Effect of Rotifer (*Brachionus plicatilis*) Bioencapsulation with SirehMAX™ on Growth and Survival of Asian Sea Bass (*Lates calcarifer*) Larvae

SHAHARAH MOHD IDRIS[1*], AHMAD BAIHAQI OTHMAN[2], NIK HAIHA NIK YUSOFF[1], SITI ZAHRAH ABDULLAH[3] and AZILA ABDULLAH[3]

[1]*Fisheries Research Institute, FRI Tanjung Demong, 22200 Besut, Terengganu*
[2]*Fisheries Research Institute, FRI Glami Lemi, 71650 Titi, Negeri Sembilan*
[3]*National Fish Health Research Division, 11960 Batu Maung, Pulau Pinang*

**Corresponding author: shaharah@dof.gov.my*

Abstract: SirehMAX™ is a product that has shown positive impact on prevention against bacterial infection in fish, in addition to the enhancement of growth performance. In this study, the effect of SirehMAX™, a plant-based product from *Piper betle* leaves on growth performance and survival of sea bass (*Lates calcarifer*) larvae against vibriosis were investigated. Cytotoxic bioassay of SirehMAX™ was assessed using rotifer. The LC_{50} value of rotifer was observed as 10 mg/L. After optimization, rotifer used in the study were enriched in 10 mg/L of herbal product for 3 h in a well aerated container and given to the fish twice daily. Survival and growth rate of the seabass larvae were monitored and compared to control. Results indicated that the addition of SirehMAX™ increased growth of seabass larvae at 47%. The survival result indicated a slightly different of 15% but there are no significant different between the group. The result presented suggested that SirehMAX™ could be used as enrichment feed for live feed to attain higher and faster growth during larval development, thus providing a new technology for mass production of fry and fingerlings.

Keywords: SirehMAX™, Rotifer, cytotoxicity, growth performance, survival, bioencapsulation

Abstrak: Kesan SirehMAX™, produk berasaskan daun sirih terhadap kadar tumbesaran dan hidup larva ikan siakap (*Lates calcarifer)* melawan jangkitan vibriosis dikaji. Kajian sitotoksik SirehMAX™ dijalankan menggunakan rotifer. Nilai LC_{50} terhadap rotifer didapati pada kepekatan 10 mg/L. selepas pengoptimuman, rotifer yang digunakan dalam kajian dikayakan dalam 10 mg/L produk herba selama 3 jam berserta pengudaraan dan diberi makan kepada ikan 2 kali sehari. Larva ikan dipantau dari segi kadar hidup dan tumbesaran berbanding dengan kawalan. Keputusan menunjukkan penggunaan SirehMAX™ meningkatkan kadar tumbesaran larva ikan, tetapi tiada perbezaan ketara dalam kadar hidup antara kumpulan rawatan. Keputusan yang dipamerkan mencadangkan SirehMAX™ boleh digunakan sebagai bahan pengkayaan untuk makanan hidup bagi mencapai kadar pertumbuhan larva yang lebih cepat, sekaligus menghasilkan teknologi baru dalam penghasilan larva dan anak ikan yang tahan penyakit.

Introduction

Live feed, rotifer is primary food for fish larvae. They are used extensively worldwide as live food for the larval stages of commercially marine fish species. The non-selective feeding behaviour makes these organisms a good biological carrier for transferring essential nutrients to predator larvae using bioencapsulation technique (Immanuel et al*.,* 2003). Aneuvo et al. (2014) pointed out that during bioencapsulation, desired essential nutrients dissolved in water are ingested by the rotifer with minimal amount of leakage when given to fish larvae. This technique is not only being used for

improving quality of live feed but also as means to deliver antibacterial agents such as oxytetracycline (Langdon et al*.,* 2008), oxolinic acid (Touraki and Niopas, 2012), florfenicol (Roiha et al*.,* 2010) and metronidazole (Rodrigue et al*.,* 2011) for treatment against bacterial diseases in larval hatcheries.

Vibriosis is a major infectious disease, affecting fry and fingerling production of marine fish and caused heavy losses to farm. These pathogens invade fish larvae and juvenile via rotifer feeding such as in Asian seabass and grouper culture (Iwata et al*.,* 1987, Masumara et al*.,* 1989, Abdullah et al., 2018). Elimination of this pathogenic bacteria in rotifer cultures using antibiotics proved effective and yielded better survival and growth of fish larvae but the use is undesirable as it could promote antibiotic resistance and threaten human safety (Battaglene et al*.,* 2006). For that reason, sustainable culture methods without applications of antibiotics are needed for mass production of fry.

