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The use of garlic in aquaculture

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Abstract
Due over the last years, marine aquaculture has grown into a very significant industry of the world, However the extensive use of antibiotics and various chemical compounds, has resulted in leftover side effects such as drug residues and resistant pathogens in the treated fish. Not only do the drug residues pollute the environment, but also threaten the health of the human consumers.
In a sharp contrast, garlic (*Allium sativum*) a natural antibiotic, has been found to have beneficial effects on the cardiovascular and immune systems of the fishes and it has shown its effectiveness in the treatment of many diseases both in humans and animals owing to its antimicrobial, antioxidant, anticancer, and antihypertensive properties.
Garlic has a wide spectrum of actions; it is antibacterial, antifungal, antiviral and antiprotozoal. Therefore in aquacultural operations, optimized doses of garlic are strongly recommended. It is one of the earliest known medicinal plants; and investigations into its mode of action are relatively recent. This review focuses on the applications of garlic in treating various fish related diseases and the prospects of using garlic preparations in aquaculture.

Key words: Garlic, aquaculture, fish, antibacterial, growth, antioxidant

1. Introduction
Industrial aquaculture is growing rapidly in many developed and developing countries due to the depletion of fisheries and market forces aimed at globalizing food sources [1, 2]. This impressive industrial development has been accompanied by certain practices that can potentially damage human and animal health [2, 3]. The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases [4]. Furthermore, there is a growing concern about the use and abuse of the antimicrobials in aquaculture, as they increase the selective pressure exerted on the microbes and encourage the emergence of resistant bacteria by transferring resistant-genes to bacteria not exposed to antibiotics. Moreover, the antimicrobials lead to drug residues in the treated fish, besides having a negative impact on the environment [5]. Antibiotics cannot be recommended for use due to their residual and other side effects, which can lead to a myriad of problems. In marine fish hatcheries, the indiscriminate use of antibiotics for prophylaxis has led to the development of resistant microbial strains [6]. Moreover, drug resistance in fish pathogens can be transferred to environmental and human pathogenic bacteria. Thus, the accumulation of antibiotics in fish can be harmful to the environment as well as consumers. Accordingly, many countries refuse to import cultured products. These problems have prompted scientists to search for an alternative to antibiotics.
Although herbal remedies have been with us for human therapy for millennia, there has been relatively little research on the medicinal plants to be used against fish diseases. Herbal drugs can be used not only as remedies but even more so, as growth promoters, stress resistance boosters and preventatives of infections. Hence, herbal drugs in disease management are gaining success, because they are cost effective, eco-friendly and have minimal side effects [7].
Garlic can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish [8, 9, 10]. 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 [11].
The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella Proteus*, *Bacillus*, and *Clostridium*. Even acid-fast bacteria such as Mycobacterium tuberculosis are sensitive to garlic. Analysis of steam

distillations of crushed garlic cloves performed over a century ago showed a variety of allyl Sulfides isolated and identified the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur compound which they termed allicin from the Latin name of the garlic plant, *Allium sativum* [12, 13, 14, 15].

In aquacultural operations, garlic promotes growth, enhances the immune system, stimulates appetite, and strengthens the control of pathogens, especially bacteria and fungi. Many reports have documented that garlic can effectively eliminate principal pathogenic bacteria in fresh water fish, *including Pseudomonas fluorescens, Myxococcus piscicola, Vibrio anguillarum, Edwardsiella tarda, Aeromonas punctata f. intestinalis, and Yersinia ruckeri.* As a food additive in fish, garlic has a food calling effect and improves flesh quality. Garlic may also prevent heavy metal-induced alterations in the lipid profile [16]. These effects of garlic are attributed to the presence of various organosulfur compounds, including *allicin* [17]. Cloves of garlic were found in the tomb of Tutankhamun and in the sacred underground temple of the bulls of Saqqara. It has long been considered that garlic (*Allium sativum*) has several beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, and antihypertensive properties [18, 19].

The content of allicin and other sulfurous chemicals in garlic varies significantly and depends on several factors. For medicinal applications, higher levels of allicin are favorable [20]. The utilization of garlic in aquaculture has developed alongside the application and popularization of Chinese herbs in aquaculture. Most aquatic garlic researches have involved fresh garlic extracts, with experimental subjects either fed a garlic-added feed or treated with a garlic juice immersion. Allicin is the most powerful component present in garlic that actively and directly kills parasites [21]. Freshly pressed garlic, liquid garlic products are made for aquarium use, and even most food items containing garlic can be effective. Some people consider garlic to be an immune system booster and compare it to Vitamin C in humans [22]. The prophylactic effect of dietary garlic aplication to rainbow trout, infected with *Aeromonas hydrophila*, was confirmed by Nya and Austin (2011) [23]. Thanikachalam *et. al.*, in 2010, showed that the embedding of garlic peel in feed enhances the hematological parameters even at a low level (0.5%) incorporation and makes *Clarias gariepinus*, fingerlings, highly immunopotent and more resistant to infection by *A.hydrophila* [24].

