Fish Physiol Biochem (2021) 47:1179–1198
https://doi.org/10.1007/s10695-021-00952-7

Exploring the garlic (*Allium sativum*) properties for fish aquaculture

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Received: 17 September 2020 / Accepted: 12 April 2021 / Published online: 23 June 2021

Abstract The aquaculture industry's rapid growth to meet commercial demand can trigger an outbreak of infectious diseases due to high-density farming. Antibiotic overuse and misuse in fish farming and its global health consequences have led to searching for more natural alternatives such as medicinal plants. In this sense, garlic (*Allium sativum*) has different bioactive compounds with biological properties for animal health. Among them are the ajoene, alliin, and allicin, which confer biological properties such as growth promotion, antimicrobial, antiviral, antioxidant, and antiparasitic. Ways to use garlic in aquaculture include oil, fresh mash, aqueous extract, and garlic powder. The powder presentation is the most used in aquaculture; it is generally applied by oral administration, adding to the feed, and the dose used ranges from 0.05 to 40 g/kg of feed. Garlic has been used in the aquaculture of different species such as rainbow trout (*Oncorhynchus mykiss*), spotted grouper (*Epinephelus coioides*), catfish (*Clarias gariepinus*), tilapia (*Oreochromis niloticus*), guppy fish (*Poecilia reticulata*), goldfish (*Carassius auratus*), and barramundi (*Lates calcarifer*). In addition to its properties, garlic's usage became popular, thanks to its low cost, easy incorporation into food, and little environmental impact. Therefore, its application can be an effective solution to combat diseases, improve organisms' health using natural supplies, and as an alternative to antibiotics. This review reports and discusses plant-derived products' beneficial properties, emphasizing garlic and its usages in fish aquaculture.

Keywords Immunostimulants · Garlic (*Allium sativum*) · Aquaculture · Fish immunology · Feed additives

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Introduction

About aquaculture

The aquaculture industry is one of the fastest-growing sectors globally; in this context, aquaculture alone contributes to 47% of the world's aquatic animal production. Of this percentage, the majority is attributed to fish farming (54.3 million tons), followed by mollusks (17.7 million tons) and crustaceans (9.4 million tons) (FAO 2020). The intensification of aquaculture is a common practice to meet the growing demand. However, animals' overcrowding favors the appearance and spread of pathogenic microorganisms, increasing disease outbreaks and consequent mortality (Reverter et al. 2020). If not managed properly, this can cause severe economic losses and increase the risk of the activity's sustainability (Alexander et al. 2010).

In fish and crustacean farming, around 40 to 60% of the production is lost due to infectious diseases (Raman 2017; Stentiford et al. 2012). For a long time, chemotherapeutic agents, including antibiotics, have been applied as effective remedies for disease outbreaks in aquatic animals; however, aquatic pathogens have become resistant to such treatments due to their excessive usage (Reverter et al. 2020). Fortunately, the modern aquaculture requires sustainable systems, and antibiotics are being replaced with environmentally friendly alternatives, including probiotics, prebiotics, postbiotics, and immunostimulants (Akhter et al. 2015; Banerjee and Ray 2017; Hai 2015; Pérez-Sánchez et al. 2018; Soliman et al. 2019; Wang et al. 2019; Zaineldin et al. 2018; Zorriehzahra et al. 2016).

Probiotics, whose term "pro-bios" means "for life" (Gismondo et al. 1999), were initially defined as the organisms that contribute to the intestinal microbial balance and which naturally help to improve the general state of health of the host organism (Parker 1974). In practical terms, probiotics are live microorganisms that confer health benefits to the host when administered in adequate amounts. These beneficial microorganisms can colonize and proliferate in the host's intestine, providing numerous beneficial effects by modulating various host's biological systems (Cross 2002), conferring nutritional advantages but, more importantly, functioning as a viable therapeutic strategy to overcome the adverse effects of antibiotics in the management of infectious diseases (Newaj-Fyzul and Austin 2015).

Prebiotics can improve the biological responses of many aquatic animals, including the host's protection against infections through stimulation of the immune system; thus, the mortality caused by the invasion of pathogens decreases (Dawood and Koshio 2016). Examples of prebiotics, also referred to as functional saccharides or immunosaccharides, include inulin, oligofructose, xylooligosaccharide, fructooligosaccharide (FOS), mannanoligosaccharide (MOS), galactooligosaccharide (GOS), and β-glucan (Akhter et al. 2015; Carbone and Faggio 2016; Dawood and Koshio 2016; Mohan et al. 2019; Song et al. 2014). Immunostimulants are diverse in origin, chemical nature, and action mode but stimulate the immune system directly, rather than through the probiotic products (Newaj-Fyzul and Austin 2015; Song et al. 2014; Tafalla et al. 2013; Vallejos-Vidal et al. 2016).

Immunostimulants are agents that activate the immune system; in this regard, these began to be used in aquaculture as enhancers of the non-specific immune response of fish, increasing resistance to diseases (Vásquez-Piñeros et al. 2012). Although some prebiotics are immunostimulants, these are excluded from the typical definition of immunostimulant, which refers to other types of compounds such as bacterial structural elements (lipopolysaccharides, glycoproteins, lipopeptides), products of β-1,3/1,6 glucans, and some other carbohydrates with complex structures, vitamins, carotenes, minerals, and plant extracts (Ganguly et al. 2013).

Plants and aquaculture

Use of plants

The use of plants for animal health is an ancestral practice, and modern science has validated some of its applications (Reverter et al. 2014). Plants and their extracts can act as enhancers of the immune system (Hernández-Contreras and Hernández 2020), providing a more natural and environmentally friendly alternative to counteract infectious diseases instead of using antibiotics and synthetic substances. Previous evidence demonstrated that plants and their derivatives have immunostimulant and health-promoting properties and also can be growth enhancers, appetite stimulators, antimicrobials, anti-inflammatory, antitumoral, efficiency enhancers of nutrient utilization, and improvers of physiological status (Cinatl et al. 2003; Wada et al. 1987; Zaki et al. 2012; Zhang et al. 1990). Some of these properties are associated with the different bioactive compounds such as terpenoids, alkaloids, tannins, saponins, flavonoids, pigments, phenols, essential oils, glycyrrhizin, polysaccharides, isoprenoids, and organosulfur compounds, among others (Awad and Awaad 2017; Chanu et al. 2012; Hernández-Contreras and Hernández 2020; Reverter et al. 2014; Van Hai 2015; Villanueva-Gutiérrez et al. 2015). Besides, garlic's bioactive compounds can inhibit or block virus transcription to reduce replication in host cells and improve innate immunity (Citarasu 2010).