Several studies have reported that herbal medicines can be used to enhance resistance of marine fish against pathogen including in the control of bacteria, fungi and viral diseases (Dhayanithi et al*.,* 2013). In addition, herbal enrichment diet was also reported to promote weight gain, immune response and recovery from stress for fish (Takaoka et al*.,* 2011). Regardless, there is little information on the effects of rotifer enrichment with medicinal herbs extract during larval rearing. Thus, in the present study, focused on the effects of SirehMAX™, an herbal product on; a) selected pathogenic bacteria (*Vibrios*); b) growth of larvae fed herb-enriched rotifer and subsequently c) survival of fingerlings after 25 days of culture. SirehMAX™, were chosen because earlier studies have shown that the product has positive impact on prevention against bacterial infection in fish (Abdullah et al., 2018). Mortality is low in fish given SirehMAX™ diet compared to control. In addition, SirehMAX™ diet was also reported to enhance growth performance (Abdullah et al., 2018).

Materials and Method

SirehMAX™

SirehMAX™, is an herbal product made from *Piper betle* leaf extract. The process for production of SirehMAX™, is patented (Patent No:MY-176273-A). The recommended dosage of this product is 1 mL for 1 kg of formulated feed.

Rotifer Toxicity Bioassay

Cytotoxicity bioassay was carried out as described by Meyer et al*.,* (1982) and McLaughlin et al*.,* (1991) on freshly harvested rotifer. Rotifer used were cultured indoor at Fisheries Research Institute Tanjung Demong (FRITD). The rotifer was disinfected following protocol of Bioencap ABI (unpublished report, 2020). Triplicate samples of SirehMAX™, were tested initially at concentrations of 1, 10, 100 and 1000 ppm (µg/mL) in vials containing 5 mL of brine solution and 10 individuals (ind.) of rotifers. Control contained only 5 mL brine solution. The rotifers were provided with sufficient light and aeration for 24 h. Survivors were counted after 24 h and the median lethal concentration (LC_{50}) with 95% confidence intervals calculated using Probit Analysis (Finney and Tattersfield, 1952)

Bacterial Counts in Rotifer by Herbal Enrichment

Rotifers were harvested from FRITD Rotifer culture system and washed with sterilized seawater. The rotifers were introduced into a 10 L container at 200 rotifer/mL containing 1 µL/mL and 10 µL/mL of SirehMAX®, 0.01 mg/mL l Elbaju (commercial name for nifurstyrenate-sodium) as positive control and brine solution as negative control. *Vibrio* sp. count in rotifer using thiosulfate citrate bile-salts sucrose (TCBS) media were compared at 1, 2, 3 and 4 h after SirehMAX®, and control enrichments. Treatments were conducted in triplicate. At each sampling, 50 mL was removed from

the containers and rotifers were filtered off using 60-micron plankton net, washed thoroughly with sterilized seawater. Then rotifers were homogenized in 1 mL of sterile seawater at 4°C. Serial dilution was performed and 1 mL of diluted homogenate were inoculated in duplicate on TCBS medium and incubated at 25°C for 24 h. Bacterial colonies obtained were counted and total bacterial number was calculated as colony forming units (CFU).

Enrichment of Rotifer with SirehMAX™,

Rotifer enrichment was carried out by following the standard procedure of Sorgeloos and Kulasekarapandian (1984). Enrichment period was conducted for 3 h as in the previous study. The enrichment was performed in 10 L containers, with continuous aeration, and a density of rotifer of approximately 600 ind/mL. The seawater had a salinity of 30% and a temperature of 23°C. Presence of the leaf extract molecules could readily be assessed by the yellowish gut.

Bioencapsulation studies on larvae growth performance

Five thousand (5,000) sea bass fish larvae with initial weight of 0.5 mg were placed in a 300-L aerated tank. Six (6) 300-L tank were prepared for the experiment. Two feeding regimes were tested; i) Larvae were fed with enriched rotifer (10 ppm SirehMAX™) once a week and unenriched rotifer twice a day at (7 and 16 h); ii) larvae fed with unenriched rotifer twice a day at (7 and 16 h) which act as a control. Three replicates were maintained for each group. The culture period was for 25 days since after this period larvae were fed with pelleted feed. At the end of the experiment, both control and experimental fish fry were randomly selected and the total body weight was measured. The remaining fish available at the end of experiment were counted and growth measurements such as weight were also recorded individually.

