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In vivo test of *Litopenaeus vannamei* infected by Vibrio using *Moringa oleifera* leaf extract

Iko Imelda Arisa[1,3,4,*], Sri Agustina[2,4,5], Cut Mutia[1], Nurfadillah Nurfadillah[1,4], Sofyatuddin Karina[2,4,5]

[1]*Aquaculture Department, Marine and Fisheries Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia.*
[2]*Marine Science Department, Marine and Fisheries Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia.*
[3]*Nutrition and Histology Laboratory, Marine and Fisheries Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia.*
[4]*Center of Marine and Fisheries Research, Universitas Syiah Kuala, Banda Aceh, Indonesia.*
[5]*Laboratory of Marine Chemistry and Biotechnology of Fisheries, Marine and Fisheries Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia.*

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ABSTRACT

This study aimed to determine the optimal concentration of *Moringa oleifera* leaf extract as an antibacterial agent on *Litopenaeus vannamei* larvae that are infected *by Vibrio sp.* It was conducted in 15 March - 15 April 2019 at PT. Global Gain Superior Seeds, Pantai Cermin, North Sumatera. The research used a completely randomized design (CRD) method with five treatments and four replications. The shrimps infected by *Vibrio sp.* were immersed into *M. oleifera* leaf extract in various concentration of (A) 0 ppm, (B) 400 ppm, (C) 600 ppm, (D) 800 ppm, and (E) 1000 ppm for ±15 minutes. The size of shrimp used was post-larvae 8 (length of 3.5-5 mm/ind). The best performance results of vannamei shrimps were obtained in treatment E (1000 ppm), namely length growth of 16.75 ± 1.16 cm, weight growth of 0.92 ± 0.89 g and survival rate of 91±7.87%.

Introduction

Vannamei shrimp (*Litopenaeus vannamei*) is an aquaculture commodity with high economic value. However, bacterial disease attacks are often observed as an obstacle in shrimp cultivation, causing big losses in both the aquaculture area and in hatcheries. Bacterial diseases caused by *Vibrio* sp. are the most serious and often lead to mass mortality on the larvae of vannamei shrimps. According to Sharma *et al.* (2010), farmers claim that the shrimp disease most frequently reported is vibriosis caused by *Vibrio* sp. infection. The research of Liu *et al.* (2019) also reported that the *Vibrio* bacteria group is the disease causing pathogens for shrimps.

Generally, *Vibrio* is a pathogenic bacteria species that cause mass mortality of panaeid shrimps in hatcheries and pond aquaculture (Amatul-Samahah *et al.* 2020). In addition, infection by *Vibrio* bacteria sometimes becomes primary infection that would then stimulate other pathogens to infect shrimp. Liu and Chen (2004); Octaviana *et al.* (2014) mentioned that shrimp production can be hampered by *Vibrio* spp. where the disease infection caused by these bacteria frequently results in secondary infection during the cultivation activities. The devastating impact of simultaneous infection by multiple pathogens can increase shrimp mortality.

Antibiotic is usually used by shrimp farmers to handle the infectious microorganisms on the site. However, the use of antibiotics would result in negative impacts, such as residue accumulation in the shrimp meat that could be harmful for consumers. It is also mentioned by Maryani *et al.*, (2002) that antibiotics given through feed, immersion, or injection would leave residues that can accumulate in the body of fishes. The frequent use of antibiotics to cope with disease caused by bacteria in shrimp aquaculture is also becoming less effective, since it generated increased resistant among the pathogens, and also leads to environment pollution (Sukenda *et al.*, 2015). Therefore, the frequent use of antibiotics is not justified in aquaculture activities. As a result, interest is increasing on the use of natural ingredients/herbs as antibacterial agents with less negative effects both

* Corresponding author.
Email address: ikoimeldaarisa@unsyiah.ac.id

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on the shrimp themselves, and on the people who consume them.

