

Application of Garlic (*Allium sativum*) as an Alternate Therapeutic for Marine Shrimp

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Abstract

Application of garlic (*Allium sativum* L.) as an alternate therapeutic for marine shrimp will be discussed. Garlic had a bactericidal property against the *Vibrio* spp., the major caused of bacterial diseases of marine shrimp, as evident from the MIC values obtained from the *in vitro* study. In addition, it had a potent to diminish the protozoa; gregarines infection in culture shrimp, however its effect on viral disease infection is limited and requires more further studies. Besides, garlic could stimulate the shrimp hemocytes as determined by the cellular immune responses (phagocytic activity, superoxide anion production and phenoloxidase activity) suggesting the immunostimulatory properties of garlic that will be useful for improvement of shrimp health. However, the optimum dosages and duration of garlic application in the real culture system on bactericidal, parasitological, virulicidal and immunostimulatory effects are needed to be approved before distribute this information to the farmers.

Keywords: Garlic, shrimp, immunomodulation, bactericidal, diseases.

Introduction

Garlic (*Allium sativum* L.) is one of the common medicinal plants possess the wide ranges of antimicrobial properties. It has been proved to be useful for therapeutic of virus, bacteria, parasites, fungi and protozoans in Human. Allicin has been identified as the major active pharmaceutical molecule found in crashed garlic, however it has a very short half-life as it react with many of the surrounding proteins. Allicin is derived from an amino acid called alliin which is a stable precursor that will be converted to allicin by the action of an enzyme allinase presented in the garlic cloves. Beside on the antimicrobial properties of allicin it also had immunomodulatory effect. Garlic was used to prevent and treat the bacterial and fungal diseases of fish in China by mixing one kilogram of garlic with powdered feed five kilograms, one kilogram sodium chloride and one kilogram of binder (Bai, 1994). The shrimp farmers in some areas of Thailand commonly use garlic in their farm to clean up the intestinal tract of shrimp. They believed that garlic help the culture shrimp to have better feeding after include the garlic paste in shrimp feed. Nevertheless, the scientific reason for this effect has not been postulated yet. This document trying to discuss some studies have been conducted in the laboratory and in the field to examine the effects of garlic on improvement of shrimp health and looking for the future research that needed to be investigated to complete the information on application of garlic in shrimp culture and other aquaculture activities in future.

Screening for efficacy of garlic

Bacteria & Fungi

The broth dilution assay and the disc or agar well diffusion assay are the commonly *in vitro* methods used for initial screening the potential antibacterial of medicinal plant (Cowan, 1999). Then more details studies of their antibiotic effects should be conducted by determining the minimum inhibitory concentration (MIC) in comparable with others currently used antibiotics. The MIC value of fresh garlic against seven *Vibrio* strains was determined by disc diffusion method in comparison with standard bacteria; *V. cholerae* ATCC 14035 and *E. coli* ATCC 25922. It showed a good bactericidal potent against all seven strains of tested bacteria and gave 0.156-0.312 mg/ml of MIC values as shown in Table 1 (Montira *et al.*, 2005).

Table 1. The MIC values of fresh garlic against different strains of bacteria (*Vibrio* spp.)

Bacterial strains	Minimum Inhibitory Concentration (MIC, mg/ml)
<i>V. harveyi</i>	0.156
<i>V. palahaemolyticus</i>	0.312
<i>V. damsela</i>	0.156
<i>V. alginolyticus</i>	0.312
<i>V. vulnificus</i>	0.156
<i>V. pelagius II</i>	0.156
<i>V. minicus</i>	0.156
<i>V. cholerae</i> ATCC 14035	0.156
<i>E. coli</i> ATCC 25922	0.312

(Montira *et al.*, 2005)

Fungal & Virus

Garlic has been reported to possess anti-fungal (Yoshida *et al.*, 1987) and antiviral (Weber *et al.*, 1992) properties. Fresh garlic extract was proved for its *in vitro* virucidal effects against viral infection in Human. However, there are no such effects of garlic has been studied in shrimp. Since virus infection is the major problem for shrimp culture therefore the virucidal effect of garlic in shrimp is attractive for future study.

Parasites & Protozoans

Chutchawanchaipan *et al.* (2004) reported the efficacy of fresh garlic paste for reducing the number of parasites; gregarines from the midgut of black tiger shrimp. Ten gram of fresh garlic paste was mixed with one kilogram of commercial feed, coated with 20 milliliters of chitosan, and fed to shrimp in three earth ponds for 5 weeks. The shrimps were sampled before start feeding diet containing garlic and every week after started feeding of garlic paste mixed diet, 20 shrimps each, to examine the number of gregarines in the intestinal tract of cultured shrimp using histological techniques. Numbers of shrimp infected with gregarines were 100 % reduced after fed garlic containing diet for 4 weeks respectively (Table 2).