2. Chemistry of Garlic

Garlic contains at least 33 sulfur compounds, several enzymes, 17 amino acids, and minerals such as selenium. It contains a higher concentration of sulfur compounds than any other Allium species. The sulfur compounds are responsible both for garlic's pungent odor and many of its medicinal effects. Dried, powdered garlic contains approximately 1% alliin (S-allyl cysteine sulfoxide). One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme alliinase, which metabolizes alliin to allicin. Allicin is further metabolized to vinyl thinner. This breakdown occurs within hours at room temperature and within minutes during cooking. Allicin, which was first chemically isolated in the 1940's, has antimicrobial effects against many viruses, bacteria, fungi and parasites. Garlic oil, aged garlic and steam-distilled garlic do not contain significant amounts of aliin or allicin, but instead contain various products of allicin transformation; none appears to have as much physiologic activity as fresh garlic or garlic powder [25].

3. Garlic as an Immunostimulant

The excess use of antibiotics has resulted in serious health and environmental problems. Consequently, the need of safe and effective alternatives of antibiotics is felt. In this context, immunostimulants have attracted significant attention. Garlic as a natural antibiotic is one of the most effective natural immunostimulants. Garlic has antioxidant properties [26] and also has beneficial effects on the cardiovascular and immune systems [27]. Generally, garlic takes effect by facilitates the function of phagocytic cells and increases their bactericidal activities; however, it can also stimulate natural killer cells, complement, lysozyme, and the antibody responses of fish. The activation of these immunological functions is associated with increased protection against infectious disease in fish. Garlic accelerates phagocytosis by macrophages [28]. Martins *et al*. [29] verified that the addition of garlic to fish diets increased the erythrocyte number, hemoglobin content, hematocrit, leukocyte number, and thrombocyte number. Garlic supplementation induced significant changes in serum total protein and globulin in rainbow trout [30]. The increases in the serum total protein, albumin, and globulin contents reflect strong innate immunity [31]. These findings are in agreement with the findings of Kyo *et al.* Iranloye, Ndong and Fall, and Nya and Austin. Aly *et al.* suggested that garlic improved the immune response of *O. niloticus* via a rapid increase in monocytes, and that over a longer time frame it enhances phagocytic activity, which affords increased protection against an immediate challenge with *A. hydrophila*, illustrating the anti-infection properties of garlic.

Cavallito and Bailey (1944) found that the antibacterial properties of crushed garlic could be attributed mainly to allicin. Allicin, the immunologically active component of garlic, has been found to affect oxidative stress and immune responses in several experimental systems. The inhibition of certain SH-containing enzymes in microorganisms by the rapid reaction of thiosulfinates with thiol groups was assumed to be the main mechanism involved in the antibiotic effect of garlic [36].

4. Effect of Garlic as an Antimicrobial Substance

Crude garlic extracts exhibited activity against both gram negative (E. coli, Proteus spp, *Salmonella, Serratia, Citrobacter, Enterobacter, Pseudomonas, Klebsiella*) and gram positive (*Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus sanguis, Group A Streptococcus, B anthracis*) bacteria at room temperature, but there were no significant effects if the garlic had been boiled for five minutes before testing [37, 38, 39, 40, 41].

Ajoene, a garlic-derived sulfur-containing compound, demonstrated antimicrobial activity against gram-positive bacteria, such as *Bacillus cereus, Bacillus subtilis, Mycobacterium smegmatis, Streptomyces griseus, Staphylococcus aureus* and *Lactobacillus plantarum* and against gram-negative bacteria, such as *Escherichia coli, Klebsiella pneumoniae*,and *Xanthomonas maltophilia*; ajoene also inhibited yeast growth at concentrations below 20 micrograms/mL [42, 43].

