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Effect of *Solanum procumbens* Lour. Extract on Survival, Growth Performances, Immune Responses and against *Vibrio parahaemolyticus* causing Acute Hepatopancreas Necrosis Disease in White Leg Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Background: Alternative materials for antibiotics used in white leg shrimp farming were extensively investigated. The promising effects of plant based extracts in controlling aquaculture diseases has been reported elsewhere. This study was conducted to examine the effect of *Solanum procumbens* Lour. extract on survival, growth performance, innate immunity and protection against acute hepatopancreas necrosis disease caused by *Vibrio parahaemolyticus* (Vp_{AHPND}) in white leg shrimp.

Methods: From March 2021 to September 2021, four difference diets were examined including the supplementation of *S. procumbens* L. extract at 0.0%, 0.5%, 1.0% and 1.5%. After 30 days of culture, the experimental shrimp was examined for survival rate, growth responses, innate immune parameters and mortality as challenged with Vp_{AHPND}.

Result: The results indicated shrimp fed diets with and without *S. procumbens* L. extract supplementation showed no significant differences in survival and growth performances. However, the innate immune parameters were likely increased in shrimp fed diets at 1.0% and 1.5% extract supplementation and shrimp fed diets at 1.0% and 1.5% extract supplementation reduced the accumulate mortality of Vp_{AHPND} challenged shrimp by 47.6%. *S. procumbens* L. could be a potential material for enhancing innate immunity of white leg shrimp and can protect shrimp from AHPND infection.

Key words: AHPND, Immune, Shrimp, *Solanum*, *Vibrio*.

INTRODUCTION

Shrimp farming plays the major role within aquaculture worldwide and provides an important source of income in coastal areas, which contributes to poverty reduction (Arthur *et al*., 2002). White leg shrimp (*Litopenaeus vannamei*) is one of the most important farmed shrimp species, accounting for 82.7% of global farmed shrimp production. The production is normally dominated by China, Thailand, Indonesia, Vietnam, Ecuador and India (Boy *et al*., 2021). Over the last few decades, shrimp diseases related to a number of opportunistic Vibrio species have caused threats to shrimp farming (Valente and Wan, 2021). Recently, acute hepatopancreas necrosis disease (AHPND) in shrimp caused by *V. parahaemolyticus* harboring *pirAB* genes (Vp_{AHPND}) has been considered as one of the most serious diseases in shrimp farming, particularly in white leg shrimp and black tiger shrimp culture (FAO, 2013; Loc *et al*., 2013). Antibiotics have been applied widely in aquaculture to control diseases in aquatic animals, yet the use of antibiotics might cause negative effects such as antibiotic resistance of bacteria and residual of antibiotics in aquaculture products (Lulijwa *et al*., 2019). Several studies demonstrated medicinal plants could be used as an alternative solution to antibiotics because they contain activate molecules such as alkaloids, terpenoids, saponins and flavonoids which can provide antibacterial, antifungal, antiviral and antiparasitic effects, growth enhancement and immunostimulation (Pu *et al*., 2017; Jha *et al*., 2016; Babikian *et al*., 2019).

Solanum procumbens Lour. distributes widely in Vietnam and tropical countries (MNHN and Chagnoux, 2022). It is known as a herbal plant used to treat some human diseases such as hepatitis, cirrhosis and high levels of liver enzymes (Hien *et al*., 2018). As reported by Hai *et al*. (2018), the extract of *S. procumbens* L. collected in

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the Mekong Delta of Vietnam contained a number of bioactive compounds including ziganein, benzoic acid, salicylic acid, hydroxybenzaldehyde, vanillic acid and indole-3-carbaldehyde. Hien *et al*. (2018) detected steroidal saponins identified as the bioactive compounds providing cardiovascular disease prevention, anti-inflammation, anti-microbial activity, as well as anti-cancer properties in *S. procumbens* L. collected in the North of Vietnam. However, up to now, no information on the effect of *S. procumbens* L. in aquaculture has been reported. Therefore, this study will examine the effect of *S. procumbens* L. extract on survival, growth and innate immunity of white leg shrimp and Vp_{AHPND} prevention in shrimp culture.