Table 2. Percentages of shrimp infected with gregarines before and after fed with garlic paste mixed diet

Duration (weeks)	% Shrimp with gregarines		
	Pond No. 1	Pond No. 2	Pond No. 3
Before feeding garlic paste mixed diet	100	90	85
	100	100	90
1	30	65	40
2	10	15	10
3	0	15	15
4	0	0	0
5	0	0	0

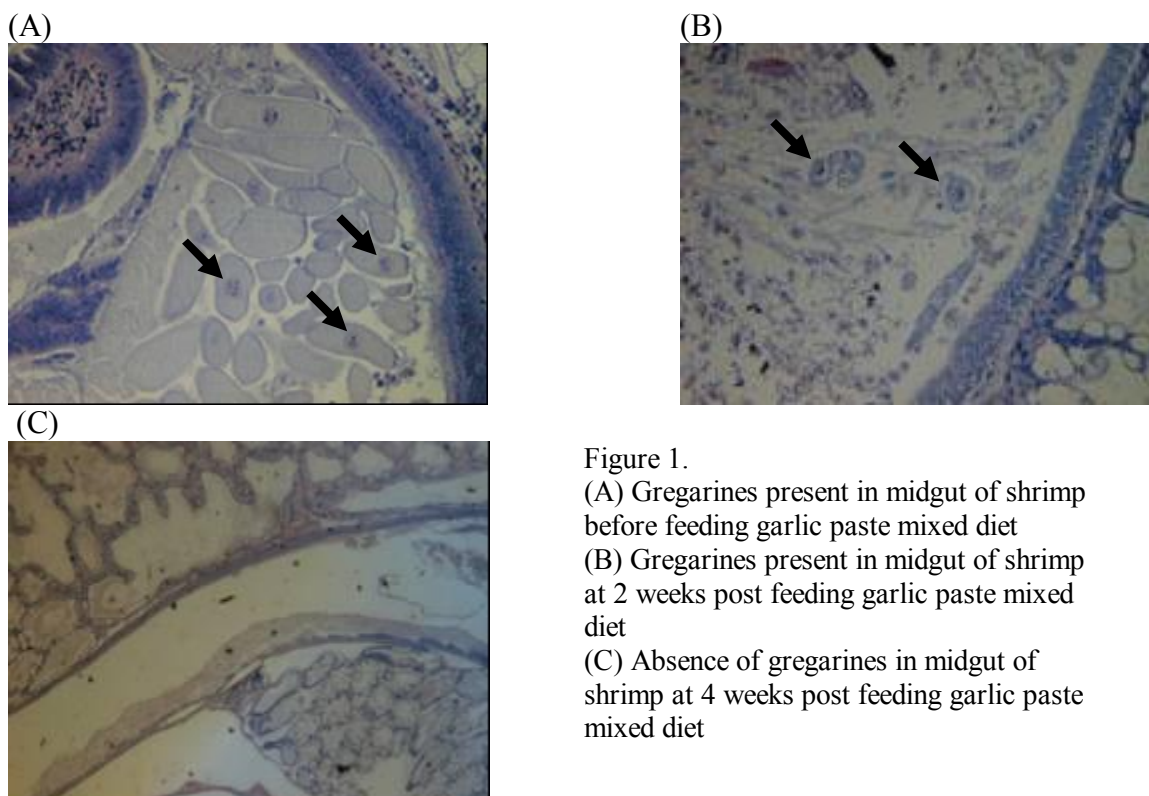


Figure 1.
 (A) Gregarines present in midgut of shrimp before feeding garlic paste mixed diet
 (B) Gregarines present in midgut of shrimp at 2 weeks post feeding garlic paste mixed diet
 (C) Absence of gregarines in midgut of shrimp at 4 weeks post feeding garlic paste mixed diet

Immunostimulatory

In vitro phagocytosis is one of screening method for the detection of immunostimulating compounds (Wagner, 1990). Effects of fresh garlic extract on phagocytic activity of *P. monodon* hemocytes was tested *in vitro* at Coastal Aquatic Animal health Research Institute. Higher phagocytic activity was found in hemocytes treated with garlic extract (78.7 %) compared to control cells without pre-incubation with garlic extract (64.1%) as shown in Figure 2. *In vitro* production of superoxide anion (O_2^-) from *P. monodon* hemocytes was also examined using the NBT reduction method to determine the potential killing of hemocytes after stimulation with zymozan. Concentrated garlic extract (100% and 10%) were toxic to the hemocytes as evident from the high number of hemocytes died under trypan blue exclusion test and this resulted in a

low production of NBT and PO activity as shown in figure 3 and 4. However, a certain dilution of garlic extract could increase the NBT activity of shrimp hemocytes (Figure 3) indicating the potential of garlic extract on killing ability of shrimp hemocytes. *In vitro* activation of prophenol oxidase activity of *P. monodon* hemocytes was performed in comparison to a well known proPO activator; trypsin as determined from the rate of optical density (OD) changed upon the dopamine formation. The garlic extract seemed not to be an effective activator for the proPO system of shrimp hemocytes as shown from the minor changed of the dopamine formation compared to control, moreover its effect was inferior to trypsin (Figure 4).

