

Short Communication

Effect of medicinal plants on the survival of white shrimp (*Penaeus vannamei*) challenged with WSSV and *Vibrio parahaemolyticus*

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ABSTRACT. Survival was investigated in *Penaeus vannamei* fed with powdered plants (PP: garlic, echinacea, ginger, and basil) and challenged with white spot syndrome virus (WSSV) and *Vibrio parahaemolyticus* by ingestion and immersion, respectively. PP was added to commercial feed at a concentration of 1, 2 and 4 g kg⁻¹. The infection with both pathogens was made at the same time. Shrimp fed with PP (4 g kg feed⁻¹) at different frequencies showed higher survival (96.7%) as compared to the positive control group not fed with PP (6.7%). WSSV prevalence in live and dead shrimp was similar in all treatments challenged with both pathogens varying from 33.3 to 55%. PP protects shrimp against WSSV and *V. parahaemolyticus*. Therefore, further research about the effect of PP is necessary for commercial shrimp farms.

Keywords: *Allium sativum*; *Echinacea purpurea*; *Ocimum sanctum*; *Zingiber officinale*; WSSV; powdered plants

The negative impact of viral (white spot syndrome virus, WSSV) and bacterial (acute hepatopancreatic necrosis disease, AHPND) diseases on shrimp production demands continuous innovation to increase productivity (Leu *et al.*, 2013; Tran *et al.*, 2013; Nunan *et al.*, 2014). Viral diseases are not curable, and there are only antibiotics to treat bacterial diseases, which cause resistance and environmental impact (Chanu *et al.*, 2012; Medina-Beltrán *et al.*, 2012). This has led to the use of natural products, such as medicinal plants, which are a good alternative in the prevention and treatment of diseases because they are deposits and sources of safe and cheap chemical products and do not generate resistance in pathogens (Chanu *et al.*, 2012).

The plants *Aegle marmelos*, *Cynodon dactylon*, *Lantana camara*, *Momordica charantia*, *Phyllanthus amarus* and fucoidan extracted from *Sargassum wightii* showed antiviral activity against WSSV and immunostimulated *Penaeus monodon* (Balasubramanian *et al.*, 2007; Immanuel *et al.*, 2012). In this sense, *Allium sativum* (garlic) contains dietary fiber, 32% sugars (included inulin), flavonoids, pectin, and the organic sulfur compounds alliin (allicin, ajoenes, vinylthiols, and sulfides), scordinin A and B, and garlicnins (Swiderski *et al.*, 2007; Nohara *et al.*, 2013). *Zingiber officinale* (ginger) contains sesquiterpene and phenolic compounds (gingerol and shogaol) (Hasan *et al.*, 2012). *Echinacea purpurea* (*Echinacea*) is one of the most

important and well-known medicinal plants in the world. It is highly composed of alkaloids, caffeic acid derivatives, and polysaccharides; however, compounds like flavonoids (quercetin, kaempferol, isorhamnetin and their free phenolic acids, including p-coumaric, p-hydroxybenzoic, and protocatechuic acids), amides, and alkaloids have also been isolated (Spelman & Wetschler, 2009; Manayi *et al.*, 2015). *Ocimum sanctum* (purple basil) contains volatile oils (eugenol, carvacrol, ursolic acid, linalool, limatrol, caryophyllene, and sitosterol) (Mondal *et al.*, 2009; Pattanayak *et al.*, 2010), phenolic compounds (apigenin, rosmarinic acid, cirsilineol, cirsimaritin, isotimisin, and isotimonin) (Yanpallewar *et al.*, 2004; Pattanayak *et al.*, 2010), flavonoids (orientin, vicenin, luteolin, and molludistin) (Gupta *et al.*, 2002), tannins, and a series of sesquiterpenes and monoterpenes (bornyl acetate, b-elemene, neral, A-pinene, b-pinene, camfeno, campesterol, and stigmasterol) (Jaggi *et al.*, 2003).

The study aimed to determine the effect of medicinal plants on the survival of *Penaeus vannamei* challenged with WSSV and *Vibrio parahaemolyticus* by ingestion and immersion.

Shrimp were obtained from a commercial farm with the hatchery, transported, and maintained at the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa. A white spot disease analysis was made in 12 shrimp, using PCR (nested PCR, Kimura, *et al.*, 1996), to know the percentage of WSSV-infected shrimp.

Farm shrimp (5-6 g) with symptoms of white spot disease were collected to obtain an inoculum in saline solution (2.5% NaCl) and a paste of macerated abdominal muscle and branchial lamella. A white spot disease analysis was made by PCR (nested and single PCR). An inoculum of *V. parahaemolyticus* was prepared according to López-León *et al.* (2016).