MATERIALS AND METHODS

The experiment was conducted from March 2021 to September 2021 at Tra Vinh University, Tra Vinh province, Vietnam. Dried *S. procumbens* L. was bought from a local traditional medicine store. The extraction of *S. procumbens* L. was applied using the protocol described by Chaweepack *et al*. (2015). Briefly, the dried *S. procumbens* L. was ground into fine powder. After that, the powder was added to a 70% alcohol solution at a ratio of 1:10 and left for 24 h before being filtered with paper. The collected liquid was evaporated at 50°C using a vacuum evaporation machine. The remaining crude extract was freeze-dried and stored at 4°C for further experiments. Shrimp at postlarvea 12 (specific pathogen free-SPF) was reared to reach required size for the experiment. Shrimp at 5.33±0.13 g was selected to place in 0.5 m^3 tanks containing 15ppt aerated seawater. The density of shrimp was 50 individuals per tank. The shrimp was fed commercial pellets supplemented with four different ratios of *S. procumbens* L. extract at 0.0%, 0.5%, 1.0% and 1.5% four times per day. Each treatment was three time replication. Before feeding, *S. procumbens* L. extract was weighted and dissolved in sterilized water before being sprayed on the pellets. After that, the pellets were coated with squid oil at 5ml per kg of feed. Water parameters such as salinity, pH, oxygen and temperature in each tank were measured daily; mean while, ammonia and hydrosulfic were measured every 3 days. Salinity was measured by master refractometer (Atago, Japan), pH and temperature by pH/Cond/Temp Pocket Meter (EZDO 7200, Taiwan), dissolved oxygen by oxygen meter (SevenGo pro; Mettler Toledo, USA), ammonia and hydrogen sulfide by sera test kit (Germany). During the experiment, environmental parameters were maintained at suitable levels for shrimp growth: dissolved oxygen was above 4 ppm, pH between 7.5-8, temperature between 28-29°C, salinity at 15 ppt and ammonia and hydrogen sulfide were undetectable. Siphoning and water exchange was carried out at 10% every 3 days. At the end of experiment, survival rate (SR), growth responses and immune parameters of experimental shrimp were examined. SR and growth responses including weight gain (WG), absolute growth rate (AGR) and specific growth rate (SGR) of experimental shrimp was calculated following formulas described by Chandran *et al.* (2016). In immune response examination, hemolymph collection for total haemocyte count (THC) and phenoloxidase (PO) activity measurement followed protocols described by Hernández López *et al*. (1996). THC was determined following the protocol described by Kakoolaki *et al*. (2011). The PO activity was determined in duplicate following the protocol described by Hernández López *et al*. (1996) and Widanarni *et al*. (2020).

To observe the effects of the treatment against Vp_{AHPND}, *pirAB* gene positive *V. parahaemolyticus* was previously isolated from AHPND shrimp stored at -80°C at the Laboratory of Aquaculture Disease Management, Tra Vinh University and it was recovered on trypsin soybean agar (TSA) (Himedia, India). The *pirAB* genes of *V. parahaemolyticus* isolate were rechecked by duplex PCR method using the protocol described by Han *et al*. (2015). Shrimp fed diets supplemented 0.0%, 0.5%, 1.0% and 1.5% after 30 days was subjected to challenge tests with three time replications. The challenge test followed the protocol described by Loc *et al*. (2013) and Hong To *et al*. (2020). Ten shrimps were placed in each plastic container containing 20L of 15 ppt seawater. *V. parahaemolyticus* harboring *pirAB* genes recovered on TSA was diluted in sterilized saline water and added into experiment containers. The density of AHPND *V. parahaemolyticus* in plastic containers was adjusted at 10^6 CFU/ml. The experiment was observed over two weeks for the cumulative mortality and clinical signs of infected shrimp. Moribund shrimp was picked-up for re-isolation of Vp_{AHPND}. The collected data was analyzed for normal distribution and variance homogeneity. One-way ANOVA was applied and Turkey HSD was employed for comparison. Significant differences were considered at P<0.05. SPSS software (version 20. IBM,USA) was used for statistical analysis.

RESULTS AND DISCUSSION

Survival, growth performance and immune responses

Fig 1 indicates that shrimp fed control diet and *S. procumbens* L. extract supplementation showed no significant difference (P>0.05) in survival rates (76%-78%) of shrimp among treatments after the period of 30 days. There were also no significant differences (P>0.05) in growth responses between shrimp fed with and without extract supplementation (Table 1). Regarding immune responses, although THC and PO activity of animals fed 1.0% was higher than that of animals fed 0.0% and 0.5% extract, they were no significant differences (P>0.05). Notably, THC (Fig 2) and PO activity (Fig 3) of animals fed 1.5% extract were significantly higher than those recorded in other treatments (P<0.05).

