Aquaculture 465 (2016) 60–64

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Short communication

Protective effect of *Aloe vera* in *Litopenaeus vannamei* challenged with *Vibrio parahaemolyticus* and white spot syndrome virus

José Vladimir Trejo-Flores [a], Antonio Luna-González [a,*], Píndaro Álvarez-Ruíz [a], Ruth Escamilla-Montes [a], Viridiana Peraza-Gómez [b], Genaro Diarte-Plata [a], Héctor M. Esparza-Leal [a], Ángel I. Campa-Córdova [a], Carina Gámez-Jiménez [a], Arturo Rubio-Castro [a]

[a] Departamento de Acuacultura, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Sinaloa–Instituto Politécnico Nacional (IPN), Boulevard Juan de Dios Bátiz Paredes #250, Guasave, Sinaloa 81101, Mexico
[b] Universidad Autónoma de Nayarit, Ciudad de la Cultura Amado Nervo, Tepic, Nayarit 62155, Mexico

ARTICLE INFO

Article history:
Received 7 July 2016
Received in revised form 23 August 2016
Accepted 24 August 2016
Available online 27 August 2016

Keywords:
Litopenaeus vannamei
Aloe vera
WSSV
Vibrio parahaemolyticus

ABSTRACT

Survival and growth were investigated in *Litopenaeus vannamei* fed with powdered whole leaf of *Aloe vera* and co-infected with white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* by ingestion and immersion, respectively. *A. vera* was added to commercial feed. The infection with both pathogens was made at the same time. *A. vera* did not affect shrimp growth. Shrimps fed with the plant (1 g kg feed^{-1}) every two days showed higher survival as compared to the control group not fed without *A. vera*. *A. vera* has the potential to be used prophylactically as antiviral and antibacterial agent in cultured shrimps.

1. Introduction

White shrimp (*Litopenaeus vannamei*) is one of the most cultivated species worldwide (Chávez and Higuera, 2003; Magallón-Barajas et al., 2007). Therefore, shrimp production demands continuous innovation to increase productivity, despite the great impact of viral and bacterial diseases such as white spot disease (WSD) and acute hepatopancreatic necrotizing disease (AHPND) (Leu et al., 2009; Lo et al., 2003; Nunan et al., 2014; Tran et al., 2013).

At present, there is no cure for viral infections in crustaceans (Medina-Beltrán et al., 2012) and the use of antibiotics in bacterial infections is not recommended due to bacterial resistance and their persistence in the aquatic environment and shrimp edible tissues (Ma et al., 2006). Therefore, prevention of disease outbreaks caused by virus and bacteria and enhancement of immunity are of primary concern (Mudagandur and Yuanan, 2009; Huynh et al., 2011). Nowadays, novel strategies such as the use of immunostimulants, probiotics, and medicinal plants to reduce mortalities would be highly desirable (Huynh et al., 2011; Medina-Beltrán et al., 2012; Mudagandur and Yuanan, 2009; Peraza-Gómez et al., 2014). *Aloe vera* (L.) Burm. f. is a perennial plant of the Xanthorrhoeaceae family that has been widely studied as a healing agent in humans (Paez et al., 2000). *Aloe* gel consists mainly of water (>98%) and polysaccharides (pectins, cellulose, hemicellulose, glucomannan, acemannan, and mannose derivatives). Glucomannan and acemannan hasten wound healing, activate macrophages, arouse the immune system and have antibacterial, and antiviral effects (Djeraba and Quere, 2000; Dotta et al., 2014; Ferro et al., 2003; Mahdavi et al., 2013; Mojtaba and Esmail, 2013; Ramamorthy and Tizard, 1998; Tan and Vanitha, 2004; Waihenya et al., 2002). Also, aloe contains anthraquinones, like aloe emodin, which generally work on viruses, preventing virus adsorption and replication (Hu et al., 2003; Reynolds and Dweck, 1999; Vander et al., 1986).

This study evaluates the effect of *A. vera* leaf powder (AVLP), added to diet, on survival and growth in *L. vannamei* challenged with WSSV and *V. parahaemolyticus*.

2. Materials and methods

2.1. Experimental animals

Shrimps were obtained from Proveedora de Larvas, S.A. de C.V. (Sinaloa, Mexico) or from a hatchery of a commercial farm (Acuícola Cuate Machado, Sinaloa, Mexico) and transported to the lab facilities of CIIDIR-IPN, Sinaloa. The outdoor culture area was covered with shadow mesh. Shrimp stock was cultured in 1000-L plastic tanks with 500-L of filtered (30 μm) seawater (30‰) and continuous aeration. Shrimps were fed twice daily at 09:00 and 17:00 h with commercial feed (Purina®, 35% protein) according to weight tables. Uneaten food and waste material were removed daily before feeding. The collected

* Corresponding author.
E-mail address: aluna@ipn.mx (A. Luna-González).

http://dx.doi.org/10.1016/j.aquaculture.2016.08.033
0044-8486/

shrimps were analyzed by PCR to verify the absence of WSSV and IHHNV.

