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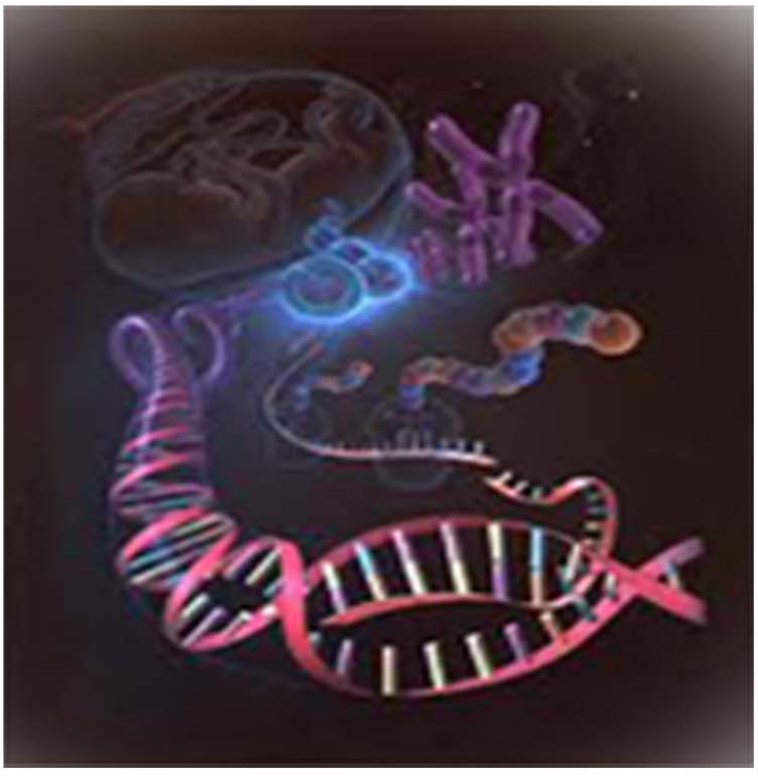

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Research Paper

EFFICACY OF GARLIC ON THE SURVIVAL, GROWTH AND HAEMATOLOGY OF *PENAEUS MONODON* POST LARVAE

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Efficacy of Garlic on the survival, growth and haematology of *Penaeus mondon* Post Larvae (PL) were investigated in the present study. Post larvae of *Penaeus monodon* were divided equally into five groups, each comprising 20 equal sized larvae to evaluate the efficiency of a garlic-supplemented diet (10 and 20 g kg^{-1} diet fed) in the performance of *Penaeus monodon*. Group (1) was the control (fed on base diet). Groups (2 and 3) were fed on garlic-supplemented diet (10 and 20 g kg^{-1} diet fed) for one month and groups (4 and 5) were fed on same doses of garlic-supplemented diet for two months. Survival, body weight and blood parameters were recorded. Significant changes in total leukocytic count in treatment group 2 and the monocytic count in groups 2-5 were also observed. There was slight increase in mean individual body weights of treatment groups 2-5 at the end of the experiment. Survival rates were significantly higher in all treatments compared to the control. Consequently garlic improved the growth performance.

Keywords: Garlic, Survival, Growth, Blood parameters, *Penaeus monodon*

INTRODUCTION

Crustaceans offer important economic, ecological and aesthetic values and also can be appreciated from the perspective of bi-level functionality. Crustaceans like shrimp, lobsters and crabs form a major food commodity (New World Encyclopedia). The cultivation of prawns and shrimps in brackish water coastal ponds has been practised in different parts of the world. Fish nutrition has an important impact on several parameters directly influencing the quality of fish, such as colour and appearance. Growth is a parameter of obvious importance in fish and shrimp culture. It has been the topic of numerous studies yet careful examination of the current scientific and technical literature shows that it is still poorly understood by many scientists and aquaculturists.

Farm-reared shrimp is increasing in popularity and profitability due to its exclusive flavor and high nutritive value (FAO, 2005). Tiger shrimp, *Penaeus monodon* is the principal type of cultured shrimp in India, which accounts the largest part of the production. It is the largest of

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all cultured species that can reach upto 36 cm in length (Nahavandi *et al.,* 2010).

