



Antimicrobial activity and immunostimulant effect of some fruit by product

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ABSTRACT

This study investigated the effects of dietary orange peels (OP), pommengrate peels (PP) and Banana peels (BP) wasting from juice industry on growth performance, feed utilization and immunity in fish. T1, T2 and T3 had 3% of Orange, Pommengrate and Banana peels respectively, T4 had mixture of 1.5% (OP) and 1.5% (PP), T5 had mixture of 1.5% (OP) and 1.5% (BP), T6 had mixture of 1.5% (OP) and 1.5% (PP), T7 mixture of 1% of each of them and T8 fish fed diet free from any additives (control group). T7 showed significantly high level of weight gain (63.07 ± 1.79 a) and feed efficacy (107.19 ± 2.06 a) followed by T1, T4, T5 FER increase respectively 83.86 ± 1.65 c, 80.76 ± 0.95 cd and 90.44 ± 3.98 b than T8 control group (76.16 ± 1.07 d). Similarly, highest values of total proteins, albumin, and globulin were significantly observed in T7 and T1 fish groups. Likewise, (T7) had a significant increase in lysozyme activity in serum 3.51 ± 0.03 a followed by T4 (3.07 ± 0.07 b), T1 (3.01 ± 0.07 b). T5 (2.73 ± 0.03 c) more than control group T8 (1.28 ± 0.03 f). All treated groups had higher level of protection than control group as Treatment 7 showed highest survival rate 90% then T1, T4, T5, T6 by 80% more than T2 and T3 show 70% survival more than control group (60%). Similarly, survival rate in *O. niloticus* challenged with *Saprolegnia spp.* were 100% in all groups except treatment 4 had a lower percent 90% more than control group (50%). So The present investigation recommended that use of mixture of 1% orange peels (OP), 1% pommengrate peels (PP) and 1% banana peels (BP) to improve the growth, immunity responses and tolerance of Nile tilapia to bacterial (*A. hydrophila*) and fungal (*Saprolegnia spp.*) infection.

Introduction

Aquaculture is facing heavy production loss on global scale. The problems in the farms are usually solved by preventing disease outbreaks or by treating the actual disease. Using of drugs or chemicals caused antibiotic resistant and drug residual, increase of residues cause toxic and allergic reaction, also increase in human infection. **(Mahesh and Satish 2008)**.

Interestingly, in some fruits, the seeds and peels are found to have even higher antimicrobial activity than the pulp **(Jain et al., 2011)**. Using peel of fruits generate safe and cheap antimicrobials as well as decrease pollution related issues due to such wastes **(ZuvaireaNazren et al., 2014)**. So, the use of some fruits by-products as (peel of banana(*Musasapientum*), peel of orange (*Citrus sinensis*) and Pomegranate (*Punicagranatum*) as immune stimulants to resist fish diseases in fish farms and increased of immunity of fish, survival rate and total fish production **(Archana Thomas and Krishnakumar, 2017)**. Where, banana peel carry most of the cellulose in fruits, Cellulose is the major complex carbohydrate in plant cell walls. Also, banana peelings considered the importance applications of probiotics and improve fish intestinal cellulolytic microbes. So, used a feed additives **(Sreeja, et al., 2013)**.

Also, Banana peel considered one of good feed additive in aquaculture where improve fish growth and resistance of disease. Banana is one of food crops which riches with minerals, vitamins, flavonoids, carbohydrates, phenolic compounds, dietary fibers, proteins, essential amino acids, polyunsaturated fatty acids, Antioxidant compounds and also, a lot of micronutrients were present in the peels of genus *Musa* and also, contains several bioactive compounds that made the peels had medicinal properties and also good immune-stimulant **Archana Thomas and Krishnakumar 2017)**.

The main component of orange peel oil consists of terpenes such as (carveol, carvone, menthol and peraldehyde) **Kanaze et al., 2008)**. Orange peels of *Citrus spp.* Where, considered are a good source of oil. Also these useful ingredients in orange peels makes it used in folk medicine to treat a lot of diseases. Orange peel extract is one of natural product where its safe recorded by **Evanepoel (2001)**. Orange peel used as anti-microbial, anti-fungal, hypotensive agent, antioxidant, antiviral, insect repellent, antibacterial, anti-mutagenic and anti-yeast agent **(Ghasemi et al. 2009, Kanaze et al., 2008)** Antibacterial effect of *Citrus sinensis* peel was evaluated against several pathogenic bacteria associated with human and fish infections and act as a growth performance, enhancement the immunity and resist a lot of pathogenic disease of tilapia fish ,so it can be used instead of antibiotics to controlling diseases in tilapia feed **(SabriKesbiç et al, 2015)**.