Purnamasari *et al.* (2010) reported that Indonesian people have long considered that natural ingredients/herbs could be used to treat various shrimp diseases and rarely cause adverse side effects compared to the use of chemicals. One of the herbal ingredients that can be used as medicine is moringa leaves (*Moringa oleifera*). According to Rani *et al.*, (2018) *M. oleifera* contains a variety of phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids and terpenes. The diversity of phytochemicals in this genus can contribute to a variety of pharmacology uses. Furthermore, Aminah *et al.*, (2015) stated that the bioactive compounds in *M. oleifera* has pharmacological properties and *M. oleifera* leaves have high antioxidant and antimicrobial content. Suhartono *et al.* (2019) reported that the ethanolic extract of *M. oleifera* leaves contains flavonoids, tannins, terpenoids, steroids, and alkaloids. Pratama *et al.* (2018) reported that using the extract of *M. oleifera* as immunostimulants can enhance the immune response of vannamei shrimp with the best conditions in the extract treatment of 40 mg/L.

Based on the reports by researchers about the content of the leaves of *M. oleifera*, there is research potential on the effect of *M. oleifera* extract through in vivo test on phatogen infected *L. vannamei*. Therefore, this study aims to determine the optimal concentration of *M. oleifera* leaf extract to act as antibacterial agent on the shrimp larvae of *L. vannamei* that has been infected *Vibrio* sp. bacteria.

Materials and Methods

Location and time of research

The research was conducted in March 15-April 15 2019 at PT. Global Gene Superior Seeds, Pantai Cermin, North Sumatra. The tools used were autoclave, magnetic stirrer, loop needle, 12 L jar, blender, hot plate, pH meter, thermometer, DO meter, scale, refractometer. While the materials used in this study were *Moringa oleifera* leaf extract, *Vibrio* sp. Bacteria, TCBS agar medium, vaname shrimp larvae (*Litopenaeus vannamei*), ethanol, feed and filter paper.

The experimental design used a completely randomized design (CRD) with 5 treatment levels and 4 replications, namely treatment A (concentration of 0 ppm *M. oleifera* extract), treatment B (concentration of 400 ppm of *M. oleifera* extract), treatment C (concentration of 600 ppm of *M. oleifera* extract), treatment D (concentration 800 ppm *M. oleifera* extract), and treatment E (concentration 1000 ppm *M. oleifera* extract).

Test shrimp

The test shrimps used were 8 days old (PL 8) size 3-5 mm, with a total of 2,000 individuals and each container was stocked with 100 shrimp. The test shrimps were taken from the same tub and were infected with the *Vibrio* sp.. The test shrimp were then soaked in their respective treatments of *M. oleifera* leaf extract for ± 15 minutes, after which they were then kept for 14 days. The calculation of *Total Vibrio Count* (TVC) on the test shrimp was carried out after soaking with *M. oleifera* leaf extract.

Extracting *Moringa oleifera* leaf

The leaves of *M. oleifera* were dried at room temperature for about 4 days under supervision. The dried samples (simplicia) were cut into pieces and then mashed using a blender to become simplicia powder. The simplicia powder was weighed at 130 g and put into an Erlenmeyer flask. Then immersion (maceration) was done with 2 liters of ethanol solution and soaked for 24 hours. After 24 hours, the solution was filtered using a filter and evaporated using an evaporator (Farika *et al.,* 2014; Kenconojati dan Rukmana, 2019).

Making TCBS media

TCBS powder was put in an Erlenmeyer flask (as much as 14,08 g), and then dissolved by adding 160 mL of distilled water and then heated until boiling on the hot plate while homogenized using a magnetic stirrer. Then, the media was sterilized using an autoclave at 121 ^{0}C with a pressure of 15 1bs for 15 minutes. TCBS media was then used to grow the *Vibrio* sp. bacteria.

