Journal of Applied and Natural Science
15(1), 422 - 428 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Effect of different concentrations of molasses and forage with *Bacillus lechniformis* on the growth of *Macrobrachium nipponense in vitro*

Tariq H. Y. Al-Maliky*
Department of Marine Biology, Marine Science Center, University of Basrah, Basrah, Iraq
Eman A. AL-Imara
Department of Biotic Evolution, Marine Science Center, University of Basrah, Basrah, Iraq
Mahmood S. Hashim
Department of Marine Biology, Marine Science Center, University of Basrah, Basrah, Iraq
P. N. Geetha
Director-India, World Alliance for Planetary Health nonprofit Crop of Louisiana, USA

*Corresponding author. Email: tariq.yasien@uobasrah.edu.iq

Article Info
https://doi.org/10.31018/jans.v15i1.4400
Received: January 9, 2023
Revised: March 7, 2023
Accepted: March 11, 2023

How to Cite
Al-Maliky, T. H. Y. *et al.* (2023). Effect of different concentrations of molasses and forage with *Bacillus lechniformis* on the growth of *Macrobrachium nipponense in vitro*. *Journal of Applied and Natural Science*, 15(1), 422 - 428. https://doi.org/10.31018/jans.v15i1.4400

Abstract
The rearing of juvenile shrimp, *Macrobrachium nipponense* is of importance in the development of aquaculture, especially by using molasses and *Bacillus lechniformis* to promote growth. The present study aimed to evaluate the rearing of juvenile shrimp, *M. nipponense* in laboratory breeding ponds without replacing the water in the presence of *B. lechniformis* using two strategies: i) Use of molasses (10=A, 50=B, and 100=C mg) with 1×10^8 $CFUml^{-1}$ *B. lechniformis* to the ponds, with shrimp in initial weight rates (650.00 ± 68.09, 639.67 ± 74.81, 639.67 ± 67.02) mg and final weight rates (695.67 ± 57.33, 825.00 ± 90.14, 761.33 ± 111.02) mg, ii) Use of feed (100=A, 250-B, and 500=C) mg, with shrimp initial weight rates (107.67 ± 21.94, 106.67 ± 20.82, 103.33 ± 18.93) mg and final weight rates (208.33 ± 16.07, 381.67 ± 36.17, 283.33 ± 15.28) mg. Results of statistical analysis for molasses showed significant differences ($p < 0.05$) in each daily growth (DG), Specific growth rate (SGR), and no significant differences ($p > 0.05$) in survival rates. As food conversion ratio (FCR), significant differences ($p < 0.05$) were found between A and B, contrary to C, which showed no significant differences. There were significant differences ($p < 0.05$) in DG among A, B, and C in using feed. While SGR showed significant differences ($p < 0.05$) between A and B but did not have significant differences with C and no significant differences ($p > 0.05$) in the survival rates. FCR showed significant differences ($p < 0.05$) between C and both A and B, which showed no significant difference ($p > 0.05$) between them. The study will help to reduce the cost and waste of water.

Keywords: Aquaculture technology, *Bacillus lechniformis*, Biofloc., Feeding, Nutrition, Shrimp farming

INTRODUCTION

Aquaculture is one fastest-developing economic sectors, its production is expected to supply 60% of the consumer's needs in 2030, and shrimp is one of its most popular products, as it contributes approximately 15% of the total production (Al-Maliky, 2013; FAO, 2016; Hoseinifar *et al.,* 2017 and FAO, 2018). Probiotics build up the gastrointestinal flora and increase host immunity (Madani *et al.,* 2018). By using biofloc, the traditional feeding rates in shrimp culture change when they are fed, so the exchange of water is zero, and thus limiting the intake of food and nutrients. The flowing particles accumulate from microorganisms, faeces and food residues. It limits the reproduction of pathogenic microorganisms and works to recycle nutrient waste, as well as the possibility of the shrimp benefiting from microbial biomass as a source of protein, thus reducing production costs. And molasses can be inoculated with *Bacillus* spp to improve water quality and increase shrimp activity (Xu *et al.,* 2012; Ekasari *et al.,* 2014; Xu *et al.,* 2014; Luna-Gonzalez *et al.,* 2017).

