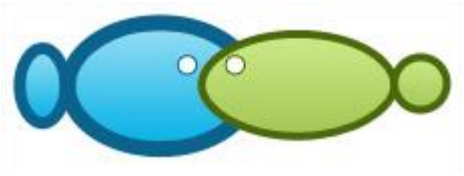

The effectiveness of *Chromolaena odorata* extract and histopathological change in tiger prawn (*Penaeus monodon*) challenged with *Vibrio harveyi*

[1]Harlina Harlina, [1]Muhammad Kasnir, [2]Rosmiati Rosmiati, [1]Andi Hamdillah, [1]Muhammad I. Wamnebo

[1] Department of Aquaculture, Faculty of Fishery and Marine Science, Muslim University of Indonesia, Makassar, Indonesia; [2] Research Institute for Coastal Aquaculture, Maros, Indonesia. Corresponding author: H. Harlina, e-mail: harlina.harlina@umi.ac.id

Abstract. The objective of the study was to investigate the clinical signs, survival rate (SR) and population growth of bacteria in the medium, post-larval *Penaeus monodon* body and also to analyze the histopathology of post-larval *P. monodon*. Samples were extracted by soaking method and the extracts were examined for their antibacterial activities against *Vibrio harveyi* MR 275 Rif. The challenge test were carried out with seven treatments, each one with three replicates. All the shrimps were soaked for 6 hours with V. harveyi MR 275 Rif 107 CFU mL^{-1}. *Chromolaena odorata* antibacterial extracts were soaked into shrimp containers in various concentrations (625, 750, 875, 1,000, 1,125, 1,250 ppm). The clinical signs were observed once a day, for 7 days. The disease status of all shrimp was also determined by mortality, population of bacteria in rearing medium and shrimp body, histology and water quality. The result of the challenge test indicated that the concentration of 1,250 ppm of *C. odorata* extract was able to decrease the mortality (with 14.44%) and the population of bacteria (to 1.40×10^{1} CFU mL^{-1}). Clinical signs of tiger prawn were restored to a normal status. The tissues and body of post-larval tiger prawn were normal (healthy) after few days of treatment. The overall results provided evidence that the extract of *C. odorata* is effective to combat vibriosis on *P. monodon* and able to serve as a promising resource for the development of therapeutic agents against bacterial infections in shrimp.
Key Words: hepatopancreas, mortality, shrimp, vibriosis, bacterial infection.

Introduction. The major issue in tiger prawn (*Penaeus monodon* Fabr.) hatchery is the mass mortality as a result of bacterial infection caused by *Vibrio harveyi* (Mohajeri et al 2011; Karami-Mohajeri & Abdollahi 2011; Lichtlen & Mohajeri 2008). It leads to major economic losses in the *P. monodon* industry. The use of chemicals and antibiotics has been widely carried out in dealing with vibriosis disease. However, it causes bacterial resistance, pollutes the environment and leaves a residue within the *P. monodon* (Hua et al 2013). Those practices have been disallowed in aquaculture due to health issues. Therefore, it is necessary to find another way of dealing with the vibriosis outbreaks, which should be safe for the aquaculture species, humans and environment.

The *Chromolaena odorata* leave has been identified as a natural antibacterial. The *C. odorata* leave extracts have been applied as antiprotozoal agent (Vital & Rivera 2009). Nevertheless, it has not been widely used as an antibacterial agent for vibriosis prevention on post-larval *P. monodon*. In addition, the methanol extract of *C. odorata* is able to inhibit the growth of *V. harveyi*. The inhibition zone of *C. odorata* is 19 mm, at a minimum concentration (MIC) of 0.625 mg mL^{-1} and a minimum bactericidal concentration (MBC) of 1.25 mg mL^{-1} (Harlina et al 2013). Previous studies found that the active methanol extract of *C. odorata* leaf did not showed any toxic effect on post-larval of tiger prawn at a concentration up to 1,250 ppm (Harlina et al 2015). The *C. odorata* leaf compounds, such as flavonoid derivatives or quercetin, have been successfully isolated using the column chromatography method. These bioactive compounds drive the anti-bacterial activity of the active methanol extract (Harlina et al

2016). Previous studies demonstrated the potential of *C. odorata* leaf as antimicrobial agent against pathogenic microorganisms, in the aquaculture business. Therefore, the study aimed to qualify the potential of the active methanol extract of *C. odorata* leaf as an antibacterial agent for the vibriosis prevention through the challenge test method applied to the post larvae *P. monodon*. Parameters observed were the clinical signs of *V. harveyi* infected post larvae, the survival rate (SR) of challenged post-larval, the bacterial population growth in the medium and the post-larval and histopathological analysis of post-larval tiger prawn.

Material and Method

Sample collection. Fresh leaves of *C. odorata* were collected from Maranak, Maros Regency, South Sulawesi, Indonesia. The samples were immediately transported to the laboratory. The plant parts were washed, air-dried in herbs dryer, chopped into small pieces and ground coarsely into a powder in a mechanical grinder. The powdered samples were stored until required for use.

Plant extraction. The powder of *C. odorata* leaves (500 g) was extracted by the soaking method using methanol (MeOH) for 72 h. The MeOH extracts were filtered using a Whatman no. 1 filter paper and they were evaporated using rotary evaporator (Heidolph) at 40°C. The collected filtrate of solvent was separately evaporated using a vacuum rotary evaporator (Buchi type) at 40°C and air-dried.

Bacterial strain. *V. harveyi* MR 275 Rif was used in the antibacterial activity assay. The bacteria were obtained from Laboratory of Environmental Health of Research Institute for Coastal Aquaculture, Maros District, South Sulawesi, Indonesia. *V. harveyi* MR 275 Rif was stored in TSB (Oxoid, UK) medium containing 20% (v/v) glycerol.

