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<b>ARABIC SUMMARY.....</b>	
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## *List of Abbreviation*

Abbreviation	Name
ACOH	Acetic acid
ADG	Average daily gain
APU	Apparent protein utilization
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
Bio-Mos <sup>®</sup>	Mannan oligosaccharide
CTLM	Camphor tree leaves meal
CoPC	Comparative papper chromatography
CSM	Caraway seed meal
DBL	Dried basil leaves
DPL	Dried peppermint leaves
EO	Essential oils
EU	Energy utilization
FAMES	Fatty acid methyl ester
FCR	Feed conversion ratio
FER	Feed efficiency ratio
F.FA	Free fatty acid
FI	Feed intake
FID	Flame-ionization detector
FW	Final weight
GTLM	Guava tree leaves meal
Hb	Hemoglobin
HPLC	High performance liquid chromatographic
HSI	Hepato somatic index
Ht	Hematocrite
K	Fulton condition factor
MTB100 <sup>®</sup>	Beta glucan
NDCs	Non digestible carbohydrates
OS	Orego-Stim <sup>®</sup>
PCs	Phenolic compounds
PER	Protein efficiency ratio
PPV %	Protein productive value
prep.PC	Preparative Paper chromatograph
RBCs	Red blood cell

RGR	Relative growth rate
RO	Roselle
Rt	Retention time
SA	Sage
SGR	Specific growth rate
SR	Survival rate
TDPC	Two-dimensional paper chromatography
VSI	Viscera-somatic index
YYS	Whole-cell <i>Saccharomyces cerevisiae</i>

## Summary and Conclusion

This study was carried out in Central Laboratory for Aquaculture Research to evaluate the use of some natural (Roselle calyx "*Hibiscus sabdariffa* L" and aerial part of Sage "*Salvia officinalis*") and biological (Bio-Mos<sup>®</sup> "mannan oligosaccharide" and MTB100<sup>®</sup> "β-glucan") as feed additives on growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L). A total number of 630 monosex Nile tilapia with an average initial weight (5.44-5.48) for roselle and sage diets, while (6.46-6.55g) for Bio-Mos<sup>®</sup> and MTB100<sup>®</sup> were randomly distributed into 42 glass aquaria 100 L each to represent fourteen treatments in triplicates i.e: control 0.0, 0.5, 1.0 and 1.5% natural additives (roselle calyx and aerial part of sage) and 0.0, 0.05, 0.1 and 0.2% biological additives (Bio-Mos<sup>®</sup> and MTB100<sup>®</sup>) as supplement in a basal diet containing 30% crude protein. During the experimental period, water samples were collected every 2 weeks to measure some water parameters such as temperature, dissolved oxygen, pH and unionized ammonia. Also, fish growth, feed utilization, fish whole body composition, Physiological parameters, and fish somatic indices were measured. After 12 week experimental period, 10 fish of each treatment were challenged by pathogenic *Aeromonas hydrophila*, which was given by interperitoneal (IP) injection and they were kept under observation for 10 days to record any abnormal clinical signs and the daily mortality rate. Lysozyme, antibody titer and bactericidal activity were measured after challenged by pathogenic *A. hydrophila*.

Results obtained are summarized in the following:

A- First part of the experimental, Natural supplementation diets;

- 1- The yellow coloured oil extracts obtained from *H. sabdariffa* calyx and *S. officinalis* were analyzed separately by gas chromatography and were shown to contain Linalool, α -terpineol, Cis, trans-linalool oxide,

Eugenol, 2-furfural, Geraniol, Hexanal and Thymoquinone for *H. sabdariffa* calyx, and  $\alpha$ -Pinene, Camphene,  $\beta$ -Pinene,  $\beta$ -Myrcene, 1,8-Cineole,  $\alpha$ -Thujone,  $\beta$ -Thujone, Camphor, Borneol,  $\beta$ -Caryophyllene,  $\alpha$ -Humulene and  $\gamma$ -Terpinene) for *S. officinal*.

