were arcsine-transformed. "GraphPad Prism" software version 9 was used to conduct the statistical analysis. The data is displayed as mean and the standard error of the mean (SEM). Treatment differences were compared using a one-way ANOVA. Pairwise comparisons were performed using Tukey's multiple comparison test. A p-value of 0.05 was considered statistically significant.

Results

Nile Tilapia fry proximate analysis

The proximate composition of Nile Tilapia fries varied significantly ($p < 0.05$) among all experimental sets. The content of protein and lipid is significantly higher in treatment groups compared to the control, Additionally, the highest values ($p < 0.05$) were recorded in S3 (0.4 g kg^{-1} Syrena Boost) followed by S1 and then S2 as compared with that of the control (CS). Moreover, the S1 and S3 had a substantially lower ash content in comparison with CS and S2. Fiber levels were higher in S1, S2, and S3 than in CS but S2 had the lowest level of fiber content. Compared to S1 and S3, the CS and S2 had substantially more carbohydrates. Compared to CS, the treatment sets (S1, S2, and S3) showed noticeably higher dry matter content and significantly lower moisture (Table 3).

Nile tilapia's growth and viability metrics

As illustrated in Table 4, fish-fed Syrena Boost exhibited significantly higher final body weight, body weight gain rate, and specific growth rate ($p \leq 0.05$) compared to the CS. There were also significant differences in feed intake and feed conversion ratio (FCR) among the experimental groups, indicating variations in feed utilization efficiency. While the final length of the fish did not differ significantly between treatments. The condition factor, reflecting overall fish well-being, was significantly higher ($p \leq 0.05$) in all treatments receiving Syrena Boost. After 21 days of Syrena Boost dietary supplementation, the survival rates of Nile tilapia fries were compared, and the CS and the other groups showed significant differences ($p \leq 0.05$). Comparing the three treatments received SB- to the control, the survival rates were significantly ($p \leq 0.05$) higher (figure 1).

Gene expression assay

Growth-Related Genes of Nile Tilapia fries:

The relative expression of growth hormone (GH) and insulin-like growth factor (IGF-1) genes in Nile tilapia fries showed significant differences ($p \leq 0.05$) between the CS and SB treatments. For GH gene expression, there was a significant upregulate ($p \leq$

0.05) in S2 (0.2 g kg^{-1} Syrena Boost) compared to the CS. Similarly, IGF-1 gene expression showed a significant upregulate ($p \leq 0.05$) in S2 compared to the CS. No other significant differences were observed among the treatments for either GH or IGF gene expression as shown in Figure 2.

Antioxidant-Related Genes of Nile Tilapia fries:

An assessment of catalase (CAT) and glutathione peroxidase (GPX) gene expression in Nile tilapia fries identified significant variations ($p \leq 0.05$) between the control set (CS) and certain treatment sets. Specifically, the S2 set (0.2 g kg^{-1} Syrena Boost) exhibited a pronounced upregulate ($p \leq 0.05$) in CAT gene expression compared to the CS. A similar pattern was observed for GPX gene expression, with both S1 (0.1 g kg^{-1} Syrena Boost) and S2 demonstrating significant upregulation ($p \leq 0.05$) relative to the CS. Nevertheless, no significant differences in GPX gene expression were detected between S1 and S2 as shown in Figure 3.

Immune-Related and Inflammatory Genes of Nile Tilapia Fries:

The results showed significant differences ($p \leq 0.05$) between the control set (CS) and some treatment sets. For IL-1β gene expression, S2 (0.2 g kg^{-1} Syrena Boost) exhibited a significant upregulate compared to the CS. Similarly, TNF-α gene expression was significantly upregulated in S1 (0.1 g kg^{-1} Syrena Boost) and S2 compared to the CS. However, no significant difference was found in TNF-α expression between S1 and S2 as shown in Figure 4.