The development of intensive freshwater aquaculture and its water drainage problems require to add nutrients (N, P), resulting in the multiplication of algae, causing an imbalance in the microbial balance in the environment and the emergence of pathogens of cultured organisms and an unprecedented deterioration of the ecosystem as a result of the use of water quality improvement factors (Wang *et al.,* 2018 and Wang *et al.,* 2019). Several methods have been used to exploit wastewater in aquaculture (Yin *et al.,* 2018), including water exchange, biofilters (Jahangiri and Esteban, 2018), and biofloc technology (Liu *et al.,* 2019). These methods have drawbacks of long duration, high cost, and toxic products (Mook *et al.,* 2012 and Hlordzi *et al.,* 2020).

The optimum use of the amounts of feed added for shrimp farming gives better growth and good water quality through the increase of plankton and nutrients that may lead to the proliferation of heterotrophic bacteria and microalgae (Alfiansah *et al.,* 2022). It confirmed that the plankton with a size between 0.5-5 µm had a positive effect on shrimp growth by 53% over the growth rates that were achieved in clear pond water, while plankton with a size of more than 5.0 µm improved the growth by an additional 36% (Moss *et al.,* 2002). Adding carbohydrates like wheat or rice bran accelerated the formation of probiotics and the growth of heterotrophic bacteria (Avnimelech, 2015). Also, adding molasses improved the biological quality of the freshwater prawn *Macrobrachium rosenbergii* (Miao *et al.,* 2017). Recently, many studies reported adding molasses to shrimp pond water with bacteria (Maia *et al.,* 2016; Madani *et al.,* 2018; Al-Maliky *et al.,* 2021; Alfiansah *et al.,* 2022). The study aimed to determine the best concentration of molasses to use in raising young shrimp in the presence of locally isolated *Bacillus lechniformis*.

MATERIALS AND METHODS

Juvenile collection of shrimps

Juvenile shrimp *M. nipponense* were collected from the water Al-Mashab near the Hummar marsh in Basra using Kufa nets. The shrimp were caught, and small juvenile sizes were selected with a number of 500 juveniles and placed in containers designated for transportation. On their way to the laboratory for about an hour, the water was replaced by one liter every 10 minutes. Upon arrival at the laboratory, they were placed in pre-prepared troughs the next day and were weighed to selected close weights.

Ethical Approval

The experiments were conducted according to the ethics approved by the Marine Science Centre, University of Basrah, Iraq.

Sampling

Five water and sediment samples were collected from fish breeding tanks in the Marine Science Center, Basra University, Basra, Iraq. For sediments: Samples were collected (20 g per sample) using sterile dry polyethylene containers, adding 1 g of each sample to a test tube containing 9 ml of distilled water and series of decimal dilutions were created from each sample until $1x10^{-6}$ by distilled water. Dilutions of $1×10^{-1}$, $1×10^{-1}$, $1×10^{-1}$ were filtered by 0.45 µm Millipore filter paper, which was put in Luria Bertani (LB) agar Petri dishes and plates incubated at 35 °C for 18 hr., then diagnosed (Al-Imara *et al.,* 2016).

Water samples 250 ml were collected by using sterile Nalgen polycarbonate conical flasks (Merck, Germany). In sterile conditions, 1 ml of each sample was added to 9 ml of sterile distilled water and this step was repeated to achieve decimal dilution series ($1 × 10^{-1} × 10^{-4}$). Then, $1×10^{-3}$ and $1×10^{-4}$ dilutions were grown in the same environment and diagnosed (Al-Imara *et al.,* 2016).

Bacterial identification

Bacterial colonies were examined from sediment and water samples under compound microscope, and gram -positive bacilli were selected for subsequent diagnosis biochemically, according to (Aruwa and Olatope, 2015). The isolate that showed the best growth was chosen to complete the study.

Bacterial inoculum

The chosen isolated *Bacillus lechniformis* (Code E1 API) was grown on LB broth overnight, and then the bacterial count was adjusted to $10.5 × 10^8$ $CFUml^{-1}$ by comparing with 0.5 McFarland tube and then put in a one-litre flask containing (LB) broth and incubated at 35 °C for 14 days, then 10 ml from it were added to each tank to accelerate the Juvenile shrimp's growth (Aruwa and Olatope, 2015).