- 2- The n. Hexane extracts which contained the fatty acids contents analyze by GC using same conditions of volatile oils analysis to prove the presence of Palmitic acid, Linoleic acid, Oleic acid, Stearic acid, Palmitoleic acid, Myristic acid, Octadeconic acid, Sterulic acid and Malvalic acid for *H. sabdariffa* calyx and Linolenic acid, Palmitic acid, Oleic acid, Eicosenoic acid, Linoleic acid, Arachidic acid and Stearic acid for aerial part of *S. officinal*.
- 3- The methanolic extract of *H. sabdariffa* calyx and *S. officinal* were shown by TDPC screening to contain a complicated mixtures of phenolics (positive response toward  $\text{FeCl}_3$  spray reagent) and flavonols glycosides (colour on paper chromatogram without and with change by ammonia vapour) prove about 4 major compounds for flavonoid, while prove about 8 major compounds (spots) phenolics and 2 major compounds (spots) flavonoid for aerial part of *S. officinal*.
- 4- Further screening the phenolic compounds contained in the different methanolic extracts were achieved through reverse phase HPLC analysis.
- 5- No significant changes in different water parameters due to both natural supplementations (*H. sabdariffa* calyx and *S. officinal*) diets.
- 6- Water temperature value ranged between 27–29°C and higher degree was in the 6<sup>th</sup> week.
- 7- The PH values were the same in all treatments (7.6–8.0).
- 8- Dissolved oxygen (DO) in water source was lower than the other treatments.
- 9- No significant change in ammonia level was observed due to natural feed additives at the same time and it ranged from 0.9–1.55 mg/L.

- 10-** Both natural supplementation (*H. sabdariffa* calyx and *S. officinal*) increased fish growth parameters and the optimum growth-related parameters were obtained at (1.0 and 1.5%, respectively) for *H. sabdariffa* calyx and *S. officinal* diets. However, there were no significant differences between (1.0% and 1.5%) *H. sabdariffa* diets in those parameters.
- 11-** No significant changes in fish survival among the different dietary treatments and it recorded 95.5% for all *H. sabdariffa* calyx supplementation diets, while it ranged from 93.3 to 95.6% for aerial part of *S. officinal* supplementation diets.
- 12-** Incorporation of *H. sabdariffa* calyx and *S. officinal* in growing Nile tilapia diets released significant affects on feed intake.
- 13-** Supplementing Nile tilapia diets with *H. sabdariffa* calyx and *S. officinal* improved the feed conversion ratio (FCR).
- 14-** Supplementation of Nile tilapia diets with *H. sabdariffa* calyx and *S. officinal* increased the feed efficiency ratio, Protein utilization and energy utilization.
- 15-** Moisture content in whole-fish body has no significant difference due to natural supplementation.
- 16-** Protein content in fish body were increased by increasing levels of *H. sabdariffa* calyx and *S. officinal* supplemented diets, however no significantly difference among the different treatments of *S. officinal* supplementation diets in comparison to the control diet.
- 17-** Total lipid content in fish body was significantly decreased with increasing levels of both *H. sabdariffa* calyx supplemented in diets, while increased by increasing levels of *S. officinal* in diets.
- 18-** Ash content not significantly affected with *H. sabdariffa* calyx supplementation in diets in comparison to the control diets, while increasing natural supplementation levels in fish diets.