Some plants' low commercial value and accessibility facilitate their uses as raw extracts or bioactive compounds on a large scale in aquaculture, either by direct application or by incorporating into commercial foods (Awad and Awaad 2017). However, plants' biological activity can vary considerably depending on the part of the plant (shoot or root systems) and the type of extract. Therefore, information about plant's bioactive compounds and their proper dosage is required to prevent toxic effects on organisms (Kavitha et al. 2012; Reverter et al. 2021). The parts of the plants commonly used for their application in aquaculture include the leaves, the pulverized plant, root, seeds, barks, fruits, and flowers (Reverter et al. 2017).

The ways of using plant derivatives in aquaculture include injection (intramuscular and intraperitoneal), oral administration, and immersion (Agus Putra et al. 2013; Ji et al. 2012; Wu et al. 2010). Although intraperitoneal injection has proven to be the fastest and most efficient administration method, it is a costly strategy as well as the immersion option; both are laborious and stressful procedures for the organisms (Syahidah et al. 2015). Besides, the immersion option has the risk of releasing exogenous molecules if the effluents are not previously treated, which can be harmful to the marine or aquatic environments (Forwood et al. 2013; Umeda et al. 2006). On the other hand, oral administration does not imply generating stress to organisms; it is cheaper and more accessible, making it a suitable aquaculture strategy (Sakai 1999; Balasubramanian et al. 2008).

There are many plants whose extracts are of interest in aquaculture. In this regard, more than 250 plant species belonging to 75 families and 32 orders have been identified (Reverter et al. 2017). Although many plants are used for specific purposes, others have been applied for various purposes; for example, garlic, an allium vegetable whose application has been almost universal in farm animals for multiple purposes. Garlic is a commonly used spice whose health benefits have been documented (Vallejo Villalobos et al. 2008; Abdel-Tawwab et al. 2020; Adineh et al. 2020). It contains various bioactive compounds, including allicin and other organic sulfur compounds, phenols, polysaccharides, and saponins (Szychowski et al. 2018). This arsenal gives garlic attributes such as antimicrobial, antioxidant, hypertensive, anti-inflammatory, anti-cancer, cardiovascular protective, antidiabetic, and anti-obesogenic properties (Guo et al. 2012; Shang et al. 2019; Szychowski et al. 2018; Yun et al. 2014).

Garlic generalities

Garlic (*Allium sativum*) is a perennial plant belonging to the family Liliaceae. It has been used for centuries as a flavoring agent, traditional medicine, and functional food to improve humans' health (Lee et al. 2014). In this regard, humans have used garlic (Order Asparagales, Family Amaryllidaceae) for over 7000 years for culinary and medicinal purposes (Reverter et al. 2017). It is a popular plant product with benefits by treating venous insufficiency, hypertension, antibacterial action, and many others (Gambogou et al. 2018b; Gbekley et al. 2018).

Garlic's proximate composition records an average of 65% water (compared to over 85% of fresh vegetables), 27.5% carbohydrates, 4.7% fiber, 2–3% organosulfurated compounds, and 2% protein (Gambogou et al. 2018a). The biochemical composition of garlic is shown in Table 1. Most of the garlic properties are attributed to organo-sulfurated compounds (Darbyshire and Henryf 1981; Gambogou et al. 2018a). The most common organosulfur compounds include S-allyl cysteine (SAC), S-allyl-mercaptocysteine (SAMC), and allicin, which are abundant in aged garlic extracts and garlic maceration (Drago Serrano et al. 2006).

Allicin is a highly unstable, volatile, cytotoxic, fat-soluble organosulfide compound (Amagase et al. 2001). It is obtained when the garlic bulb or clove is cut or crushed, and the alliin (another organo-sulfurated compound) comes in contact with the enzyme alliinase (it smooths the cysteines of cytosolic sulfoxide). Thereafter, allicin is obtained (Fig. 1), giving the garlic its characteristic odor and some of its pharmacological and therapeutic properties (Gökalp 2018). Also, it is the most active compound in garlic, and it is associated with antiseptic, antiviral, antifungal, antiparasitic, and antibacterial activities (Guo et al. 2015; Lee et al. 2014). For example, garlic has been demonstrated to be an anti-ectoparasitic agent; its use as oil is effective against protozoa such

Table 1 Biochemical composition of garlic (*Allium sativum*) reported by Anton (2016) and Gambogou et al. (2018a)

Carbohydrates	Monosaccharides (fructose, glucose) Disaccharides (sucrose, lactose) Trisaccharides (raffinose) Tetrasaccharides (tetrafructose, escorodose) Depolysaccharides (starch, dextrin, inulin, fructosan) D-galactane L-arabinose Pectinsfructane D-fructan
Lipids	Fatty acids (linoleic acid, linolenic acid, oleic acid, palmitic acid) Triglycerides Phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine) Prostaglandins (prostaglandin A, prostaglandin E, prostaglandin F)
Proteins	Proteins and amino acids (lysine, threonine, valine, methionine, isoleucine, tryptophan, phenylalanine, leucine, histidine, arginine, aspartic acid, serine, glutamine, proline, glycine, alanine, and cysteine)
Vitamins	Vitamin A Vitamin B1 Vitamin B2 Vitamin B6 Vitamin C Vitamin E
Sulfur compounds	Allicin and allicin derivatives (various trisulfides, ajoene, dialyl-disulfide) Aliin (S-allylcysteine-sulfoxide) Glutamyl-S-allylcysteine Methin (S-methylcysteine-sulfoxide) Isoaliin (S-trans-1-propenylcysteine-sulfoxide)
Minerals	Phosphate Potassium Magnesium Copper Iron Manganese Zinc Selenium (dimethylselenide, methyl-ester-metanosulfenoselenoic acid, dimethyldiselenide, bi-(methylnethyon)-selenide, allylmethylsulfide acid, methylster-2-propensulfenoselenoic acid, propilester-1-propenic alylethylethylethylselenid acid)
Pigments	Chlorophyll Carotenoids Anthocyanins (these are water-soluble pigments which give a red color, purple or blue)
Other compounds	Phenol acid Organic acid Saponósidos Flavonoids Fitohemaglutininas Gibberellins A3 and A7