Data analysis

Research data from the in vivo test of *Litopenaeus vannamei* infected with Vibrio using *Moringa oleifera* leaf extract were analyzed by ANOVA using the SPSS software program and if the effect was significant, it was followed by a Duncan test. Data were calculated based on the formula in Effendie (1979), which includes weight growth (GR), length growth (P) and survival (SR). In addition, the total bacterial colony (Total Vibrio Count) was also calculated using the formula from Wiyatanto *et al.* (2020).

Weight growth

$$GR = (W_t - W_0) / t$$

Where: GR = Weight growth, Wt = Final average weight, W_0 = Initial average weight, t = Time (day).

Length growth

$$P = P_t - P_0$$

Where: P=Length growth (cm), P_t= Final length (cm), P_0=Initial length (cm)

Survival rate

$$SR = (N_t - N_0) \times 100\%$$

Where: SR= Survival rate (%), N_t = Number of shrimp death during study (ind), N_0= Number of shrimp at the start of study (ind).

Total vibrio count (TVC)

$$TVC = \frac{\sum c}{1 \times n_1 + (0.1 \times n_2) \times d}$$

Where: $\sum c$ = Number of colonies on all plates count; n_1 = The number of plates at the first dilution count; n_2 = The number of plates at the second dilution count d = The first dilution calculated.

Results

The result of the research on the growth of vannamei shrimp larvae treated with different concentrations of Moringa leaf extract (*M. oleifera*) were tested by ANOVA and showed no effect (P>0.05). while the survival test and the total vibrio count of vannamei shrimp larvae showed an effect (P<0.05). The research data can be seen in Table 1.

Table 1. The results of the study length growth, weight growth, survival rate and total vibrio count of vannamei shrimp larvae (*Litopenaeus vannamei*).

Transect Names	Length Growth (mm)	Weight Growth (g)	Survival Rate (%)	TVC (10^6 Cfu/ml)
A (0 ppm)	16.63±0.01	0.16±0.01	72.00±8.52^a	700.00±86.66^b
B (400 ppm)	15.68±1.47	0.11±0.02	73.75±7.50^a	686.67±122.80^b
C (600 ppm)	16.55±0.86	0.15±0.03	85.50±4.04^{ab}	621.67±150.19^b
D (800 ppm)	16.66±0.27	0.92±0.89	87.50±9.00^{ab}	603.33±177.68^b
E (1000 ppm)	16.75±1.16	0.30±0.33	91.00±7.87^b	410.00±29.56^a

*Values not sharing a common superscript letter on each column are siginificantly different according to treatment (p<0.05)

Discussion

The use of antimicrobial therapy is considered to prevent existing microbial diseases, usually used for healing, where testing is carried out on infected animals in the laboratory (Ibrahim *et al.,* 2020). Result of in vivo test of vannamei shrimp (*Litopenaeus vannamei)* soaked in *Moringa oleifera* leaf extract can be seen in Table 1. Maslang *et al.* (2018) research states that feed substitution of *M. oleifera* powder in feeds on the survival growth and feed conversion for tilapia resulted in an increase of survival rate and decreased feed conversion.

The results of the immersion study of *M. oleifera* extract showed an effect on the survival of vannamei shrimp larvae (P<0.05), where the highest shrimp survival rate was 91% in treatment E (concentration of 1000 ppm *M. oleifera* extract). The increase in survival rate of shrimp larvae is suspected to have been cause by the antibacterial compounds contained within the *M. oleifera* extract that plays a role in inhibiting the growth of *Vibrio* sp. pathogen. According to Savitri *et al.* (2018) *M. oleifera* leaves contain antibacterial compounds such as suckedonins, triterpenoids and tannins which have action mechanisms that damages bacterial cell membranes. Research by Dima *et al.* (2016) said that *M. oleifera* leaves have compounds that can inhibit bacterial growth and the concentration differences in Moringa leaf extract affect the inhibition of growth of *Escherichia coli* and *Stapylococcus aureus* bacteria, where the higher the concentration given, the greater the antibacterial activity that inhibit bacterial growth.