Commercial cultivation of the black tiger shrimp, *Penaeus monodon* has been an important industry in many tropical countries and widespread domestication and selective breeding programs for this species have resulted in improved commercial performance (Withya chumnarnkul *et al*., 2001).

Recently aquaculturists have been showing interests in developing technologies to induce prawn growth, reproductive performance and larval quality by feeding supplemented diets enriched with various feed additives and growth promoters (Vatheeswaran and Ali, 1986; Jayaprakas and Sambhu, 1996, 1998; Sambhu and Jayaprakas, 1997, 2001). Many scientists believe that the word "consumption of medical plants" is growing rapidly (Aliyu *et al*., 2007).

Garlic, *Allium sativum L*., has been used for the treatment of many diseases since ancient times as reported in the Codex Ebers (1550 BC), where an Egyptian medical papyrus described several therapeutic formulas based on the garlic as a useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumors (Block, 1985). It has long been considered that garlic (*Allium sativum*) has several beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, and antihypertensive properties (Konjufca *et al*., 1997; Sivam, 2001). Previous research suggested that those functions are mainly attributed to the bioactive components of garlic, including sulphur containing compounds, such as allin, diallylsulphides and allicin (Amagase *et al*., 1993). Many beneficial health properties of garlic are attributed to organo sulphur compounds, particularly to thiosulfinates (Block, 1992). Allicin (diallythiosulfinate) is the most abundant compound representing about 70% of all thiosulfinates present, or formed in crushed garlic (Block, 1992; Han *et al.,* 1995). Garlic has proven to be hypolipidemic (Sumiyoshi, 1997), antimicrobial (Kumar and Berwal, 1998), antihypertensive (Suetsuna, 1998), hepato protective and insecticidal (Wang *et al.,* 1998).

Garlic extract has also been shown to reduce serum cholesterol levels (Bordia *et al.,* 1975; Augusti, 1977) and increase blood coagulation time (Bordia *et al.,* 1975). An antifungal activity has been identified in garlic bulbs (Fromthing and Bulmer, 1978). S-allyl cysteine, present in the crushed garlic, was found to inhibit tumor metabolism and enhance the immune response (Sumiyoshi, 1997). The allyl sulfides enhance the glutathione S-transferase enzyme systems, which through their dependent biochemical pathways enhance the liver's detoxification of carcinogenic substances. The allium species show immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo*et al.*, 1998).

The present study was conducted to evaluate the efficiency of the garlic (*Allium sativum* L) in improving the survival, growth and hematology in black tiger shrimp (*Penaeus monodon*) post larvae.

MATERIALS AND METHODS

Shrimps

Post larvae of *Penaeus monodon* (PL-6) weighing about 12 ± 0.25 g were obtained from local shrimp farm in Chennai, Tamil Nadu. The PLs were divided into five groups each comprising 20 larvae, including the control. The whole

experiment was performed in 3 replicates for 60 days.

Garlic

Garlic (*Allium sativum L.*) was procured from the local market, crushed and two doses, i.e., 10 and 20 g of garlic kg^{-1} feed were mixed with the balanced diet in pellets. The pellets were prepared biweekly, air-dried at room temperature for 24 h and stored in a refrigerator (4ºC). The garlic cloves were analyzed for its chemical study which revealed sulfer-containing amino acids (1-3%) named alliin which is the stable precursor that is converted to allicin by the action of allinase enzyme which represent 10 mg/g garlic cloves fresh weight (Ellmore and Feldberg, 1994).

Diets

Basal diet is a control diet, containing 45% crude protein, 10% crude fat, 3% crude fibre, 13% crude ash, 88% dry matter. The basal diet was prepared by grinding the corn to granules using 0.5 mm mesh (Thomes-Willey Laboratory Mill Model 4). The ingredients were mixed mechanically by horizontal mixer (Hobart model D300T) at a low speed for 30 min. Oil (vegetable and cod liver) was added gradually to assure the homogeneity of the ingredients. The mixing speed increased for 5 min during the addition of water (600 mL) until the mixture began to clump. Pellets were then prepared using a pellet machine (CPM California pellet mill Co.) with 0.5 cm diameter. Batches of feed were prepared every two weeks and pellets left for 24 h to air dry, and stored in a refrigerator (4°C) for daily use.