Experimental design. A completely randomized design was applied with seven treatments and three replicates for each treatment. This test was carried out by challenging the post-larval of *P. monodon* using soaking with *V. harveyi* MR 275 Rif at the density of 10^7 CFU mL^{-1}. The cell density was estimated based on the McFarland standard, using a spectrophotometer. The soaking process lasted 6 hours until clinical sign appeared. During the challenge test, the feed remaining and feces were siphoned out of the container every day. Shrimp were fed their respective diet at a daily rate of 10% of the body weight, at 08.00 and 16.00. Then *C. odorata* leave extracts were soaked into the post-larval tiger prawns' container, with different concentrations (treatments): A. 0 ppm (control), B. 625 ppm, C. 750 ppm, D. 875 ppm, E. 1,000 ppm, F. 1,125 ppm and G. 1,250 ppm, according to Goulden et al (2012). The clinical signs were observed after 6-24 hours of soaking. The disease status of all shrimp was also determined by evaluating the mortality, bacterial population density, histology, and water quality.

Histopathology on hepatopancreas. Tissue samples were also collected at 7 days after exposure to different concentrations of bacteria according to the study method. Shrimp tissues were fixed in Buffer Neutral Formalin (BNF) for 48 hours. The standard histology processing and procedures were applied as described by Zhang (2008) and Maftuch (2007) with some modification.

Water quality. Water samples were taken from the investigated indoor and measured every 12 hours. Dissolved oxygen (DO) and water temperature were determined by a DO meter (YSI 58, Yellow Spring Instrument co. Inc., USA), pH was determined by a portable pH meter (Meterlab PPM 201), Salinity was determined by a hand refractometer and total ammonia was determined by spectrophotometer.

Statistical analysis. Statistical analysed performed SPSS 16.00. A one way ANOVA test was applied to find the differences, with a significance of $P<0.05$, and a Duncan's test was performed for further analysis.

Results

Clinical signs. The observation of clinical signs in post-larval tiger prawn during the challenge test against *V. harveyi* MR 275 Rif indicated behavioral and morphological changes. The behavioral changes of post-larval *P. monodon* which were challenge-tested against *V. harveyi* MR 275 Rif before and after the medication are listed in the Table 1.

Table 1

Clinical signs of *Vibrio harveyi* infected post-larvae *Penaeus monodon* after using *Chromolaena odorata* extract

Day	*Motion*	*Appetite*	*Body color*	*Morphology*
1	Indolent, irregular	Loss of appetite	Slimy, dark, reddish hepatopancreas	Tail cramping, broken antenna
2	Indolent, irregular	Decreased appetite	Reddish, dark hepatopancreas	Broken antenna
3	Begin to be more active	Decreased appetite	Red colored in the tail edge	Broken antenna
4	Begin to be more active	Begin to eat	Begin to be bright	Back to normal, red color begins to disappear
5	Active motion	Begin to eat	Bright color	Hepatopancreas is back to normal
6	Active motion	Begin to eat	Bright color	Back to normal
7	Active motion	Begin to eat	Bright color	Back to normal

Mortality. The mortality observations of post-larval *P. monodon*, after being challenge-tested with *V. harveyi* MR 275 Rif at a density of 10^7 cell mL^{-1} and treated with different concentrations of *C. odorata* extract during the rearing period are illustrated in Figure 1.

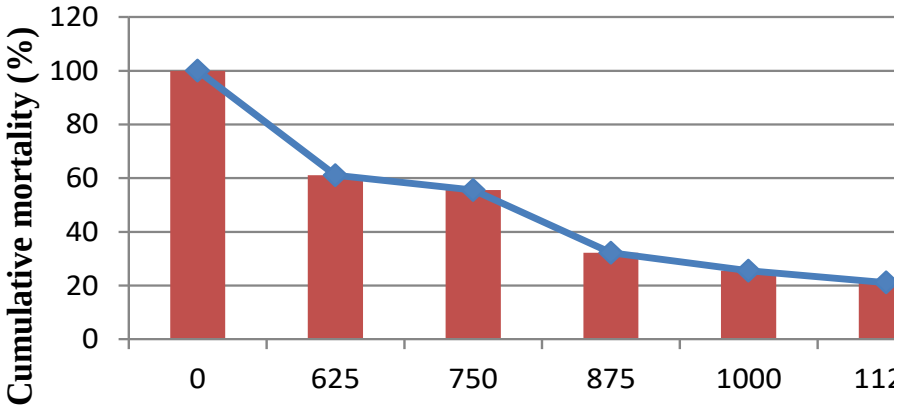

Figure 1. Cumulative mortality of post-larval *Penaeus monodon* challenged with *Vibrio harveyi* and treated with the different concentrations of *Chromolaena odorata*.

Based on the mortality statistical analysis, the six *C. odorata* extract concentrations had different effects on the survival rate of post-larval *P. monodon*, as shown in Table 2.

Table 2

The post-larval *Penaeus monodon* mortality statistical analysis after being challenged with *Vibrio harveyi* and treated with different concentrations of *Chromolaena odorata* extract

Concentration of C. odorata (ppm)	*Cumulative mortality (%)*
0	100.00[a]
625	61.11[b]
750	55.55[b]
875	32.22[c]
1,000	25.55[cd]
1,125	21.11[de]
1,250	14.44[e]

Superscript letters ([a,b,c,d, e]) in the same column represents significant differences (P<0.05).

V. harveyi population total bacteria in the rearing medium of the post larvae *P. monodon* after being challenged with *V. harveyi* MR 275 Rif and treated with *C. odorata* extracts for 6 hours of rearing time period is illustrated in Figure 2.