Experience design

It took place in two phases, as the breeding tanks were prepared on 31-3-2021: The first stage: Determining the best concentration of molasses for shrimp growth. The average weight of shrimp were (650.00 ± 68.09, 639.67 ± 74.81, 639.67 ± 67.02) mg. Three concentrations of molasses (10=A, 50=B and 100=C) mg were added respectively and for one time to the pond water with three replicates for each treatment, and $1 × 10^8$ $CFUml^{-1}$ *B. lechniformis* was added. The experiments lasted for 28 days. The second stage, in which the best feed quantity for shrimp growth was determined at an average weight of (107.67 ± 21.94, 106.67 ± 20.82, 103.33 ± 18.93) mg by adding three quantities (100=A, 250=B and 500=C) mg in the pond water with three replications for each treatment, with the addition of one time each of 50 mg molasses and $1 × 10^8$ *Bacillus* spp. It also lasted for 28 days. In both cases, 14 shrimp were placed in each pond (30×20×15 cm). Nitrates and nitrites were measured by Coulomb method (APHA, 1998; Dutra *et al.,* 2017). Temperature, salinity and dissolved oxygen were measured in laboratories at the Marine Science Center. Determining the amount of water added during the experiment period. A European -made NRD12 type diet intended used for feeding fish larvae with proportions of protein 60%.

Growth metrics were used based on Madani *et al.* (2018) and Widanarni *et al.* (2019):

Weight gain (mg)= Final average weight (gm) - Initial average weight (gm). Eq. 1

Daily growth (mg)= Final average weight (gm) (Nt) - Initial average weight (gm) (Wo)/Time (t) Eq. 2

Specific growth rate (SGR) (% per day) =100 (In W2–InW1)/t. Eq.3

survival rates (%)= 100 (Number of final shrimp) / Number of initial shrimp. Eq. 4

The amount of feed consumed by shrimp was determined = the amount of feed before the experiment - the remaining feed at the end of the experiment. Eq. 5

Food conversion ratio (FCR)= Total feed given (mg)/ weight gian (mg). Eq.6

Molasses and components

The local molasses was used with the specifications shown in Table 1. And water samples were taken from all basins after sterilization to examine them and diagnose the species of bacteria.

Statistical analysis

For statistical analysis, SPSS version 15 program was used, and One-way ANOVA method with LSD levels less than 0.05 to refer to the results of the rates of each of survival rates (SR), initial and final weights, DG, SGR and FCR, for a period of 28 days in both stages od the experiments.

RESULTS AND DISCUSSION

The addition of beneficial bacteria such as *Bacillus* with molasses to the shrimp pond may increase the important biomass as a natural food for shrimp that is used in the different cultivation stages (Alfiansah *et al.,* 2022). The environmental conditions of the research experiment breeding tanks were according to the appropriate ranges for the growth of juvenile shrimp *M. nipponense*, as well as the quality of water quality was monitored during the culture period in both phases of the research, that is because adding a number of beneficial bacteria and use the optimal feeding ranges (Table 2).

In a study on shrimps *Litopenaeus vannamei*, the water of the culture tanks had a temperature 23 C^o, which was lower than in the present study due to the time of rearing, while the oxygen values were close to the present study and the acidity values are somewhat higher, while the nitrate and nitrite were in agreement compared to the values of the study (Luna-Gonzalez *et al.,* 2017). In a study on rearing *L. vannamei*, the temperatures measured in the morning and evening were in agreement with their values in the present study. In contrast, the values of measured dissolved oxygen were lower in the morning and higher in the evening. The values of nitrate 0.58-0.61 mgL^{-1} and nitrite 1.71-1.73 mgL^{-1} were somewhat higher compared with the present study, and this may be due to the difference in the length of the study period, as well as the difference in the shrimp species farmed (Maia *et al.,* 2016). Using the biofloc technique may result in a weight gain of the produced shrimp *Penaeus vannamei* ranging between 8-43% (Manan *et al.,* 2020).

