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Research Article

Dietary administration of aqueous *Zingiber officinale* extract on growth performance, antioxidant activity and resistance of shrimp *Litopenaeus vannamei* against *Photobacterium damselae*

Shahraki N.[1]; Imanpour M.R.[1*]; Akbary P.[2]; Safari R.[1]; Jafari V.[1]

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Abstract

This study was conducted to evaluate the effect of aqueous *Zingiber officinale* extract (ZE) on growth performance, antioxidant activity and resistance of shrimp (*Litopenaeus vannamei*) challenged with *Photobacterium damselae* bacteria. A total number of 600 post larvae shrimps (initial weight, 1.06±0.7 g) were randomly distributed into 12 fiberglass tanks representing four treatments each tank containing of 50 shrimp. The control shrimp group (ZE0) was fed a ZE free basal diet. Other groups were fed the basal diet supplemented with 0.5 (ZE 0.5), 1.0 (ZE1) and 1.5 (ZE 1.5) g kg^{-1} diet. Diets were offered to shrimp at a feeding rate of 10% of live body weight for 8 weeks. After 8 weeks of experimental trial 30 shrimp from each group was infected with LD70 bacteria *P. damselae* over 10 days to evaluated disease resistance of infected shrimp. Results revealed that growth performance (WG, SGR, FW and PER), the antioxidant activity (SOD, PO, GPx and CAT) of shrimp have significantly increased and cumulative mortality rate decreased (p<0.05) in the ZE1 group compared with the other groups. Meanwhile, the lowest FCR and MDA value were observed in shrimp fed ZE1 supplemented diet. It can be concluded that *Z. officinale* extract at the level of 1g kg^{-1} (ZE1) diet seems to be the most appropriate level for increasing growth performance, antioxidant activity and disease resistance of *L. vannamei*.

Keywords: *Litopenaeus vannamei, Zingiber officinale,* Growth, Antioxidant activity, Shrimp, *Photobacterium damselae*

1-Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural, Science and Natural Resources, Gorgan, Iran

2-Department of Fisheries, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran

*Corresponding author's Email: imanpour_m@yahoo.com

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Introduction

Diseases as one of the major constraint are recognized to sustainable animal production which can cause significant economic loss especially in aquaculture. However, in farmed fish and shrimp, various chemotherapeutic agents have been traditionally used in the treatment and prevention of diseases but using these kind of materials are not recommended due to continuous and improper use of antibiotics, may lead to environmental pollution, accumulation of toxic residues and potential development of antibiotic resistant bacteria in aquatic animals and shrimp (Citarasu *et al*., 2010). Therefore, researchers discover natural products such as medical herbs as dietary supplements which increase feed utilization, growth performance and immune system of shrimp and cultured fish, as these products are safe, inexpensive, effective, and can be easily prepared and are biodegradable (Ali *et al*., 2008; Goda, 2008).

Antioxidant systems can counterbalance negative effects of free radicals. The interaction of free radicals with component of living organisms is clearly negative and should be avoided. Oxidizing of lipids through peroxides formation (LPO) has been called lipids peroxidation (LPO) which is usually used in free radical research field. Aquatic animals possess high quantity of substrates for oxidant such as residues of polyunsaturated fatty acids and lipids. Measuring of the final products of LPO such as malondialdehyde (MDA) is the most attractively used to assay LPO concentration (Lushchak, 2011). To eliminate reactive oxygen species, aquatic animals possess the antioxidant system comprising both low molecular mass and high molecular mass antioxidants (Livingstone, 2001). High molecular mass antioxidant group includes antioxidant enzymes superoxide dismutases (SOD), catalases (CAT), glutathione peroxidases (GPx) (Livingstone, 2001).

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) as a spice and herbal medicine is generally used in food around the world (Weidner and Sigwart, 2000). Ginger contains flavonoids, saponin, alkaloids, polyphenols, tannin, vitamins, steroids, fiber, minerals, carbohydrate and carotenoids (Otunola *et al*., 2010), natural antioxidants as Zingerone, shogaols and gingerols (Hori *et al*., 2003); anti-inflammatory effects as essential oils (Zarate and Yeoman, 1996).