Garlic has antibacterial activity against *A. hydrophila* in freshwater, as reported by Diab [44, 45]. Nya and Austin (2009) reported that the use of garlic-supplemented diets for 14 d led to a marked reduction in mortality after challenge with *A. hydrophila*. Only 4% mortalities were recorded in groups fed 0.5 and 1% garlic-mixed feed compared to 88% mortality in the control group [30]. Sahu *et al*. (2007) obtained similar results for controlling *A. hydrophila* infection in Labeo rohita fingerlings, and they noted that the 0.1 and 0.5% added groups showed the highest level of survival (85%) compared to the control group (57%) [46]. Aly and Mohamed (2010) also found that *O. niloticus* fed a 3% garlic-supplemented feed showed a significantly increased survival rate (85%) after a challenge with *A. hydrophila* [35]. Zhang (2003) [47], surveyed the inhibitory effects of garlic on two isolates of *A. hydrophila*, AH_1 and AH_2, in vitro and found that the minimum inhibitory concentrations (MICs) were 15.6 and 1.95 mg/ml respectively. When examined with infected *Silurus soldatovi meridionalis* Chen (1977) at 50 mg garlic/kg diet, there was an obvious inhibitory and controlling effect on AH_2, but neither isolate was eradicated. This indicates that a higher concentration or longer time may be needed to obtain a healing effect in practice. Rahman *et al*. (2009) [48], evaluated the efficacies of antibiotics and medicinal plants on three common bacterial fish pathogens: *A. hydrophila*, *P. fluorescens*, and *E. tarda*. They found that young Thai silver barb (*Barbonymus gonionotus*) fed a diet supplemented with 8 mg/mL garlic showed the best recovery rate (90%) during the 10-d experimental period. This is almost in agreement with Lee and Musa (2008) [49], who reported that the MIC of 18 isolates of *E.tarda* ranged from 7.81 to 31.25 mg/mL, within which ET_{18} had an MIC value of 7.81 mg/mL.

Many reports have documented that garlic can effectively eliminate principal pathogenic bacteria in freshwater fish, including *Pseudomonas fluorescens, Myxococcus piscicola, Vibrio anguillarum, Edwardsiella tarda, Aeromonas punctate* and *f. intestinalis* [49, 50].

5. Garlic as an Antiprotozoal Agent

Garlic is effective in treating intestinal parasites has been known for a long time. Many folks healers and herbalists worldwide recommend garlic as a treatment for intestinal parasites. In some cultures, children infested with helminthes are treated with enemas containing crushed garlic.

An extract of garlic as effective against a host of protozoa such as *Opalina ranarum, Opalina dimidicita, Balantidium entozoon, Entamoeba histolytica, Trypanosoma, Leishmania, Leptomonas,* and *Crithidia* [51]. In addition, it was efficacious at killing wild-type amoebae isolated from the diseased fish, slowing the clinical signs of amoebic gill disease (AGD). However, it is necessary to study the toxicity and pathological effect of garlic on Atlantic salmon before using garlic to treat AGD in farmed Atlantic salmon.

6. Garlic and Fish Growth

In aquaculture, feed and feeding are among the most important factors influencing growth, feed utilization and tissue composition of the fish in intensive culture [51].

Garlic (*Allium sativum*) has been used to improve the growth and resistance of a number of livestock and fish. The use of garlic in fish farming has become popular for enhancing the activity of non-specific defense systems and conferring protection against diseases and it was used as a growth promoter in *O. niloticus* culture [45, 53], also it increased body gain, feed intake and feed efficiency ratio [53, 54]. Many reports have documented the effect of allicin as a growth promoter. Fo *et al*. (1990) [55], mixed a 1% garlic residue premix with the feed of grass carp, *C. idellus*, and common carp, *C. carpio*, in a polyculture system. After 3 mo of breeding, the feed intake rates of the grass carp and common carp were improved, and the feed coefficient decreased 23.5%. Zeng *et al*. (1996) [56], reported that when 50 mg/kg synthesized allicin was added to tilapia feed, the weight gain and survival rates increased by more than 2 – 3% after 45 d, the feed conversion ratio increased 11%, and biological appraisal was 12% higher than in the control group. Jia *et al*. (1999) found that the addition of 50 and 100 mg/kg allicin to soft-shelled turtle feed increased the weight gain rate by 26.97 and 45.355% ($P<0.01$), the feed conversion ratio by 15.18 and 17.37%, and the survival rate by 2.44 and 2.96%, respectively, compared to the control group [57]. Similar results were obtained in common carp when 100 mg/kg synthesized allicin and iodized allicin were added to carp feed [57, 58, 59].

Shalaby *et al*. (2006) [60], reported that the final weight and specific growth rate of *O. niloticus* increased significantly with increasing levels of *A. sativum*. These results are in agreement with those obtained by Khattab *et al*. (2004) [61]. In addition, Aly *et al*. (2008) and Aly and Mohamed (2010) [35, 62], examined the growth rates of Nile tilapia after feeding with garlic (10 and 449 20 g/kg diet fed), and found statistically non-significant increases after 1 or 2 mo, but a significant increase only after 8 mo, indicating that high doses or a long period was needed to enhance the growth rate. The most effective ingredient in garlic (allicin) is unstable, so the efficacy of garlic may vary considerably by species and preparation. A similar finding was reported by Huang *et al*. [63], who reported that rice field eel, *Monopterus albus* fed with 800 mg kg-1 composed allicin died within 3 days. Xiang and Liu found that the increased growth rate in red bellied pacu, *Colossoma barchypomum* followed by decreased with increasing amount of allicin [64].