Plasmodium spp., *Trypanosoma* spp., *Leishmania* spp., and *Giardia* spp. (Anthony et al. 2005). Aqueous garlic extracts are also useful against hymenolepiasis and giardiasis (Soffar and Mokhtar 1991). Evidence has demonstrated that garlic is effective against nematodes; for instance, aqueous extracts can control *Trichuris muris* and *Angiostrongylus cantonensis* (Klimpel et al. 2011). As a thiosulfinate, allicin is a reactive sulfur species causing a redox reaction with thiol groups in glutathione and proteins, a mechanism that is essential for its biological activity (Borlinghaus et al. 2014).

Allicin and ajoene are the primary active compounds conferring antiparasitic properties to garlic

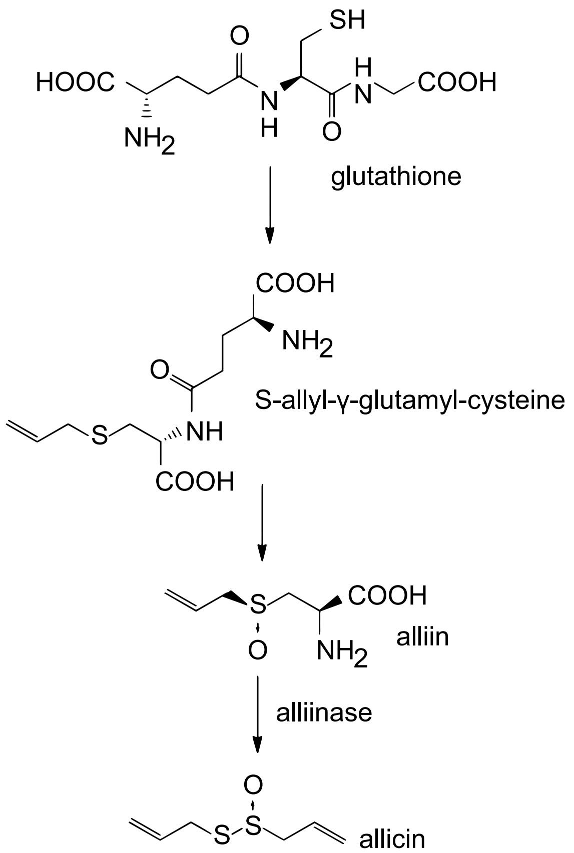

Fig. 1 Allicin biosynthesis. It starts from glutathione which is hydrolyzed to γ-glutamyl-S-allylcysteine, and this is subsequently oxidized to alliin (inactive precursor of allicin) by the enzyme Y-glutamyl transpeptidase. Finally, alliin is hydrolyzed by the action of the enzyme alliinase to produce allicin. Modified image of Borlinghaus et al. (2014)

and serve veterinary purposes (Ankri and Mirelman 1999; Reverter et al. 2017). Allicin has antiparasitic activity in humans and animals against *Trypanosoma brucei*, *Plasmodium falciparum*, *Giardia lamblia*, *Entamoeba histolytica*, and *Leishmania* spp. (Saif et al. 2020).

Garlic's curative properties have been used to treat bacterial infections (Gambogou et al. 2018a). The antibacterial activity of some garlic extracts has been attributed to several thiosulfinates, including allicin (Ankri and Mirelman 1999; Karimi Pashaki et al. 2020). For example, alliinase inhibition, an enzyme that catalyzes alliin to allicin, eliminates the garlic extract's antibacterial activity (Jonkers et al. 1999). A wide diversity of bacteria is killed or inhibited by garlic extracts, including one of the most relevant that is the inhibition and bactericidal activity against *Helycobacter pylori*, which is the most common bacterial infection in the world and prevalent gastric bacterial pathogen in humans (Zardast et al. 2016).

Garlic has also been shown to have antiviral activity (Mikaili et al. 2013). Ajoene (allicin condensation product) is known to be responsible for the antiviral activity of garlic, and its mechanism of action relies on blocking the processes dependent on integrins in an infected cell system, in this case, by the virus of human immunodeficiency (El-dougdoug et al. 2018; Gökalp 2018). Also, in vitro and in vivo approaches have demonstrated the antiviral activity of garlic extracts. Particularly the sulfur constituents have antiviral activity against human-relevant viruses, including influenza B, parainfluenza virus type 3, herpes simplex virus types 1 and 2, *Coxsackie virus* spp., vaccinia virus, vesicular stomatitis virus, human immunodeficiency virus type 1, and human rhinovirus type 2 (Sharma 2019; Singh and Singh 2019). In this regard, Singh and Singh (2019) reported that the order for virucidal activity is ajoene > allicin > allyl methyl thiosulfinate > methyl allylthiosulfinate.

Another important garlic property is its immunostimulant activity attributed to the organosulfur compounds, polysaccharides, and fructans (Chandrashekar and Venkatesh 2012; Saif et al. 2020). Such compounds increase immunity, stimulate lymphocyte proliferation and macrophage phagocytic activity, have immunomodulatory effects on T cells and the molecules of adhesion, and participate in the inhibition of NF-kappaB (Bruck et al. 2005). The above garlic compounds can also increase the activity of natural killer cells, IL-2 (interleukin-2), TNF (tumor necrosis factor), and interferon-gamma (Gurley et al. 2005). Also, allicin has an inhibitory effect on intestinal epithelial cells, resulting in decreased intestinal inflammation caused by pathogenesis (Lang et al. 2004). Furthermore, garlic can promote phagocytosis by macrophages. Still, it is unclear if these compounds facilitate the phagocytosis process or accelerate the process by an indirect mechanism (Shakya and Labh 2014).