This study showed the weight rates of juvenile shrimp *M. nipponense* were in continuous improvement during the experiment periods in the presence of bacteria and the quantities of molasses, and the characteristics of the juveniles were their rapid growth with an amount of 50 mg of molasses, and this may indicate the appropriate extent of molasses to meet the juveniles' need for food and improve their environment during the experiment period (Fig. 1). The feed conversion rate for *M. nipponense* during 28 days of rearing with molasses in different quantities showed a positive interaction, So was the best consumption of feed in the treatment C (1.15 ± 0.21), while the lowest feed consumption in treatment D (0.65 ± 0.04), which is in contrary to what it says that with the increase of probiotics the consumption of feed decreases (Sado *et al.,* 2008). Feed conversion means the ratio of conversion, 1 kg of feed to the production, 1 kg of shrimp (Widanarni *et al.,* 2019). The lower the feed conversion ratio, the higher the feeding efficiency and, thus, the farmers' financial return. The temperatures were (27.89-27.91 ˚C), pH (7.19-7.43), salinity (31-33 ppt) and dissolved oxygen between (5-7 mgL^{-1}). The fluctuation in the values of these factors is due to the activity of the algae used in

Table 1. Chemical composition of the Iraqi sugar cane molasses according to the book of the General Company for Food Products/Sugar Cane Factory and Farm/Maysan - Iraq

Ingredien	Percentage %	Matter	Value
$C_{12}H_{22}O_{11}$	**50**	solids	≥%78
Organic & inorganic soluble salts	**30**	Purity	≥ 45%
Water	**20**	pH	≥%5.5
		Ash	9-15 %

Table 2. Some environmental factors in shrimp culture pond water during the two research stages of molasses treatments and feed quantity treatments

*Treatments	Parameters					
	Temperature (˚C)	Salinity (ppt)	Dissolved Oxygen (mgL^{-1})	pH	NO_3-N (mgL^{-1})	NO_2-N (mgL^{-1})
First (i)						
A	26.27±1.52	2.21±0.20	6.67±0.50	7.64±0.39	0.08±0.04	0.38±0.30
B	26.00±1.73	2.31±0.27	6.47±0.50	7.66±0.50	0.08±0.05	0.37±0.13
C	26.33±1.52	2.31±0.30	6.47±0.35	7.73±0.40	0.08±0.04	0.55±0.14
Second (ii)						
A	27.33±2.08	2.57±0.15	6.23±0.25	7.73±0.25	0.20±0.08	0.65±0.40
B	27.00±2.00	2.50±0.17	6.27±0.68	7.87±0.31	0.25±0.06	0.71±0.16
C	26.67±2.08	2.53±0.15	5.80±0.53	7.93±0.25	0.26±0.05	0.84±0.15

*10=A, 50=B, and 100=C) mg with 1×10^8 $CFUml^{-1}$

the biofloc with Bacteria during the experiment period of 100 days (Manan *et al.,* 2020).

The present study was compared with Luna-Gonzalez *et al.* (2017), who used slightly higher weights of juvenile shrimp, and the final weights were higher, and the reason was the difference between the culture period and the species of shrimp. In comparing it with Maia *et al.* (2016), which gave close survival rates for the second treatment, but higher growth rates reached 11 g, and feed conversion rates ranged between 1.51- 1-53, as it is higher due to the length of the culture period of 16 weeks.

Research shows that adding Bacillus as a probiotic to *Penaeus monodon* increased growth, survival, and stimulated immunity (Rengpipat *et al.,* 2000). Like this one, an increase in the survival and growth rate of *Fenneropenaeus indicus* treated with Bacillus sp. was observed, and it showed that *Bacillus* sp. secretes several enzymes that degrade slime and biofilm and allow their antibiotics to penetrate slime around gram-negative bacteria (Ziaei-Nejad *et al.,* 2006).

From Table 3, it was noted that the survival rates were rather good, and the reason of the deaths during the experiment may be attributed to the fatigue and weakness of the juveniles from the source of their hunting. The averages of the initial weights were close among the adolescents, which is important for comparing the research results. The final weight averages were the most valuable within treatment B indicating the appropriate amount of molasses that had been added with the beneficial bacteria during the experimental period. Also, both daily and qualitative growth rates were high in treatment B, whereby juvenile shrimp gave more weight gain compared to other treatments. The food conversion rates were less valuable within treatment B, which is a positive result indicating the optimal use of juveniles for food and a high ability to convert food into meat, and this is important for the success of the farm and the economic feasibility of it.