There are a lot of uses of Pomegranate (*Punica granatum*) as treated various human diseases **(Jayaprakasha et al., 2006)**. The pomegranate extract from leaves, barks, roots, peels, juice and seeds have high antimicrobial and

antioxidant activity (**Tehranifar et al., 2011**). The dried peels of pomegranate contain higher levels of hydrolysable tannins (such as punicalin, punicalagin, pedunculagin, and punigluconin); flavonoids and alkaloids. Pomegranate peel had health-promoting, wound- healing, antimicrobial, anticancer and antioxidative properties .Pomegranate peel enhanced health status of tilapia, immunological and biochemical parameters (**Badawi et al, 2014**) .

The present study the effect of orange peel, banana peel and pomegranate peel and mixture of each on blood parameters, growth performance and survival rate to tilapia fish (*Oreochromis niloticus*) when challenged with both bacterial infection (*Aeromonas hydrophyla*) and fungal challenge by *Saprolegnia spp.*.

Material and methods

Feed collection and preparation

Collection of fruits by-products:

Orange peels, Pomegranate peels and banana peels collected from a local market in zagazig city, Egypt.

Preparation of ripened fruit peel powder

The peel of ripened fruit was rinsed very well by water and placed on white papers under shade for drying for 2 weeks, to prevent degradation and then crushed individually and finely grind to fine powder using a mixer grinder. The powder was stored at room temperature before weight the proper amount for each group diet (**Saravana et al., 2013**).

Feed preparation

Fish diets Ingredients and chemical analysis (on dry matter (The experimental diets containing various component of fruit by-product.

A commercial diet contained 30% protein was prepared and mix with concentrated fruits peel powder as additives.

Experimental fish

Apparently healthy Nile tilapia (*O. niloticus*; total n=240) obtained from a local commercial fish farm with average body weight of 20 ± 5 g transported alive to the laboratory of Fish Diseases Dept., Central Laboratory for Aquaculture Research , El-Abbassa, Egypt. They were randomly distributed in 24 glass aquaria filled with de-chlorinated tap-water supplied with adequate aeration and under water internal power filters for 2 weeks under observation for acclimatization before the start of the experimental diet. Thirty percent of the water was weekly exchanged to maintain good water quality. They fed a commercial diet containing 30% crude protein twice daily.

Table 1- The experimental diets containing various component of fruit by-product:

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8
Treatments	3% O P	3% P.P	3% B P	1.5% O P 1.5% P.P	1.5% O P 1.5% B P	1.5% P P. 1.5% B.P	1% O P. 1% P.P. 1% B.P.	0%
Fish meal	6	6	6	6	6	6	6	6
Soybean meal	43.8	43.8	43.8	43.8	43.8	43.8	43.8	43.8
Ground corn	21.3	21.3	21.3	21.3	21.3	21.3	21.3	21.3
Wheat bran	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4
Cod fish oil	2.65	2.65	2.65	2.65	2.65	2.65	2.65	2.65
Corn oil	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Vitamins premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Minerals Premix	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Starch	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dry matter	91.01	91.01	91.01	91.01	91.01	91.01	91.01	91.01
Crude protein	30.21	30.21	30.21	30.21	30.21	30.21	30.21	30.21
Crude fat	3.48	3.48	3.48	3.48	3.48	3.48	3.48	3.48
Ash	8.65	8.65	8.65	8.65	8.65	8.65	8.65	8.65
Fiber	5.10	5.10	5.10	5.10	5.10	5.10	5.10	5.10

Note: OP: orange peels, PP :pommengrate peels and BP: Banana peels

Feeding experiment

There are eight groups, each group had three replicates (10 fish per replicate) and fish was fed with their respective diets at the rate of 3% of their body weight per day for the period of the experiment. Group 1 (T1) commercial diet supplemented with 3.0% orange peel powder, Group 2 (T2) commercial diet supplemented with 3.0% pomegranate peel powder, Group 3 (T3) commercial diet supplemented with 3.0% banana peel powder, Group 4