Compared to other medicinal plants, garlic's application has proven to be the most effective in animal health at the laboratory level (Guo et al 2012). This is due to its various therapeutic properties, especially its broad spectrum against pathogens and its immunostimulating effect (Guo et al. 2015; Lee et al. 2014).

Also, it is easy to obtain, has low cost, and is easy to administrate by the oral route, injection, and immersion. Oral administration is the most used because it does not generate stress in animals (Agus Putra et al. 2013; Ji et al. 2012). On the other hand, as mentioned above, garlic contains various active components, the most prominent being the organosulfur compounds (López 2007; Campelo et al. 2020). The presence and proportion of these compounds depend on the different processing methods (López 2007).

Application and bioactivity of garlic in aquaculture

Garlic has been used in different presentations in aquaculture (Table 2). Among the most used presentations are garlic powder, essential oil, garlic macerated in alcohol, and aged garlic extract (Amagase et al. 2001; López 2007; Subramanian et al. 2020).

Garlic powder is commonly used as a flavoring for seasonings and processed foods (Amagase et al. 2001; Miron et al. 2004). The composition of garlic powder and raw garlic is similar; however, the proportions of various compounds can vary significantly (Iberl et al. 1990b; Amagase et al. 2001; Subramanian et al. 2020). For example, raw garlic has 8 g/kg of alliin, and a dehydration process without loss of ingredients would result in an amount of alliin of 20–25 mg/g in the powder. However, garlic powder only has 1% alliin at most (Lawson and Hughes 1992). This implies that most of the alliin is lost during the dehydration process. As for garlic powder, the allicin content is well below average, reflecting its instability in the processes (Freeman and Kodera 1995). Although garlic powders contain compounds similar to raw garlic, their proportions do not, which can vary significantly (Amagase et al. 2001).

On the other hand, garlic oil used for therapeutic purposes is obtained through the steam distillation process (Subramanian et al. 2020). The essential oil content in garlic cloves is 0.2–0.5%, and it contains sulfide groups such as DADS and diallyl trisulfide (Yan et al. 1992). The steam distillate contains allyl methyl, diallyl, and dimethyl mono parahexa sulfide (Subramanian et al. 2020). Water-soluble compounds are completely removed during this process, such as allicin (Amagase et al. 2001).

The products macerated in oil are made from raw garlic cloves ground in vegetable oil and packed in gel capsules (Amagase et al. 2001). During the manufacturing process, some alliin is converted to allicin (Lawson 1998). Because allicin is unstable and decomposes rapidly, oil mash preparations contain decomposed allicin compounds such as dithiins, ajoene, sulfides, residual amounts of allicin, and other garlic constituents (Block 1985; Iberl et al. 1990a). However, the standardization to obtain ingredients in mass has not been widely explored.

The aged garlic extract (AGE) is processed differently from the other presentations of garlic. It is allowed to age up to 20 months (Amagase et al. 2001). During this process, the odorous, harsh, and irritating garlic compounds are naturally converted into stable and safe sulfur compounds (Lawson 1993; Amagase et al. 2001). AGE mainly contains water-soluble components such as SAC and SAMC and also contains stable fat-soluble allyl sulfides, flavonoids, phenolic compounds, saponins, and other essential nutrients (Weinberg et al. 1993; Imai et al. 1994; López 2007).

All these presentations in which we can find garlic have been used in aquaculture. For instance, Metwally (2009) used garlic diets in their different presentations (natural, oil, and powder), concluding that adding garlic in any presentation to the diet improved the growth rate, decreased mortality rate, and increased antioxidant activity in fish. On the other hand, Prieto et al. (2005) suggest that the most effective presentation is fresh crushed garlic. The presence of sulfur atoms in the molecules, both in the fat-soluble fraction (alein) and in the water-soluble one (allicin), is known to be fungicidal and bactericidal. This presentation has been used as a fungicide against *Saprolegnia parasitica* in doses of 200 mL/L, having effectiveness of 100%. By contrast, when subjected to a process such as dehydration, its effectiveness drops to 80%. This can be explained by the loss of garlic ingredients when exposed to any process (Amagase et al. 2001; Subramanian et al. 2020). Abd El-Galil and Aboelhadid (2012) reported that the application of garlic oil and freshly crushed garlic cloves in the treatment of trichodiniasis and gyrodactylosis in tilapia (*Oreochromis niloticus*) is effective for use in hatcheries and are promising treatments for field application.

The efficacy of freshly crushed garlic compared to other presentations is due to the interaction of the alliin compound and the allinase enzyme that results in the formation of the allicin compound (Gökalp

Table 2 Garlic (*Allium sativum*) presentations used in fish farming experiments

Presentation	Species	Application method	Results	Reference
Raw garlic	Tilapia (*Oreochromis niloticus*)	Diets (0.5 and 1 g/kg raw garlic)	No significant differences between treatments were detected. Garlic diets improved the immune response	Ndong and fall 2011
Oil and cloves of garlic	Tilapia (*Oreochromis niloticus*)	Bath (2, 2.5, and 3 ppm garlic oil) (300 mg/L $^{x\,1}$ of crushed garlic cloves)	Tilapia recorded parasite recovery rates of 65% with garlic oil treatment and 75% with the treatment of crushed garlic cloves	Abd El-Galil and Aboelhadid 2012
Aqueous extract of garlic and garlic powder	Guppy fish (*Poecilia reticulata*)	Bath aqueous garlic extract (7.5–and 12.5 mL L^{-1}) and diets (10 and 20% garlic powder) in *G. turnbulli* infected guppies	The prevalence and intensity of parasites were significantly reduced compared to control However, histopathology revealed elevated muscular dystrophy in the garlic-fed group at 20%, compared to control	Fridman et al. 2014
Garlic extract	Barramundi (*Lates calcarifer*)	Bath (1, 2, 10, and 20 mL/L)	Garlic significantly decreased the hatching of the eggs (only 5% hatched) contrasting with the high percentage of hatching in the control (95%)	Militz et al. 2014
Casfaces and garlic cloves	African Catfish (Clarias gariepinus)	Diets (0, 10, 20, and 30 g/kg^{-1})	Significant increase in weight, food conversion, and survival parameters (64%) fish-fed garlic cloves	Eirna-liza et al. 2016
Aqueous garlic extract	Common carp (*Cyprinus carpio*)	Bath (200 mg/L garlic aqueous extract)	Aqueous garlic extract has a low toxicity in common carp; therefore, it can be used safely in this species for any experimental purpose	Syngai et al. 2016
Garlic extract	Rainbow Trout (*Oncorhynchus mykkis*)	Diets (1, 1.5, and 2% garlic extract)	Weight gain and growth rate of fish improved significantly with diets containing garlic	Etyemez Büyükdeveci et al. 2018
Garlic powder	Red Tilapia (*Oreochromis*sp.)	Diets (1, 1.5, and 2% garlic powder)	Adding 1% to 1.5% garlic powder in the diet improved food utilization and survival rate of red tilapia	Samson 2019
Raw garlic polysaccharide	African catfish (*Clarias gariepinus*)	Diets (0, 0.5, 1.0, 2.0, and 4.0%/kg)	The addition of raw garlic polysaccharid increases growth parameters, hematological indices and food consumption, food conversion index, and protein efficiency index	Gabriel et al. 2019