The growth and development of vibrio bacteria did not properly occur in treatment E. It was observed that the number of *Total Vibrio Count* (TVC) in this treatment reached the least growth compared to the other treatments, namely 410 x 10^6 Cfu mL^{-1} (Table 1). Low TVC in this treatment shows that the immersion of the vannamei shrimp larvae infected with the *Vibrio* sp. with high concentrations of *M. oleifera* leaf extract have resulted in the inhibition of bacterial growth. Futhermore Valdez-Solana *et al.* (2015) said that the leaves of *M. oleifera* contain falvonoids and phenolic acids which act as antioxidants.

In addition, *M. oleifera* leaf extract is known to inhibit the formation of vibrio bacteria biofilm. Research by Suhartono *et al.* (2019) reported that *M. oleifera* leaf extract significantly inhibited *V. alginolyticus* biofilms formation in vitro. Biofilms are nutrient traps for the growth of microorganism populations and help prevent the release of cells from the surface of living or inanimate objects. The surface where the biofilm attached is an important habitat for microorganisms because nutrients can be trapped on this surface. Microorganism or biofilms in a system can be destroyed chemically. Other studies regarding the use of *M. Oleifera* leaves that have been carried out include Farika *et al.* (2014) who stated that *M. oleifera* leaf extract can be used as a control for *Argulus* sp. in *Carrasius auratus auratus* with the best treatment at the highest concentration 62,5 mgL^{-1}. Shailemo *et al.* (2016), reported that *M. oleifera* extract has antibacterial activity against *Bacillus cereus*, *Enterococcus faecalis* and *Escherichia coli*, with an inhibition zone between 7 – 9 mm, at a concentration of 50 mg mL^{-1}. Futhermore Widowati *et al.* (2014) also tested the antibacterial activity of *M. oleifera* leaves extract against spoilage bacteria of fresh fish (*Pseudomonas aeruginosa*) where the best tratment was shown to be at the highest concentration of 100%.

Clinical symptoms of vannamei shrimp larvae stricken with vibriosis disease at the time of study were body weakness, dull appearance of shrimps, inactive movement and decreased appetite. According to research by Sarjito *et al.* (2015) clinical symptoms of shrimps affected by vibriosis are the reddening of the body (carapace), melanosis of the skin, necrosis of the tail, flushed swimming and walking legs and a reddened hepatopancreas that tends to darken. Vibrio bacteria in aquaculture media are normal flora and they generally thrive when there is an imbalance. Arias-Moscoso *et al.* (2018) said that when the balance between bacterial communities is disturbed, vibrio bacteria become opportunistic pathogens.

Water quality parameters during shrimp rearing (temperature, pH, DO and salinity are still within the tolerance of optimal shrimp growth. The results of observations in all treatments showed that the temperature ranged between 26-28ºC, pH 8.0-8.3, DO 6-6,6 mgL^{-1} and salinity 29-30 ppt. Research by Fuady *et al.* (2013) stated that the management of water quality on survival rates and growth rates of vannamei shrimp needs the range of temperature 26-29 ºC, pH 6-8, DO 5-6 mgL^{-1} and salinity 30 ppt.

Conclusion

Soaking *Moringa oleifera* leaf extract in vannamei shrimp larvae (*Litopenaeus vannamei)* infected with *Vibrio harveyi* at a concentration of 1000 ppm can inhibit bacterial growth and increase the survival of shrimp larvae

References

Amatul-Samahah, A.M., W.H.H.W. Omar, N.F.M. Ikhsan, M.N.A. Azmai, M. Zamri-Saad, M.Y. Ina-Salmany. 2020. Vaccination trials against vibriosis in shrimp: A review. Aquaculture Reports, 18: 100471.