(T4) commercial diet supplemented with mix of (1.5% orange peel powder and 1.5% Pomegranate peel powder) , Group 5 (T5) commercial diet supplemented with mix of (1.5% orange peel powder and 1.5% banana peel powder) , Group 6(T6) commercial diet supplemented with mix of (1.5% banana peel powder and 1.5% Pomegranate peel powder) , Group 7 (T7) commercial diet supplemented with mix of (1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powder) , Group 8(T8) control group non-treated group. The water of the aquaria was changed daily. The fish were weighted at 20th, 40th and 60th from the beginning of the feeding experiment for growth performance and serum blood samples were collected for immunological parameters.

Growth performance

Weight gain (WG) = W2-W1;

Daily gain (DG) = W2 - W1 / T;

Weight gain % = [(W2 – W1) / W1] X 100.

Where: W2 = average final body weight (g), W1 = average initial body weight (g), and T = experimental period (days).

Immunological Studies:

Serum samples collection:

The whole blood was collected from the caudal vein of fish (randomly ten fish in each groups) at the 20th, 40th and 60th day of the feeding experiment, where, transferred to Eppendorf tubes without anticoagulant and centrifuged at 3000 round for 15 min. The serum was collected and stored at -20°C until used for biochemical blood parameters and lysozyme activity.

Biochemical blood parameters.

Serum total protein , serum albumin levels were estimated , Serum globulin level was calculated by subtracting albumin level form total protein concentration and In the ratio of albumin to globulin was calculated .according to **Henry 1964 and Dumas et al. 1971** ,also Creatinine concentrations were estimated by **Pattonand Crouch 1977** and **Henry1974** methods. using reagent kits produced by Diamond Diagnostics (Egypt).

7. c- Serum lysozyme activity:

Lysozyme was determined by the turbidometric assay according to (**Parry et al., 1965**) methods. Briefly, the lysozyme substrate was 0.75 mg/ml of gram positive bacterium *Micrococcus lysodeikticus* (Sigma, St. Louis, MO), suspended substrate in 0.1 M sodium phosphate/citric acid buffer, pH 5.8. Plasma (25 µl) was placed, in triplicate, into a microtiter plate and 175 µl of substrate solution was added to each well at 25° C. Where, determined by using microplate ELISA reader (Bio TEC, ELX800G, and USA) which was

read the reduction absorbance at 450 nm after 0 and 20 minutes. The units of lysozyme in plasma or mucus ($\mu\text{g/ml}$) were obtained from standard curve made with lyophilized hen-egg-white-lysozyme (Sigma).

Challenge test:

Bacterial challenge

At the end of the feeding experiment, the fishes of each groups were collected and randomly stocked at density level of 10 fish per 100-L tanks in duplicates. The challenge test was carried out using *A. hydrophyla* which was isolated previously from fish health and management department, Central laboratory for aquaculture research, Agriculture research center, preliminary challenge experiment was performed to determine the LD50 (lethal dose) of the pathogenic bacteria. Then, fish were challenged with pathogenic *A. hydrophyla*. Where was grown on nutrient broth for 24 hr at 30°C in an incubator, then centrifuged at 3,000 g for 30 min to collect bacterial cells form pellets. Which, were re-suspended in 1.0 ml of 0.1% peptone water and using a sublethal dose as recorded by **Schäperclaus (1992)**, the dose of IP injected was 0.1 ml of 24-hr broth from virulent *A. hydrophyla* (5×10^5 CFU/ml).

The fish group was IP injected with 0.1 ml of saline solution and considered as a negative control and all fish groups were IP injected, then, kept under observation for 10 days to record any abnormal clinical signs and recorded the daily fish mortality. *Aeromonas hydrophyla* was re-isolated from liver, kidneys and spleen of the moribund and recently dead fish.