Experimental Design

PLs were divided into five equal groups. Group 1 was fed a base diet (control). Garlic supplemented base diets (10 and 20 g kg^{-1} diet fed) were given to groups 2 and 3 for one month and to groups 4 and 5 for two months, respectively. The experiment was performed in 3 replicates for a period of 60 days altogether. PLs were observed daily and dead or wasted feed were removed. The base diet was fed to all treatment and control groups throughout the experiment. At the end of the feeding period, the PLs were examined in the laboratory for the various parameters.

Laboratory Tests

Body Weight Gain: PLs from all treatment replicates and control were weighed individually at the end of the experiment.

Survival: Survival rate was recorded during the course of the feeding experiment for all treatment replicates.

Hematological Parameters

Hemolymph was collected and divided into two portions one mixed with Alsever's solution according to FAO (2002) with slight modification (Citric acid 0.055 g, Sodium Citrate 0.8 g, Glucose 2.05 g, Nacl 0.64 g, EDTA 4 g, distilled water 100 mL) for estimation of Total Hemocytic Count (THC), Hemocyte Viability (HV) and Phagocytic activity, while the other portion was mixed with 10% formalin for determination of Differential Haemocytic Count (DHC). THC and HV were measured following the method of Cheng *et al. (*2003). DHC was measured following the method followed by Abdel Hameed (2008).

Phagocytic Index (PI) was measured according to Itami *et al.* (1994) with minor modification as follows: PI = 100 * (Number of haemocytes engulfed beads/ Total number of haemocytes) * (Number of beads that were engulfed by the haemocytes/ Total number of haemocytes).

PR = (Phagocytic haemocytes/ Total haemocytes) * 100

Statistical Analysis

The data were analyzed with one-way ANOVA and significant differences were determined at 0.05 probability by Scheff.'s multiple comparison test after the normality and homogeneity (Bartletto's test) of the data were checked with Minitab Statistical Package.

RESULTS

In the first phase of the experiment, groups 2 and 3 revealed a slight increase in the hematocrit values when compared with the control (group 1) (Table 1). There is less significant change in total leukocytic counts in groups 2 and 3 when compared with the control group. However, a significant increase in monocytes were observed in both the treatment groups. In the second phase, groups 4 and 5 revealed a significant increase in hematocrit values when compared to control (group 1). The total leukocytic count of groups 4 and 5 also showed significant change, with slight increase in monocytes, compared to controls (Table 2).

Table 1: Effect Of Garlic Supplemented Diets on Total Hemocyte Count and Hemocyteviability (%) of *Penaeusmonodon* at 30 and 60 Days

Treatments	Total Hemocyte Count		Hemocyte Viability	
	30 days	60 days	30 days	60 days
Group 1 (Control)	7.5 ± 0.25	5.4 ± 0.20	48.7 ± 0.081	65.20 ± 0.21
Group 2	20.25 ± 0.21	23.13 ± 0.49	99.92 ± 0.19	82.44 ± 0.23
Group 3	12.6 ± 0.16	15.43 ± 0.32	59.70 ± 0.33	74.22 ± 0.24
Group 4	12.56 ± 0.40	9.56 ± 0.32	54.43 ± 0.16	84.29 ± 0.15
Group 5	16.53 ± 0.16	14.43 ± 0.26	100.2 ± 0.24	82.14 ± 0.25

Table 2: Heamatological Parameters of Penaeusmonodon Fed with Different Concentration of Garlic Supplemented Diets