2.2. Shrimp acclimation to culture conditions

For each bioassay, shrimps maintained in an outside culture system were acclimated to culture conditions for 3 days in 6-L glass tanks containing 4 L of filtered (20 μm) sea water (30‰) and under continuous aeration. Shrimps were fed as above. Uneaten food and waste material were removed daily before feeding.

2.3. Preparation of the experimental diet with powdered A. vera

Sun dried *A. vera* leaves were ground in a Waring® blender. The powder obtained was then pulverized in a hammer mill (Thomas Scientific 3383-L60, GE Motors & Industrial Systems, USA) to obtain a fine powder. Besides, commercial feed (CF, Purina®, 35% protein) was pulverized in a coffee grinder to obtain a fine powder.

A homogeneous paste with CF, AVLP, gelatin, and distilled water was prepared. The concentrations of the aloe in feed were based on the works of Medina-Beltrán et al. (2012) and Luna-González et al. (2012). For each kilogram of feed, 40 g of gelatin and 410 mL of distilled water were used. In the control diet, cellulose (Sigma®, St. Louis, MO, USA) was used to replace the highest concentration of aloe used in the other treatments. Subsequently, the paste was pelletized in a meat grinder (Torrey, model M-22 R, Monterrey, Nuevo León, Mexico) and then dried at room temperature with a fan for 24 h. The pellets were stored at −20 °C. Consumption of prepared feed was determined before the first bioassay.

2.4. Preparation of V. parahaemolyticus inoculum

V. parahaemolyticus, the causative agent of AHPND, was isolated and characterized (including bacterial count) by López-León et al. (2016). Bacteria was grown in tryptic soy broth (TSB) supplemented with 2.5% NaCl and incubated at 30 °C for 18 h. Aliquots of 50 mL were centrifuged at 2379*g* (Sigma 2-6E) for 20 min and the cell pellet was resuspended in 1 mL of sterile saline solution (2.5% NaCl). The bacterial solution was adjusted spectrophotometrically to an optical density of 1.0 at 580 nm. Bacterial count at this optical density was that provided by the authors mentioned above.

2.5. Preparation of white spot syndrome virus (WSSV) positive shrimp tissue

Fifty microliters of a WSSV inoculum, obtained from shrimps of Guasave (Sinaloa, Mexico) was injected to juvenile shrimps (5–8 g) in the second abdominal segment with an insulin syringe. After 24 h, the moribund shrimps were euthanized, after that gills and muscle were removed and stored at −80 °C until used for tissue preparation. A gill lamellae was tested to verify WSSV load by single or nested polymerase chain reaction (PCR) with Kimura et al. (1996) primers. To prepare shrimp infected tissue as food, shrimps were thawed and cut into fine pieces with a scalpel.

2.6. Initial prevalence of WSSV

PCR is a powerful tool for the detection of WSSV in shrimps showing no overt signs of disease. Sensitivity of single PCR is about 1000 viral particles whereas sensitivity of nested PCR is 10–50 viral particles (Lo et al., 1996a, 1996b; Takahashi et al., 1996). Twelve shrimps from stock were used for WSSV prevalence determination using primers of Kimura et al. (1996) for one-step and nested PCR, which amplified genome fragments of 982 and 570 bp, respectively. When negative samples were found, they were tested with an internal control that amplified a fragment of 298 bp of shrimp GAPDH gene, using primers of Tang and Lightner (2000). Shrimps from the stock were WSSV negative.

2.7. Initial prevalence V. parahaemolyticus

Shrimps from WSSV prevalence were used for *V. parahaemolyticus* prevalence using primers of Nunan et al. (2014) that amplify a 470 bp fragment. Shrimps from the stock were *V. parahaemolyticus* negative.

2.8. Experimental design

2.8.1. V. parahaemolyticus LC_{50}

Before each *V. parahaemolyticus* (*Vibrio*) challenge, a bioassay was conducted to determine the median lethal concentration (LC_{50}) using animals weighing 80 ± 5 mg. Each treatment had three replicates (30 shrimps, 10 per tank). The bioassay was conducted for 4 days. Every bioassay consisted of five treatments: I) Control without *Vibrio*; II) *Vibrio* (1×10^3 CFU mL^{-1}); III) *Vibrio* (1×10^4 CFU mL^{-1}); IV) *Vibrio* (1×10^5 CFU mL^{-1}); V) *Vibrio* (1×10^6 CFU mL^{-1}). Shrimps were fed (35% protein feed) twice daily at 09:00 and 17:00 h. Bioassays were conducted under the natural photoperiod. Mortality was recorded three times a day. No cleaning of the tanks was made during the challenging period and temperature was maintained between 28 and 30 °C to favor shrimp infection.