Water quality analysis

Table 5 illustrates significant differences ($p \leq 0.05$) between experimental groups for total ammonia nitrogen (TAN) and unionized ammonia (NH3) levels. Specifically, the control set (CS) exhibited significantly higher ($p \leq 0.05$) TAN and NH3 levels compared to all other sets.

Discussion

In Nile tilapia aquaculture, a hormonal treatment is typically administered for 21 days to induce transformation to male sex in fries. However, during this crucial period, optimizing fry growth and health may necessitate the inclusion of additional dietary supplements alongside hormone treatment. This research focuses on the use of Syrena Boost®, a natural probiotics mixture consisting of *Quillaja Saponaria*, Star anise oil, and capsaicin, on the Nile tilapia (*Oreochromis niloticus*) fries growth performance, feed utilization, body composition, water quality, and expression of genes related to growth, immunity, and antioxidant capacity in this crucial period.

Numerous natural phytobiotic products are utilized as feed additives to enhance fish husbandry and production. The phytobiotics mixture contains *Quillaja Saponaria*, Star anise oil, and capsaicin (Syrena Boost®) significantly improved the proximate chemical composition of fish compared to the control set. Hence, the S3 set exhibited elevated protein and lipid levels, while the S1 and S3 sets had higher fiber content. In contrast, the control and S2 sets displayed higher carbohydrate content. These results corroborate previous findings [32, 33]. This notable improvement in the chemical composition of fish returned to the structural diversity of steroidal saponins enables them to regulate carbohydrate and lipid metabolism by influencing the activity of lipoprotein lipase and hepatic lipase [34, 35]. Additionally, it has been demonstrated that dietary capsaicin intake increases the activity of antioxidant enzymes in the gastrointestinal mucosa, shielding the gastrointestinal tract against sepsis and systemic inflammation [10]. Capsaicin improves the small intestine's absorptive surface by encouraging the formation and elongation of microvilli, triggers the production of calcitonin gene-related peptides, and activates gastroprotective cyclooxygenase-1 [36].

The sets receiving Syrena Boost demonstrated superior performance in terms of final body weight, weight gain, growth rate, feed intake, feed conversion efficiency, condition factor, and survival rate compared to the control set. These results corroborate previous findings [32, 37, 38]. Thus, the improved growth performance in fish-fed QS is linked to the presence of saponins, which enhance cell membrane permeability, thereby facilitating nutrient absorption [12, 39]. In addition, Star Anise Oil, a potent flavoring agent containing compounds like limonene, acetic acid, and anethole, can stimulate fish growth by increasing appetite, activating digestive enzymes, and promoting protein synthesis [20, 40]. Moreover, dietary capsaicin has been shown to increase intestinal permeability by expanding the absorptive surface area of the gut and stimulating digestive juice secretion, leading to increased food intake [41].

The findings from the gene expression analysis conducted in this research indicated an increase in the expression of genes related to fish growth (GH and IGF-1), immune-related cytokines (IL-1β, and TNF-α), and antioxidant enzyme (GPX and CAT) in fish acquired a Syrena Boost-enriched diet beyond to fish acquired control diet, with the most significant results observed in the group receiving S2. The elevated expressions of GH and IGF-1 serve as key indicators of enhanced growth performance in Nile tilapia. This upregulation could contribute to the enhanced growth rate observed in fish-fed Syrena Boost, as it may increase intestinal permeability by expanding the absorptive surface area of the gut and stimulating digestive juice secretion, as previously discussed. These findings align with previous studies [17, 42].

The expressions of GPX and CAT are essential markers for assessing antioxidant activity in animals, as these genes help neutralize free radicals and decrease lipid peroxidation [43]. The S2 and S1 sets showed a substantial upregulation of both CAT and GPX gene expression compared to the CS. These results corroborate previous research [14, 18]. This indicates that star anise oil, saponin, and capsaicin can enhance fish antioxidant status by upregulating antioxidant gene expression (CAT and GPX) and enzyme activities [14, 44]. While oxidative stress often accompanies inflammation [18], the addition of Syrena Boost demonstrated protective effects on the fish's antioxidant status. This was evident in the increased mRNA levels of antioxidant genes (CAT and GPX) leading to enhanced antioxidant enzyme activities and this may contribute to strengthening the antioxidant system [18, 44].