Table 2 (continued)

Presentation	Species	Application method	Results	Reference
Aqueous garlic extract	Guppy fish (*Poecilia reticulata*)	Diets (0, 0.10, 0.15, and 0.20 mL kg^{-1} aqueous garlic extract)	Increased immune parameters of skin mucus, which is the first barrier to pathogens. However, the addition of aqueous extract did not have a significant effect on final body weight and weight gain	Motlagh et al. 2020

2018), which, as mentioned above, is an active agent against parasites (Ankri and Mirelman 1999; Reverter et al. 2017). Furthermore, it can penetrate living tissue, which has implications for its potent and prolonged effect (Miron et al. 2000).

Garlic properties for their application in aquaculture

As mentioned, garlic has bioactive compounds providing various biological properties that increase fish resistance to aquaculture diseases (Shakya and Labh 2014; Erguig et al. 2015; Foysal et al. 2019). In fish aquaculture, garlic presents antibacterial, antiparasitic, antioxidant, immunostimulatory, and growth-promoting activities (Bender-Bojalil and Bárcenas-Pozos 2013; Lee 2012); however, its use is mostly limited to the experimental level, while its use as an ingredient at an industrial level is still awaiting.

Antibacterial activity

The antibacterial activity results from the modification of lipid biosynthesis and RNA synthesis in bacteria, inhibiting gram-positive and gram-negative bacteria (Bender-Bojalil and Bárcenas-Pozos 2013). The antibacterial activity of garlic conferred by allicin has demonstrated the ability to inhibit gram-positive growth like *Staphylococcus aureus* (Li et al. 2011) and gram-negative bacteria as *Escherichia coli* and *Aeromonas salmonicida* (Nya et al. 2010; Oosthuizen et al. 2018). Allicin is a sulfur-containing compound and a recognized quorum sensing cooling molecule inhibiting biofilm formation and virulence (Bayan et al. 2014; Jakobsen et al. 2012). Musa et al. (2008) studied the minimum inhibitory concentration (MIC) of aqueous garlic extracts (500, 250, 125, 62.5 mg mL^{-1}) to inhibit the growth of *Staphylococcus aureus*, *Streptococcus agalactiae* (gram-positive), *Citrobacter freundii*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* (gram-negative), and *Edwardsiella tarda*. Results revealed that all garlic extracts were effective against the pathogenic bacteria tested.

The activity against pathogen bacteria is manifested by the higher survival rates of diverse infected fish when garlic extracts are used as dietary ingredients. Robust evidence about this has been documented. For example, Nya and Austin (2009) tested diets added with different concentrations of garlic for rainbow trout (*Oncorhynchus mykiss*) infected with *Aeromonas hydrophila*, and the experiment lasted 14 days and revealed a reduction in mortality of at least 4% compared to controls (88%). Thanikachalam et al. (2010) evaluated the use of garlic husk (*Allium sativum*) in infected African catfish (*Clarias gariepinus*) with *Aeromonas hydrophila*. Powdered garlic peel was incorporated into the diets (0%, 0.5%, 1.0%, and 1.5%) and fed to catfish fry for 20 days. Results revealed higher survival rates in fish fed with any garlic concentrations than in the control group. Also, Talpur and Ikhwanuddin (2012) evaluated the resistance of the Asian sea bass (*Lates calcarifer*) to the *V. harveyi* infection when fed with diets containing garlic (*Allium sativum*). Diets supplemented with garlic favored survival, particularly in those consuming 10 g kg^{-1} of feed, registering 83.4% compared to 33.3% in control.

In general, improvements in the productive response and resistance to pathogen challenges have been widely documented in a variety of fish when raw garlic, extracts, or plant parts are used as dietary ingredients (Guo et al. 2012, 2015; Breyer et al. 2015; Eirna-liza et al. 2016; Foysal et al. 2019). The antimicrobial and antioxidant activities seem to play a role in the physiological status of fish.

Antioxidant activity

Garlic's phenols and saponins are compounds that provide antioxidant activity (Oosthuizen et al. 2018; Szychowski et al. 2018; Shang et al. 2019). Such compounds can inhibit the formation of free radicals, reinforce the uptake mechanism of endogenous radicals, and increase cellular antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) (Liu et al. 2018). The modifier subunit glutamate-cysteine ligase (GCLM) protects low-density lipoproteins from oxidation caused by free radicals (González Maza et al. 2014; Liu et al. 2018). Likewise, using garlic as a feed supplement can also improve the antioxidant system of fish (Metwally 2009; Chanu et al. 2012). For example, Metwally (2009) tested the effect of garlic on the various antioxidants present in Nile Tilapia (*Oreochromis niloticus*); for 3 months, four fish groups were fed with diets containing different garlic sources: natural garlic (a diet of 40 g kg^{-1}), capsules of garlic oil (Strongus®, capsules of pure garlic oil; diet of 250 mg kg^{-1}), and garlic powder tablets (32 g kg^{-1} diet), and a control group without garlic. Results revealed enhanced activities of GPx, SOD, and CAT in fish consuming any of the garlic-based products.