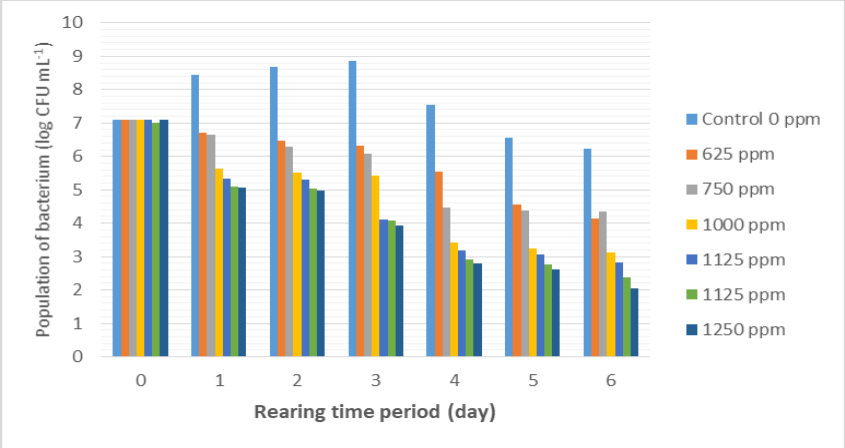

Figure 2. Population of *Vibrio harveyi* (Log[10] CFU mL[-1]) in the rearing water treated with different concentrations of *Chromolaena odorata* extract during the rearing time period.

The population of *V. harveyi* MR 275 Rif in the *P. monodon* post-larval challenged with *V. harveyi* MR 275 Rif and treated with *C. odorata* extract, after different rearing time periods, is illustrated in Figure 3.

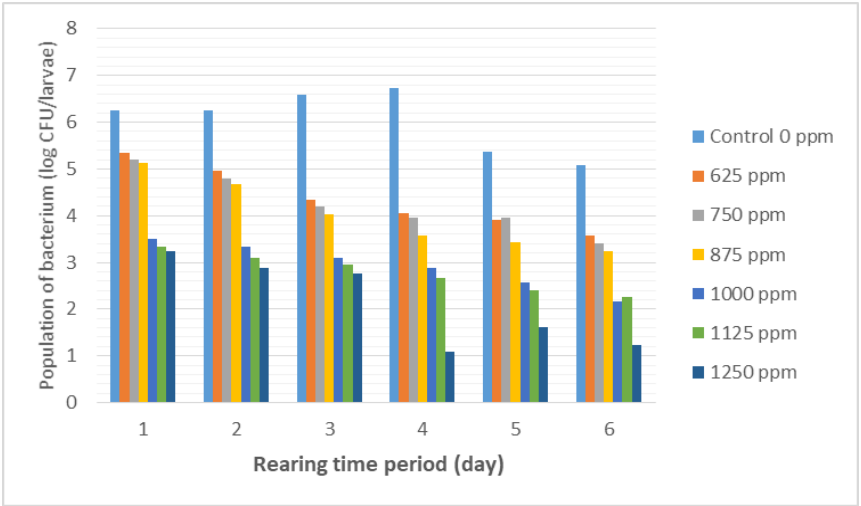

Figure 3. Population of *Vibrio harveyi* in the *Penaeus monodon* post larvae treated with different concentrations of *Chromolaena odorata* extract during the rearing time period.

The regression analysis of the relationship between the concentration of *C. odorata* extract and the population of *V. harveyi* MR 275 Rif indicated a linear curve type: Y=5.442–0.003 X, with R^2=0.917. The equation represented a strong relationship in which R^2>0.5. The statistical tests result is listed in Table 3.

Table 3

Statistical analysis of the population of *Vibrio harveyi* MR 275 Rif within the *Penaeus monodon* larvae's body after being challenged with *Chromolaena odorata* extract at different concentrations

Concentration of extract (ppm)	*Population of V. harveyi MR 275 Rif (CFU mL^{-1})*
0	$1.23\times10^{5.a}$
625	$3.70\times10^{3\ b}$
750	$2.60\times10^{3\ c}$
875	$1.73\times10^{3\ d}$
1,000	$1.43\times10^{2\ e}$
1,125	$2.20\times10^{1\ f}$
1,250	$1.40\times10^{1\ g}$

Superscript letters ($^{a, b, c, d, e}$) in the same column represents significant differences (P<0.05).

Histopathological observation. The histopathological observation on the hepatopancreas of post-larval *P.monodon* challenged with *V. harveyi* MR 275 Rif and treated with *C. odorata* extract at different concentrations was described in Figure 4a and Figure 4b.