Pro-inflammatory cytokines secreted by immune cells, particularly TNF-α, and IL-1β, have an essential function in modulating the innate immune response [25]. In this trial, S2 (0.2 g kg^{-1} Syrena Boost) significantly upregulated IL-1β gene expression compared to the control set (CS). Similarly, TNF-α gene expression was significantly upregulated in both S1 (0.1 g kg^{-1} Syrena Boost) and S2 sets compared to the CS. These findings align with previous research [17, 45]. This outcome may be returned to QS saponins' abilities to stimulate the formation of inflammasomes, which subsequently leads to the release of IL-1β and IL-6 from antigen-presenting cells (APC) [46]. The inflammasome consists of various sensors and receptors that initiate inflammation via activating caspase-1. Additionally, saponins can enhance the expression of TLR

interferon-α, resulting in the generation of active forms of IL-18 and IL-1β [47]. Therefore, the upregulation of pro-inflammatory genes is a normal response to Syrena Boost dietary inclusion.

The generation of antibodies and the transition of the immune response to a cell-mediated response are two biological roles of QS saponins [48]. Furthermore, saponins can activate IL-8 and IL-1β by stimulating the expression of TLR-2 and interferon-α and inducing the in vitro production of IL-1β and IL-6 [45, 48]. Accordingly, the current findings showed that dietary Syrena Boost boosted the expression of genes associated with somatotropic axis growth-mediation (IGF-1 and GH), antioxidant enzyme activity (CAT, GPX), and immune-related pro-inflammatory cytokines (TNF-α and IL-1β). Consequently, this improved the Nile tilapia larvae's growth performance, immunological response, and general health. To confirm this, more research is needed to examine the precise gene expression profile of fish given Syrena Boost.

Water is an essential factor for all aquatic species throughout their life cycle. In aquaculture systems, fish rely on water for fundamental physiological processes like feeding, reproduction, and respiration [49]. The integration of Syrena Boost in the feed had a non-significant effect on most water quality parameters. However, supplementation of Syrena Boost in treatment sets (S1, S2, S3) exhibited significantly lower levels of total ammonia nitrogen and unionized ammonia compared to (CS). These findings align with previous research [17]. This outcome may be attributed to the power of saponins to bind ammonia and aid in its conversion to nitrite and nitrate may be responsible for the improvement in water quality metrics [50].

Conclusion

The present study demonstrates that incorporating Syrena Boost® into the feed enhanced growth performance, feed utilization efficiency, water quality, and the expression of growth, immune, and antioxidant-related genes in Nile tilapia fries. The most significant improvements were observed in fish fed a diet containing 0.2 g kg^{-1} Syrena Boost (S2). A novel area of research should explore the combined application of Syrena Boost and hormonal sex reversal to enhance growth performance, feed conversion, and overall health in Nile tilapia fry.

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Conflicts of interest

There are no conflicts to declare. The authors declared no competing interests.

Author Contributions

All authors contributed equally to this work (conception, acquisition, samples analysis, statistical analysis, data interpretation, manuscript drafting, and manuscript revision).

TABLE 1. Ingredients of diets and his proximate analysis (g kg^{-1}).

Ingredients (g kg^{-1})	Experimental diets			
	CS	S1	S2	S3
Soybean meal	100	100	100	100
Fishmeal	300	300	300	300
Corn meal	280	280	280	280
Fish oil	50	50	50	50
Rice bran	100	100	100	100
Wheat bran	93	92.9	92.8	92.6
Gluten	50	50	50	50
Di calcium phosphate	9	9	9	9
Premix[◈]	18	18	18	18
Syrena Boost[◈◈]	0.00	0.1	0.2	0.4
Chemical analysis (g kg^{-1})				
Dry matter	959.19	967.2	938.8	960.94
Crude protein	414.6	418.6	427.55	431.7
Ether extract	220.34	213.8	225.43	245.6
Fiber	75.6	29.6	26.13	27.55
Ash	107.5	102.3	105.36	109.1
Carbohydrate	141.15	128.9	115.33	130.99

The experimental groups were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.01, 0.02, and 0.04 g kg^{-1} Syrena Boost, respectively.
[◈]premix content of vitamins and minerals was described in full by [21].
[◈◈] SYRENA BOOST® (a mix of *Quillaja saponins* (QS), capsaicin from pepper, star anise essential oil).