Mortality results from the bioassay were used to calculate the LC_{50} by using Probit analysis (Finney, 1971) with StatPlus® 2009 professional 5.8.4.

2.8.2. Bioassay 1 (A. vera concentrations)

The bioassay was conducted for 26 days with shrimps weighing 80 ± 5 mg in glass aquariums with 4 L of seawater. The challenge was done adding bacteria ($LC_{50} = 6.16 \times 10^4$ CFU mL^{-1}) and infected shrimp tissue (WSSV, 500 mg $tank^{-1}$) to each tank at day five. The bioassay consisted of five treatments each one in triplicate (10 shrimps per tank): I) Control group without pathogens; II) control with WSSV + *Vibrio* LC_{50}; III) AVLP (1 g kg $feed^{-1}$) + WSSV + *Vibrio* LC_{50}; IV) AVLP (2 g kg $feed^{-1}$) + WSSV + *Vibrio* LC_{50}; V) AVLP (4 g kg $feed^{-1}$) + WSSV + *Vibrio* LC_{50}. Shrimps were fed (35% protein feed) twice daily at 09:00 and 17:00 h. Bioassay was conducted under the natural photoperiod. Uneaten food and waste material were removed (with exception of days 5–8 of the challenge) by gravity siphoning every 3 days before feeding, and 50% of the water was exchanged. During the bioassay, mortality was recorded daily. Shrimps from the stock were WSSV-free.

At the end of the bioassay, survival and weight were determined. Temperature ranged from 28.6 ± 2.3 to 29.0 ± 2.7 °C; salinity was between 30.3 ± 1.2 and 30.6 ± 1‰; dissolved oxygen ranged from 5.02 ± 0.5 to 5.3 ± 0.7 mg mL^{-1}; and pH was between 8.0 ± 0.11 and 8.2 ± 0.13.

The specific growth rate (SGR) was determined using the following equation:

$$\mathrm{SGR}\left(\%\mathrm{day}^{-1}\right) = 100 \times [(\mathrm{Ln\ Wf} - \mathrm{Ln\ Wi})]/t$$

where Wf = mean weight at the end of the period, Wi = mean weight at the beginning of the period, and t = time in days of the period (Ricker, 1979).

2.8.3. Bioassay 2 (feed frequency with A. vera)

The bioassay was conducted for 11 days with shrimps weighing 104.75 ± 2.5 mg in glass tanks with 4 L of seawater. Bacteria ($LC_{50} = 7 \times 10^4$ CFU mL^{-1}) and shrimp tissues with WSSV (500 mg $tank^{-1}$) were added to each aquarium at day 5. The bioassay consisted of five treatments each one in triplicate (10 shrimps per tank): I) Control group with WSSV from the stock; II) control group with

WSSV + *Vibrio* LC_{50}; III) AVLP (1 g kg feed^{-1}) daily + WSSV + *Vibrio* LC_{50}; IV) AVLP (1 g kg feed^{-1}) every 2 days + WSSV + *Vibrio* LC_{50}; V) AVLP (1 g kg feed^{-1}) every 3 days + WSSV + *Vibrio* LC_{50}. Shrimps were fed (35% protein feed) twice daily at 09:00 and 17:00 h. Bioassay was conducted under the natural photoperiod. Uneaten food and waste material were removed (with exception of days 5–8 of the challenge) by gravity siphoning every 3 days before feeding, and 50% of the water was exchanged. During the bioassay, mortality was recorded daily. Shrimps from the stock were WSSV-positive (100%). Temperature ranged from 27.2 ± 1.8 to 28.7 ± 2.5 °C; salinity was between 31.4 ± 1.2 and 30.7 ± 1.2‰; dissolved oxygen ranged from 4.9 ± 0.5 to 5.5 ± 0.4 mg mL^{-1}; and pH was between 8.0 ± 0.2 and 8.2 ± 0.1.