The relative percent of fish survival (RPFS) was calculated at 10 days post challenge according to **Amend (1981)** as follows

$$\text{RPFS} = 100 \left[1 - \left(\frac{\% \text{ mortality in treated fish}}{\% \text{ mortality in control fish}} \right) \right].$$

The relative level of protection (RLP) calculated according to the equation of **(Newan and Majnarichsm1982)**

$$\text{RLP} = 1 - [\text{percentage of treated mortality} / \text{percentage of control mortality}] \times 100.$$

Fungal challenge test: The treated groups as well as the control group redistributed in five fish in duplicate were challenged by *Saprolegnia sp.* Obtained from fish health and management department, Central laboratory for aquaculture research, Agriculture research center. The end of the study at October and this time is suitable for *Saprolegnia spp.* to induce infection. The treated groups distributed into 20 L glass aquaria used for exposure to zoospores of *saprolegnia spp* to induce infection, all fish groups were subjected to net-shake, fish, and dead and moribund fish were removed for

examination. All fish remaining at the end of the 10-day period were removed for examination (Mortada, *et al.* , 2002).

Statistical analysis:

The data will be analyzed using **State View 4.01 (1993)**

Result

Feed utilization and growth performance: -

supplemented with mix of (1.5% banana peel powder and 1.5% Pomegranate peel powder) ,, Group 7 (T7) commercial diet supplemented with mix of (1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powder) ,, Group 8(T8) fish fed diet free from any additives (control group). RBWG: relative body weight gain.

Results by table(2) revealed that FER in all tested groups was significantly increased group 7 (T7) which fed by mixture of 1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powder had the highest WG 63.07 ± 1.79 a and FER 107.19 ± 2.06 a. followed by T5 which fed commercial diet supplemented with mix of (1.5% orange peel powder and 1.5% banana peel powder then T1 which fed commercial diet supplemented with 3.0% orange peel powder at the other side the groups T2, T3 and T6 slightly increase in the value of WG and FER than T8 control group fed by commercial diet without addition of fruit by product.

Table 2. Weight gain, feed intake, feed conversion ratio and feed efficacy ratio of Nile tilapia *Oreochromis niloticus* at 60th day of feeding experiment fed commercial diet supplemented with fruits peel powder.

Treatment	Initial weight(g)	Final weight(g)	Waight gain(g)	RBWG	Feed intake	FCR	FE
T1	184.93 ± 1.52 ab	228.10 ± 5.19 bc	48.38 ± 1.12 b	26.14 ± 0.60 b	57.69 ± 0.62 a	1.19 ± 0.02 b	83.86 ± 1.65 c
T2	185.00 ± 1.15 ab	223.13 ± 2.37 c	38.17 ± 1.24 d	20.62 ± 0.54 d	48.93 ± 0.96 c	1.28 ± 0.02 a	77.95 ± 1.11 cd
T3	184.33 ± 1.20 ab	225.00 ± 2.89 c	40.67 ± 1.76 cd	22.05 ± 0.83 cd	51.61 ± 1.77 bc	1.27 ± 0.01 ab	78.75 ± 0.72 cd
T4	185.07 ± 1.21 ab	229.47 ± 0.29 bc	44.40 ± 1.40 bc	24.00 ± 0.91 bc	55.02 ± 1.21 ab	1.24 ± 0.02 ab	80.76 ± 0.95 cd
T5	187.17 ± 0.44 a	234.00 ± 2.31 b	46.83 ± 2.74 b	25.03 ± 1.52 bc	51.71 ± 0.76 bc	1.11 ± 0.05 c	90.44 ± 3.98 b
T6	184.97 ± 0.52 ab	245.07 ± 0.64 a	44.90 ± 1.73 bc	24.29 ± 1.01 bc	56.77 ± 2.92 a	1.26 ± 0.02 ab	79.18 ± 1.12 cd
T7	182.00 ± 1.53 b	234.00 ± 2.31 b	63.07 ± 1.79 a	34.64 ± 1.28 a	57.94 ± 1.27 a	0.93 ± 0.02 d	107.19 ± 2.06 a
T8	183.17 ± 0.84 b	212.33 ± 1.79 d	29.17 ± 1.19 e	15.91 ± 0.62 e	38.27 ± 1.09 d	1.31 ± 0.02 a	76.16 ± 1.07 d

Note: Means having different letters in the same row indicate significant difference at $p < 0.05$.