Mohebbi et al. (2012) analyzed the antioxidative effect of dietary garlic on rainbow trout (*Oncohorynchus mykkis*). Trout fingerlings were fed on diets containing 10, 20, 30, 40, and 50 g of garlic powder per kilogram of formulated feed. Serum lipid peroxides and activities of antioxidant enzymes were measured, revealing that garlic consumption resulted in a significant decrease of lipid peroxidation and substantial reductions in the catalase activity. By contrast, serum alanine aminotransferase, aspartate aminotransferase levels, and superoxide dismutase activity increased in all garlic-treated groups compared with the control. Also, Altinterim and Aksu (2019) evaluated the antioxidant activities of CAT, glutathione reductase (GR), glutathione peroxidase (GPx), and malondialdehyde (MDA) in the rainbow trout (*Oncorhynchus mykiss*) sera. Fish were fed diets containing garlic (*Allium sativum*), garlic Tunceli (*Allium tunceli-anum*), and onion (*Allium cepa*) oils at a concentration of 10 g kg^{-1} for 21 days; at the end of the trial, the GPx activity of the macerated garlic oil group and the CAT, GR, and MDA activities of the onion group registered significant increases.

Antiviral activity

Fresh garlic extracts in which allicin is known to be the main active component have antiviral activity (Ankri and Mirelman 1999). In comparison with garlic's antibacterial action, scarce information has been published regarding its antiviral properties (Bayan et al. 2014). Karimi Pashaki et al. (2020) evaluated the influence of garlic extract (*Allium sativum*) on the survival rate of the common carp (*Cyprinus carpio*) after exposure to spring viremia of carp virus (SVCV). The fish were fed 1 and 5 g of garlic extract per kilogram of the basal diet for 8 weeks. Results showed that the survival rate of carps fed with both garlic extract concentrations increased significantly compared to the control group, especially the diet containing 5 g. The results indicated that the addition of garlic extract in the fish diet has led to a higher immunity and survival rate of common carp exposed to SVCV. It is possible that the antibacterial activity is not the best characteristic of garlic and its extracts and that its positive effect is mainly due to its antimicrobial and antioxidant activity. However, this is the least explored field on the use of garlic in aquaculture.

Antiparasitic activity

In aquatic organisms, ectoparasite infestations' occurrence is not the exception; the presence of parasites in fish aquaculture is a recurring issue that affects production. Abd El-Galil and Aboelhadid (2012) exposed tilapia (*Oreochromis niloticus*) infested with *Trichodina* and *Gyrodactylus* to garlic oil dip baths. Herein, five tilapia groups were exposed to baths with garlic oil (1, 1.5, 2, 2.5, and 3 ppt) and a control group (0% garlic oil) for 24 h. Results revealed that tilapia registered recovery rates of $\geq$55% when exposed to garlic oil treatment.

Militz et al. (2014) infected the barramundi fish (*Lates calcarifer*) with *Neobenedenia* sp. and exposed the organisms to a therapeutic garlic extract bath using four concentrations (1, 2, 10, and 20 mL L^{-1}) and a control (freshwater with formalin). The garlic extracts significantly prevented the hatching of the eggs (only 5% hatched) and the longevity of the larvae (< 2 h), contrasting with the high hatching percentage in the control (95%). Low concentrations of garlic extract (1 and 2 mL L^{-1}) decreased *Neobenedenia* sp. infection's success down to 25 and 11%, respectively. In another approach with the guppy fish (*Poecilia reticulata*). Fridman et al. (2014) exposed infected fish with *Gyrodactylus turnbulli* to therapeutic baths with garlic extracts (7.5 to 30 mL L^{-1}) and fed other infected fish (*Gyrodactylus turnbulli* and *Dactylogyrus*) with formulated diets containing garlic powder (10 and 20%) for 14 days. The results of this approach revealed that the exposure to the garlic baths caused the detachment of the parasite in the fish, showing a positive correlation between the garlic concentration and the time of detachment and death of the parasites which, at the highest concentration (30 mL L^{-1}), occurred at 4.1 and 8.6 min, respectively. For the groups of fish fed with diets containing garlic, a lower prevalence and intensity of parasites were observed compared to the control. Saha and Bandyopadhyay (2017) exposed the goldfish (*Carassius auratus*) infected with Trichodinid to therapeutic baths with different concentrations of garlic extract (5, 10, 15, 20, 25, and 30 mg L^{-1}), demonstrating that 15 mg L^{-1} of garlic extract was more effective for the reduction of tricodinides, highlighting the antiprotozoal activity of garlic.

The antiparasitic activity of garlic as a potential advantage for aquaculture is undeniable; moreover, using garlic is not only applicable to diseased fish but also as a prevention measure. For example, Militz et al. (2013) developed a preventive treatment against *Neobenedenia* sp. (Platyhelminthes) infection using food supplemented with garlic for the barramundi fish (*Lates calcarifer*); two garlic diets (50 and 150 mL L^{-1}) and a control diet (0% garlic) were administered for 10 and 30 days. Results indicated that feeding with the garlic-added diets for 30 days was effective prevention because the infection decreased by 70% compared to the control and did not affect the food's palatability.

Immunostimulant activity

The immunizing effect of garlic has been used in the rainbow trout, hybrid tilapia, and Asian sea bass. Increases in the cellular (phagocytic activity) and humoral responses (total proteins, lysozymes, anti-proteases, and bactericidal activities) were registered after using raw garlic or garlic extracts as additives in formulated feeds (Ndong and Fall 2011; Nya and Austin 2009; Talpur and Ikhwanuddin 2012). In guppy fish (*Poecilia reticulata*), the administration of garlic extract in food increased the skin mucus' immune parameters, which is the first barrier against pathogens (Motlagh et al. 2020). In crustaceans, the inclusion of garlic in the diet increased the expression of the Penaeidin, Crustin, Lysozyme, Toll-like, and tumor necrosis factor genes in the kuruma shrimp (*Marsupenaeus japonicus*) (Tanekhy and Fall 2015), while in the red crab (*Procambarus clarkii*), the total hemocytes and SOD activity increased (Mona et al. 2015). Also, the addition of garlic increased food consumption and improved protein and amino acid utilization in shrimp (*Litopenaeus vannamei*) compared to organisms fed with the fishmeal-based diet (Tazikeh et al. 2020).