2.8.4. Bioassay 3 (validation of bioassays 1 and 2)

The bioassay was conducted for 7 days with shrimps weighing 110 ± 5 mg in glass aquariums with 4 L of seawater. Bacteria (LC_{50} = 6.5 × 10^4 CFU mL^{-1}) and infected shrimp tissue WSSV (500 mg) were added to each tank at day 4. The bioassay consisted of three treatments each one in triplicate (10 shrimps per tank): I) Control without pathogens; II) control with WSSV + *Vibrio* LC_{50}; III) AVLP (1 g kg feed^{-1}) every 2 days + WSSV + *Vibrio* LC_{50}. Shrimps were fed (35% protein feed) twice daily at 09:00 and 17:00 h. Bioassay was conducted under the natural photoperiod. Uneaten food and waste material were removed by gravity siphoning daily before the challenge, and 50% of the water was exchanged. During the bioassay, mortality was recorded daily. Shrimps from the stock were WSSV-free. Temperature ranged from 28.4 ± 1.2 to 28.9 ± 2.7 °C; salinity was between 29.8 ± 1.2 and 30.2 ± 1.3‰; dissolved oxygen ranged from 5.2 ± 0.4 to 5.1 ± 0.6 mg mL^{-1}; and pH was between 8.0 ± 0.1 and 8.2 ± 0.3.

2.9. Statistical analysis

One-way variance analysis (ANOVA) was applied to examine the differences in survival (%) and growth. Survival data were arcsine transformed according to Daniel (1997). Where significant ANOVA differences were found, a Tukey's HSD test was used to identify these differences at $P < 0.05$.

3. Results

3.1. Bioassay 1 (A. vera concentrations)

3.1.1. Survival and SGR

In dying shrimp, lethargy, and empty stomach and midgut were observed. Survival in treatments I and II, without aloe, was 100% and 60%, respectively. Survival in treatments III, IV, and V with 1, 2, and 4 g aloe kg feed^{-1} showed high values (90, 83, and 80%, respectively). No significant differences were found in survival but *A. vera* showed some protective effect against *V. parahaemolyticus* and WSSV (Fig. 1).

A. vera did not affect shrimp growth since no significant differences were observed among treatments (Fig. 1).

3.2. Bioassay 2 (feed frequency with A. vera)

3.2.1. Survival

Clinical signs were similar to those observed in the bioassay 1. No mortality was recorded in treatment I (negative control). Conversely, survival was of 36.7% in treatment II (positive control). Survival in treatments with aloe reached 66.7, 90, and 53.3% in treatments III, IV, and V, respectively. Treatment I was significantly different as compared with treatments II ($P = 0.003$) and V ($P = 0.027$). Treatment IV was significantly different as compared with treatment II ($P = 0.012$) (Fig. 2). Results showed a clear protective effect of *A. vera* against *V. parahaemolyticus* and WSSV in treatment IV, where shrimps were fed with 1 g aloe kg feed^{-1}, every 2 days.

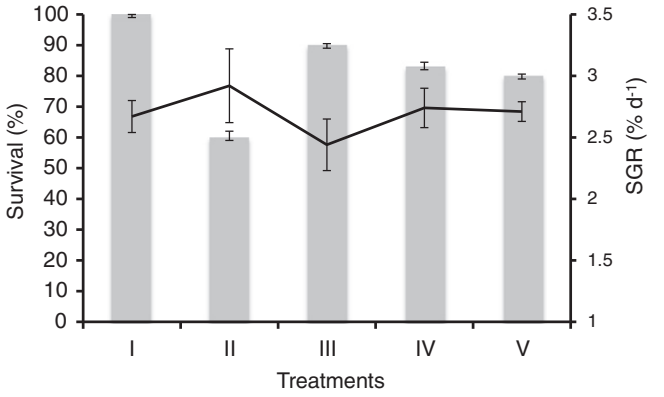

Fig. 1. Survival and SGR in *L. vannamei* fed *A. vera* and challenged with WSSV and *V. parahaemolyticus*. Treatments: I) CF + cellulose (4 g kg feed^{-1}) (negative control); II) CF + cellulose (4 g kg feed^{-1}) + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (6.16 × 10^4 CFU mL^{-1}); III) CF + AVLP (1 g kg feed^{-1}) + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (6.16 × 10^4 CFU mL^{-1}); IV) CF + AVLP (2 g kg feed^{-1}) + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (6.16 × 10^4 CFU mL^{-1}); V) CF + AVLP (4 g kg feed^{-1}) + WSSV (500 mg tank^{-1}) + *V. parahaemolyticus* (6.16 × 10^4 CFU mL^{-1}). CF = commercial feed. SGR = specific growth rate. Data represent mean ± SE.

3.3. Bioassay 3 (validation of bioassays 1 and 2)

3.3.1. Survival

Bioassay 3 validates results of bioassay 1 (*A. vera* concentrations) and bioassay 2 (feed frequency with aloe). Survival in treatment I (negative control) was 100%. On the other hand, survival in treatment II without aloe and challenged against pathogens (positive control) was 50%. Finally, survival in treatment III with shrimps fed aloe was 93.3% (Fig. 3). Survival in treatment II was significantly lower than treatment I ($P = 0.000462$) and III ($P = 0.00118$).