- 19- Fish fed diets containing 1.0% exhibited higher Hb, RBCs and Ht values than the other treatments in both natural supplementation diets.
- 20- *H. sabdariffa* calyx supplementation increased total protein, creatinine, glucose, AST and globulin values at 1.5%, and decreased total lipids, urea, albumin, and ALT by increased levels of *H. sabdariffa* calyx supplementation diets.
- 21- *S. officinal* supplementation increased total protein, glucose, albumin, globulin values at 1.5%, and decreased total lipids, urea, creatinine, AST and ALT by increased levels of *S. officinal* supplementation diets.
- 22- Fulton condition factor (K), hepato somatic index (HSI) and viscera somatic index (VSI) of Nile tilapia fingerling were significantly affected ( $P > 0.05$ ) with *H. sabdariffa* calyx treatments in comparison to the control.
- 23- Fulton condition factors (K), Hepato somatic index (HSI) and viscera somatic index (VSI) of Nile tilapia fingerling were increased significantly with increasing levels of aerial part of *S. officinal*.
- 24- Total fish mortality 10-days after IP injection with *A. hydrophila* decreased with increase *H. sabdariffa* calyx and *S. officinal* supplementations diets.
- 25- Lysozyme activity of tilapia serum increased with increase of *H. sabdariffa* calyx and *S. officinal* supplemented levels in fish diets, while the highest value was recorded at 1.0% *H. sabdariffa* calyx diet (3.295,  $\mu\text{g}/\text{ml}$ ) and 3.350 $\mu\text{g}/\text{ml}$  for 1.5% *S. officinal* in comparison to fish group fed with diet control (1.850,  $\mu\text{g}/\text{ml}$ ).
- 26- Antibody titer of Nile tilapia fingerlings had not been detected at 1/100, 1/200, 1/400 and 1/600 for the serum fish fed with *H. sabdariffa* calyx and *S. officinal* (0.5, 1.0 and 1.5%), while cut point appeared at (1/800, 1/800 and 1/400) for (0.5, 1.0 and 1.5%, respectively).

- 27-** Serum bactericidal activity of Nile tilapia fingerlings fed with both natural supplementations (*H. sabdariffa* calyx and aerial part of *S. officinal*) against *A. hydrophila* was higher than control. The viable bacterial counts were significantly lower in all treatments groups of *H. sabdariffa* calyx with values (2, 15 and  $9 \times 10^6$  respectively), while (25, 29 and  $30 \times 10^6$ , respectively) for *S. officinal* when compared with control group  $35 \times 10^6$ .
- 28-** The reduction in feed cost compared with the control diet to produce one kg fish gain of treatment containing 1.0 % *H. sabdariffa* calyx diet levels was 4.80 %, while 9.94%.for 1.5 % *S. officinal* diet.

**B- Second part of the experimental;**

- 1-** No significant changes in different water parameters due to both biological supplementations (Bio-Mos<sup>®</sup> and  $\beta$ -gluan) diets.
- 2-** Water temperature value ranged between 27–29°C and higher degree was in the 6<sup>th</sup> week.
- 3-** The PH values were the same in all treatments (7.6–8.0).
- 4-** Dissolved oxygen (DO) in water source was lower than the other treatments.
- 5-** No significant change in ammonia level was observed due to biological feed additives at the same time and it ranged from 0.9–1.55 mg/L.
- 6-** Both biological supplementations (Bio-Mos<sup>®</sup> and  $\beta$ -gluan) increased fish growth parameters and the optimum growth-related parameters were obtained with 0.2%.
- 7-** No significant changes in fish survival among the different treatments and ranged from 93.3 to 95.6% for Bio-Mos<sup>®</sup> supplemented diets with control, while it ranged from 93.3-100% for  $\beta$ -gluan supplemented diets with control.