TABLE 2. Primers used for qRT-PCR.

Genes		Primer sequence (5′-3′)	GenBank accession no.	Reference
β-actin	Forward (5′–3′)	TAATAACAGAACGCAGCGCC	EU887951.1	[24]
	Reverse (5′–3′)	AGTGCGGCGATTTCATCTTC		
GH	Forward (5′–3′)	CGATGGCAGAAAAACTGACTCG	M97766.1	[25]
	Reverse (5′–3′)	GCTGCACTGAGGTCTAGCAG		
IGF-1	Forward (5′–3′)	CATCGTGGACGAGTGCTG	XM_019346352.2	[26]
	Reverse (5′–3′)	ACAGGTGCACAGTACATCTCAAG		
GPX	Forward (5′–3′)	CCAAGAGAACTGCAAGAACGA	EF206801	[27]
	Reverse (5′–3′)	CAGGACACGTCATTCCTACAC		
CAT	Forward (5′–3′)	CGTCATATGAACGGATACGG	GQ376154	[27]
	Reverse (5′–3′)	TCAGCCTGCTCAAAGGTCAT		
IL_1B	Forward (5′–3′)	CAAGGATGACGACAAGCCAACC	XM_003460625.2	[28]
	Reverse (5′–3′)	AGCGGACAGACATGAGAGTGC		
TNF-α	Forward (5′–3′)	GGAAGCAGCTCCACTCTGATGA	JF957373.1	[25]
	Reverse (5′–3′)	CACAGCGTGTCTCCTTCGTTCA		

Where "GH" is growth hormone gene, "IGF-1" is insulin-like growth factor gene, "GPX" is Glutathione Peroxidase gene, "CAT" is Catalase gene, "IL_1B" is Interleukin-1 beta gene, and "TNF-α" is Tumor Necrosis Factor-alpha

TABLE 3. Nile Tilapia proximate analysis in different Sets of Nile tilapia fed different dietary dose of Syrena Bost.

Items	Sets				p-value
	CS	S1	S2	S3	
Protein	54.21±0.076^{a}	55.09±0.015^{b}	54.30±0.015^{c}	55.82±0.060^{d}	0.0001
Lipid	7.473±0.129^{a}	9.677±0.096^{b}	8.410±0.110^{c}	10.14±0.490^{d}	0.0001
Ash	21.60±0.025^{a}	19.641±0.117^{b}	21.10±0.061^{a}	20.17±0.200^{b}	0.0010
Fiber	2.523±0.049^{a}	2.777±0.015^{b}	2.633±0.038^{c}	2.867±0.035^{b}	0.0001
Carbohydrate	3.837±0.038^{a}	2.747±0.105^{b}	3.530±0.425^{a}	2.140±0.105^{b}	0.0001
Moisture	10.850±0.078^{a}	10.045±0.450^{a}	9.893±0.567^{b}	9.203±0.060^{b}	0.0007
Dry Matter	89.60±0.010	89.931±0.055	89.97±0.036	90.91±0.779	0.0727

Means within each raw that lack common superscripts differ significantly at $P < 0.05$. The experimental groups were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.

TABLE 4. Growth performance parameters of Nile tilapia fed different dietary dose of Syrena Bost.