4. Discussion

The negative effects of AHPND and WSD in shrimp culture can be found worldwide. Therefore, novel strategies such as the use of medicinal plants need to be taken to reduce production losses (Huynh et al.,

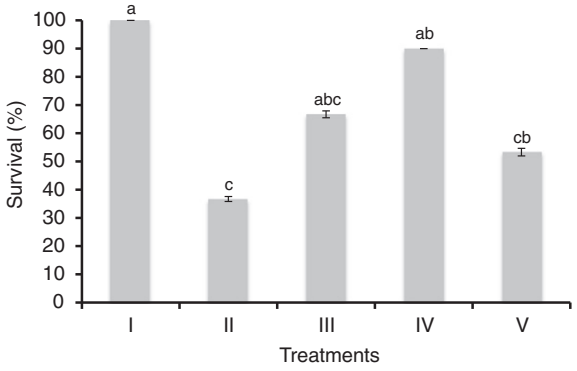

Fig. 2. Survival of in *L. vannamei* fed with *A. vera* and challenged with WSSV and *V. parahaemolyticus*. Treatments: I) CF + cellulose (1 g kg feed^{-1}) (negative control); II) CF + cellulose (1 g kg feed^{-1}) + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (7 × 10^4 CFU mL^{-1}) (positive control); III) CF + AVLP (1 g kg feed^{-1}) daily + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (7 × 10^4 CFU mL^{-1}) daily; IV) CF + AVLP (1 g kg feed^{-1}) every 2 days + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (7 × 10^4 CFU mL^{-1}); V) CF + AVLP (1 g kg feed^{-1}) every 3 days + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (7 × 10^4 CFU mL^{-1}). Data represent mean ± SE. Different letters indicate significant difference ($P < 0.05$).

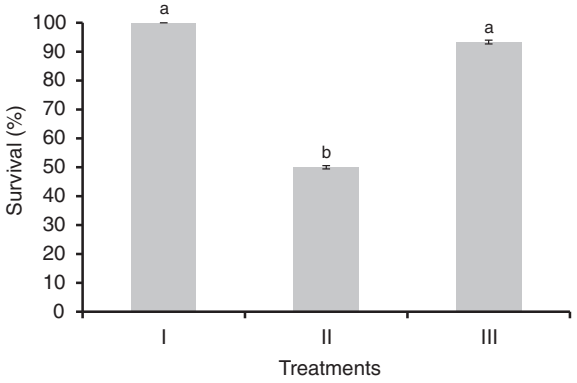

Fig. 3. Survival of *L. vannamei* fed with *A. vera* and challenged with WSSV and *V. parahaemolyticus*. I) CF + cellulose (1 g kg feed^{-1}) (negative control); II) CF + cellulose (1 g kg feed^{-1}) + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (6.5×10^4 CFU mL^{-1}) (positive control); III) CF + AVLP (1 g kg feed^{-1}) every 2 days + WSSV (500 mg of infected tissue tank^{-1}) + *V. parahaemolyticus* (6.5×10^4 CFU mL^{-1}). Data represent mean ± SE. Different letters indicate significant difference ($P < 0.05$).

2011). The herbology medicine in humans is an ancient practice but the use of medicinal plants has become an alternative for the control and prevention of diseases of aquatic organisms only in recent years (Citarasu et al., 2006; Huynh et al., 2011; Medina-Beltrán et al., 2012; Peraza-Gómez et al., 2009, 2011). However, there are no reports on the effect of *A. vera* on survival and growth of *L. vannamei* infected with pathogens such as *V. parahaemolyticus* and WSSV. In this work, *A. vera* powder was used as recommended by Prieto et al. (2005), who mentioned that plants for veterinary medicine should be used in their natural stage to reduce costs.