Biochemical blood parameters: -

Results of table (3): showed biochemical blood parameters of *O. niloticus* fed commercial diets supplemented with fruits peel powder 60th day of feeding experiment. Creatinine was high in control group than the other treatments (40.01±0.12 a). Albumin, Globulin and total protein high in T7 (1% orange peel powder ,1% Pomegranate peel powder and 1% banana peel powder than other treatments followed by T1 then T4 and T5 higher than control group. On the other hand blood parameters of T3 (3% pommengrate powder) show no significance by the control group

Table 3. Effect of fruits peel powder supplemented commercial diets on creatinine, total protein, albumin and globulin of *Oreochromis niloticus* serum at 60th day of feeding experiment.

	Creatinine (mg/dl)	Total protein(mg/dl)	Albumin (mg/dl)	Globulin(mg/dl)
T1	31.03±0.09 b	26.67±0.09 b	12.37±0.09 b	14.30±0.12 a
T2	27.43±0.23 c	18.53±0.09 d	11.37±0.09 c	7.53±0.09 c
T3	24.93±0.23 d	15.50±0.06 f	10.10±0.06 d	5.67±0.09 d
T4	18.00±0.12 f	17.40±0.21 e	10.20±0.15 d	7.67±0.99 bc
T5	21.28±0.15 e	13.23±0.12 g	8.43±0.03 e	8.73±0.07 b
T6	18.04±0.15 f	19.27±0.15 c	6.77±0.13 g	3.61±0.03 e
T7	24.78±0.40 d	28.40±0.23 a	12.67±0.09 a	15.17±0.18 a
T8	40.01±0.12 a	11.67±0.09 h	7.77±0.03 f	6.21±0.06 d

Note: Means having different letters in the same row indicate significant difference at $p < 0.05$

Immunity parameter and survival of fish:

From table (4) results of lysozyme activity in fish serum fed commercial diet supplemented with fruits peel powder significant increase more than control group. Treatment (T7) a significant increase in lysozyme activity in serum 3.51±0.03a followed by T4 (3.07±0.07b), T1 (3.01±0.07b).T5 (2.73±0.03c) then decreased in T2 (2.33±0.12 d) and T6 (2.19±0.06de) but still more than control group T8 (1.28±0.03 f).

Bacterial Challenge by *Aeromonas hydrophyla*

O. niloticus fed commercial diet supplemented with fruit peel powder increased its resistance to artificial infection with live *A. hydrophila*. Treatment 7 showed the highest survival rate 90% then T1, T4, T5, T6 by 80% more than T2 and T3 show 70% survival more than control group (60%). All

treated groups had higher level of protection than control group but treatment 7 showed the highest level of Relative Percent of Fish Survival 75%.

Fungal challenge by *Saprolegnia sp.*

Fruits peel powder supplemented diets gave very high protection of *O. niloticus* against *Saprolegnia sp.* as in figure (2). Survival rate in *O. niloticus* fed commercial diet supplemented with fruits peel powder and challenged with saprolegnia were 100% in all groups except treatment 4 had a lower percent 90% more than control group (50%) so fish fed on fruit by product showed high level of protection than control group.

Table 4. Lysozyme activity of *Oreochromis niloticus* serum at 60th day of feeding experiment fed commercial diet supplemented with fruits peel powder.

	T1	T2	T3	T4	T5	T6	T7	T8
Lysozyme (mg/l)	3.01 ±0.07b	2.33 ±0.12d	2.19 ±0.06de	3.07 ±0.07b	2.73 ±0.03c	2.08 ±0.04e	3.51 ±0.03a	1.28 ±0.03f

Note: Means having different letters in the same row indicate significant difference at p < 0.05