Growth promoter

The inclusion of garlic in fish feed can also influence growth performance due to organosulfur compounds such as allicin, which is a potent stimulant for the "smell" or chemoreception of most aquatic animals, which increases the intake of food in fish and crustaceans (Lee 2012). The effect on growth performance from the incorporation of garlic in food has been tested in different aquatic species. Aly and Atti (2008) fed tilapia (*Oreochromis niloticus*) with a diet supplemented with garlic (10 and 20 g kg^{-1} diet) for 2 months and reported increases in the survival rate, quality, and shelf life of tilapia. Thanikachalam et al. (2010) fed catfish fry (*Clarias gariepinus*) with diets containing different concentrations of garlic husk powder (0%, 0.5%, 1.0%, and 1.5%) for 20 days, reporting higher survival rates in all groups consuming garlic peel. Manoppo Gpeogoc et al. (2016) used diets with granules containing garlic as an ingredient for feeding the common carp (*Cyprinus carpio*) for 1 month, documenting a significant effect in the growth compared to the control without garlic. Finally, Etyemez Büyükdeveci et al. (2018) studied the impact of using diets with garlic extract to feed the rainbow trout (*Oncorhynchus mykiss*), finding that the weight gain and the specific growth rate of the fish were significantly improved when the fish consumed the diets containing garlic. Whether research is required to comprehend the mechanisms through which garlic's use improved the growth performance, it could be hypothesized that the protective role of garlic against diseases could favor the bioenergetics of fish, spending more energy on somatic growth than trying to eradicate pathogens.

The use of garlic has also been tested to improve the production performance of crustaceans. Mona et al. (2015) assayed different experimental diets containing with supplementation of garlic, Biogen (as probiotic), bermudagrass (as immunostimulant at 1%, 2%, and 3%), and one concentration (3 g L^{-1}) of sodium alginate (as prebiotics) to produce red crab (*Procambarus clarkii*) for 6 weeks. The results revealed that the diet supplemented with garlic increased the survival rate, wet weight, and SOD activity compared to organisms consuming the control diets. In another approach, Tazikeh et al. (2020) tested diets with MBM (meat and bone meal), and MBM enriched with garlic powder at 0, 25, and 50%, for 60 days, to produce white shrimp (*Litopenaeus vannamei*). The addition of garlic improved the growth performance of shrimp and had a positive effect on muscle composition.

Effect of garlic on the intestinal microbial composition of fish

Garlic has been used in aquaculture as a natural agent to obtain advantages because of its various properties. The addition into formulated diets influences the immune system with a positive response of the host. For example, the resistance to disease and stress is increased (Talpur and Ikhwanuddin 2012; Guo et al. 2015; Foysal et al. 2019; Abdel-Tawwab et al. 2020; Adineh et al. 2020). However, the use of garlic could also influence the composition of the gut microbiota (Etyemez Büyükdeveci et al. 2018; Foysal et al. 2019; Rimoldi et al. 2020). Although this topic's information is still minimal, this is relevant, considering the gut microbiota as an annex organ of animals (Pérez et al. 2010; Etyemez Büyükdeveci et al. 2018; Hoseinifar et al. 2019).

Some approaches have been conducted. For example, Etyemez Büyükdeveci et al. (2018) fed rainbow trout (*Oncorhynchus mykiss*) with diets containing different concentrations of garlic: 0% (control), 1% (Group 1), 1.5% (Group 2), and 2% (Group 3), for 120 days. The abundance and species richness in the treatments were different in the control and group 3, while these indexes decreased as the garlic concentration increased. The fish's general ecosystem was dominated by four phyla: Actinobacteria, Firmicutes, Proteobacteria, and Tenericutes. The most abundant genera were *Deefgea* and *Aeromonas* in the control group, *Deefgea* and *Mycoplasma* in groups G1 and G2, and genera *Aeromonas*, *Deefgea*, and *Exiguobacterium* in group 3 that received the highest concentration of garlic in the diet. The genus *Deefgea* is associated with healthy trout skin (Carbajal-González et al. 2011). Members belonging to the genus *Exiguobacterium* were found in greater abundance in the group receiving the highest garlic amount. Studies indicate that this genus is involved in forming lipid droplets (Semova et al. 2012). Lipid droplets are involved in diverse cellular functions with energetic implications such as lipid metabolism (Walther and Farese 2012). Furthermore, a significant correlation was found between the abundance of the genus *Clostridium* and the garlic amount in the diets. It is suggested that this genus can improve fish nutrition by providing essential fatty acids and vitamins (Sakata 1990; Ringo et al. 1995).

Bacterial composition changes may be related to garlic's antibacterial properties, containing a wide range of compounds such as tannins, alkaloids, saponins, fatty acids, and essential oils (Harris et al. 2001; Saha and Bandyopadhyay 2017). This may imply the proliferation of certain groups of bacteria because garlic has a wide spectrum of antibacterial activity in gram-negative bacteria such as *Aeromonas*, *E. coli*, *Pseudomonas*, and *Vibrio* species, as well as gram-positive bacteria such as *Bacillus*, *Streptococcus*, and *Staphylococcus* (Ankri and Mirelman 1999; Bakri and Douglas 2005; Sasmal et al. 2005; Vuddhakul et al. 2007).

Torrecillas et al. (2019) tested the effect of diets with garlic oil and Labiatae oil mixture against commercial diets in the intestinal microbiota of the European bass (*Dicentrarchus labrax*), before and after the infection with *Vibrio anguillarum*, combined with the stress of crowding. The administration of the diet with garlic oil and labiate oil had a protective function in the fish's intestine by reducing the density of the goblet cells, compared to the control (inflamed goblet cells). This photogenic diet probably intervened in buffering the effects of commercial diets on the intestinal microbiota by stabilizing the microbiota by altering the detection of the quorum of pathogenic bacteria and modulating the local immune system by eliminating free radicals (Cunha et al. 2018; Lillehoj et al. 2018). Also, it improved mucus coverage, which is the first line of defense against pathogens (Lazado and Caipang 2014), which significantly enhanced resistance to infection against *V. anguillarum*. Possibly, the antibacterial properties of garlic could prevent the colonization of pathogenic bacteria in the intestine and influence the immune responses by providing an ideal environment for the proliferation of beneficial bacteria for the body and by manipulating the host's intestinal microbiota in favor of a microbial community beneficial (Nayak 2010; Pérez et al. 2010).