The mixture of powdered plants (PP), *A. sativum* (40%), *Z. officinale* (20%), *E. purpurea* (20%), and *O. sanctum* (20%) was added to commercial feed Camaronina® (Purina, 35% protein), which was reconstituted in a meat mill, dried at room temperature with a fan for 24 h, and stored at 4°C. This proportion of powdered plants was based on previous works and on the cost of the plants since *Echinacea* is very expensive.

Before each *V. parahaemolyticus* challenge (bioassays one and two), a bioassay (4 d) was conducted to determine the LC₅₀ (lethal concentration, 50%) using animals weighing 528 ± 31.7 mg and 452 ± 50 mg. Experimental glass aquariums (6 L) contained 4 L of filtered sea water under constant aeration. Every bioassay consisted of five treatments, each one in

triplicate (30 shrimps, 10 per tank): I) control without *Vibrio*; II) *Vibrio* (1×10³ CFU mL⁻¹); III) *Vibrio* (1×10⁴ CFU mL⁻¹); IV) *Vibrio* (1×10⁵ CFU mL⁻¹); and V) *Vibrio* (5×10⁵ CFU mL⁻¹). Shrimp were fed (35% protein feed) twice daily at 09:00 and 17:00 h. Bioassays were conducted under the natural photoperiod. No cleaning of the tanks was made during the challenge period, and the temperature was maintained between 29.5 and 30.5°C to favor shrimp infection. Salinity was maintained at 30. Mortality was recorded three times daily, and final data were used to calculate the LC₅₀ using Probit analysis (Finney, 1952) with StatPlus® 2009 professional 5.8.4.

The first bioassay was conducted for 10 d with 10 shrimp (528 ± 31.7 mg) per glass aquarium with 4 L of seawater under constant aeration. The challenge was done by adding bacteria (LC₅₀ = 15.9×10⁴ CFU mL⁻¹) and infected shrimp tissue (WSSV, 500 mg, low viral load) to each glass aquarium at day seven once only. The bioassay consisted of five treatments each one in triplicate (10 shrimps per tank): I) control without pathogens; II) control with WSSV + *Vibrio* LC₅₀; III) PP (1 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀; IV) PP (2 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀; and V) PP (4 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀. Shrimp were fed (35% protein feed) twice daily during 10 d (09:00 and 17:00 h) with commercial feed alone or commercial feed with PP. The bioassay was conducted under the natural photoperiod. The temperature was maintained between 29.5 and 30.5°C. Uneaten food and waste material were removed (except days 7-10 of the challenge to avoid eliminating the vibrio) by siphoning every three days before feeding, and 50% of the water was exchanged. During the bioassay, mortality was recorded daily. Shrimp from the stock were WSSV-free.

The second bioassay was conducted for 20 d with 10 shrimp (452 ± 50 mg) per glass aquarium with 4 L of seawater and constant aeration. The challenge was done by adding bacteria (LC₅₀ = 6.5×10⁴ CFU mL⁻¹) and infected shrimp tissue (WSSV, 200 mg, high viral load) to each glass aquarium at day nine once only. The bioassay consisted of five treatments each one in triplicate (10 shrimps per tank): I) control without pathogens; II) control with WSSV + *Vibrio* LC₅₀; III) PP (4 g kg feed⁻¹) daily + WSSV + *Vibrio* LC₅₀; IV) PP (4 g kg feed⁻¹) every 2 days + WSSV + *Vibrio* LC₅₀; and V) PP (4 g kg feed⁻¹) every 3 days + WSSV + *Vibrio* LC₅₀. Shrimp were fed previously mentioned. The bioassay was conducted under the natural photoperiod. The temperature was maintained between 29.5 and 30.5°C. Uneaten food and waste material were removed (except days 9-12 of the challenge to avoid eliminating pathogens) by siphoning every three days before feeding, and 50% of the water was exchanged.

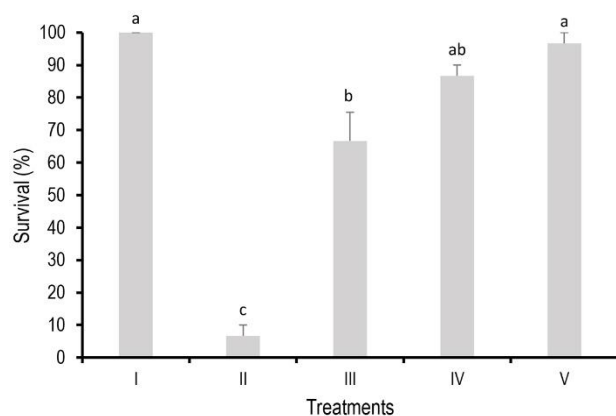


Figure 1. Survival of *Penaeus vannamei* fed at different concentrations of PP. Treatments: I) control without pathogens; II) control with WSSV + *Vibrio* LC₅₀; III) PP (1 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀; IV) PP (2 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀; V) PP (4 g kg feed⁻¹) + WSSV + *Vibrio* LC₅₀. Data are mean ± SE (standard error). Different letters indicate significant differences ($P < 0.05$). PP: powdered plants, WSSV: white spot syndrome virus.