Parameters	30 days			60 days		
	Control (Group 1)	Garlic (10 g/kg) Group 2	Garlic (20 g/kg) Group 3	Control (Group 1)	Garlic (10 g/kg) Group 4	Garlic (20 g/kg) Group 5
HCV (%)	30.23 ± 0.20	32.6 ± 0.33	32.43 ± 0.24	28.46 ± 0.36	31.2 ± 0.16	32.83 ± 0.16
NBT (mg/ml)	0.26 ± 0.04	0.24 ± 0.05	0.26 ± 0.03	0.11 ± 0.02	0.13 ± 0.04	0.13 ± 0.06
TLC (10^3 /μl)	36.33 ± 0.28	36.46 ± 0.16	32.6 ± 0.08	41.2 ± 0.20	37.3 ± 0.32	41.3 ± 0.21
Neutrophils (10^3 /μl)	11.78 ± 0.02	11.58 ± 0.02	11.80 ± 0.04	12.45 ± 0.03	12.31 ± 0.03	12.06 ± 0.04
Lymphocytes (10^3 /μl)	23.3 ± 0.29	24.41 ± 0.03	20.24 ± 0.02	27.67 ± 0.04	23.77 ± 0.03	27.21 ± 0.02
Eosinophils (10^3 /μl)	0.34 ± 0.02	0.36 ± 0.01	0.14 ± 0.03	0.54 ± 0.32	0.46 ± 0.04	0.53 ± 0.04
Basophils (10^3 /μl)	0.04 ± 0.02	0.18 ± 0.02	0.04 ± 0.02	0.08 ± 0.03	0.2 ± 0.02	0.26 ± 0.01
Monocytes (10^3 /μl)	0.39 ± 0.35	1.19 ± 0.02	0.95 ± 0.02	0.92 ± 0.02	1.31 ± 0.04	1.19 ± 0.03

A trend of increased growth rates among experimental shrimps (groups 2 and 3) was apparent. At the end of the second phase of the experiment, significantly increased growth rate was observed in groups 4 and 5 (Figure 1). A significant increase, in survival rate was observed in shrimps of groups 2-5 during the experimental period (Figure 3). Significant increase in body weight also observed in animals fed with garlic supplemented diet (Figure 2).

DISCUSSION

The hematocrit values of the experimental shrimps showed a slight increase during the first and second phases of the experiment, which support the contention that the garlic doses used in this experiment were both safe and efficacious. Sahu (2004) also observed an increase in erythrocytic count after administering garlic. A marked increase in the total leukocytic count was

Figure 1: Specific Growth Rate of *Penaeusmonodon* post larvae treated with different concentration of garlic supplemented diets

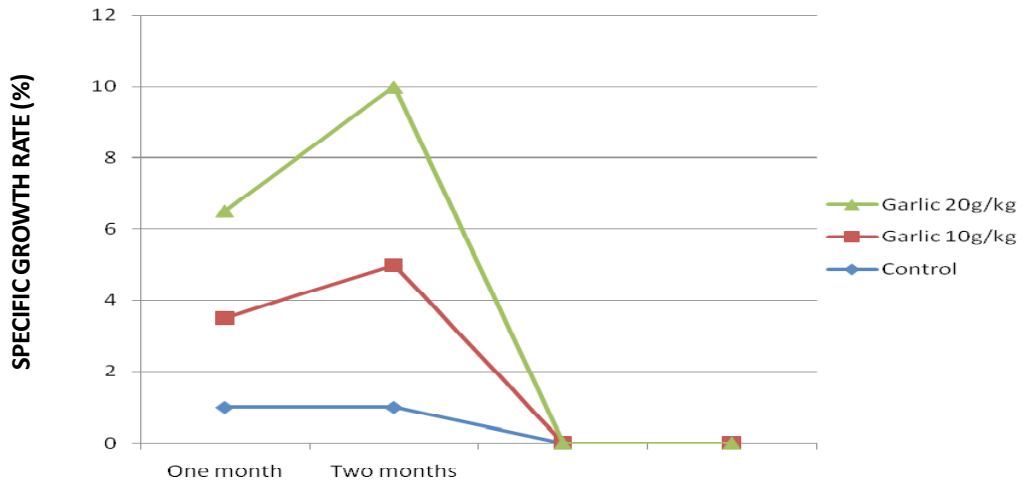


Figure 2: Weight Gain Rate of *Penaeusmonodon* post larvae fed with different concentrations of garlic supplemented diets