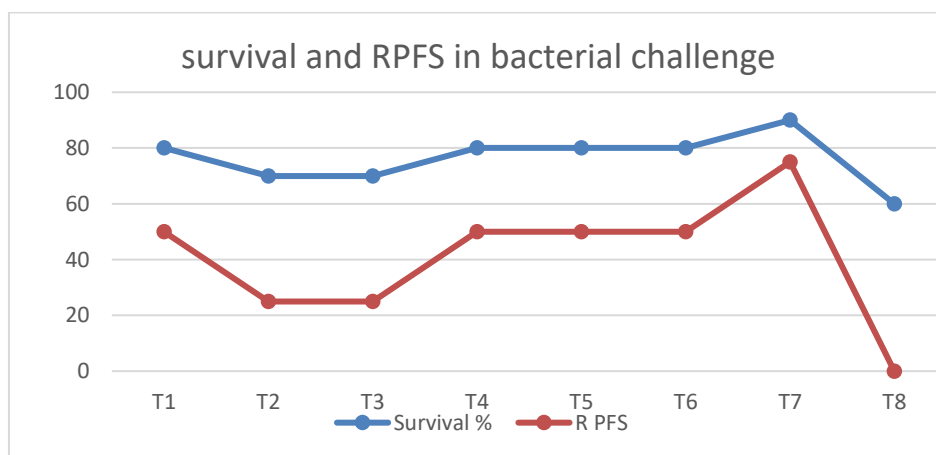


Fig. (1) Survival rate and RPFS and relative level of protection of *O. niloticus* due to challenge IP with *Aeromonas hydrophila* 5×10⁵ CFU/ ml saline after feeding experiment with fruits peel powder for 60 days

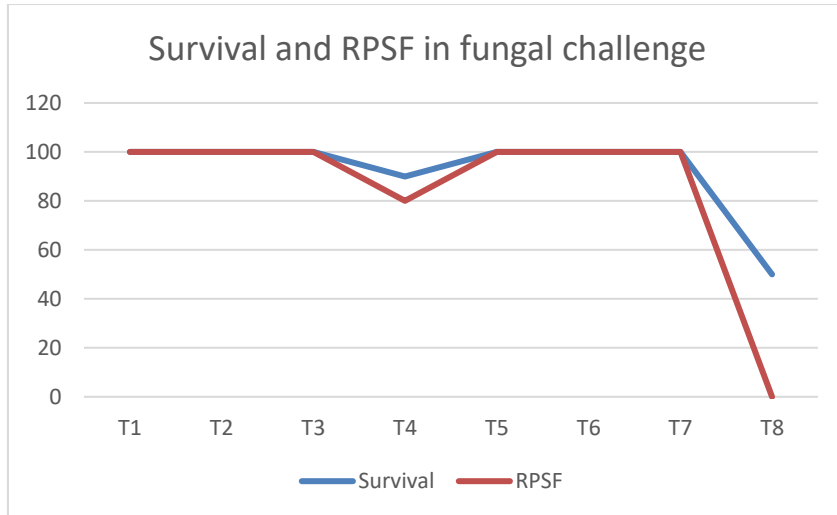


Fig. (2) Survival rate and relative percent of fish protection of *O. niloticus* challenged with *Saprolegnia sp.* after feeding experiment with fruits peel powder for 60 days.

Discussion

Pomegranate peels had noted to have some pharmacological effects in aquatic species (Vidal *et al.* 2003; Badawi and Gomaa, 2016) and considered one of the useful fruits because it has high phenolic content and antioxidant activity (Tabaraki *et al.* 2012) on the other hand, its antioxidant activity, it has antimicrobial, antibacterial, antiviral, antifungal and antimutagenic effect (Cook and Samman 1996). Antioxidant effect of pomegranate peels is better than the pomegranate seeds (Pan *et al.* 2011). The orange peel basic content are phenolic compounds, flavonoids, dietary fiber, alkaloids, saponins, terpenes, resins and tannins that have strong effective properties (Al-Saadi, Ahmad, & Sa'eed, 2009). The antioxidant effect of flavonoids regulates the cellular response to multiple stresses by stimulating antioxidant protection (Virgili & Marino, 2008). Ascorbic acid (Vitamin C) is contained in the peel with a considerable concentration much more than in the juice (Hakim & Harris, 2001). Vitamin C has a water soluble free radical scavenger properties with regeneration of vitamin E from vitamin E free radicals (Oh *et al.*, 2010), vitamin E as a lipidsoluble antioxidant with a chain breaker properties that it intercepts lipid peroxy radical during lipid peroxidation reactions in cell membrane (Nimse & Pal, 2015). Orange peel contains 23 different carotenoids such as zeaxanthin, violaxanthin, α - and β -carotene, phytoene, lutein and β -cryptoxanthin (Rodrigo, Marcos, Alférez, Mallent, & Zacarías, 2003; Rodrigo, Marcos, & Zacarías, 2004). These carotenoids possess a peroxy radical scavenging activity, which protects lipoproteins and cellular membrane (Stahl & Sies, 2003). Banana peel is contain highly