Some researchers in aquaculture have shown an effective role of medicinal plants on growth parameters (Citarasu *et al.*, 1999, 2003; Venkataramalingam *et al*., 2007; Balasubramanian, 2009; EI-Desouky *et al*., 2012; Shubha, 2015), antioxidant activity (Cheng *et al*., 2004, 2012 Scalbert *et al*., 2005, Kim *et al*., 2007; Hsich *et al.*, 2008; Islam *et al*., 2016;Akbary and Aminikhoei, 2018) and disease resistance (Yogeeswara *et al*., 2012; Sharif Rohani *et al*., 2013;

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Jahanjoo *et al*., 2018; Jafarinejad et al., 2020) in aquatic animals and shrimp. Studies on *P. monodon* and *P. indicus* revealed that the *Z. officinalis* Artemia enriched and different herbs such as *Hygrophila spinosa, Withania somnifera, Z. officinalis, Solanum trilobatum, Andrographis paniculata, Psoralea corylifolia, Eclipta erecta, Ocimum sacnctum, Picrorhiza kurooa, Phyllanthus niruri, Tinospora cordifolia*, purified Silajit and cod liver oil added to the feed reduced the feed conversion ratio while increased protein, specific growth rate, weight gain and final weight (Citarasu *et al.*, 1999, 2003; Venkataramalingam *et al*., 2007). Cheng *et al*. (2012) reported that PO activity in *L. vannamei* fed various doses of zingerone (1, 2.5 and 5 mg kg^{-1}) significantly increased compared to control group. However, no studies were carried out on the effect of *Z. officinalis* extract on antioxidant activity and disease resistance of *Litopenaeus vannamei*.

Hence, in the present research, we aimed to assess the effects of different levels of *Z. officinalis* extract on growth performance, antioxidant activity and resistance of *L. vannamei* challenged with *Photobacterium damselae* bacteria.

Materials and methods

Preparation of water extract of Z. officinale and experimental diets.

Z. officinale root was collected and identified in herbarium of faculty of botany from Shiraz province, Iran, at mid November 2015. Then, it was completely washed by the distilled water, and air-dried at 60ºC. *Z. officinale* extract (ZE) was prepared according to Choi *et al*. (2015). Briefly, 30 g of dried *Z. officinale* was grounded, sieved (pore size<0.5 mm) and added to 750 ml of deionized water and boiled for 4h, followed by centrifugation at 18,500×g for 10 min at 10º C. The supernatant was concentrated under reduced pressure at 60ºC. To obtain four experimental diets at inclusion level of 0, 0.5, 1.0 and 1.5 g kg^{-1}ZE extract, the ZE extract was mixed with ingredients of the basal diet (Table 1), then oil and 30% distilled water were added and further mixed. The wet dough was pelletized at a particle size of 1 mm using a handmade modified grinder (National, Japan). The experimental diets were air-dried and kept at 4ºC until use.

Shrimp and experimental design

This study was conducted in Offshore Fisheries Research Center (Chabahar, Iran) in May 2015. A total number of 600 post larvae *L. vannamei* (mean 1.06±0.7 g) were obtained from a private hatchery (Chabahar, Iran). The shrimp were stocked into two 300 L rearing fiberglass tanks for 2 weeks as an adaptation period and fed with a basal diet. One third of the water in each tank was replaced every day. Wastes were removed from tanks by siphoning.

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Table 1: Composition (g kg^{-1} diet) and proximate analysis (% as fed basis) of the basal diet.

Ingredients	**g kg^{-1} diet**
Fish meal	200
Soybean meal	333
Wheat meal	295
Squid meal	38
Shrimp meal	38
Yeast	15.5
Fish oil	40
Lecithin	14
Vitamins and minerals [a]	26.5
Proximate composition	(%)
Protein	36.7
Lipid	9.7
Moisture	8.3
Ash	9.5
Fiber	0.9
Nitrogen free extract	34.9

[a] Vitamins and minerals: Vitamins: Vitamin A, 3000 IU g^{-1}; vitamin D, 2500 IU g^{-1}; vitamin E,50000 mg kg^{-1}. Minerals: 20000 mg kg^{-1} CuSO4, 40000 mg kg^{-1} ZnSO4; 15000 mg kg^{-1} MnSO4 2000 mg kg^{-1} CoSO4 ; 1 mg kg^{-1} Fe, 100 mg kg^{-1} Se