Items	Sets				p-value
	CS	S1	S2	S3	
Initial body weight (g)	0.01233±0.0006	0.01233±0.0015	0.01200±0.0020	0.01200±0.0010	0.9804
Final body weight (g)	0.4300±0.030^{a}	0.5200±0.017^{b}	0.6200±0.017^{c}	0.5500±0.017^{b}	0.0001
Weight gain rate (g)	0.4180±0.030^{a}	0.5090±0.017^{b}	0.6100±0.017^{c}	0.5380±0.017^{b}	0.0001
Feed intake (g)	0.6200±0.056^{a}	0.7100±0.026^{ab}	0.7333±0.025^{b}	0.7067±0.032^{ab}	0.0248
FCR	1.497±0.021^{a}	1.400±0.010^{ac}	1.207±0.074^{b}	1.323±0.025^{c}	0.0001
Specific growth rate (% day^{-1})	17.03±0.335^{a}	18.36±0.162^{b}	19.64±0.133^{c}	18.21±0.156^{b}	0.0001
Final length of Fish (cm)	2.860±0.078	2.807±0.067	2.660±0.070	2.733±0.111	0.0782
Condition Factor	1.840±0.193^{a}	2.353±0.228^{ac}	3.297±0.222^{b}	2.703±0.336^{bc}	0.0007

Means within each raw that lack common superscripts differ significantly at $P < 0.05$. The experimental groups were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.

TABLE 5. Water quality parameters of Nile tilapia (O. niloticus) that feed different dietary dose of Syrena Bost.

Items	Sets				p-value
	CS	S1	S2	S3	
Temperature (^{o}C)	27.91±0.720	27.89±0.619	27.83±0.679	27.78±0.490	0.9935
DO_2 (ppm)	6.390±0.431	6.703±0.629	6.587±0.710	6.197±0.404	0.7367
pH	7.957±0.873	8.080±0.708	7.880±0.634	7.997±0.801	0.9901
TAN (ppm)	0.630±0.021^{a}	0.505±0.032^{b}	0.424±0.031^{b}	0.421±0.018^{b}	0.0001
NH_3 (ppm)	0.101±0.011^{a}	0.045±0.002^{b}	0.041±0.016^{b}	0.036±0.002^{c}	0.0001

Means within each raw that lack common superscripts differ significantly at $P < 0.05$. The experimental groups were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.

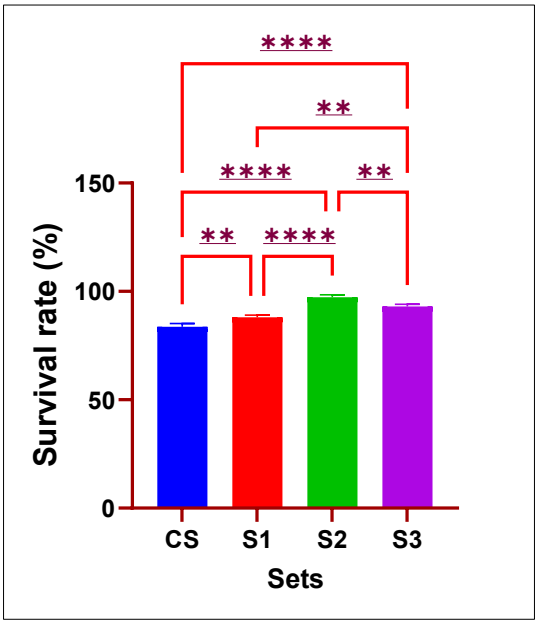

Fig.1. Effect of dietary natural Phytobiotics mixture (Syrena Boost) on Nile Tilapia fries survival rate. All sets were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.
Significant differences between sets were indicated by asterisks (*, **, ***, ****) at p < 0.05, p < 0.01, p < 0.001, and p < 0.0001, respectively.