- 8-** Bio-Mos<sup>®</sup> and  $\beta$ -gluan enhanced the feed intake which was the highest at 0.2%.
- 9-** Supplementing Nile tilapia diets with Bio-Mos<sup>®</sup> and  $\beta$ -gluan improved the feed conversion ratio (FCR) and the optimum FCR was obtained at 0.2%.
- 10-** Protein utilization and energy utilization increased significantly with increasing levels of Bio-Mos<sup>®</sup> and  $\beta$ -gluan.
- 11-** Moisture content in whole-fish body has no significant difference due to Bio-Mos<sup>®</sup> and  $\beta$ -gluan supplemented diets.
- 12-** Protein content in fish body was increased by increasing levels of Bio-Mos<sup>®</sup> and  $\beta$ -gluan.
- 13-** Total lipid content in fish body was significantly decreased with increasing levels of both biological supplementations (Bio-Mos<sup>®</sup> and  $\beta$ -gluan). However, no significant difference in lipid content among all treatments of  $\beta$ -gluan in comparison to the control diet.
- 14-** Ash content in whole-fish body has no significant difference due to Bio-Mos<sup>®</sup> and  $\beta$ -gluan supplemented diets.
- 15-** Fish fed diets containing 0.2% exhibited higher Hb, RBCs and Ht values than the other treatments in Bio-Mos<sup>®</sup> and  $\beta$ -gluan supplemented diets.
- 16-** Bio-Mos<sup>®</sup> supplementation diets increased total protein, total lipid, albumin, and globulin values at 0.2%, and decreased urea, creatinine, glucose, AST and ALT by increased levels of Bio-Mos<sup>®</sup> supplementation diets.
- 17-**  $\beta$ -gluan supplementation diets increased total protein, total lipids, albumin and globulin values at 0.2%, and decreased, glucose, urea, creatinine, AST and ALT by increased levels of  $\beta$ -gluan supplementation diets.
- 18-** Fulton condition factors has no significant difference due to Bio-Mos<sup>®</sup> supplemented diets, while Hepato somatic index and viscera somatic



index were significantly increased by increasing levels of Bio-Mos<sup>®</sup> supplemented diets.

- 19-**Fulton condition factors, Hepato somatic index and viscera somatic index have no significant difference due to  $\beta$ -gluan supplemented diets.
- 20-**Total fish mortality 10-days after IP injection with *A. hydrophila* was 0.0% for fish fed Bio-Mos<sup>®</sup> and  $\beta$ -gluan supplemented diets, while it was recorded 90% in fish control diet.
- 21-** Lysozyme activity of tilapia serum increased with increase Bio-Mos<sup>®</sup> and  $\beta$ -gluan levels in fish diets.
- 22-**Antibody titer of Nile tilapia fingerlings had not been detected at 1/100, 1/200, 1/400 and 1/600 for the serum fish fed with Bio-Mos<sup>®</sup> (0.05, 0.1 and 0.2%), while cut point appeared at (1/800, 1/800 and 1/400) for (0.05, 0.1 and 0.2%, respectively).
- 23-**Antibody titer had not been detected at 1/100, 1/200, 1/400 and 1/600 for the serum fish fed with  $\beta$ -gluan 0.05, 0.1 and 0.2%, while cut point appeared at (1/200, 1/100, 1/100, respectively) for (0.05, 0.1 and 0.2%, respectively).
- 24-**Serum bactericidal activity of Nile tilapia fingerlings fed with Bio-Mos<sup>®</sup> and  $\beta$ -gluan supplemented diets against *A. hydrophila* was higher than control.
- 25-**The reduction in feed cost compared with the control diet to produce one kg fish gain of treatment containing 0.2 % Bio-Mos<sup>®</sup> diet levels was 20.36 %, while 23.16% for 0.2 %  $\beta$ -gluan.

Conclusion:

1- Using 1.0% *H. sabdariffa* L calyx and 1.5% aerial part of *S. officinal* as feed additives in diet for Nile tilapia , *O. niloticus* can improve the growth performance feed utilization, body composition due to the presence of a mixture of essential fatty acids, vitamins, minerals and protein which essential for growth performance.

2- 1.0% *H. sabdariffa* L. and 1.5% *S. officinal* have highly immune response against pathogenic *Aermonas hydrophila* which related to the presence of phenolic and flavonoid compounds which participate on fish metabolism helping to improve health. In addition to the presence mixture of essential oil which documented to have wide range of antibacterial effects.

3- The prebiotic (Bio-Mos<sup>®</sup> and  $\beta$ - glucan<sup>®</sup>) at 0.2% of the diet were responsible for significantly the best obtained results related to the parameters of growth performance, survival, body composition and physiological parameter.

4- Bio-Mos<sup>®</sup> and  $\beta$ -glucan<sup>®</sup> supplementation could increase the nonspecific immune system of Nile tilapia resulting in a fish resistance to *A. hydrophila* infection.