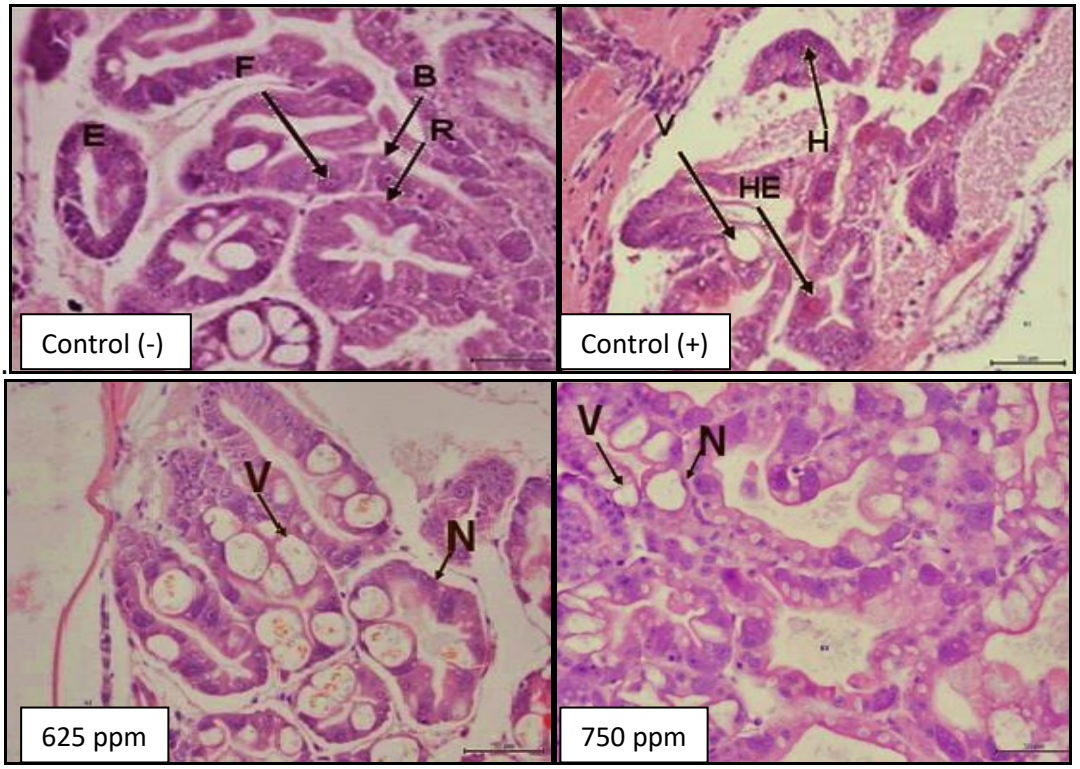

Figure 4a. Histological appearances of hepatopancreas of post-larval *Penaeus monodon* after medication with the *Chromolaena odorata* extracts: control treatment (-), control treatment (+), concentration of 625 ppm and 750 ppm (E-embryonic cell; N-necrosis; B-blasenzellen cell; H-hyperplasia; R-restzellen cell; HE-hemorrhagic; F-fibrillazellen cell; V-vacuola). Haematoxylin and eosin staining, magnification=400.

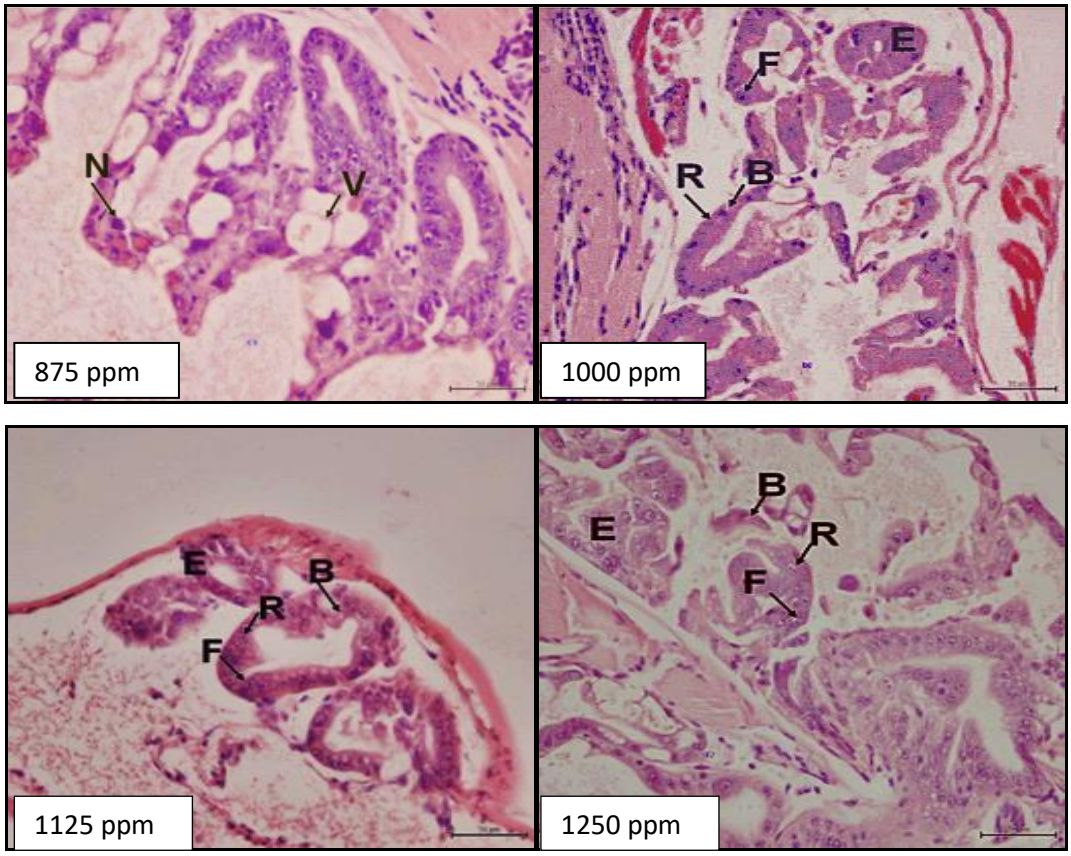

Figure 4b. Histological appearances of hepatopancreas of post-larval *Penaeus monodon* after medication with *Chromolaena odorata* extract at concentrations of: 825 ppm; 1,000 ppm; 1,125 ppm and 1,250 ppm (N-necrosis; E-embryonic cell; F-fibrillazellen cell; R-restzellen cell; V-vacuola).

The observation of water quality during the study was carried out twice per day at 8 AM and 4 PM and presented in Table 4.