In comparison with a study by Luna-Gonzalez et al. (2017), which is similar to the current study, they found a positive effect of molasses with bacilli bacteria on the feeding rate and growth of juvenile shrimp in the biofloc system, which gave an SGR between 5.24-5.79 and survival rates of 100%. In the present study, shrimp survival rates after 28 days of rearing were similar across all treatments, ranging from 76%-83%, while the daily growth rates differed in all transactions and this

Table 3. Preferred measures of the effect of molasses on shrimp growth in the presence of bacteria

Parameters	*Treatments		
	A	B	C
Survival (%)	78.57 ± 14.29 ^{a}	83.33 ± 10.91 ^{a}	76.19 ± 14.87 ^{a}
Initial Weight (mg)	650.00 ± 68.09	639.67 ± 74.81	639.67 ± 67.02
Final Weight (mg)	695.67 ± 57.33	825.00 ± 90.14	761.33 ± 111.02
DG (mg)	1.63 ± 0.65 ^{a}	6.62 ± 0.61 ^{b}	4.35 ± 1.57
SGR (% per day)	0.11 ± 0.06 ^{a}	0.40 ± 0.61 ^{b}	0.27 ± 0.06
FCR	3.21 ± 1.61 ^{a}	0.65 ± 0.04 ^{b}	1.15 ± 0.21 ^{b}

*10=A, 50=B, and 100=C) mg with 1×10^8 $CFUml^{-1}$; Means not sharing a common superscript letter between column values for each indexes differ significantly ($p < 0.05$). Daily growth (DG), Specific growth rate (SGR), Food conversion ratio (FCR).

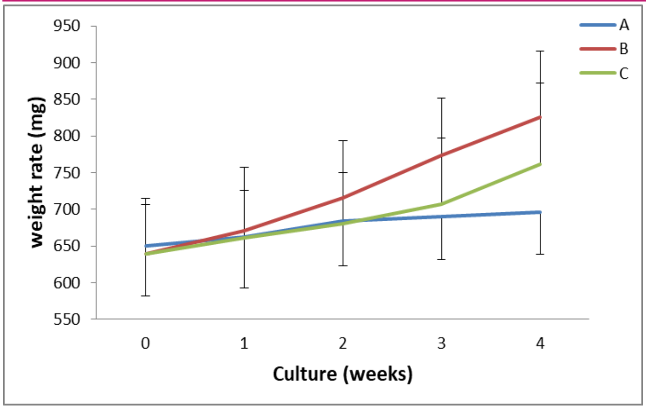

Fig. 1. *Effect of three concentrations of molasses (10= A, 50=B, 100= C) mg on the growth of juvenile M. nipponense during 28 days*

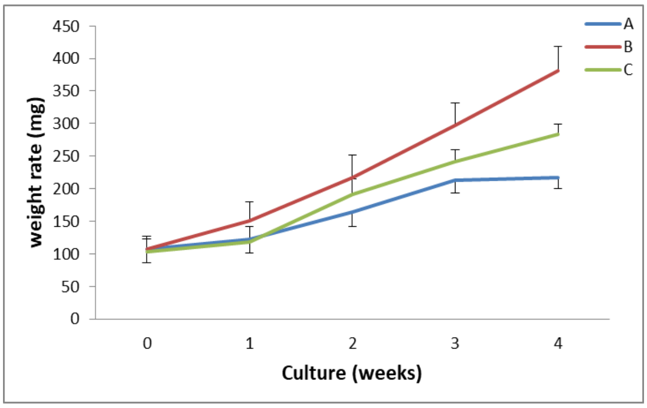

Fig. 2. *The effect of the added diet (100 = A, 250 = B, 500= C) mg on the growth of juvenile M. nipponense with concentration molasses (50 mg) during 28 days*

corresponds to survival rates and daily growth for *L. vannamei* (Widanarni *et al.,* 2019). Adding molasses and bacteria to the daily feed for shrimp *Macrobrachium rosenbergii* without changing the water, increased the beneficial microbial mass and consequently increased shrimp weight and survival rates and improved water quality (Miao *et al.,* 2017).

From the Fig. 2, it was noticed that the growth rates of juvenile shrimp recorded the highest growth in treatment B, indicating that the amount of feed added with molasses and bacteria is the most suitable for the needs of juveniles compared with other quantities of added feed, which gave treatment A better growth values compared to treatment C.

Widanarni *et al.* (2019) tested some growth rates by rearing postlarvae shrimp with a food that had 40% protein ration with the use of probiotic honey at residual percentages of 0.06%=C which gave the best daily growth, SGR and FCR, adding the best amount of the best bacterial growth, while survival rates were high and similar in all treatments. The differences may be due to some experimental procedures from their duration, age stage of shrimp, water use, type of feed and environmental conditions. A review study was conducted on the uses of probiotics in shrimp farms, especially carpentry species *Macrobrachium* sp., to increase knowledge (Cienfuegos *et al.,* 2020).