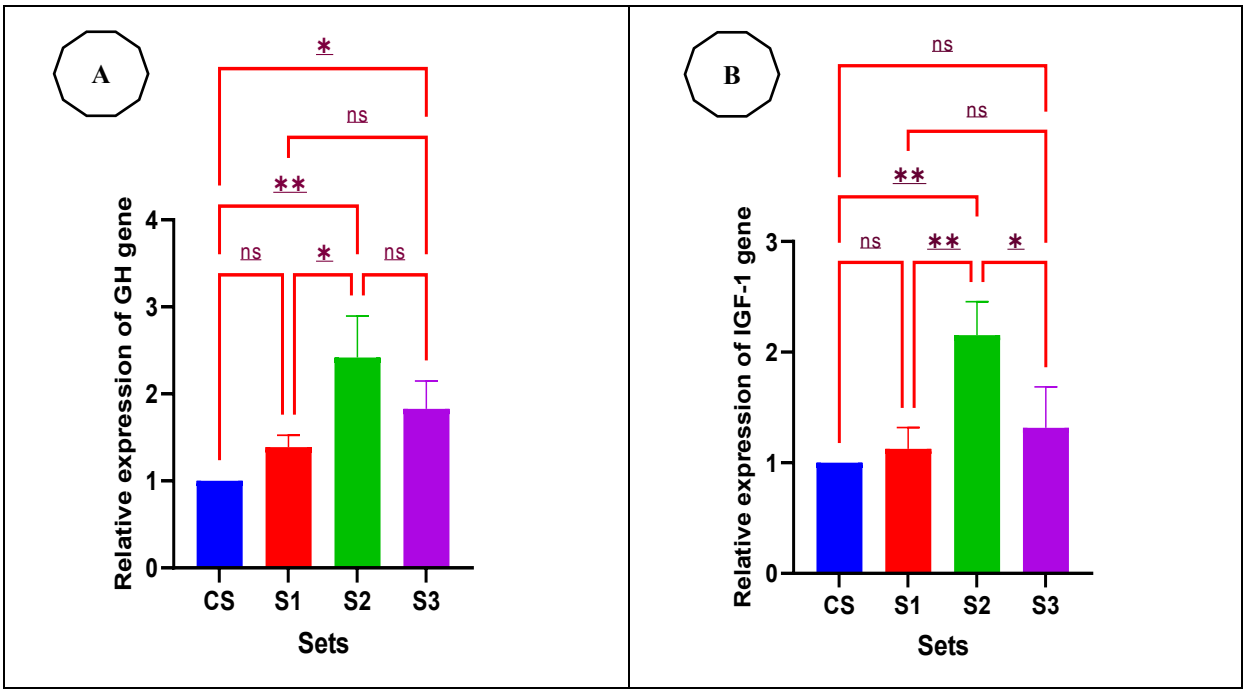

Fig. 2. Effect of dietary natural Phytobiotics mixture (Syrena Boost) on Growth-related genes of Nile Tilapia larvae, where "A" is growth hormone gene (GH), and "B" is insulin-like growth factor (IGF-1).
All sets were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.
Significant differences between sets were indicated by asterisks (*, **, ***, ****) at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