During the bioassay, mortality was recorded daily. Shrimps from the stock were WSSV-free.

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

In shrimp fed PP at different concentrations, the highest survival was observed in treatment IV ($96.67 \pm 3.33\%$) and the lowest in treatment II ($6.67 \pm 3.33\%$). The negative control group and treatments with PP showed significant differences ($P < 0.05$) as compared with treatment II in which the shrimps were only challenged with pathogens (Fig. 1).

High survival (96.7-100%) was observed in shrimp fed PP at different frequencies. The negative control group and treatments with PP showed significant differences ($P < 0.05$) as compared with treatment II in which the shrimps were only challenged with pathogens. Live and dead shrimp positive to WSSV showed low viral load (nested PCR). WSSV prevalence was similar in all treatments challenged with both pathogens (Fig. 2).

Plants are a source of secondary metabolites (Chanu *et al.*, 2012) of interest for aquaculture to prevent and treat diseases and it is known that combinations of medicinal plants can show a better biological effect than an individual medicinal plant since the bioactive compounds of each one can act in synergy (Martínez *et al.*, 2013; Más Toro *et al.*, 2017). For the above, we worked with a combination of garlic, ginger, basil, and

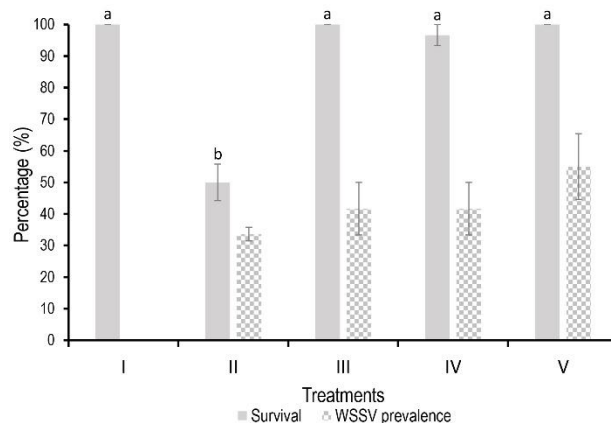


Figure 2. Survival and WSSV prevalence in *Penaeus vannamei* fed PP at different frequencies. Treatments: I) control without pathogens; II) control with WSSV + *Vibrio* LC₅₀; III) PP (4 g kg feed⁻¹) daily + WSSV + *Vibrio* LC₅₀; IV) PP (4 g kg feed⁻¹) every 2 days + WSSV + *Vibrio* LC₅₀; V) PP (4 g kg feed⁻¹) every 3 days + WSSV + *Vibrio* LC₅₀. Data are mean ± SE. Different letters indicate significant differences ($P < 0.05$). PP: powdered plants, WSSV: white spot syndrome virus.

Echinacea to know their effect on the survival of *P. vannamei* challenged with pathogens such as *V. parahaemolyticus* and WSSV. However, it is important to mention that plants, in addition to antimicrobial and immunostimulant molecules, contain molecules such as phytic acid and tannins that can affect cultured organisms (Bairagi *et al.*, 2002; El-Adawy, 2002; Flores-Miranda *et al.*, 2014); thus, the amount and frequency of application in the feed must be determined.

In this work, survival of shrimp challenged with WSSV and *V. parahaemolyticus* and fed with 4 g kg feed⁻¹ of PP at different frequencies was higher (50-90%) than the survival of shrimp not fed with PP. Results were consistent with those reported by Huynh *et al.* (2011) who found that *P. vannamei* showed increased resistance to infection with *V. alginolyticus* and WSSV when they were immersed in seawater containing *Sargassum hemiphyllum* var. *chinense* powder and its extract. In the same way, Trejo-Flores *et al.* (2016) found a protective effect of *Aloe vera* powder (1 g kg feed⁻¹ every two days) in *P. vannamei* challenged with the same pathogens of this work.

Regarding WSSV prevalence in live and dead shrimp, it was similar in all treatments challenged with both pathogens, indicating that in shrimp challenged with the low viral load, mortality occurred mainly due to *V. parahaemolyticus* (Rubio-Castro *et al.*, 2016), even though, Medina-Beltrán *et al.* (2012) and Peraza-Gómez *et al.* (2014) observed that *E. purpurea* and *Uncaria tomentosa* have antiviral (WSSV) effects that

contribute to obtaining better survival in cultured white shrimp infected with WSSV.

Results showed that the tested PP protects shrimp against WSSV and *V. parahaemolyticus*. Therefore, further research about the effect of garlic, ginger, *Echinacea*, and basil is necessary for commercial shrimp farms.

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