From Table 4, it was noticed that the survival rates of shrimp juveniles were similar in all treatments, and the rates of the initial weights were also close to each other; as a result, they gave different final weight rates and were the highest when neglected B, which may be an evidence to the appropriate amount of feed added needed by juvenile shrimp in the presence of molasses and bacteria, as well as the rates of both daily growth and specific growth were of high values in treatment B because it gave higher growth, while the FCR gave acceptable values of 2, but it is necessary to reduce them to reach to the values one to be more economical. In comparison, treatment A showed the best growth compared to treatment C.

Several factors influence the bacterial density in the gut of cultured organisms, including feed consumption and absorption, environmental conditions, nutrients, protein digestion, and digestive enzymes (Tzuc *et al.,* 2014). A study on the effect of biofloc on the growth and survival of prawn *P. vannamei* with molasses confirmed that the biofloc improved the growth of shrimp, so the weight rates were the best during the molasses treatments, and thus showing specific growth rates of 3-4 %

Table 4. Preferred measures of the effect of feed quantities on shrimp growth in the presence of bacteria

Parameters	*Treatments		
	A	B	C
Survival (%)	76.19 ± 10.91 ^{b}	83.33 ± 10.91^{a}	78.07 ± 7.14 ^{a}
Initial Weight (mg)	107.67 ± 21.94	106.67 ± 20.82	103.33 ±18.93
Final Weight (mg)	208.33 ± 16.07	381.67 ± 36.17	283.33 ± 15.28
DG (mg)	3.07 ± 0.77 ^{a}	8.27 ± 0.57 ^{b}	5.65 ± 0.20
SGR (% per day)	1.04 ± 0.42 ^{a}	1.99 ± 0.20 ^{b}	1.58 ± 0.20 ^{a}
FCR	2.97 ± 1.33 ^{a}	2.23 ± 0.39 ^{a}	6.74 ± 0.93 ^{b}

*100=A, 250=B, and 500=C) mg with 1 × 10 ^{8} CFUml^{-1}; Means not sharing a common superscript letter between column values for each indexes differ significantly ($p < 0.05$). Daily growth (DG), Specific growth rate (SGR), Food conversion ratio (FCR).

per day and survival rates of 23- 99 % during one hundred days of rearing (Manan *et al.,* 2020). Comparing the results of the present study with Cienfuegos *et al.* (2022), who studied *M. rosenbergii* postlarvae, it was noticed a difference, as they had a low FCR in all transactions and rising values of survival rates, final weights, and SGR, and were high in treatments fed on beneficial bacteria due to the increase in microbial mass. These differences may be due to the species of shrimp, the experiment's duration, the type of ponds, and the novelty of the current experiment.

Conclusion

The present study showed that in the first stage, the river shrimp *M. nipponense* interacted with the quantities of molasses used and preferred treatment 50=B mg and 1 × 10 ⁸ $CFUml^{-1}$ *B. lechniformis*. The juveniles gave good growth in the presence of beneficial bacteria, indicated by growth measures. The second stage showed determining the optimal amount of forage required for growth and survival. Thus treatment B was the best in the presence of both specific molasses and beneficial bacteria. These results have important implications that river shrimp in inland waters gave positive results with modern methods used in aquaculture, especially shrimp, thus reducing water waste and reducing feed and thus reducing production cost. Thus, reducing the dependence on industrial feed (the best quantity was determined by cultivation) and water wastage leads to reducing the cost of shrimp production and overcoming the water crisis that Iraq and the world are exposed to in general. Therefore, it will encourage Iraqi farmers and investors to establish a shrimp farm.

ACKNOWLEDGEMENTS

We extend our deep thanks to our professional, scientific institution represented by the Marine Sciences Center in all its departments, Basrah, Iraq. Especially the Department of Marine Biology and the Department of Marine Chemistry for their active contribution to the completion of our research.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCES

1. Alfiansah, Y.R., Harder, j., Slater, M.J. & Gardes, A. (2022). Addition of Molasses Ameliorates Water and Bio-Floc Quality in Shrimp Pond Water. Trop Life Sci Res., 33 (1), 121-141. doi: 10.21315/tlsr2022.33.1.8. Epub.
2. Al-Imara, E.A. & Al-Gazzawy, G.J. (2016). Antibacterial activity of secondary metabolites from Bacillus spp. Isolated from Basrah, Iraq. Arab Gulf. J. Sci. Res., 34(1-2), 35–42.
3. Al-Maliky, T.H.Y. (2013). Manual and characters of common shrimp species in Southern Iraqi waters. Deposit number in the House of Books and Documents in Baghdad 756, 2013 - Publications of the Center for Marine Sciences - Basra University – Iraq. 192 pp.
4. Al-Maliky, T.H.Y., Al-Maliky, A.M., Al-Maliki, G.M.J. & Boyd, C.E. (2021). Effects of prebiotic and molasses on water quality, growth and survival of Metapenaeus affinis and *Macrobracium nipponense* in vitro, without changing water or adding pellets. Egyp. J. Aqua. Bio. Fish., 25(4), 767 – 783.
5. APHA. (1998). Standard Methods for the Examination of Water and Waste Water, 20th Edition. American Public Health Association, Washington DC.
6. Aruwa, C.E. & Olatope, S. (2015). Characterization of Bacillus Species from Convenience Foods with Conventional and API Kit Method: A Comparative Analysis.
7. Avnimelech, Y. (2015). Biofloc technology: A practical guide book. 3rd ed. Baton Rouge, LA: World Aquaculture Society.
8. Cienfuegos, M.K., Monroy-Dosta, M.D.C., Hamdan-Partida, A., Hernández-Vergara, M.P., Aguirre-Garrido, J.F. & Bustos-Martínez, J. (2022). Effect of the probiotic *Lactococcus lactis* on the microbial composition in the water and the gut of freshwater prawn (*Macrobrachium rosenbergii*) cultivate in biofloc. Aquac. Res., 00, 1–13. DOI: 10.1111/are.15889.
9. Cienfuegos, M.K., Monroy-Dosta, M.D.C., Hamdan-Partida, A., Hernández-Vergara, M.P., Becerril-Cortés, D. & E López-García, E. (2020). A review of the use of probiotics in freshwater prawn (*Macrobrachium* sp.) culture in biofloc systems. La. Ameri. J. Aquat. Res., 48 (4), 518-528. DOI: 10.3856/vol48-issue4-fulltext-2464.
10. Dutra, F.M., Ronnau, M., Sponchiado, D., Fornek, S.C., Freire, C.A. & Ballester, E.L.C. (2017). Histological alterations in gills of *Macrobrachium amazonicum* juveniles exposed to ammonia and nitrite. Aquatic Toxicology.187, 115-123.
11. Ekasari, J., Azhar, M.H., Surawidjaja, E.H., Nuryati, S., Schryver, P.D. & Bossier, P. (2014). Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish Shellfish Immunol. 41(2), 332-339.
12. FAO. (2016). The State of World Fisheries and Aquaculture. FAO.
13. FAO. (2018). The State of World Fisheries and Aquaculture. FAO.
14. Hlordzi, V., Kuebutornye, F.K.A., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S. & Anokyewaa, M.A. (2020). The use of *Bacillus* species in maintenance of water quality in aquaculture: A review. Aquac. Rep., 18, 10053.
15. Hoseinifar, S.H., Sun, Y-Z. & Caipang, C.M. (2017). Short chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res., 48, 1380–1391.
16. Jahangiri, L. & Esteban, M. (2018). Administration of probiotics in the water in finfish aquaculture systems: A review. Fishes., 3, 33.
17. Liu, H., Li, H., Wei, H., Zhu, X., Han, D., Jin, J., Yang, Y. and Xie, S. (2019). Biofloc formation improves water quali-

ty and fish yield in a freshwater pond aquaculture system. Aquacul., 506: 256-269.