Aminah S., T. Ramdhan, M. Yanis. 2015. Kandungan nutrisi dan sifat fungsional tanaman kelor (*Moringa oleifera*). Buletin Pertanian Perkotaan, 5(2): 35-44.

Arias-Moscoso, J.L., L.G. Espinoza-Barrón, A. Miranda-Baeza, M.E. Rivas-Vega, M. Nieves-Soto. 2018. Effect of commercial probiotics addition in a biofloc shrimp farm during the nursery phase in zero water exchange. Journal Aquaculture Reports, 11: 47-52.

Dima, L.L.R., Fatimawali, W.A. Lolo. 2016. Uji aktivitas antibakteri ekstrak daun kelor (*Moringa oleifera* L.) terhadap bakteri *Escherichia coli* dan *Staphylococcus aureus*. Pharmacon Jurnal Ilmiah Farmasi – UNSRAT, 5(2): 282-289.

Effendie M.I. 1979. Metode Biologi Perikanan. Yayasan Dewi Sri. Bogor, 112 hlm.

Farika, E.Y., N.A. Suratma, I.M. Damriyasa. 2014. Ekstrak daun kelor (*Moringa oleifera*) sebagai pengendali infestasi *Argulus* sp. pada ikan komet (*Carassius auratus auratus*). Jurnal Ilmu dan Kesehatan Hewan, 2(1): 1-11.

Fuady, M.F., M.N. Supardjo, Haerudin. 2013. Pengaruh pengelolaan kualitas air terhadap tingkat kelulusanhidupan dan laju pertumbuhan udang vaname (*Litopenaeus vannamei*) di PT. Indokor Bangun Desa Yogyakarta. Diponegoro Journal of Maquares Management of Aquatic Resources, 2(4): 155-162.

Ibrahim, M., F. Ahmad, B. Yacub, A. Ramza, A. Imran, M. Afzaal, S. A. Mirza, I. Mazhar, M. Younus, Q. Akram, M.S.A. Taseer, A. Ahmad, S. Ahmed. 2020. Current trends of antimicrobials used in food animals and aquaculture. Journal Antibiotics and Antimicrobial Resistance Genes in the Environment, 1: 39-69.

Kenconojati H., N.R. Rukmana. 2019. Daya hambat ekstrak daun kelor (*Moringa oleifera*) terhadap *Aeromonas hydrophila*: Studi awal untuk pengobatan Aeromoniasis. Journal of Aquaculture Science, 4(1): 12-20.

Liu, C.H., C.J. Chen. 2004. Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. Journal Fish and Shellfish Immunol, 16(3): 321-334.

Liu K., J. Han, S. Li, L. Liu, W. Lin, J. Luo. 2019. Insight into the diversity of antibiotic resistance genes in the intestinal bacteria of shrimp *Penaeus vannamei* by culture-dependent and independent approaches. Journal Ecotoxicology and Environmental Safety, 172: 451-459.

Maryani, D., Dana, Sukenda. 2002. Peranan ekstrak kelopak dan buah mangrove *Sonneratia caseolaris* (L) terhadap infeksi bakteri *Vibrio harveyi* pada udang windu (*Panaeus monodon* FAB.). Jurnal Akuakultur Indonesia, 1(3): 129-138.

Maslang, M., A.A. Malik, Sahabuddin. 2018. Subtitusi pakan tepung daun kelor terhadap pertumbuhan sintasan dan konversi pakan benih ikan nila. Jurnal Galung Tropika, 7(2): 132-138.

Oktaviana, A., Widanarni, M. Yuhana. 2014. The use of synbiotics to prevent IMNV and *Vibrio harveyi* co-infection in *Litopenaeus vannamei*. HAYATI Journal of Biosciences, 21(3): 127-134.