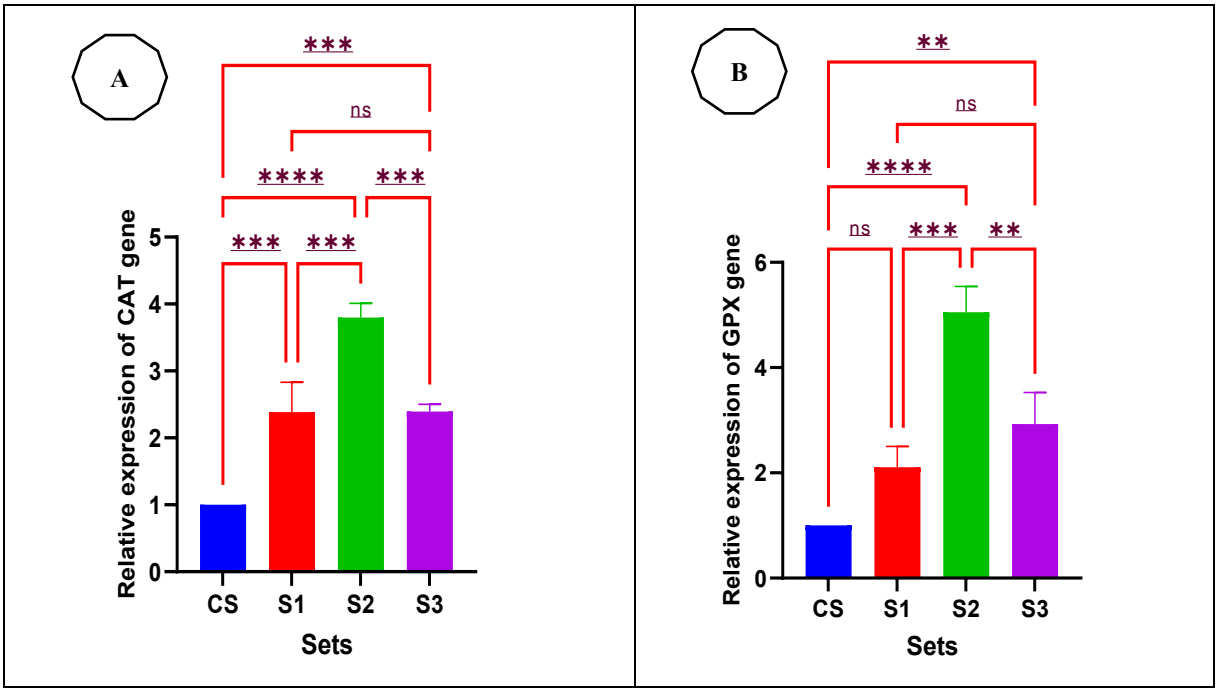

Fig. 3. Effect of dietary natural Phytobiotics mixture (Syrena Boost) on Antioxidant -related genes of Nile Tilapia larvae, where "A" is the Catalase gene and "B" is the Glutathione Peroxidase gene. All sets were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.
Significant differences between sets were indicated by asterisks (*, **, ***, ****) at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