Thereafter, shrimps were randomly divided into four groups (at three replications for each group) at a stocking density of 50 shrimp in each replication. The control shrimp group (ZE0) was fed the basal diet free of supplemented ZE. Other shrimp groups were fed the basal diet supplemented with ZE at levels of 0.5 (ZE 0.5), 1 (ZE1) and 1.5 (ZE 1.5) g kg^{-1} diet. Every two weeks, all shrimps in each tank were weighted and the amount of food was adjusted based on the actual body weight changes. Experimental diet was introduced manually three times a day, at 8:00, 13:00 and 17:00 h. During the experimental trial, average values of the water salinity, pH, dissolved oxygen, temperature and total ammonia nitrogen were maintained at 37 g L^{-1}, 8±0.2, 7.5±0.65 mg L^{-1}, 28.4ºC±0.7 and 0.1±0.03 mg L^{-1}, respectively, which were suitable for shrimp rearing (Akbary and Aminikhoei, 2018).

Growth performance analysis

The growth parameters of shrimp were evaluated in terms of weight gain percentage (WGP), protein efficiency ratio (PER), feed conversion ratio (FCR) and specific growth rate (SGR) according to following equations (Harikrishnan *et al*. 2011):

WG (Weight gain) = [(final body weight - initial body weight)/ initial body weigh] × 100

SGR (Specific growth rate) = [(ln final body weight - ln initial body weight) /days] × 100

FCR (Feed conversion ratio) = Wet weight gain × 100/feed intake

PER (protein efficiency ratio) = WG (g, body weight gain)/protein fed (g)

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Antioxidant activity assay

At the end of the experimental trial, three shrimp from each tank were randomly collected for antioxidant activity assay. Then, six shrimp were frozen immediately in liquid nitrogen and stored at 70º C. For assays, we defrosted the shrimps, homogenized in 10 (w/v) phosphate buffer solutions (NaCl 8 g, KCl 0.2 g, Na2HPO4 1.42 g, KH2PO4) on ice. The homogenates were centrifuged (3000 rpm, 10 min) at 4ºC. The supernatants were kept at -70º C until analysis (Akbary and Aminikhoei, 2018). The biochemical levels of MDA, SOD, GPx, and CAT were determined colorimetrically by using commercial kits provided by ZellBio GmbH, Ulm, Germany. MDA was determined according to Akbary and Aminikhoei (2018) at 535, 550, 340 and 405 nm, respectively. Total soluble proteins have been measured via Bradford's method (1976) through bovine serum albumin as a standard. The activities of enzymes have been stated as certain activities (U mg^{-1} protein). Each enzymatic assay has been performed in triplicate (Bradford, 1976). Centrifugation of the total shrimp homogenate has been done at 700×g at 4°C for twenty minutes to measure phenoloxidase (PO) activity. Afterwards, the supernatants have been eliminated and pellet has been washed, resuspended slowly in cacodylate citrate buffer (0.45 M sodium chloride, 0.01 M sodium cacodylate, pH 7.0, 0.10 M trisodium citrate) and centrifuged once more. Next, resuspension of the pellet has been done with 200 µL cacodylate buffer (0.26 M magnesium chloride, 0.45 M sodium chloride, 0.01 M calcium chloride, pH 7.0, 0.01 M sodium cacodylate), and incubation of a 100 µL aliquot has been done by 50 µL trypsin (1 mg mL^{-1}), serving as an activator, for ten minutes at 25 to 26°C. Next, 50 µL of DOPA has been included, followed by 800 µL of cacodylate buffer five minutes later. Measurement of optical density at 490 nm has been performed by a spectrophotometer.

Challenge test

After 8 weeks of experimental trail, the effect of *Z. officinale* extract incorporated feed for the disease resistance (cumulative mortality percentage) on shrimp (n=30/ group) were investigated. Sk7 strain of *Photobacterium damselae* primarily was separated from the suspected juvenile shrimp by Iran Veterinary Organization (IVO), Chabahar province and then grown on brain heart infusion broth (BHI, Sigma) at 30ºC for 24-48 h. Bacterial cells were washed twice with sterile phosphate buffered saline (PBS) solution and then re-suspended in the same solution to obtain bacterial suspension. The bacteria concentrations were adjusted to LD70=7.2×10 CFU mL^{-1} through the suspension optical density (Austin and Austin, 2007). Ultimately, the shrimps were immersed into aquarium water which inoculated with bacteria for 4 hours. Furthermore,

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over a 10 days challenge test, the data for cumulative mortality were recorded.