Pratama, A.F., Tarsim, O. Susanti. 2018. Kajian ekstrak daun kelor (*Moringa oleifera Lam*) sebagai imunostimulan untuk meningkatkan imunitas non spesifik udang vannamei (*Litopenaeus vannamei).* Jurnal Sains Teknologi Akuakultur, 2(2): 16-21.

Purnamasari, A.D., E. Munandziroh, R.M. Yugiartono. 2010. Konsentrasi ekstrak biji kakao sebagai material alam dalam menghambat *Streptococcus* mutans. Jurnal PDGI, 59(1): 14-18.

Rani, N.Z.A., K. Husain, E. Kumolosasi. 2018. Moringa Genus: A review of phytochemistry and pharmacology. Journal Frontiers in Pharmacology, 9(108): 1-26.

Sarjito, M. Apriliani, D. Afriani, A.H.C. Haditomo. 2015. Agensia penyebab vibriosis pada udang vaname (*Litopenaus vannamei*) yang dibudidayakan secara intensif di Kendal. Jurnal Kelautan Tropis, 18(3): 189-196.

Savitri, E., Fakhrurrazi, A. Harris. 2018. Uji antibakteri ekstrak daun kelor (*Moringa oleifera* L.) terhadap pertumbuhan bakteri *Staphylococcus auretus*. Jurnal Ilmiah Mahasiswa Veteriner, 2(3): 373-379.

Sharma, S.R.K., K.M. Shankar, M.L. Sathyanarayana, A.K. Sahoo, R. Patil., H.D. Narayanaswamy, S. Rao. 2010. Evaluation of immune respone and resistance to diseases in tiger shrimp, *Penaeus monodon* fed with biofilm of *Vibrio alginolyticus*. Journal Fish and Shellfish Immunology, 29(5): 724-732.

Shailemo, D.H.P., H.M. Kwaambwa, M. Kandawa-Schulz, T.A.M. Msagati. 2016. Antibacterial activity of *Moringa ovalifo* and *Moringa oleifera* methanol, N-Hexane and water seeds and bark extracts against pathogens that are implicated in water borne diseases. Journal Green and Sustainable Chemistry, 6: 71-77.

Suhartono, S., Y.S. Ismail, S.R. Muhayya, M. Husnah. 2019. Ethanolic extracts of *Moringa oleifera* leaves inhibit biofilm formation of *Vibrio alginolyticus* in vitro. Proceeding of the The 2ndICFAES 2019 International Conference on Fisheries, Aquatic and Environmental Sciences 2019 In Conjunctions with The 6th ASI 2019 Annual Conference of The Asian Society of Ichthyologist 2019 IOP Conf. Series: Earth and Environmental Science, 348(1): pp 012018.

Sukenda, R. Praseto, Widanarni. 2015. Efektivitas sinbiotik dengan dosis yang berbeda pada pemeliharaan udang vaname di tambak. Jurnal Akuakultur Indonesia, 14(1): 1-8.

Valdez-Solana, M.A., V.Y. Mejía-García, A. Téllez-Valencia, G. García-Arenas, J. Salas-Pacheco, J.J. Alba-Romero, E. Sierra-Campos. 2015. Nutritional content and elemental and phytochemical analyses of *Moringa oleifera* grown in Mexico. Journal of Chemistry, (860381): 1-9.

Widowati, I.,S. Efiaty, S. Wahyuningtyas. 2014. Uji aktivitas antibakteri ekstrak daun kelor (*Moringa oleifera*) terhadap bakteri pembusuk ikan segar (*Pseudoonas aeruginosa*). Jurnal PELITA, 9(2): 146-157.

Wiyatanto M.T., A. Setyawan, B. Putri. 2020. Efektivitas pemberian pakan alami *Artemia* specifik pathogen free (SPF) *Vibrio* sp. terhadap insidensi vibriosis dan pertumbuhan pada pemeliharaan post-larva udang vaname (*Litopenaeus vannamei*). Jurnal Sains Teknologi Akuakultur, 3(1): 42-51.

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