Table 4

Water quality of post-larvae *Penaeus monodon* challenged-with *Vibrio harveyi* MR 275 Rif and treated with *Chromolaena odorata* extract at different concentrations, during the rearing period

Concentration (ppm)	*Salinity (ppt)*	*pH*	*Temperature (^oC)*	*Dissolved oxygen (mg L^{-1})*	*Ammonia (NH_3)*
K+0	28.68±0.21	7.14±0.06	26.76±0.32	5.87±0.10	0.061±0.028
A. 625	28.65±0.29	7.12±0.06	26.74±0.39	5.87±0.26	0.049±0.003
B. 750	28.72±0.19	7.18±0.12	26.77±0.33	5.83±0.16	0.044±0.026
C. 875	28.68±0.25	7.16±0.13	26.76±0.31	5.84±0.12	0.054±0.027
D. 1000	28.69±0.19	7.11±0.05	26.71±0.19	5.83±0.28	0.054±0.029
E. 1125	28.71±0.30	7.14±0.06	26.82±0.38	5.92±0.23	0.051±0.028
F. 1250	28.59±0.29	7.13±0.06	26.73±0.32	5.84±0.30	0.044±0.034

Discussion

Clinical signs. Post-larval *P. monodon* was reared in the experimental medium and challenged with *V. harveyi* MR 275 Rif for 6 hours until the population density of *V. harveyi* reached MR 275 Rif 10^7 cell mL^{-1}. In the present study, the symptoms of *V. harveyi* MR 275 Rif infection started showing after 3 hours, being indicated by the swimming behavior and by the body color changes, and eventually *V. harveyi* MR 275 Rif infection appears after 6 hours. In many cases, the bacterial infection appears after 3

hours to several days, depending on the *P. monodon* size and immunity after *V. harveyi* challenge test (Rengpipat et al 2000). Clinical signs of *V. harveyi* MR 275 Rif against post-larval *P. monodon* (PL 13) may emerge in shell, gills, muscle, alimentary canal and hepatopancreas of prawn (Kannapiran et al 2009; Manilal et al 2010). External signs appeared in certain parts of the body, for instance: abdominal inflammation, pale morphological appearance, body color change in the abdomen and swimmeret, body color darkening, weakening swimming balance and whirling swimming motions, deficient or absent motility, decreasing appetite, oozing body (mucosa), reddish color changes on the antenna and swimmerets of prawn.

Body color was one of the clinical signs observed in the present study. The post larvae *P. monodon* challenged with *V. harveyi* MR 275 Rif and treated with the *C. odorata* extract at the concentrations of 625, 750 and 875 ppm showed that the body color of the experimental post-larval *P. monodon* turned to reddish, indicating that the larvae were unhealthy (vibriosis-infected *P. monodon*). Similar symptoms were also observed in the control treatment (post-larval *P. monodon* challenged with *V. harveyi* without plant extract). On the contrary, when treated with a *C. odorata* extract at the concentrations of 1,000, 1,125 and 1,250 ppm, the *P. monodon* larvae did not show similar symptoms, their body colors tending to be brighter, which suggested that the larvae in this group of treatment were healthier than in control (+). As reported by Sung et al (1991), that reddish body surface and the gills that turn red to brown are the clinical signs shown when the *P. monodon* is infected by vibriosis. Furthermore, Saeed (1995) and Kannapiran et al (2009) reported that *V. harveyi* infected *P. monodon* body present reddish spots, incomplete walking legs structure and swimmeret, damaged tail and turbid-milky or murky carapace color. In addition, the *V. harveyi*-infected tiger shrimp has a slow response to stimulation, remains motionless at the bottom of the water and swims irregularly.

Mortality. Figure 1 illustrated that treatment with *C. odorata* extract with different concentrations showed the different effect on decreasing mortality of post-larval *P. monodon*. The lowest mortality rate was obtained with the concentration of 1,250 ppm (14.44%) followed by the treatments at 1,125, 1,000, 875, 750 and 625 ppm, with a mortality of 21.11, 25.55, 32.22, 55.55 and 61.11%, respectively. In contrast, the larvae in the control treatment showed the highest mortality (100%). The highest mortality was showed for the *C. odorata* extract concentration of 625–875 ppm treatment, indicating a lack of effectiveness against the vibriosis. Although the *C. odorata* extract was reported to be able to reduce the *V. harveyi* population, the extracts were incapable of avoiding bacterial infection in the post-larvae *P. monodon*. The mechanism of the bacterial pathogenic into the host body is initiated by the *V. harveyi* attachment to the host body surface by means of flagella and fimbria. The bacterium is multiplied in the host's tissue. During the process, bacteria need iron, commonly known as siderophora membrane proteins. The proteins used by bacteria are heme, transferrin, and lactoferrin or ferritin. If the proteins are fulfilled, bacteria tend to more pathogenic and resistant to the host's defense, through a defensive system existing in a capsule, containing extracellular polysaccharides. Bacterial resistance leads to damaging the tissue, by producing exotoxin enzyme (hemolysin, protease and phospholipase), endotoxin enzyme (LPS) and extracellular toxin complex. Finally, the attack causes the mortality of post-larvae tiger shrimp. The increasing of the post-larvae survival rate was driven by the active flavonoid compounds obtained in *C. odorata* extract. The bioactive compounds had a function as antibacterial agents capable of hampering and combating the bacterium. The mechanism of the active substance in killing the bacteria is by protein denaturation and bacterium cell membrane damage, such as dissolving the fat existing in the cell wall. The cell membrane damage caused a delay in the biosynthesis activity of the specific enzymes which are involved in the metabolism. Consequently, this condition leads to the cell mortality.

V. harveyi population. The decrease of the *V. harveyi* MR 275 Rif population in the rearing water of the post-larvae *P. monodon* treated with *C. odorata* extract at all the

tested concentrations, during the rearing period, is illustrated in Figure 2. The densities of the population of *V. harveyi* MR 275 Rif under the treatment effect of the *C. odorata* extract at concentrations of 625 and 750 ppm were of 5.26×10^6 and 4.57×10^6 CFU mL^{-1}, respectively, after 2 days of infection, while in the untreated post-larvae the bacterial density was of 7.46×10^8 CFU mL^{-1} after 4 days of infection. Conversely, a drastic decreasing population density of *V. harveyi* MR 275 Rif occurred in the post-larvae treated with *C. odorata* extract at the concentrations of 1,125 and 1,250 ppm, at 1.23×10^5 and 1.16×10^5 CFU mL^{-1}, after 2 days of infection. In general, the population of *V. harveyi* MR 275 Rif can be decreased in the rearing water of the post-larvae treated with the bioactive compounds at all concentrations. The decreasing of *V. harveyi* population in the rearing water of the post larvae treated with *C. odorata* extract indicated that the bioactive compound was capable of hampering the *V. harveyi* growth. This antibacterial capacity was highly affected by the stability of protein, lipid and acidity (pH) in the rearing medium (Wu et al 2016). According to Maddox et al (2010), in general, antibacterial compounds are phenols and their derived compounds. The compounds can penetrate the cell wall and damage the cell system. The permeability of the cytoplasm membrane functioning to hold the cell's internal components in place can be disturbed by some antibacterial compounds existing in *C. odorata* extract, resulting in the cell permeability. The leakage of the bacterial cell cytoplasm membrane can be caused by antiseptic and disinfectant compounds.