Many medicine plants could be accepted as antibiotic alternative solution in aquaculture because they contain activate molecules that could benefit for growth and immune system of animals (Pu *et al*., 2017; Kaur *et al*. 2022; Bharathi *et al*., 2021; Jha *et al*., 2022). Previous studies demonstrated the effectiveness of herbal extract or herbal products on survival and immune system of shrimp (Chandran *et al*., 2016). In this study, shrimp fed *S. procumbens* L. extract displayed an improvement in immune parameters such as THC and PO value. Similarly, Chandran *et al*. (2016) reported that *Penaeus monodon* postlarvae fed polyherbal formulation (aqualmmu) showed better performances of immune parameters such as THC, superoxide anion activity, PO activity, lysozyme activity, plasma protein content and bactericidal activity in comparison to shrimp without herbal supplementation. Recent study by Xie *et al*. (2021) also indicated that white leg shrimp fed diets supplemented Tian-Dong-Tang-Gan powder increased the activities of PO, acid phosphatase, superoxide dismutase and alkaline phosphatase in the shrimp hemolymph during the 28-day feeding trials. As reported by AftabUddin *et al*. (2021), the administration of *Padina tetrastromatica* extract improved

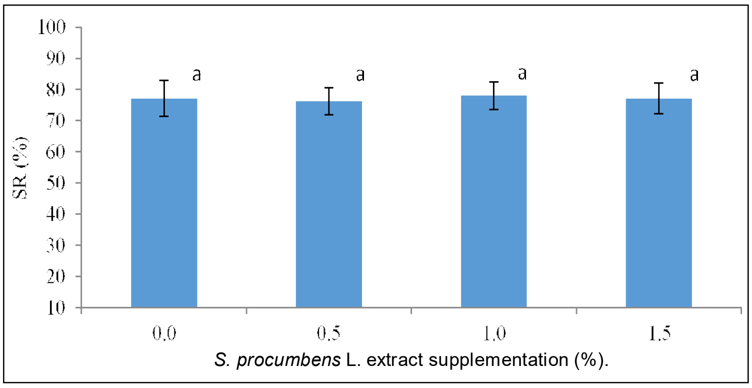

Fig 1: SR of shrimp fed four different diets.
Data are presented as mean±SD. Different lowercase indicate statistically significant differences (P<0.05).

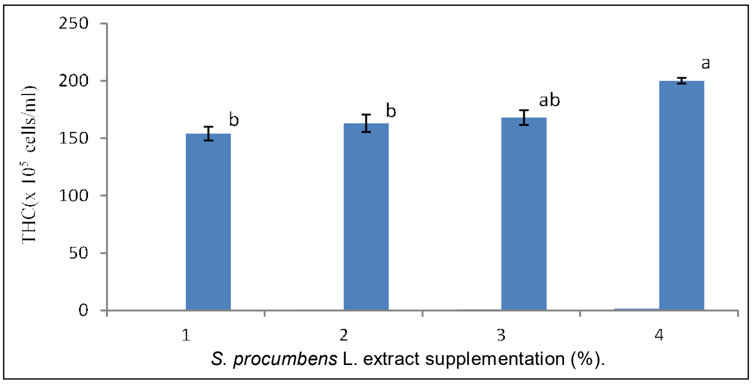

Fig 2: THC of white leg shrimp in 4 treatments after 30 day period.
Data are presented as mean±SD. Different lowercase letters in the same row indicate statistically significant differences (P<0.05).

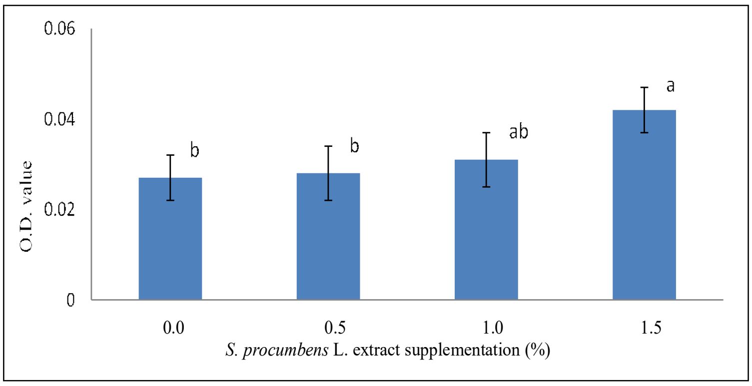

Fig 3: PO activity of white leg shrimp fed 4 different diets.
Data are presented as mean±SD. Different lowercase indicate statistically significant differences (*P*<0.05).