amount of dietary proteins, essential amino acids, vitamins, polyunsaturated fatty acids, fiber, and potassium (**Emaga et al., 2007**), Bioactive component as flavonoids, tannins, alkaloids, glycosides, anthocyanin and terpenoids were presented in banana peels, and these compounds have been important biological and pharmacological role as (antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities) (**Pereira and Maraschin, 2015**). Also, antioxidant compounds (e.g., prodelphinidins, polyphenols, catecholamines, and carotenoids) (**Rebello et al., 2014**) and rich with micronutrients (**Sundaram et al., 2011**).

This result investigated the effects of orange peel, pomengrate peels banana peels and mixture of them which are obtained as a fruit by-product from juice industry, on growth performance and immune system of Nile tilapia (*Oreochromis niloticus*) fingerlings. revealed that WG and FER in T4, T1, T5 increase respectively 80.76 ± 0.95 cd, 83.86 ± 1.65 c, 90.44 ± 3.98 b then significantly increase in group 7 (T7) which fed by mixture of 1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powder had the highest WG 63.07 ± 1.79 a and FER 107.19 ± 2.06 a. at the other side the groups T2, T3 and T6 slightly increase in the value of WG and FER than T8 control group fed by commercial diet without addition of fruit by product. The results of **Badawi and Goma (2016)** as they recorded that pomengrate peels has no significant role in growth performance and may not have influence the health status of the monosex *O. niloticus*.

Orange peels have highly amount of alkaloids, saponins, terpenes, resins, flavonoids, phenols, and tannins (**Al-Saadi, Ahmad, & Sa'eed, 2009**). It has been recorded that herbs which have potent bioactive component that effect on digestive processes by stimulating enzyme activity, improving digestibility of nutrients and feed absorption. So, improvement in fish growth obtained (**Immanuel et al., 2009; Citarasu, 2010; Kaleeswaran, Ilavenil, & Ravikumar, 2010; Hashemi & Davoodi, 2011**).

Hematological and biochemical investigations have a vital role in evaluating of nutritional status, health status and the capacity for fish adaptation to the external environment among others (**Faggio et al., 2014**) (**Burgos-Aceves, Lionetti, and Faggio, 2019**).

The result of biochemical blood parameters of *O. niloticus* fed commercial diets supplemented with fruits peel powder 60th day of feeding experiment showed, Creatinine was high in control group than the other treatments (40.01 ± 0.12 a). Albumin, Globulin, and total protein high in T7 (1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powder than other treatments followed by T1 then T4 and T5 higher than control group. On the other hand, blood parameters of T3 (3% pomengrate powder) show no significance by the control group. This agreed with **badrey et al., 2019** Nile tilapia supplemented with different doses of pomengrate peel exhibited

significant increases in blood total protein levels after 90 days of feeding, confirming good growth performance. Blood glucose levels have been used as Influences of pomegranate peel on biochemistry of Nile tilapia 141 indicators of environmental stress, as they reflect changes in carbohydrate metabolism under stress conditions (**Kamal and Omar, 2011**). Glucose levels significantly increased with increasing pomengrate peel concentrations over the 90-day feeding trial. Increased levels of glucose have previously been recorded in the blood of stressed fish (**Levesque et al. 2002; Poléo and Hytteørd, 2003; Sayed et al. 2007; Adedeji et al. 2009; Mekkawy et al. 2010; Osman et al. 2010**).