Figure 3 showed the population of *V. harveyi* MR 275 Rif within post-larvae *P. monodon* body after being treated with *C. odorata* extract. The population of *V. harveyi* MR 275 Rif in the post-larvae *P. monodon* body treated with *C. odorata* extract decreased at a significantly lower level than in the positive control. The maximum decrease of the *V. harveyi* MR 275 Rif population in all larvae treated with the bioactive compound was obtained in 2 days post-infection and continued to decrease gradually till the end of rearing time period. The lowest population density of *V. harveyi* MR 275 Rif was found in the post-larvae body treated with the bioactive compound at the concentration of 1,250 ppm (1.74×10^3 CFU mL^{-1}). On the one hand, the population density of *V. harveyi* MR 275 Rif was reached in the post-larvae body treated with *C. odorata* extract at the concentrations of 1,125 and 1,000 ppm, namely 2.19×10^3 CFU mL^{-1} and 3.12×10^3 CFU mL^{-1}, respectively. On the other hand, the highest *V. harveyi* MR 275 Rif population density was observed in the untreated post-larvae: 1.82×10^6 CFU mL^{-1}.

The decreasing of *V. harveyi* population occurred in the post-larvae body and in the rearing water showed that the bioactive compound of *C. odorata* extract was able to hamper the population growth of *V. harveyi.* The *C. odorata* extract at the concentrations of 625, 750, and 850 ppm could also decrease population of *V. harveyi* MR 275 Rif. Nevertheless, these concentrations could not prevent the bacterium infection in the post-larvae treated. The application of *C. odorata* extract at the concentrations of 1,000, 1,125, and 1,250 ppm showed a strong antibacterial activity of the bioactive compound, presumably caused by the presence of the flavonoid compounds higher concentrations. Flavonoid compounds have the capacity to hamper the bacterium growth by different pathways, causing the permeability of the cell wall, microsome and lysosome, due to the interaction between flavonoids and bacterium DNA. Xie et al (2015) and Kumar et al (2013) suggested that the hydroxil group existing in the flavonoid compounds' structure changed the organic components and the nutritional transport, causing atoxic effect on the bacteria.

The hepatopancreas has an important role in the *P. monodon* digestive system. In the vertebrate animal, the hepar and pancreas have the following functions: 1) to contribute, together with the midgut, to the nutrients absorption, 2) to secrete trypsin and lipase proteolytic enzymes, and related enzymes carbohydrase and chitinase, 3) to process the carbohydrates (glycogenesis and glycolysis), protein (gluconeogenesis) and fat, and 4) to store organic and inorganic substances like Ca, Mg, and PO_4, which are important for the shell turnover. The results of the histo-pathological examination on the hepatopancreas of the post-larval *P. monodon* (challenged with *V. harveyi* and treated with different concentrations of *C. odorata* extract), 7 day post-treatment (Figure 4) showed the changes of the cell and tissue duo to the pathogens. The cells necrosis was

still exhibited by the treatment of *C. odorata* extract with the concentration of 625 ppm (B) and 750 ppm (C) nevertheless, more necrotic cells were observed on the treatment B compared to C. Meanwhile, the fat degeneration observed in the treatment B was not caused by the pathogenicity of *V. harveyi* MR 275 Rif but it was due to the malnutrition. The severe hepato-pancreatic pathology typical for the enteric vibriosis is the cell necrosis, haemocytic nodule formation, haemorrhage and atrophy. In contrast, the observation of treatments D (1,000 ppm), E (1,125 ppm) and F (1,250 ppm) showed a normal haepato-pancreas, with the lumen containing a granular material and the lumen-facing surface on the tubule being covered with a microvillus border. Besides the tubular apex differentiated embryonic cells (E cells) presence, R cells, B cells and F cells were also observed and F cells nuclei were larger than those of R cells. *C. odorata* extract at the concentrations of 1,000, 1,125 and 1,250 ppm was able to stimulate the post-larval *P. monodon* immune system of against the bacterial infection. The antibacterial features of the bioactive compounds of *C. odorata* extract were presumably due to their flavonoid compounds (derived from the phenolic compounds). According to Kumar et al (2013), some chemical compounds have an antimicrobial nature, such as the phenols. Phenolic compounds have the capability to damage and penetrate into bacterial cell wall and then to precipitate the microbial-cell protein, toxic to the protoplasm (Ravikumar et al 2010). As reported by Guil-Guerrero et al (2016), phenols have pharmacological effects against the gram-negative bacteria, including *V. harveyi.* Moreover, the tannin-derived compounds can cure the wounds. According to Iketani et al (2011), Nadella et al (2018) and Soto-Rodriguez et al (2012), the histo-pathological observation of the post-larval *P. monodon* suffering of vibriosis indicates that the systemic vibriosis is typically forming hemostatic nodules in lymphoid, liver and hepatopancreas organs. Vibriosis-infected hepatopancreas suffers a vacuolation indicating a low level lipid and atrophy of multifocal hepatopancreas due to the necrosis and inflammation (Mohajeri et al 2011). Furthermore, the vibriosis in the body of *P. monodon* is associated with the formation of "spheroids" growing in the lymphoid organ (Wu et al 2016).