Statistical Analysis

Data obtained were subjected to one-way ANOVA followed by Duncan's multiple range tests to calculate differences in growth rates among the different experimental treatments and survival rate.

Results and Discussion

Determination of the Median Lethal Concentration (LC_{50})

The lethal concentration (LC_{50}) of the standard SirehMAX™, was found to be at 10 ppm. At this concentration, rotifer could survive more than 24 h. At higher dose rotifer could survived only for 0.167 to 0.5 h in SirehMAX™. Rotifer could survive for more than 160 hours at low dose (1 ppm) (Figure 1). This correspond the findings by Krishnaraju et al., (2005) that the degree of lethality was found to be directly proportional to the concentration of the extract. They observed that the maximum and least mortalities of brine shrimp were at 1000 µg/L and 10 µg/L respectively when placed in herbal solution. Final optimization of 10 µL/g SirehMAX™ solution was selected for enrichment of rotifer. The maximum uptake of SirehMAX™ by rotifer was observed at 180 and 240 minutes after immersion in the herbal solution (Figure 2). The photomicrograph shows that the increment of intensity of the color in the gut of rotifer which indicate the present of SirehMAX™ in there. As stated by Rico-Martínez et al., (2016), rotifer is sensitive to organic matter and pesticide which can lead to their mortality when expose to high concentration for long period of time.

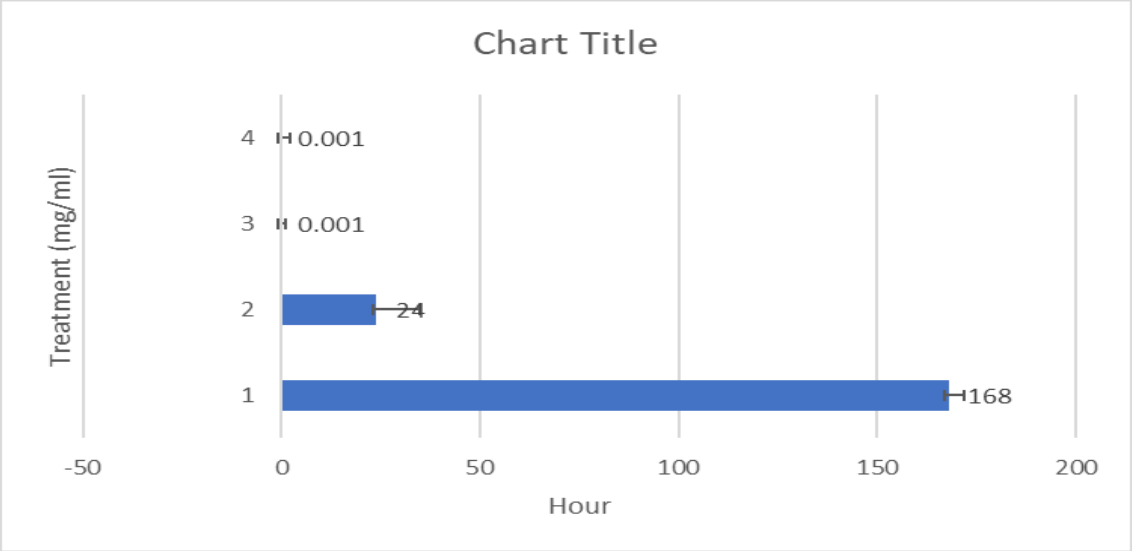

Figure 1. Mortality of rotifer (*Brachionus plicatilis*) against time and treatment concentration