The negative effects have also been reported. Ndong and Fall (2007) [34], reported that hybrid tilapia fed a garlic-supplemented diet at 0.5 and 1% exhibited no improvement in growth compared to those fed a control diet after 2 – 4 wk. This may be due to the brief experimental period, or the fact that the fish used in the experiment were larger (25.5 ± 1.0 g) than those used in the aforementioned two experiments (7±1 and 6.5±1.0 g, respectively), so the growth performance was not obvious. A negative effect was also observed in Manila clam, *Ruditapes philippinarum* [65].

The above results indicate that extremely high concentration of garlic extract or allicin does not improve fish growth; instead, they are harmful to fish health. This may be due to much alkyl sulfide that reaches the intestine, interfering with normal metabolism and suppressing mitosis, resulting in slow growth and even death. Therefore, garlic as a feed additive is not optimal for all fish species, and the optimal feeding amount is species-specific. Further study is needed to determine the optimum garlic concentration for specific fish species.

7. Effect of Garlic on Flesh Quality

Despite many studies on the effects of garlic in aquaculture, little is known about the effect of garlic on flesh quality. Currently, there is no perfect system for evaluating the flesh

quality of cultured fish. Nonetheless, the contents of crude protein, crude lipids, amino acids, water loss rate, and folding endurance of muscle may reflect the flesh quality to some extent. Long-term garlic supplemented feeding may lower the lipid and cholesterol content of fish. Moreover, allicin could activate intestinal proteases, which help to convert feed protein into fish protein, increasing the content of palatable amino acids. Luo *et al*. (2008) [66], found that a compound from *Eucommia ulmoides* and garlic could improve the flesh quality of grass carp, *C. idellus*. Aly *et al*. (2008) reported that the post-harvest flesh quality and shelf-life of fish fed a garlic-supplemented diet were improved [35]. Metwally (2009) found that the protein content in whole fish increased significantly in the group fed a garlic-containing diet, whereas the total lipid and ash contents decreased significantly in the same group [53]. These results are in agreement with those obtained by Xiang and Liu (2002) [64], Abdelhamid *et al*. (2002) [67], Khattab *et al*. (2004) [61], Shalaby *et al*. (2006) [60], and El-Dakar *et al*. (2007) [68].

Most fish feed is lacking in amino acids that generate an aroma and palatable taste, such as histidine, leucine, aspartic acid, and valine. The fragrant ingredients of fish are generally sulfur-containing base groups. Biochemical analysis has indicated that garlic contains various alkyl sulfide compounds and the C3H6S (O)-base group, which relates to flesh aroma. Therefore, the addition of garlic to feed could make up for the shortage and improve flesh quality.

8. Antioxidant Effect of Garlic on Stress

Among its many uses, garlic appears to have the fortunate capacity for protecting against the ravages of stress that affects the autonomic nervous and neuroendocrine system. Rats that were trained with endurance exercises to physical fatigue enjoyed improved parameters of aerobic glucose metabolism, attenuated oxidative stress, and vasodilation, when given garlic at a dosage of 2.86 g/kg 30 minutes before exercise [69]. In rats exposed to psychologically stressful situations, aged garlic extracts significantly prevented the decreases in spleen weight seen in control animals. Additionally, the garlic significantly prevented the reduction of hemolytic plaque-forming-cells in spleen cells and anti-SRBC antibody titer in serum caused by this psychological stress**.**

Moreover, a reduction in NK activities was observed in the psychological stress-exposed mice as compared with normal mice (non-stress), whereas NK activities in the garlic administered mice were almost equivalent to the mice not exposed to stressors [70].Garlic was able to block the lipopolysaccaride induced immune cytokine and plasma corticosterone and catecholamine changes following cold water immersion [71]..Aged garlic extract is also effective to prevent adrenal hypertrophy, hyperglycemia and elevation of corticosterone in hyperglycemic mice induced by immobilization stress [72]. Fishes from the intensive culture systems are continuously exposed to a form of stress, so it can lead in organism significant changes of biochemical and physiological level. Stress factors include: repeated handling, high density, therapeutic treatments, improper water chemistry, temperature changes etc. These results indicate that using Garlic in fish feed, can reduce levels of stress.

9. Conclusions

Aquaculture is currently facing a major challenge: how to relieve fishing pressure that is exerted on stocks and how, in the same time, meet the growing demand for aquatic food products, all without causing an ecological disaster and without endangering public health. It is currently believed that in the near future aquaculture will largely develop in Morocco. To avoid the potential environmental impact it is important that the aquaculture sector uses the simplest and healthiest techniques, concise and based on scientific research to also ensure the sustainable development of this activity. Garlic can also be used as an alternative to antibiotics or chemotherapeutic agents; however, more research is needed under practical conditions.

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