Statistical analyses

Differences among dietary groups were evaluated with one-way ANOVA test using SPSS software (version 22, Armonk, NY, USA). Duncan's multiple range tests was conducted for comparison of differences among the groups. The results were considered as significant at $p<0.05$. Data was presented as mean ± SD.

Results

Growth performance

Data of growth performance was illustrated in Table 2. Dietary supplementation with ZE at level of 1 g kg^{-1} diet led to significant ($p<0.05$) increase of WG, SGR, PER and FW compared to the other experimental groups and control group. Meanwhile, the lowest FCR was observed in shrimp fed with ZE1 compared to the other groups.

Table 2: Growth performance of *Litopenaeus vannamei* fed the experimental diets containing different levels of *ZE* for 8 weeks.

Parameters	Experimental diets ZE (mg kg^{-1} diet)			
	0	**50**	**100**	**150**
Initial body weight (g)	1.06± 0.12^{a}	1.07± 0.10^{a}	1.09 ± 0.09^{a}	1.06 ± 0.07^{a}
Weight gain (%)	428.30±16.8 ^{b}	426.16 14.72 ± ^{b}	476.20 ± 8.17^{a}	376.41 ± 24.30^{c}
Specific growth rate (%)	1.61± 0.31^{b}	1.60 ± 0.40^{c}	1.69 ± 0.55^{a}	1.51± 0.36^{c}
Feed conversion ratio (%)	2.06± 0.06^{c}	1.85 ± 0.01^{b}	1.65± 0.02^{a}	2.03± 0.03^{c}
Final weight (g)	5.60 ± 0.30^{b}	5.63± 0.40^{b}	6.28± 0.48^{a}	5.05 ± 0.23^{c}
Protein efficiency ratio (%)	1.68 ± 0.54^{b}	2.64± 0.14^{a}	2.61± 0.3^{a}	1.21± 0.24^{c}

Values (mean±SE) with different superscripts in the same row are significantly different ($p<0.05$).

Antioxidant activities

Dietary supplementation with ZE at 1g/kg diet led to significant ($p<0.05$) decrease in malondialdehyde (MDA) values. However, superoxide dismutase (SOD), glutathione peroxidase (GPx), phenoloxidase (PO) and catalase (CAT) activities were significantly increased ($p<0.05$) compared with the other levels of ZE or the control group (Table 3).

The resistance of L. vannamei challenged with P. damselae

Supplemented diet with the level of 0.5 and 1 g kg^{-1} ZE significantly decreased mortality in comparison to ZE1.5 and the control group ($p<0.05$). The lowest cumulative mortality percentage with 40±8.5% were observed in ZE 1 supplemented diet at 10 days after challenging with LD70 *P. damselae* . After 10 days of inoculation, cumulative mortality percentage in ZE0.5 and ZE1.5 supplemented diets was 65.3±11.90% and 90±8.01%, respectively, which significant ($p>0.05$) difference was showed among them. The cumulative mortality percentages for ZE0 group were recorded from 5.06± 1.3% on the 3rd day to 75.2±6.5% on the 10th day (Fig.1).

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Table 3: Total antioxidant capacity and antioxidant enzyme activities of *Litopenaeus vannamei* fed the experimental diets containing different levels of ZE for 8 weeks.

Antioxidant enzyme (U mg protein)	Experimental diets ZE (mg kg^{-1} diet)			
	0	50	100	150
PO	23.99 ± 1.07 ^{c}	34.16 ± 2.01 ^{b}	38.45± 1.62 ^{a}	23.91 ± 1.21 ^{c}
SOD	38.14± 0.54 ^{c}	45.42 ± 0.25^{a}	38.18 ± 0.78 ^{b}	45.48 ± 0.45 ^{a}
GPX	187.53 ± 8.12 ^{c}	234.69 ± 7.24 ^{b}	262.47 ± 3.15 ^{a}	121.13 ± 8.06 ^{d}
CAT	3.73 ± 0.21 ^{c}	3.86 ± 0.41^{b}	4.17 ± 0.17^{a}	3.32 ± 0.43^{d}
MDA	7.14 ± 0.33^{b}	6.68 ± 0.24^{c}	6.32 ±0.54^{d}	7.66 ± 0.25^{a}
PO	23.99 ± 1.07 ^{c}	34.16 ± 2.01 ^{b}	38.45± 1.62 ^{a}	23.91 ± 1.21 ^{c}

Values (mean±SE, n =6 in each tank, triplicate) with different superscripts in the same row are significantly different (p<0.05). Malondialdehyde (MDA), Superoxide dismutases (SOD), Catalases (CAT), Glutathione peroxidases (GPx), Phenoloxidase (PO).