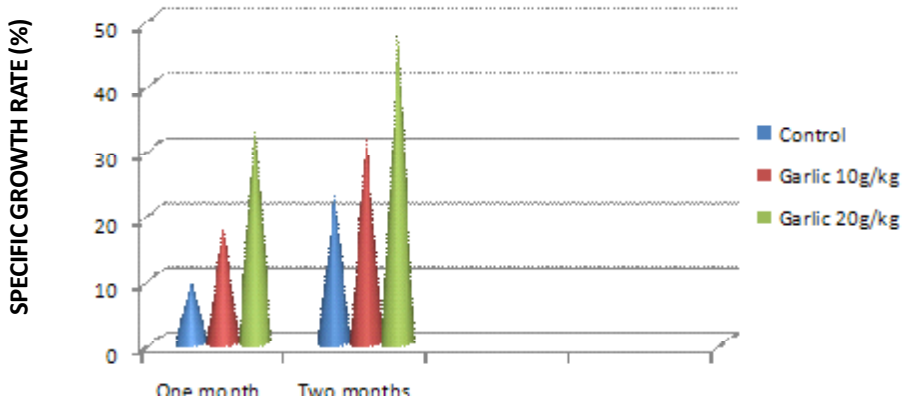
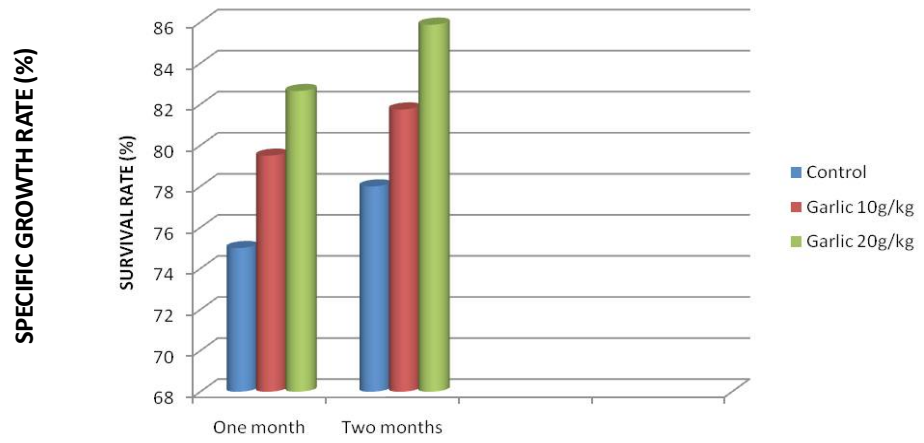


Figure 3: Survival Rate of *Penaeusmonodon* Post Larvae Fed With Different Concentrations Of Garlic Supplemented Diets



seen in group 2 and was associated with a high number of monocytes and lymphocytes during the first phase. Iranloye (2002) also reported increases in total white blood cell count, neutrophils, lymphocytes and monocytes following 30-day of feeding garlic, illustrating the anti-infection properties of garlic. In the present study shrimps that are sampled during the second phase showed a non significant change in TLC as well as significant increases in monocytes. This suggests that the feeding of garlic-supplemented diet for 60 days may be effective in improving the TLC but may improve the monocytes. Hematological studies showed that small doses of garlic (10 g kg^{-1}) administered for 30 days were sufficient to induce the most promising results. Heo *et al. (*2000) reported that the only changes in fish blood profiles, observed when l-carnitine was included in diet, were increased concentrations of WBC and lymphocytes, although there is no direct evidence from the literature on the effect of l-carnitine supplementation on immune-related blood cell counts. However, many previous reports have suggested that l-carnitine supplement may influence lipid metabolism. Dietary lipid may affect a great number of immune parameters, such as lymphocyte proliferation, cytokine synthesis, natural killer cell activity and phagocytosis (De Pablo and De Cienfuegos, 2000). The NBT test is used to determine phagocytic activity which indicates the immune response, especially neutrophils and monocytes (Jabs *et al.,* 1980). A significant increase in NBT values was detected in shrimps during the second phase of the experiment when compared with the control group. This shows the effect of garlic in enhancing the phagocytic activity of the leukocytes and this activity is dependent on the duration of garlic supplementation (Ally *et al.,* 2008). Many investigators have reported enhanced bactericidal activity by the phagocytic cells of different fish species treated with immunostimulants (Jorgensen *et al.,* 1993). For example, serum bactericidal activity was enhanced in groups treated with garlic (Sahu*et al.,* 2007).