Table 1: Growth performances of white leg shrimp fed four different diets after 30 day period.

Parameters	*S. procumbens* L. extract supplementation (%)			
	0.0	0.5	1.0	1.5
Initial weight (g)	5.33±0.13	5.33±0.13	5.33±0.13	5.33±0.13
Final weight (g)	15.53±1.12[a]	15.49±2.10[a]	15.64±0.37[a]	15.75±1.03[a]
WG (g)	191.4[a]	190.8[a]	197.2[a]	195.5[a]
AGR (g/body weight/day)	0.36[a]	0.36[a]	0.37[a]	0.37[a]
SGR (%/day)	3.82[a]	3.81[a]	3.84[a]	3.87[a]

Data are presented as mean±SD. Different lowercase letters in the same row indicate statistically significant differences (P<0.05).

Table 2: Accumulate mortality and clinical symptoms of Vp_{AHPND} challenged shrimp.

S. procumbens extract suplementation (%)	Challenge	Accumulate mortality (%)	Clinical signs of infected shrimp
0	-	0[c]	
0	+	100[a]	Pale and atropied hepatopancreas, empty stomach and migut
0.5	+	90.0[a]	Pale hepatopancreas, empty stomach and migut
1	+	52.4[b]	Pale hepatopancreas, empty stomach and migut
1.5	+	52.4[b]	Pale hepatopancreas, empty stomach and migut

Different lowercase indicate statistically significant differences (P<0.05).

the immune capacity of *P. monodon* such as increasing THC, PO activity and superoxide anion concentration. Other research stated that a combination of enrofloxacin and Chinese herbal product (San-Huang-San) can enhance immune parameters of white leg shrimp including THC and PO activity (Zhai and Li, 2019).

Against Vp_{AHPND}

Table 2 indicates that after 10 days challenged with $Vp\text{-}_{AHPND}$, the accumulate mortality of shrimp fed *S. procumbens* L. extract at 1.0% and 1.5% (52.4%) was significantly lower (P<0.05) than that observed on animals fed diet supplemented *S. procumbens* L. extract at 0.0% and 0.5% (100% and 90%, respectively). The infected shrimp showed the clinical symptom of AHPND such as pale and atrophied hepatopancreas, empty stomach and midgut. *V. parahaemolyticus* harboring *pirAB* genes was also isolated from moribund shrimp in the challenge experiment.

It is known that *V. parahaemolyticus* is the causative agent of AHPND in shrimp and has caused large economic losses for shrimp farming worldwide (Loc *et al*. 2013). In this study, *S. procumbens* L. extract was shown to reduce the mortality of Vp_{AHPND} challenged shrimp. Other research also noted that plant extracts can protect shrimp from AHPND infection. Jha *et al*. (2016) stated that white leg shrimp fed a mixer of natural essential oils extracted from various herb species *Lavandula latifolia, Pinus sylvestris, Jasminum officinale, Citrus limon, Prunus avium, Viola odorata, Gardenia jasminoides, Cocos nucifera, Rosa damascene* and *Eucalyptus globulus* showed no mortality as challenged with Vp_{AHPND}. Babikian *et al*. (2019) also indicated the Pondguard (Registration no. D 16060285-HBC) consisting of natural oils, lavender oil, eucalyptus oil and pine oil can help to improve survival rate by approximately 50% of shrimp from Vp_{AHPND} challenge. According to Gamboa-Barraza *et al*. (2021), white leg shrimp fed macroalgae *Gracilaria vermiculophylla* and *Ulva flexuosa* extract provided a survival rate at 60%-67% which was twice the amount of survival in the positive control at 24 h post-infection with Vp_{AHPND}.

CONCLUSION

In conclusion, although *S. procumbens* L. extract did not enhance survival rate and growth performances of white leg shrimp in this study, the extract can improve innate immune parameters such as increasing THC and PO value and reducing the mortality of shrimp challenged with Vp_{AHPND}. Therefore, *S. procumbens* L. could be a potential material for enhancing innate immunity of white leg shrimp and protecting shrimp from AHPND infection.

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Conflict of interest: None.

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