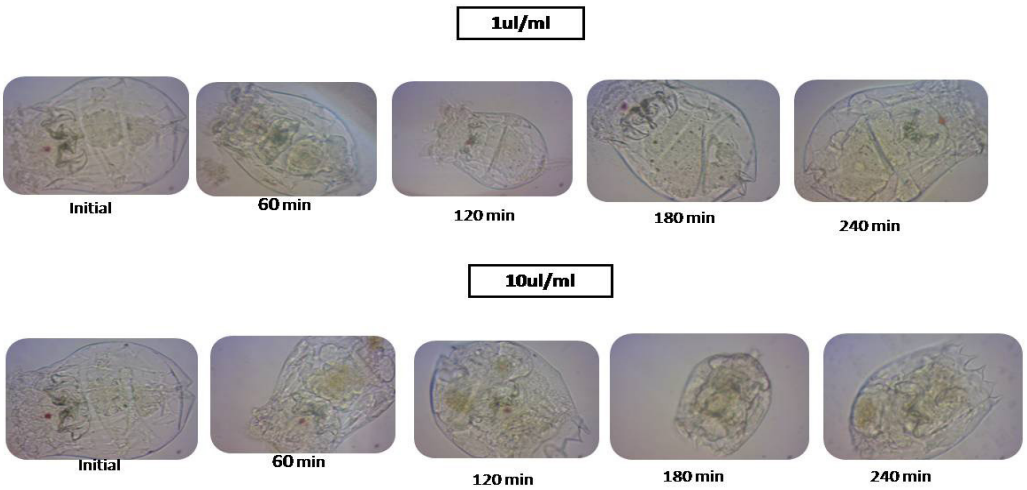

Figure 2. Photomicrograph showing the gut region of rotifer *(Brachionus plicatilis*) enriched with *SirehMAX™*

Bacterial Counts in Rotifers' body after SirehMAX™, enrichment

The bacterial load of rotifer in control and 1 µL/mL treatment with SirehMAX™ appeared relatively constant for 1 to 4 h ranging from 2.2 x 10^3 to 7.2 x 10^6 CFU/mL. However, in 10 µL/mL treatment with SirehMAX™, bacterial load was completely eliminated after 2 h. (Table 1). Treatment with Elbaju can completely remove the bacterial load in rotifer after 1 h treatment. This result indicated that SirehMAX™ can eliminate bacteria in rotifer by using doses of 10 µL/mL for 2 h treatment time.

Table 1. Number of bacterial loads in rotifer ***(****Brachionus plicatilis)* after received SirehMAX™

Treatment	1 h	2 h	3 h	4 h
Control	$2.4x10^6$	$1.8x10^6$	$5x10^6$	$7.2x10^6$
SirehMAX™ 1 µL/mL	$5.2x10^5$	$6x10^3$	$2.1x10^4$	$2.2x10^3$
SirehMAX™ 10 µL/mL	$5.5x10^2$	$3.2x10^2$	-	-
Elbaju 0.01 mg/mL	-	-	-	-

Growth Performance and Survival of Fish Larvae

The effect of herbal treatment on growth of seabass larvae are shown in Figure 3. Significant difference ($p<0.05$) was observed in seabass received with SirehMAX™, compared to control. The highest total length observed was 15 cm compared to 8 cm in control. This is consistent with findings by Daga et al. (2013) in turbot (*Scophthalmus maximus*) enriched with probiotics and Arulvasu et al., (2012) in *Poecilia sphenops* enriched with *Vitex negundo* leaf extract.