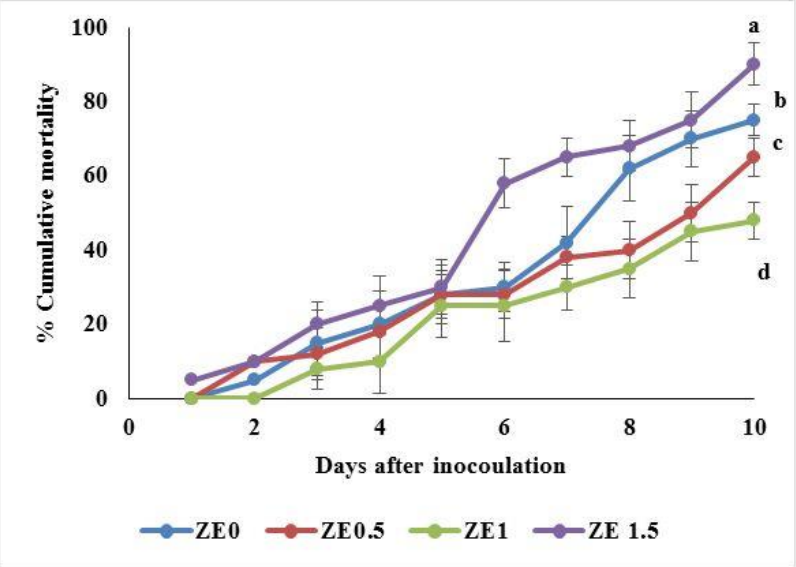


Figure 1: Cumulative mortality (%) of *Litopenaeus vannamei* fed the experimental diets containing different levels of ZE for 8 weeks after exposure to *P.damselae.*

Discussion

Plant extracts have been introduced to appropriate different activities such as growth promotion, appetite stimulation, immune stimulation, anti-pathogen and anti-stress properties in shrimp and fish aquaculture (Reveter *et al*., 2014).

Feeding shrimp with the ZE at the level of 1 g kg^{-1} diet showed the highest WG (476.20±8.17), SGR (1.69±0.55 %), FW (6.28±0.48 g) and PER (2.61±0.3 %) during the trial among the other groups. According to some researches, dietary *Z. officinalis* has positive impacts on the growth performance and feed utilization of aquatic animals and shrimp (Balasubramanian, 2009; EI-Desouky *et al*., 2012; Shubha, 2015). Our results are in line with the other research. Venkataramalingam *et al*. (2007) reported that *Penaeus monodon* post larvae received 75 and 100 % *Z. officinalis Artemia* enriched had significantly higher weight gain, condition factor and specific growth rate than those of the control group. Similarly, Chang *et al*. (2012) pointed out that all diets supplemented with zingrone (1, 2.5 and 5 mg kg^{-1}) showed

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significant higher weight gain and feed efficiency and use of 5 mg zingerone (kg^{-1} diet) increased growth. This proved that zingerone from ginger plays positive roles in the growth and food efficiency of shrimp. Also, Citarasu *et al*. (1999; 2003) reported that the use of different herbs such as *Hygrophila spinosa*, *Withania somnifera*, *Z. officinalis*, *Solanum trilobatum*, *Andrographis paniculata*, *Psoralea corylifolia*, *Eclipta erecta*, *Ocimum sacnctum*, *Picrorhiza kurooa*, *Phyllanthus niruri*, *Tinospora cordifolia*, purified Silajit and cod liver oil in *Penaeus indicus* larviculure had a positive role on growth performance, non-specific immune responses and stress/or disease resistance. It is mentioned that an increase in the growth performance can be attributed to the active ingredients of *Z. officinalis* suspected to stimulate digestive enzymes, increase appetite and improve the growth and the overall digestive process (Platel and Srinivasan, 2004). Also, ginger contains phytochemical constituent carbohydrate, mineral, elements and vitamins which increase the growth and health of animals (Iheanacho *et al*., 2017). However, the results of this study showed that the lowest SGR, WG and PER was reported in the shrimp fed with ZE 1.5 supplemented diet. This decrease can be probably resulting from the high fiber content or anti-nutrient ingredients in this concentration of ZE (Cho *et al*., 2007).