A. vera leaf contains molecules such as glucomannan and acemannan that in humans hasten wound healing, activate macrophages, arouse the immune system and have antibacterial, and antiviral effects (Djeraba and Quere, 2000; Dotta et al., 2014; Ferro et al., 2003; Mahdavi et al., 2013; Mojtaba and Esmail, 2013; Ramamorthy and Tizard, 1998; Tan and Vanitha, 2004; Waihenya et al., 2002). Also, *A. vera* contains the anthraquinone aloe emodin that prevents virus adsorption and replication (Hu et al., 2003; Reynolds and Dweck, 1999; Vander et al., 1986). In this work, survival of shrimps infected with pathogens and fed with 1 g of *A. vera* per kilogram, every two days, was higher than the survival of shrimps not fed with *A. vera*. Results were consistent with those reported by Medina-Beltrán et al. (2012) and Peraza-Gómez et al. (2014) who observed that medicinal plants *Echinacea purpurea* and *Uncaria tomentosa*, alone or in combination with microbial immunostimulants, have antiviral and immunostimulatory effects that contribute to obtain better survival in cultured white shrimps infected with WSSV. In the work of Rameshthangam and Ramasamy (2006), the survival of *Penaeus monodon* infected with WSSV was significantly higher when shrimps were fed with feed impregnated with the ethanolic leaf extract of *Pongamia pinnata* at 300 mg g^{-1} body weight. Furthermore, it has been found that extracts of marine plants, such as extracts added to feed (200 mg kg^{-1} body weigh per day) from *Sargassum polycystum* (Chotigeat et al., 2004) and *S. wightii* (0.1, 0.2, and 0.3%) (Immanuel et al., 2012), have virucidal effects and reduce the impact of WSSV in *P. monodon*. Regarding co-infections *virus*-bacteria, Huynh et al. (2011) found that *shrimps (L. vannamei) showed increased resistance to infection with Vibrio alginolyticus* and WSSV when they were treated with powder or extract of *S. hemiphyllum* var. Chinense in the water. Therefore, results of this work suggest that *A. vera* protects shrimps from *V. parahaemolyticus* and WSSV due to antimicrobial compounds like acemannan, glucomannan, and aloe emodin (Hu et al., 2003; Reynolds and Dweck, 1999; Vander et al., 1986).

In this kind of works, the possible negative effect of plants on growth should be taken into account because of anti-nutritional factors (alkaloids, tannins) that may affect the utilization of nutrients (Soetan, 2008). *A. vera* had no negative effect on growth, which is very important since Courvalin (2006) mentions that the substances applied to culture systems or added to the diet should not affect the productive performance.

5. Conclusion

Results showed that *A. vera* does not affect shrimp growth and protects it against WSSV and *V. parahaemolyticus*. Therefore, *A. vera* has the potential to be used prophylactically as an antiviral and antibacterial agent in cultured shrimp.

Acknowledgements

Authors are grateful to *Secretaría de Investigación y Posgrado, Instituto Politécnico Nacional (SIP-IPN)* for financial support (20150461). José Vladimir Trejo Flores acknowledges support from the *Consejo Nacional de Ciencia y Tecnología* (CONACyT-Mexico) (337336) and SIP-IPN (664) for grants to pursue graduate studies.