Several studies demonstrated that herbs like *Z. officinalis* and *C. dactylon* is beneficial to

growth and immune systems in aquatic animals and shrimp (Balasubramanian, 2009). Since immunostimulants can increase non-specific immunity by promoting phagocytosis, bactericidal activity, PO activity and respiratory bursts (Kim *et al.,* 1999). Rutin extract from *Toonasinensis* administered by injection for *L. vannamei* significantly improved survival rates (Hsieh *et al.,* 2008).

The significant increases in total leukocytic count and monocytes of group 2 and 3 (10 g/kg of garlic for 1 month), in the present study, did not seem to be associated with much significant increase in the NBT values, suggesting that the feeding of garlic-supplemented diet for one month may promote the phagocytic activity of neutrophils more than that activity of monocytes. But there was slight changes in TLC and increased monocytes in groups 4 and 5 (20 g/kg of garlic for 2 months). Neutrophil activity can be an indicator of fish response, as they adhere to the endothelium by adhesion molecules, thereby facilitating their emigration from the capillaries to the site of injury (Kishimoto *et al*., 1989; Magnuson *et al*., 1989). Neutrophils also exhibit increased production of oxygen radicals which are potentially capable of destroying invading pathogens (Hassett and Cohen, 1989). While limited significant increase in total leukocytic count was seen in groups 4 and 5, the monocyte numbers significantly increased. The latter seem to be associated with the significant increase in NBT values, suggesting that the application of garlic for two months may be more effective on the phagocytic activity of the monocytes. However, other studies claim that the protective effect of garlic may be associated with its antioxidant properties (Pedraza-Chaverri*et al*., 2000; Rahman, 2003).

Several herbs have been tested for their growth-promoting activities in aquatic animals (Jayaprakas and Eupharsia, 1996; Citarasu*et al*., 2002; Sivaram*et al*., 2004). Our observations showed significant increases in growth rates after one or two months of feeding with garlic. Horton *et al*. (1991) reported no effects of feeding 1 or 10 g garlic kg^{-1} diet on the growth performance of pigs. Other studies have shown that garlic did not affect growth performance of broilers (Freitas *et al*., 2001) or growing lambs (Bampidis *et al*., 2005). The pungent smell of garlic, may lead to lower diet palatability. However, in the present study a marked increase in growth rate was noticed in the second phase in groups 4 and 5. Cullen *et al*. (2005) also found improved feed conversion rates when garlic was added to the diet for grower-finisher pigs at the level of 1 or 10 g kg^{-1} diet. Khalil *et al. (*2001) mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy, leading to improved growth. Reduced mortalities following pathogenic challenges in the presence of a low dose of herbal principals, have been reported by Kim *et al*. (2001) and Jain and Wu (2003).

Immunostimulants can increase non-specific immunity by either increasing the number of phagocytes or activating phagocytosis (Shoemaker *et al.,* 1997). Many defense mechanisms activated by garlic counteract the challenge infection including the production of superoxide anions against the *A. hydrophila* infection. It has been found that the aqueous extract of raw garlic and dried powder scavenge hydroxyl radicals (Yang *et al*., 1993; Kim *et al*., 2001), and superoxide anions (Kim *et al.,* 2001). Gildberg and Mikkelsen (1998) fed Atlantic cod

(*Gadusmorhua*) fry on a commercial feed, supplemented with *Carno bacterium divergens* alone or combined with immuno-stimulating peptides for 3 weeks, then challenged with *Vibrioanguillarum* (10^7 mL^{-1}, for 1 h). The fry showed reduced mortality.