18. Luna-Gonzalez, A., Avila-Leal, J., Fierro-Coronado, j., Alvarez-Ruiz, P., Esparza-Leal, H., Escamilla-Montes, R., Flores-Mirana, M.D.C., Montiel-Montoya, J. & Lopez-Alvarez, ES. (2017). Effects of bacilli, molasses, and reducing feeding rate on biofloc formation, growth, and gene expression in *Litopenaeus vannamei* cultured with zero water exchange. Lat. Am. J. Aquat. Res., 45 (5), 900-907.
19. Madani, N.S.H., Adorian, T.J., Farsani, H.G. & Hoseinifar, S.H. (2018). The effects of dietary probiotic Bacilli (*Bacillus subtilis* and *Bacillus licheniformis*) on growth performance, feed efficiency, body composition and immune parameters of whiteleg shrimp (*Litopenaeus vannamei*) post larvae. Aquac. Res., 49(1), 1-8.
20. Maia, E.P., Modesto, G.A., Brito, L.O., Galvez, A.O. & Gesteira, TCV. (2016). Intensive culture system of *Litopenaeus vannamei* in commercial breeding tanks with zero water exchange and addition of molasses and probiotics. Rev. Biol. Mari Ocean., 51(1), 61- 67.
21. Manan, H., Amin-Safwan, A., Kasan, N.A. & Ikhwanuddin, M. (2020). Effects of biofloc application on survival rate, growth performance and specific growth rate of pacific whiteleg shrimp, *Penaeus vannamei* Culture in Closed Hatchery System. Pak. J. Biol. Sci., 23(12), 1563-1571.
22. Miao, S., Sun, L., Bu, H., Zhu, J. & Chen, G. (2017). Effect of molasses addition at C:N ratio of 20:1 on the water quality and growth performance of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquac. Inter., 25,1409-1425. Doi: 10.1007/s10499-017-0124-3.
23. Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam, M.S. & Abu Hassan, M.A. (2012). Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: A review. Desalination. 285, 1-13.
24. Moss, S. (2002). Marine shrimp farming in the western hemisphere: Past problems, present solutions, and future visions. Rev. Fish. Scie., 10(3–4), 601–620. doi: 10.1080/20026491051820.
25. Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. & Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (*Bacillus* S11). Aquac., 191, 271-288. Doi: 10.1016/S0044-8486(00)00440-3.
26. Sado, R.Y., Almeida, B. & Cyrino, J.E.P. (2008). Feeding dietary mannan oligosaccharides to juvenile nile tilapia, *Oreochromis niloticus* has no effect on haematological parameters and showed decreased feed consumption. J. World Aquac. Soci., 39, 821-826.
27. Tzuc, J.T., Escalante, D.R., Herrera, R.R., Cortes, G.G. & Ortiz, M.L.A. (2014). Microbiota from *Litopenaeus vannamei*: digestive tract microbial community of pacific white shrimp (*Litopenaeus vannamei*). Springer Plus., 3, 280, 1-10. Doi: 10.1186/2193-1801-3-280.
28. Wang, X., Daigger, G., de Vries, W., Kroeze, C., Yang, M., Ren, N-Q., Liu, J. & Butler, D. (2019). Impact hotspots of reduced nutrient discharge shift across the globe with population and dietary changes. Nat. Commun., 10(1), 2627.
29. Wang, R., Zhang, Y., Xia, W., Xin, W., Guo, C., Bowker, J. & Chen, Y. (2018). Effects of aquaculture on lakes in the Central Yangtze River Basin, China, I. water quality. N. Am. J. Aquac., 80, 369-378.
30. Widanarni, W., Putri, F.N. & Rahman. (2019). Growth performance of white shrimp *Litopenaeus vannamei* fed with Various dosages of prebiotic honey. IOP Conf. Series: Earth Enviro. Scie., 278, 11 p. Doi:10.1088/1755-1315/278/1/012079.
31. Xu, W.J. & Pan, L.Q. (2014). Dietary protein level and C/N ratio manipulation in zero-exchange culture of *Litopenaeus vannamei*: evaluation of inorganic nitrogen control, biofloc composition and shrimp performance. Aquac. Res., 45, 1842-1851.
32. Xu, W.J., Pan, L.Q., Sun, X.H. & Huang, J. (2012). Effects of bioflocs on water quality, and survival, growth and digestive enzyme activities of *Litopenaeus vannamei* (Boone) in zero-water exchange culture tanks. Aquac. Res., 44, 1093-1102.
33. Yin, H., Yang, C., Jia, Y., Chen, H. & Gu, X. (2018). Dual removal of phosphate and ammonium from high concentrations of aquaculture wastewaters using an efficient two-stage infiltration system. Sci Total Envir., 635, 936-946.
34. Ziaei-Nejad, S., Rezaei, M.H., Takami, G.A., Lovett, D.L., Mirvaghefi, A.R. & Shakouri, M. (2006). The effect of Bacillus spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquac., 252, 516 - 524. DOI:10.1016/J.AQUACULTURE.2005.07.021.