References

Chávez, S.M.C., Higuera, C.I., 2003. Manual de buenas prácticas de producción acuícola de Camarón para la inocuidad alimentaria. SENASICA.CIAD A.C., Mazatlán, Sinaloa, Mexico (87 pp.).
Chotigeat, W., Tongsupa, S., Supamataya, K., Phongdara, A., 2004. Effect of fucoidan on disease resistance of black tiger shrimp. Aquaculture 233, 23–30.
Citarasu, T., Sivaram, V., Immanuel, G., Rout, N., Murugan, V., 2006. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, *Penaeus monodon* with reference to haematological, biochemical, and immunological changes. Fish Shellfish Immunol. 21, 372–384.
Courvalin, P., 2006. Antibiotic resistance: the pros and cons of probiotics. Dig. Liver Dis. 38, S261–S265.
Daniel, W.W., 1997. Bioestadística. Base para el análisis de las ciencias de la salud. Editorial Limusa, México, pp. 639–693.
Djeraba, A., Quere, P., 2000. In vivo macrophage activation in chickens with acemannan, a complex carbohydrate extracted from *Aloe vera* L. Immunopharmacology 22, 365–372.
Dotta, G., Alves de Andrade, J.I., Tavares-Gonçalves, E.L., Brum, A.J.J., Mattos, A.A., Maraschin, M., Laterça-Martins, M., 2014. Leukocyte phagocytosis and lysozyme activity in Nile tilapia fed supplemented diet with natural extracts of propolis and *Aloe barbadensis*. Fish Shellfish Immunol. 39, 280–284.
Ferro, V., Bradbury, B., Cameron, P., Shakir, E., Rahman, S., Stimson, W., 2003. In vitro susceptibility of *Shigella flexneri* and *Streptococcus pyogenes* to inner gel of *Aloe barbadensis* Miller. Antimicrob. Agents Chemother. 3, 1137–1139.
Finney, D.J., 1971. Probit Analysis. 3rd edition. Cambridge University Press, Cambridge, England.
Hu, Q., Xu, J., Hu, Y., 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis* Miller) extracts. J. Agric. Food Chem. 51, 7788–7791.
Huynh, T.G., Yeh, S.T., Lin, Y.C., Shyu, J.F., Chen, L.L., Chen, J.C., 2011. White shrimp *Litopenaeus vannamei* immersed in seawater containing *Sargassum hemiphyllum* var. Chinense powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. Fish Shellfish Immunol. 31, 286–296.
Immanuel, G., Sivagnanavelmurugan, M., Marudhu pandi, T., Radhakrishnan, S., Palavesam, A., 2012. The effect of fucoidan from *Sargassum wightii* on WSSV resistance and immune activity in shrimp *Penaeus monodon* (Fab). Fish Shellfish Immunol. 32, 551–564.
Kimura, T., Yamano, K., Nakano, H., Momoyama, K., Hiraoka, M., Inouye, K., 1996. Detection of penaeid rod-shaped DNAvirus (PRDV) by PCR. Fish Pathol. 31, 93–98.
Leu, J.H., Yang, F., Zhang, X., Xu, X., Kou, G.H., Lo, C.F., 2009. Whispovirus. Curr. Top. Microbiol. Immunol. 328, 197–227.
Lo, C.F., Leu, J.H., Ho, C.H., Chen, C.H., Peng, S.E., Cheng, Y.T., Chou, C.M., Yeh, P.Y., Huang, C.J., Chou, H.Y., Wang, C.H., Kou, G.H., 1996a. Detection of baculovirus associated with white spot syndrome (WSBV) in penaeid shrimps using polymerase chain reaction. Dis. Aquat. Org. 25, 133–141.
Lo, C.F., Ho, C.H., Peng, S.E., Chen, C.H., Hsu, H.C., Chiu, Y.L., Chang, C.F., Liu, K.F., Liu, M., Su, S., Wang, C.H., Kou, G.H., 1996b. White spot syndrome baculovirus (WSBV) detected in cultured and captured shrimp, crabs and other arthropods. Dis. Aquat. Org. 27, 215–225.
Lo, C.F., Chang, Y.S., Peng, S.E., Kou, G.H., 2003. Major viral diseases of *Penaeus monodon* in Taiwan. J. Fish. Soc. Taiwan 30, 1–13.
López-León, P., Luna-González, A., Escamilla-Montes, R., Flores-Miranda, M.C., Fierro-Coronado, J.A., Álvarez-Ruiz, P., Diarte-Plata, G., 2016. Isolation and characterization of infectious *Vibrio parahaemolyticus*, the causative agent of AHPND, from the whiteleg shrimp (*Litopenaeus vannamei*). Lat. Am. J. Aquat. Res. 44 (3), 470–479.

Luna-González, A., Almaraz-Salas, J.C., Fierro-Coronado, J.A., Flores-Miranda, Ma. del C., González-Ocampo, H.A., Peraza-Gómez, V., 2012. The prebiotic inulin increases the phenoloxidase activity and reduces the prevalence of WSSV in whiteleg shrimp (*Litopenaeus vannamei*) cultured under laboratory conditions. Aquaculture 362–363, 28–32.

Ma, D., Hu, Y., Wang, J., Ye, S., Li, A., 2006. Effects of antibacterials use in aquaculture on biogeochemical processes in marine sediment. Sci. Total Environ. 367 (1), 273–277.

Magallón-Barajas, F.J., Villarreal-Colmenares, H., Arcos-Ortega, F., Avilés-Quevedo, S., Civera-Cerecedo, R., Cruz-Hernández, P., González-Becerril, A., Gracia-López, V., Hernández-Llamas, A., Hernández-López, J., Ibarra-Humphries, A.M., Lechuga-Deveze, C., Mazón-Suáztegui, J.M., Muhlia-Melo, A.F., Naranjo-Páramo, J., Pérez-Enríquez, J., Porchas-Cornejo, R., Portillo-Clark, G., Pérez-Urbiola, J.C., 2007. Orientaciones estratégicas para el desarrollo sustentable de la acuicultura en México. Publicaciones especiales del Centro de Investigaciones Biológicas del Noroeste, S. C. Cámara de Diputados (LX Legislatura).

Mahdavi, M., Hajimoradloo, A., Ghorbani, R., 2013. Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). World J. Med. Sci. 9 (1), 55–60.

Medina-Beltrán, V., Luna-González, A., Fierro-Coronado, J.A., Campa-Córdova, A.I., Peraza-Gómez, V., Flores-Miranda, Ma. del C., Gutiérrez-Rivera, J.N., 2012. *Echinacea purpurea* and *Uncaria tomentosa* reduce the prevalence of WSSV in whiteleg shrimp (*Litopenaeus vannamei*) cultured under laboratory conditions. Aquaculture 358, 164–169.