Results of lysozyme activity in fish serum fed commercial diet supplemented with fruits peel powder significant increase more than control group. Treatment (T7) a significant increase in lysozyme activity in serum $3.51\pm 0.03a$ followed by T4 ($3.07\pm 0.07b$), T1 ($3.01\pm 0.07b$), T5 ($2.73\pm 0.03c$) then decreased in T2 ($2.33\pm 0.12 d$) and T6 ($2.19\pm 0.06de$) but still more than control group T8 ($1.28\pm 0.03 f$). The non-specific immune system of fish is considered to be the first line of defense against invading pathogens. Lysozyme is important indices of non-specific immunity the results indicated that the highest survival rate was in fish groups fed on diet contained mixture of orange and pomengrate peels compared with other dietary treatments or the control. These results reported that the cumulative mortality was high, 80%, in fish fed non-pomegranate enriched diet against bacterial infection (10). The pomegranate enriched diet enhanced the innate immune response that has a vital role in reducing the percentage cumulative mortality thereby protecting the fish from bacterial infection. In conclusion, this study declared that the addition of pomegranate peel extract in *Oreochromus niloticus* diets without any adverse effects on growth parameters or health status. In addition, it improved immune status, lipid profile and functions of liver and kidney. Bioactive compounds like flavonoids, tannins, alkaloids, glycosides, anthocyanins, and terpenoids were present in banana peels, and these components have been different biological and pharmacological influence (antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities) that have beneficial effects on human health (**Pereira and Maraschin, 2015**). Further, antioxidant compounds (e.g., prodelphinidins, polyphenols, catecholamines, and carotenoids) (**Rebello et al., 2014**). The peels of genus *Musa* contains a lot of micronutrients recorded by (**Sundaram et al., 2011**).

Conclusion

The overall data of the present investigation showed that the 1% orange peel powder, 1% Pomegranate peel powder and 1% banana peel powders were significantly stimulate the performance, health and immunity responses of Nile tilapia. Also, this mixture enhanced the resistance of fish against both

pathogenic bacteria *A. hydrophila* and pathogenic fungal infection *Saprolegnia* sp.

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إستخدام مخلفات الفاكهة كمحفز مناعي ومضاد للميكروبات فى الأسماك المستزرعة

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الملخص العربي

تهدف هذه الدراسة لمعرفة تأثير اضافة مخلفات الفاكهة من قشور البرتقال والموز والرمان علي صحة ونمو ومناعة الاسماك المعاملات الاولي والثانية والثالثة تحوي علي ٣٪ من مسحوق قشور البرتقال والرمان والموز بالترتيب و المعاملة الرابعة ١,٥٪ قشوربرتقال و ١,٥٪ قشوررمان والخامسة ١,٥٪ قشوربرتقال و ١,٥٪ قشورموز والسادسة ١,٥٪ قشورموز و ١,٥٪ قشوررمان والسابعة ١٪ قشوربرتقال و ١٪ قشوررمان و ١٪ قشورموز المجموعة الثامنة بدون اي اضافات . اوضحت النتائج ان المعاملة السابعة تعطي اعلي النتائج في معدل نمو الاسماك (63.07 ± 1.79 a) ومعدل كفاءة الغذاء (107.19 ± 2.06 a) يليها المعاملة الاولي (83.86 ± 1.65 c) والرابعة (80.76 ± 0.95) والخامسة (90.44 ± 3.98 b) بالترتيب عنها في المجموعة الثامنة (76.16 ± 1.07 d). وايضا اعلي قيمة من بروتينات الدم في المعاملة الاولي والسابعة اما بالنسبة للانزيمات المناعية الليزوزيم تكون اعلي في المجموعة السابعة 3.51 ± 0.03 a يليها المجموعات الاولي (3.07 ± 0.07 b) والرابعة (3.01 ± 0.07 b) والخامسة (2.73 ± 0.03 c) عن المجموعة الثامنة (1.28 ± 0.03 f) اما نسبة الحماية بعد الحقن بالبكتريا ابروموناس هيدروفيللا والفطريات سابرو لجنيا كل المعاملات لديها نسبة حماية اكثر من مجموعة الكنترول المعاملة السابعة ٩٠٪ يليها الاولي والرابعة والخامسة والسادسة ٨٠٪ اكثر من المعاملة الثانية والثالثة ٧٠٪ اكثر من المجموعة الثامنة ٦٠٪ وايضا كل المعاملات تظهر اعلي نسبة حماية ضد سابرو لجنيا ١٠٠٪ اكثر من المعاملة الثانية والثالثة ٩٠٪ اكثر من المجموعة الثامنة ٥٠٪ ولذلك فالدراسة السابقة توضح ان استخدام خليط من مسحوق قشور البرتقال والموز والرمان بنسبة ١٪ لكل منهما يعمل علي تحسين النمو والمناعة ومقاومة الامراض البكتيرية والفطرية في الباطي النيلبي