According to the histological observation (Figure 4a and 4b), hemorrhage (HE), hyperplasia (H), B cell loss due an increased vacuola count (V) and cell necrosis (N) occurred in post-larvae. The condition occurred when the *V. harveyi* challenge test was carried out and the bioactive compound of *C. odorata* extract was not applied. Kannapiran et al (2009) suggested that *V. harveyi* infection of the hepatopancreas organ was marked by tissue swelling and cell damage (necrosis). The hepatopancreas organ color became darken at the center, where bacterial colony grows. At the *C. odorata* extract concentration of 625 ppm, the number of vacuola increased within the cell (V). In addition, at *C. odorata* extract concentrations of 750 and 825 ppm the hyperplasia (H) occurred in the infected hepatopancreas of the post-larvae challenged with *V. harveyi*. Necrosis was observed at the *C. odorata* extract concentrations of 625, 750 and 825 ppm, at which abnormal tissue and cell change, such as hyperplasia, vacuolation and cell necrosis, also occurred in the post-larvae hepatopancreas, indicating that those concentrations cannot cure the infection with *V. harveyi*. The damage of the nucleus cell, caused by an acute bacterial attack, leads to a sudden death. But *C. odorata* extract at concentrations of 1,000, 1,125, and 1,250 ppm maintained the post-larval tiger prawn in good health till the end of the study, as indicated by histological observations of hepatopancreas (i.e. cells B, E, R and F). Table 5 shows that all water quality parameters during the research were appropriate for the post-larval *P. monodon* survival. Accordingly, the mortality found in the study is not caused by the water quality parameter. Water salinity ranged from 28.59 to 28.72‰, being suitable for *P. monodon* aquaculture, based on the recommended degree of salinity, i.e. 5–35 ppt, while for an optimum growth of the prawn, the salinity should be 15-25 ppt (Rosmiati & Suryati 2014). Water temperature, as a limiting factor, has a decisive role in the *P. monodon* survival and growth, and for all treatments in the study it averaged 26.71±0.19°C. According to Soundarapandian & Gunalan (2008), the most suitable water temperature for the *P. monodon* growth and living ranges between 28 and 30°C, but the *P. monodon* can still be alive at temperatures between 18 and 36°C. Temperatures above 32°C may induce stress, while 35°C is critical a temperature for *P. monodon* and *P. merguensis.*

The optimal temperature for the *P. monodon* growth is 29-30°C (Kasnir et al 2014). Dissolved oxygen in all treatments ranged from 5.54 ppm to 7.50 ppm. The pH ranged from 7.02 to 7.86, the water temperature was between 27.49 and 29.05°C, DO was 5.54–7.50 and ammonia was 0.06–0.71, all being suitable for prawn's survival. Compared to the rearing optimal range for post-larvae tiger prawn, the water quality parameters set in the study were close to the optimum values for the post-larval tiger prawn's survival.

Conclusions. The presented study demonstrated that the active methanol extract of *C. odorata* extract was effective in decreasing the population density of the *V. harveyi* up to 99%, either in the rearing medium or in the post-larval *P. monodon* body. The *C. odorata* extract decreased the mortality rate up to 14.55%, besides normalizing the tissue of *P. monodon* and the post-larval hepatopancreas cells infected with *V. harveyi*. The use of this natural compound can increase the in vitro survival rate of the post-larval *P. monodon*. It can be concluded that the *C. odorata* extract can be the source of active compounds for developing a natural antibacterial agent against vibriosis.

Conflict of interest. The authors declare no conflict of interest.

References

Goulden E. F., Hall M. R., Bourne D. G., Pereg L. L., Hoj L., 2012 Pathogenicity and infection cycle of *Vibrio owensii* in larviculture of the ornate spiny lobster (*Panulirus ornatus*). Applied and Environmental Microbiology 2841-2849.

Guil-Guerrero J. L., Ramos L., Moreno C., Zúñiga-Paredes J. C., Ruales P., Carlosama-Yepez M., Ruales P., 2016 Antimicrobial activity of plant-food by-products: A review focusing on the tropics. Livestock Science 189:32–49.

Harlina H., 2016 Isolation and identification of bioactive compound of kopasanda (*Chromolaena odorata* L.) leaf to fight *Vibrio harveyi* on post-larval tiger prawn (*Penaeus monodon* Fabricius). International Journal of Tropical Medicine 11(4):72–79.

Harlina H., Prajitno A., Suprayitno E., Nursyam H., Rosmiati, 2015 Potential study of kopasanda (*Chromolaena odorata* L.) leaves as antibacterial against *Vibrio harveyi*, disease causative agent of tiger shrimp (*Penaeus monodon* Fabricius) post larvae. Aquaculture Research & Development Research 6(10):1-5.

Harlina H., Prajitno A., Suprayitno E., Nursyam H., 2013 The identification of chemical compound and antibacterial activity test of kopasanda (*Chromolaena odorata* L.) leaf extract against vibriosis -causing *Vibrio harveyi* (MR 275 Rif) on tiger shrimp. Aquatic Science and Technology 1(2):15–29.

Hua, Mui L., Apun K., 2013 Antimicrobial susceptibilities of *Vibrio parahaemolyticus* isolates from tiger shrimps (*Penaeus monodon*) aquaculture in Kuching, Sarawak. Research Journal of Microbiology 8(1):55–62.

Iketani, Gabriel, Pimentel L., Silva-Oliveira G., Maciel C., Valenti W., Schneider H., Sampaio I., 2011 The history of the introduction of the giant river prawn, *Macrobrachium* cf. *rosenbergii* (Decapoda, Palaemonidae), in Brazil: New insights from molecular data. Genetics and Molecular Biology 34(1):142-151.

Kannapiran E., Ravindran J., Chandrasekar R., Kalaiarasi A., 2009 Studies on luminous, *Vibrio harveyi* associated with shrimp culture system rearing Penaeus monodon. Journal of Environmental Biology 30(5):791–95.