Mojtaba, A., Esmail, A., 2013. Effects of different levels of *Aloe vera* L. extract on growth performance, hemato-immunological indices of *Cyprinus carpio* L. Iranian J. Vet. Sci. Technol. 5 (2), 33–44.

Mudagandur, S.S., Yuanan, L., 2009. Application of nucleic-acid-based therapeutics for viral infections in shrimp aquaculture. Mar. Biotechnol. 11, 1–9.

Nunan, L., Lightner, D., Pantoja, C., Gómez-Jiménez, S., 2014. Detection of acute hepatopancreatic necrosis disease (AHPND) in Mexico. Dis. Aquat. Org. 111, 81–86.

Paez, A., Gebre, G.M., Gonzalez, M.E., Tschaplinski, T.J., 2000. Growth, soluble carbohydrates, and aloin concentration of *Aloe vera* plants exponed to three irradiance levels. Environ. Exp. Bot. 44, 133–139.

Peraza-Gómez, V., Luna-González, A., Campa-Córdova, A.I., López-Meyer, M., Fierro-Coronado, J.A., Álvarez-Ruiz, P., 2009. Probiotic microorganisms and antiviral plants reduce mortality and prevalence of WSSV in shrimps (*Litopenaeus vannamei*) cultured under laboratory conditions. Aquac. Res. 40, 1481–1489.

Peraza-Gómez, V., Luna-González, A., Campa-Córdova, A.I., Fierro-Coronado, J.A., González-Ocampo, H.A., Sainz-Hernández, J.C., 2011. Dietary microorganism and plant effects on the survival and immune response of *Litopenaeus vannamei* challenged with the white spot syndrome virus. Aquac. Res. 42, 559–570.

Peraza-Gómez, V., Luna-González, A., González-Prieto, J.M., Fierro-Coronado, A., González-Ocampo, H.A., 2014. Protective effect of microbial immunostimulants and antiviral plants against WSSV in *Litopenaeus vannamei* cultured under laboratory conditions. Aquaculture 420–421, 160–164.

Prieto, A., Auró de Campo, A., Fernández, A., Pérez, M.B., 2005. El empleo de medicina natural en el control de enfermedades de organismos acuáticos y potencialidades de uso en Cuba y México. Tip Revista Especializada en Ciencias Químico-Biológicas 8 (1), 38–49.

Ramamorthy, L., Tizard, I., 1998. Induction of apoptosis in a macrophage cell line RAW 264.7 by acemannan, a beta-(1,4)-acetylated mannan. Mol. Pharmacol. 53 (3), 415–421.

Rameshthangam, P., Ramasamy, P., 2006. Antioxidant and membrane bound enzymes activity in WSSV-infected *Penaeus monodon* Fabricius. Aquaculture 254, 32–39.

Reynolds, T., Dweck, A.C., 1999. *Aloe vera* leaf gel: a review update. J. Ethnopharmacol. 68, 3–37.

Ricker, W.E., 1979. Growth rates and models. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), Fish Physiology, Volume VIII, Bioenergetics and Growth. Academic Press, New York, USA.

Soetan, K.O., 2008. Pharmacological and other beneficial effects of antinutritional factors in plants - a review. Afr. J. Biotechnol. 7 (25), 4713–4721.

Takahashi, Y., Itami, T., Maeda, M., Suzuki, N., Kasornchandra, J., Supamattaya, K., Khongradit, R., Boonyaratpalin, S., Kondo, M., Kawai, K., Kusuda, R., Hirono, I., Aoki, T., 1996. Polymerase chain reaction (PCR) amplification of bacilliform virus (RV-PJ) DNA in *Penaeus japonicas* Bate and systemic ectodermal and mesodermal baculovirus (SEMBV) DNA in *Penaeus monodon* Fabricius. J. Fish Dis. 19, 399–403.

Tan, B.K., Vanitha, J., 2004. Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr. Med. Chem. 11, 1423–1430.

Tang, K., Lightner, D.V., 2000. Quantification of white spot syndrome virus DNA through a competitive polymerase chain reaction. Aquaculture 189, 11–21.

Tran, L., Nunan, L., Redman, R.M., Mohney, L.L., Pantoja, C.R., Fitzsimmons, K., Lightner, D.V., 2013. Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Org. 105, 45–55.

Vander, D.A., Vlietinick, A.J., Hoof, L.V., 1986. Plant products as potential antiviral agents. Bull. Inst. Pasteur 844, 101–105.

Waihenya, R., Mtambo, M., Nkwenguliga, G., 2002. Evaluation of the efficacy of the crude extract of *Aloe secundiflora* in chickens experimentally infected with Newcastle disease virus. Ethnopharmacol. 79, 299–304.