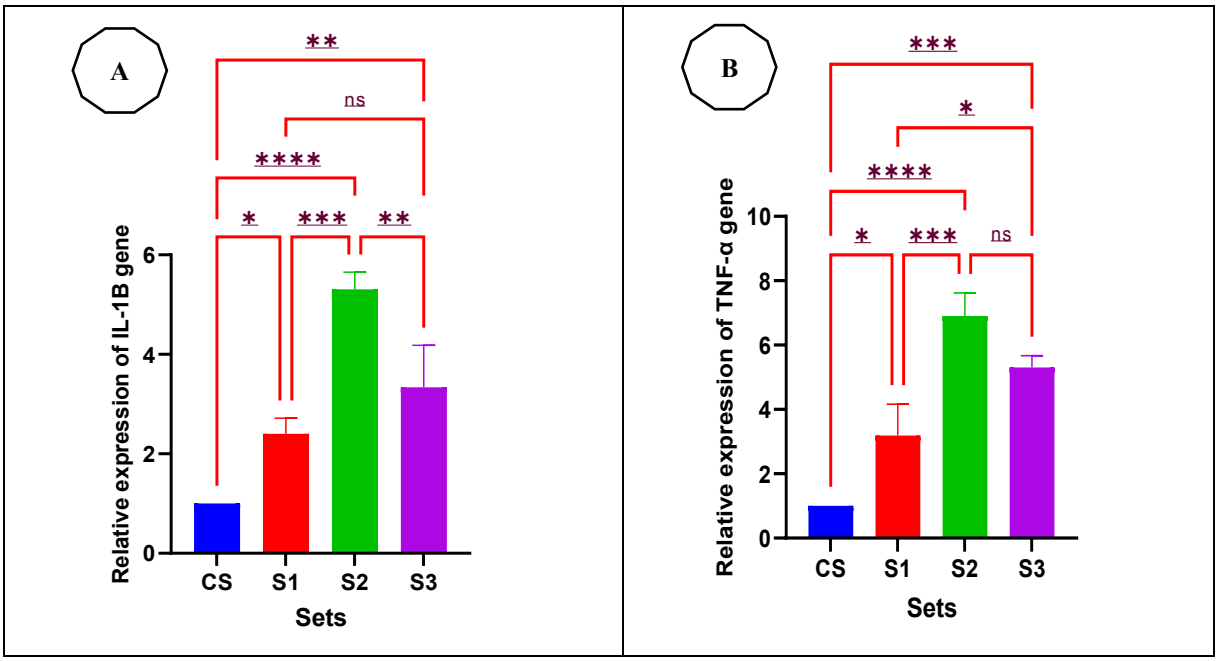

Fig. 4. Effect of dietary natural Phytobiotics mixture (Syrena Boost) on Immune-related and inflammatory genes of Nile Tilapia larvae, where "A" is Interleukin-1 beta and "B" is Tumor Necrosis Factor-alpha. All sets were as follows: a control group (CS) fed a basal diet, and three treatment groups (S1, S2, and S3) fed the basal diet supplemented with 0.1, 0.2, and 0.4 g kg^{-1} Syrena Boost, respectively.
Significant differences between sets were indicated by asterisks (*, **, ***, ****) at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

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تأثير خليط من المواد الحيوية النباتية الطبيعية الغذائية على النمو وتكوين الجسم والمناعة والتعبير الجيني المرتبط بمضادات الأكسدة في صغار أسماك البلطي النيلي (*Oreochromis niloticus*)

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الملخص

هدف هذا البحث إلى دراسة تأثير خليط من المواد الحيوية النباتية الطبيعية (Syrena Boost®) على النمو، والاستفادة من الأعلاف، وتكوين الجسم، وجودة المياه، والتنظيم الجيني لأداء النمو، والجهاز المناعي، ومسارات مضادات الأكسدة في صغار أسماك البلطي النيلي (*Oreochromis niloticus*). يحتوي خليط المواد الحيوية النباتية على نبات الصابوناريا *Quillaja Saponaria* وزيت اليانسون النجمي والكابسيسين. تم توزيع الصغار (ن = 6000، متوسط الوزن 0.01 ± 0.012 جم) بشكل عشوائي على 12 حجرة مائية (500 زريعة لكل حجرة). تم تقسيم الصغار إلى أربع مجموعات: مجموعة التحكم (CS) التي تلقت نظامًا غذائيًا أساسيًا وثلاث مجموعات S1 وS2 وS3، تم تدعيم النظام الغذائي الأساسي بـ 0.1 جم/ كجم و0.2 جم /كجم و0.4 جم /كجم من Syrena Boost على التوالي، وتم تغذية الزريعة لمدة 21 يوم. أظهرت هذه الدراسة تحسنات كبيرة ($p < 0.05$) في أداء نمو الأسماك وكذلك تكوين الجسم. بالإضافة إلى ذلك، أدى تناول مكملات Syrena Boost الغذائية إلى زيادة تنظيم جينات عامل النمو الشبيه بالأنسولين (IGF-1) وهرمون النمو (GH) والإنزيمات المضادة للأكسدة (CAT، GPX)، والسيتوكينات المناعية (TNF-V، IL-1) التي يمكن أن تؤثر على النمو وسعة مضادات الأكسدة والجهاز المناعي للزريعة على التوالي. تم تقليل إجمالي النيتروجين الأمونيا والأمونيا غير المتأينة (NH3) بشكل كبير بواسطة Syrena Boost مقارنةً بـ CS. وبالتالي، فإن المكملات الغذائية من Syrena Boost تعمل على تحسين نمو صغار البلطي النيلي واستخدام الأعلاف وتكوين الجسم وجودة المياه. علاوة على ذلك، فهو يعزز النمو ومضادات الأكسدة والتعبير الجيني المناعي. وجد أن أفضل جرعة هي 0.2 جرام/ كجم (S2) Syrena Boost.

الكلمات الدالة: الصابوناريا، صغار البلطي النيلي، التعبير الجيني، مضادات الأكسدة، الاستجابة المناعية.