Garlic has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections. The recent chemical characterization of their sulphur compounds has promoted claims that such compounds are the main active antimicrobial agents (Rose *et al*., 2005). The survival rate was significantly greater in all garlic-supplemented groups when compared with the control group at the end of the experiment. However, it was significantly higher in group 5 than the other groups. Although the use of garlic resulted in good survival rates, feeding the higher doses of garlic for extended periods gave better results. Using a combination of five herbs developed an Artemia-enriched herbal diet for *Penaeus monodon*, which significantly increased survival rate during stress conditions (Citarasu *et al.,* 2002). The current results revealed improved survival rate in the garlic supplemented groups which may be due to the enhanced immune response resulting from increased numbers of monocytes, increased phagocytic activity or other defense mechanisms. Immunostimulants can also increase serum lysozyme activity by increasing the number of phagocyte-secreting lysozyme, or by increasing the amount of lysozyme synthesized per cell (Engstad *et al.,* 1992).

Dietary Garlic extract supplementation improved growth performance, protein level, body weight and feed utilization by juvenile Sterlet Sturgeon (*Acipenserruthenus*) (Dong-Hoon Lee *et al.,* 2012). A study on ethanolic extract of turmeric, showed inhibition effect of *Vibrio* spp. No significant differences were observed in survival rate, feed conversion ratio and growth rate of white shrimp (*L. vannamei*) fed with ethanolic turmeric extract. The efficacy of ethanol turmeric extract as an immunostimulant for white shrimp showed that the total hemocyte count, phenoloxidase activity and bactericidal activity increased as the levels of ethanol turmeric extract in feed increased (Ong-ardLawhavinit *et al.,* 2011).

A lot of studies were carried out by supplementation of garlic feed, only very few reports are available stating its effect on flesh quality. Kwon *et al. (*2005) reported that garlic improved the meat quality of growing-finishing pigs. Garlic is known to have medicinal properties and is useful in combating bacteria (Resse*t al.,* 1993) and fungi (Adetumbi *et al*., 1986). Garlic contained 1-3% alliin which converted to allicin by the action of allinase (10 mg/g garlic cloves) (Ellmore and Feldberg, 1994). It is also known that, garlic powder contain 99% pure dimethyl trisulfide and a mixture of diallylsulfides, DASS (33% diallylsulfides DADS, 16% diallyltrisulfide DATS and 17% diallyltetrasulfide DATTS). Many efficient organization ensures that clove defense mechanism is only activated in a very small location and for a short duration, whereas the rest of the alliin and allinase remain preserved in their respective compartments and are available for interaction in case of subsequent microbial attacks. These phenomena explain the efficiency of garlic in improving the quality and shelf life, as it is well accepted that allicin which possesses a variety of biological activities (Miron *et al.,* 2004), is the bioactive substance. The present study

also suggests that garlic is very much efficient in improving shrimp quality, its growth and survival rate. However, several reports suggest that garlic also has a lipid-lowering action. On the other hand, Yeh and Liu (2001) suggested that garlic inhibits the synthesis of cholesterol and fatty acids in the liver; however, the exact mechanisms are not well understood. Therefore, further studies should be done to establish the dosage used, period of application and time of withdrawal to obtain the best evaluation of the shrimp quality.

CONCLUSION

It can be concluded that garlic improves the immune response of *Penaeus monodon* through a rapid increase in monocytes and over a longer time frame enhanced phagocytic activity which may provide increased protection against immediate infection by pathogens. High doses also appear to enhance growth rate. A significant improvement was seen in the survival of all treatment. It seems beneficial to use garlic at lower doses for one month, in short-term operations, such as in the hatchery, and for two to four months to improve shrimp production in large scale shrimp industry.

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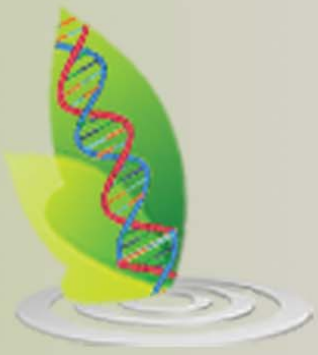
ABBREVIATION

PL	- Post Larvae
CPM	- California pellet mill
EDTA	– Ethylene Diamine Tetra Acetic acid
THC	- Total Haemocytic Count
HV	- Haemocyte Viability
DHC	- Differential Haemocyte Count
TLC	- Total Leucocyte Count
NBT	- Nitro Blue Tetrazolium



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