Karami-Mohajeri, Somayyeh, Abdollahi M., 2011 Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: A systematic review. Human and Experimental Toxicology 30(9):1119–40.

Kasnir M., 2014 Water quality parameter analysis for the feasibility of shrimp culture in Takalar Regency, Indonesia. Journal of Aquaculture Research & Development 5(6):5-7.

Kumar, Shashank, Abhay K., Pandey, 2013 Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal, pp. 1-18.
Lichtlen P., Mohajeri M. H., 2008 Antibody-based approaches in Alzheimer's research: safety, pharmacokinetics, metabolism, and analytical tools. Journal of Neurochemistry 104(4):859-74.
Maddox C. E., Laur Lisa M., Tian L., 2010 Antibacterial activity of phenolic compounds against the phytopathogen xylella fastidiosa. Current Microbiology 60(1):53–58.
Manilal A., Sujith S., Selvin J., Shakir C., Gandhimathi R., Kiran G. S., 2010 Virulence of vibrios isolated from diseased black tiger shrimp, *Penaeus monodon*, Fabricius. Journal of the World Aquaculture Society 41(3):332–43.
Mccann T., John A., Ozolek, Carlos A., Castro, Michael T., McCann J. A., Ozolek C. A., Castro, Parvin B., Kovačević J., 2015 Automated histology analysis IEEE Signal Processing Magazine, pp. 78–87.
Mohajeri, Afsharnasab J. M., Jalali B., Kakoo S., Sharifrohani M., Haghighi A., 2011 Immunological and histopathological changes in *Penaeus semisulcatus* challenged with *Vibrio harveyi*. Iranian Journal of Fisheries Sciences 10(2):254-265.
Nadella, Kumar R., Prakash R. R., Dash, Ramanathan S., Velayudhan L., Kuttanappilly, Mothadaka M. P., 2018 Histopathological changes in giant freshwater prawn *Macrobrachium rosenbergii* (de Man 1879) fed with probiotic Bacillus licheniformis upon challenge with *Vibrio alginolyticus*. Aquaculture Research 49(1):81-92.
Soundarapandian P., Gunalan B., 2008 Recent technology for the survival and production of giant tiger shrimp *Penaeus monodon* along South East Coast of India. International Journal of Zoological Research 4(1):21-27.
Rengpipat, Sirirat, Rukpratanporn S., Somkiat P., Menasaveta P., 2000 Immunity enhancement in black tiger shrimp (*Penaeus monodon*) by a probiont bacterium (Bacillus S11). Aquaculture 191:271-288.
Rosmiati, Suryati E., 2014 Toxicity of *Aaptos suberitoides* extract on tiger shrimp Penaeous monodon post larvae. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 9(2):75-84.
Saeed M. O., 1995 Association of *Vibrio harveyi* with mortalities in cultured marine fish in Kuwait. Aquaculture 136(1995):21–29.
Soto-Rodriguez, Sonia A., Gomez-Gil, Lozano R., del Rio-Rodríguez R., Diéguez A. L., Jesús L., Romalde, 2012 Virulence of *Vibrio harveyi* responsible for the 'Bright-Red' syndrome in the pacific white shrimp *Litopenaeus vannamei*. Journal of Invertebrate Pathology 109(2012):307-317.
Sung H. H., Song Y. L., Kou G. H., 1991 Potential uses of bacterin to prevent shrimp vibriosis. Fish and Shellfish Immunology 1:311-312.
Vital, Pierangeli G., Windell L., Rivera, 2009 Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L. f.) King and Robinson and *Uncaria perrottetii* (A. Rich) Merr. extracts. Journal of Medicinal Plants Research 3(7):511–518.
Wu, Yanping, Bai J., Zhong K., Huang Y., Qi H., Jiang Y., Gao H., 2016 Antibacterial activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of Cedrus deodara, against Staphylococcus aureus. Molecules 21:1-12.
Xie, Yixi, Yang W., Tang F., Chen X., Ren L., 2014 Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current Medicinal Chemistry 22(1):132–149.
Zhang J. Z., 2008 An approach to analyzing histology segmentations using shape distributions. MSc Thesis, Drexel University, US, 81 p.

Received: 21 January 2021. Accepted: 22 April 2021. Published online: 04 May 2021.
Authors:
Harlina Harlina, Muslim University of Indonesia, Faculty of Fishery and Marine Science, Department of Aquaculture, Jl. Urip Sumoharjo Km. 5, 90231 Makassar, Indonesia, e-mail: harlina.harlina@umi.ac.id
Muhammad Kasnir, Muslim University of Indonesia, Faculty of Fishery and Marine Science, Department of Aquaculture, Jl. Urip Sumoharjo Km. 5, 90231 Makassar, Indonesia, e-mail: muhammadkasnir66@gmail.com
Rosmiati Rosmiati, Research and Development Institute for Coastal Aquaculture, Jl. Makmur Daeng Sitakka 129, 90512 Maros, Indonesia, e-mail: emirosmiati@yahoo.com
Andi Hamdillah, Muslim University of Indonesia, Faculty of Fishery and Marine Science, Department of Aquaculture, Jl. Urip Sumoharjo Km. 5, 90231 Makassar, Indonesia, e-mail: andi.hamdillah@umi.ac.id
Muhammah Ikhsan Wamnebo, Muslim University of Indonesia, Faculty of Fishery and Marine Science, Department of Aquaculture, Jl. Urip Sumoharjo Km. 5, 90231 Makassar, Indonesia, e-mail: ikhsanwamnebo25@gmail.com

How to cite this article:
Harlina H., Kasnir M., Rosmiati R., Hamdillah A., Wamnebo M. I., 2021 The effectiveness of *Chromolaena odorata* extract and histopathological change in tiger prawn (*Penaeus monodon*) challenged with *Vibrio harveyi*. AACL Bioflux 14(3):1138-1149.