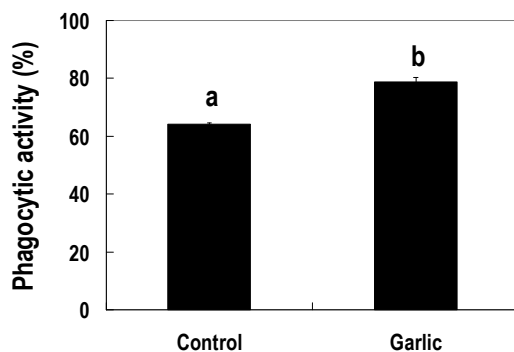


Figure 2.

In vitro phagocytic activity of shrimp hemocytes with and without garlic stimulation.

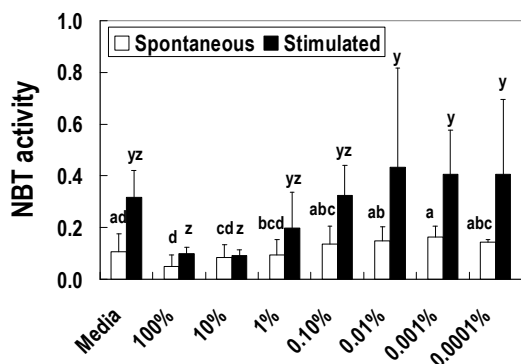


Figure 3.

The NBT activity of shrimp hemocytes after 30 min incubation with different concentration of garlic extract with (stimulated) and without (spontaneous) zymosan activation.

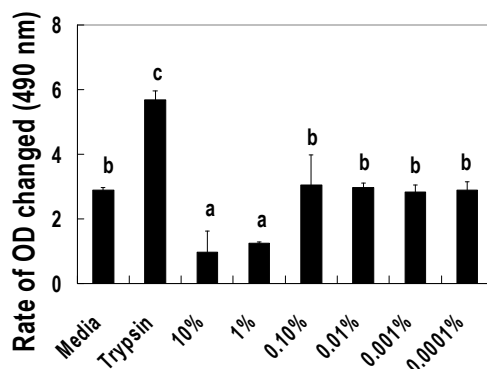


Figure 4.

Rate of dopamine formation (OD changed at 490 nm) without adding of ProPO activator and after activated with trypsin and garlic extract at different concentrations.

In vivo study the effect of fresh garlic extract on phagocytic activity of shrimp hemocytes was conducted by feeding shrimp with diets containing different dosages of fresh garlic extract prior to sample for determination of phagocytic activity at 7 and 14 days post feeding (Figure 5). The phagocytic activity of shrimp fed garlic supplemented diet were higher than control indicating an effective of garlic on enhancement of the cellular immune response of shrimp.

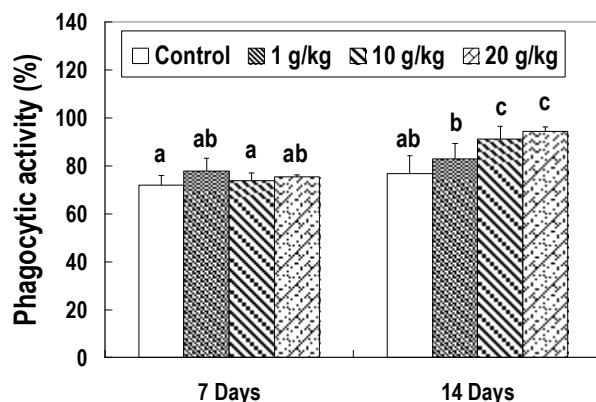


Figure 5. *In vivo* phagocytic activity of shrimp hemocytes after fed diets containing different amounts of garlic for 7 and 14 days.

Conclusion

Garlic had a potent to treat the parasites; gregarines in culture shrimp, however its effective on other parasites and protozoans are needed to be examined in the same manner as done for the gregarines to elucidate further potentiality of garlic. From the *in vitro* study, garlic had an antibacterial potent against bacteria *Vibrio spp.* which are the major caused of bacterial disease of marine shrimp. However, more information on optimum dosages and treating duration of garlic in the real culture system are need to be clarified in order to introduce this kind of knowledge to the farmers to use in their farms which is the final goal of the research work. Information on virulicidal effects of garlic in shrimp is reared and the research on this topic is required under either *in vitro* or *in vivo*.

In addition, garlic seemed to be benefit to use as an immunostimulant for shrimp as well since it could activate the function of shrimp hemocytes both *in vitro* and *in vivo*. However, dosages and duration of garlic used in shrimp pond also needed to be fulfilled as in the case of the above mentioned for bacteria, parasites, and viral diseases.

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