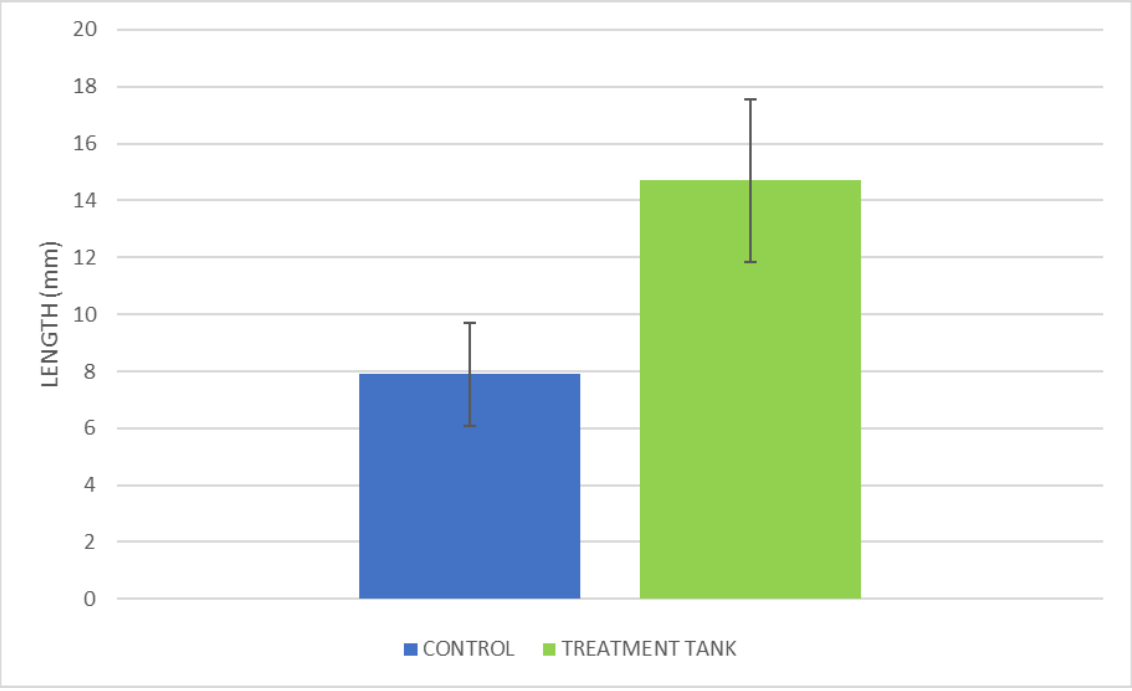

Figure 3. Graph showing growth of seabass (*Lates Calcarifer*) fry cultured for 25-days

At the end of the experiment, there were no significant differences in survival of larvae between both group (Figure 4). This corroborates findings by Takaoka et al., (2011) with no significant difference in survival of red sea bream larvae fed with rotifer enriched with *Crataegu fructus* and mixture of *Crataegu fructus*. Contrarily, Arulvasu et al., (2012) observed maximum survival in *Poecilia sphenops* treated with *Artemia* nauplii enriched with *Vitex negundo* leaf extract.

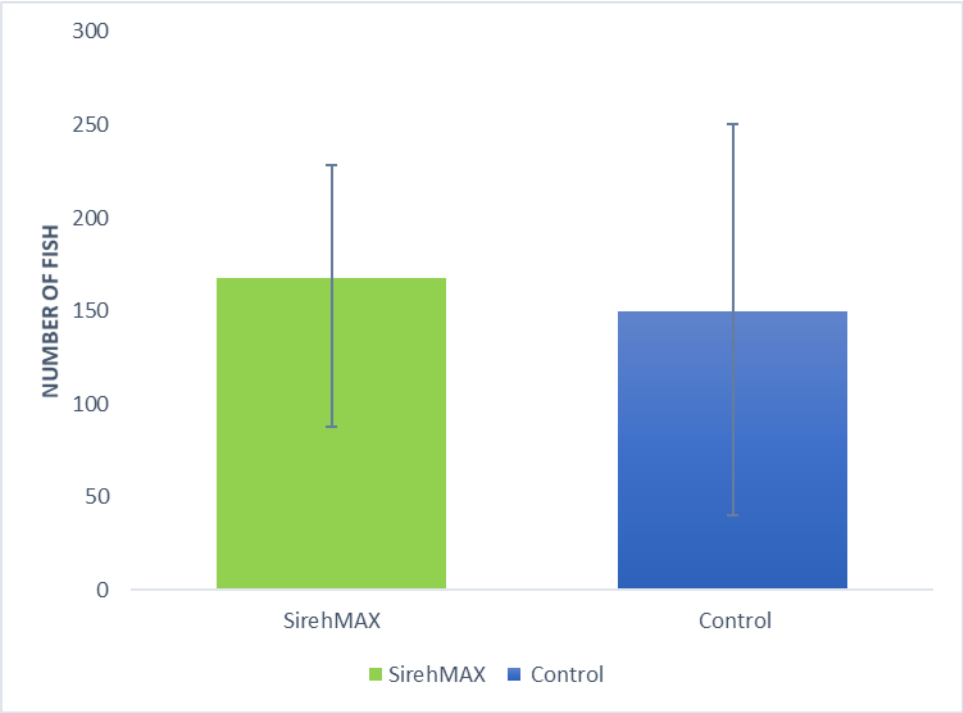

Figure 4. Number of fish fry fed with unenriched and enriched rotifer after 25 days of culture period.

Conclusion

The recommended concentration of SirehMAX™ use for bioencapsulation is at 10 ppm which seabass larvae fed with enriched rotifer with optimized dose herbal extract showed overall increase in growth and survival when compared to that of unenriched control. Further studies should be conducted to infer which ingredients in medicinal herbs promoted the growth and immune response of fish larvae.

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