Zingerone, phenolic acids gingerol, shogols and flavonoids are bioactive molecules of ginger (Ghasemzadeh *et al*., 2010) which were assessed for its anti-apoptotic, anti-inflammatory, immune modulatory, antimicrobial, antioxidant and antiulcer activities (Ali *et al*., 2008). Phenolic compounds of ginger (gingerols, shogaols, volatile oils, flavonoids, and phenolic ketone derivatives) encourage antioxidant activity against free radicals and prevent lipid peroxidation (Kim *et al*., 2007). In the present study, shrimps fed with ZE1 supplemented diet showed significant increase in PO, SOD, GPx and CAT activity than those of the other experimental groups and control group which is in line with the previous study of Cheng *et al.* (2004) who reported that PO activity in *L. vannamei* fed all doses of the zingerone (1, 2.5 and 5 mg kg^{-1}) diets significantly increased compared to control group. It is reported that ginger extract as an immunostimulant can increase nonspecific immunity by promoting PO, SOD, CAT and GPx activity and increasing resistance against pathogens (Cheng *et al*., 2004; Hsieh *et al*., 2008). Moreover, bioactive compounds of ginger such as polyphenols and flavonoids due to antioxidant properties directly affect shrimp health by activating immune mechanisms and plays an important role in the prevention of infections (Scalbert *et al*., 2005). Similar tendency of increasing SOD, CAT and GPx activity have been achieved in *L. vannamei* fed diets

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containing 1 and 1.5g water polysaccharides extract of *Ulvae rigida* kg^{-1} diet (Akbary and Aminikhoei, 2018). These results contrary with the study of Vahedi *et al*. (2017) who showed that there was no significant difference in SOD activity in *Huso huso* fed diets containing 0.5% ,1 and 1.5% ginger extract compared to control group. This possibly attributed to the effect of each plant may differ depending on the dose of additive, size of fish or shrimp, nutritional status, physiological status and rearing conditions. Also, in this study, MDA content significantly decreased in the shrimps fed with ZE at the level of 0.5 and 1 g kg^{-1} diets compared to control group after 8 weeks experimental trial. Malondialdehyde as a toxic by-product is produced by polyunsaturated fatty acids peroxidation. Furthermore, induced intracellular oxidative stress by MDA is led to membrane lesions in erythrocyte. So, its decrease than the normal level indicate good health condition, which is in line with the previous work of Islam *et al*. (2016) who reported that antioxidant enzymes including GPx and SOD in *Orechromis niloticus* showed significant increase in ginger treated groups in relation with control. Concerning the effect of ginger extract in disease resistance against *P. damselae* bacteria, the results revealed that the shrimps fed with 0.5 and 1 g ZE kg^{-1} diets showed a decrease in cumulative mortality percentage compared to control group. The lowest mortality percentage was observed in ZE1 fed shrimps (40%). These results supported by the results of Jahanjoo *et al*. (2018) who showed after a challenge with *P. damselae* survival of Sea bream (*Sparidentex hasta*) fed with medicinal herb adjuvants (*Allium sativum, Z. officinale* and *Thymus vulgaris*) was improved when compared with the control group. A similar result was reported that *Zatraria mutiflora´*s essential oil had a significant anti-fungal effect and eliminated *Candida albicans* and *Fusarium salani* in culture shrimp, *L. vannamei* (Sharifi Rohani *et al*., 2013). Also, Yogeeswaran *et al*. (2012) showed that shrimps fed with diets containing methanolic extracts of *Acalypha indica, Cynodon dactylon, Picrorrhiza kurrooa, W. somnifera* and *Z. officinalis* for 60 days after vaccination, successfully protected them from WSSV. This probably could be also attributed to that *Z. officinale* contains gingerols and shogaols and over 50 components of the oils have been characterized these are mainly biasbolene (10-15%), sesquiphellandrene (15-20%), and monoterpenoids, the main pharmacological actions of ginger and compounds isolated from it and those are reported as antihyperglycemic, anti–inflammatory, immune–modulatory, anti- apoptotic, antimicrobial, anti-platelet, anti–ulcer, anti-oxidant and antitumourgenic (Ali *et al*., 2008).

In conclusion, the present study documents that *Z.officinalis* extract as an appetizer and immunostimulant at the level of 1 mg kg^{-1} diet could greatly

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enhance the growth performance, non-specific immune responses in *L. vannamei* and remarkably decreases the mortality against *P. damselae*

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