Rimoldi et al. (2020) tested the effect of the dietary inclusion of garlic + Labiatae oil mixture on the composition of the intestinal microbiota of European bass (*Dicentrarchus labrax*) fed a diet low in fishmeal (FM) and fish oil (FO). The autochthonous (adherent to the mucosa) and allochthonous (transient) microbial communities were analyzed. Results revealed that garlic and Labiatae's dietary inclusion induces changes in the European bass's intestinal microbiota composition. A reduction of coliforms, specifically the genus *Escherichia* and vibrional bacteria that include several species potentially pathogenic for fish, were reduced, while the order Clostridiales resulted enriched.

The importance of some members of the order Clostridiales has been highlighted since they can improve fish nutrition by providing essential fatty acids and vitamins (Ringo et al. 1995; Pryde et al. 2002; Esquivel-Elizondo et al. 2017), while including several butyrate producers. Butyrate is the end product of prebiotic fermentation; it is considered a source of energy for invertebrates, including fish. It plays a critical role in maintaining general intestinal health, morphology, and intestinal function (Wong et al. 2006; Roberfroid et al. 2010; Rimoldi et al. 2016; Guerreiro et al. 2018). Among the butyrogenic genera, *Ruminococcus* and *Faecalibacterium* stand out, found in the intestines of fish fed garlic and labiate. The genus *Ruminococcus* plays an essential role in the breakdown of indigestible carbohydrates, such as starch and dietary fibers, thus contributing to enhanced efficiency in the use of food energy and the host's intestinal health (Walker et al. 2011; Ze et al. 2012; Bonder et al. 2016). Similarly, *Faecalibacterium* is the most important butyrate-producing commensal bacterium in the human colon and is considered a bioindicator of human health (Ferreira-Halder et al. 2017).

Finally, Foysal et al. (2019) demonstrated that the use of garlic (0.5 and 1.0 g of garlic per 100 g of commercial food) for 14 days in tilapia (*Oreochromis niloticus*) infected with *Streptococcus iniae*. Results revealed improved survival rates of fish against infection, immunostimulation observed by the gene expression profile of gut cytokines, and gut microbial structure modulation. The bacterial diversity index increased, specifically the abundance of the phyla Proteobacteria and Tenericutes.

The phylum Proteobacteria presence in the intestine improves the fish's health status (Gajardo et al. 2017; Wang et al. 2017; Michl et al. 2017; Nyman et al. 2017). The phylum Tenericutes has positive effects on the growth and suppression of harmful bacteria causing diseases and is used as a novel additive as a probiotic to improve fish's health status (Dehler et al. 2017; Wang et al. 2017). Therefore, the inclusion of garlic in diets for tilapia is encouraged due to the positive effect on fish's health, including the beneficial modification of the intestinal microbiota. Therefore, garlic as a therapeutic strategy to treat dysbiosis should be addressed in further studies.

Other benefits

Garlic has been used to improve aquaculture production and the shelf life and post-harvest quality of aquaculture products. In this regard, Kumolu-Johnson et al. (2012), tested the effect of fresh garlic on lipid oxidation and shelf life in the meat of African catfish (*Clarias gariepinus*). Three concentrations of garlic (10, 30, and 50 g of garlic per kilogram of fish) were prepared, smoked hot, and stored for 28 days at room temperature of 20–26 °C. The highest lipid oxidation was determined by thiobarbituric acid (TBA) and peroxide concentration in control (without garlic). By contrast, the lowest concentration of peroxide occurred in the sample with 50 g of garlic per kilogram of fish, and the lowest TBA was observed in a sample containing 30 g of garlic per kilogram of fish. These results showed that fresh garlic has antioxidant properties that can extend the life of catfish.

Conclusions

Several studies have been carried out to evaluate the effect of using garlic as a feed additive to produce different species of fish and crustaceans of aquaculture importance. Most of the approaches reported improvements in survival rates, growth performance, feeding efficiency, and increased resistance to pathogen diseases. However, the use of garlic is not a common ingredient in formulated diets used in aquaculture. Nonetheless, garlic can play a role as an antioxidant, antiviral, antibacterial, antiparasitic, immunostimulant, and growth promoter. Therefore, it can be a substitute for many of these products, representing a cheaper alternative at an industrial scale. However, some research is required to respond to this hypothesis.

Regarding the cultivation of mollusks such as snails, oysters, clams, and other bivalves, which are similarly affected by viral or parasitic diseases, there are no studies about using any naturally occurring antimicrobial in their cultivation (Ibrahim et al. 2020). Measures are generally taken to prevent the spread of diseases in shellfish culture, including the mass crop reduction, regulation of salinity and temperature changes, and avoiding the transfer of nonendemic organisms (Rodgers and Furones 2009); however, exploring the use of garlic in these systems remains as a pending task.

Evidence has demonstrated that garlic (*Allium sativum*) has multibiological properties, including antioxidant, antimicrobial, antiviral, antiparasitic, immunostimulatory, anti-stress, appetite stimulation, and more recently, intestinal microbiota modulator. These properties are due to bioactive compounds such as allicin, ajoene, and alliin, among others. Especially allicin, an organosulfur compound, provides most of the garlic's biological properties and has been extensively studied as a supplement in fish diets, highlighting its antimicrobial activity, where it has been possible to eradicate pathogenic microorganisms from fish. Garlic properties can also be associated with better production performances.

The growth promotion effect of garlic can also be associated with its flavor that increases food intake, improves digestion, and the availability of nutrients, leading to higher growth rates. However, research about the mechanisms through which the different garlic components induce the host's responses and affect pathogens is still required.

In addition to its properties, garlic is easily accessible and easy to apply as it can be administered orally and does not generate stress. It can also substitute several chemotherapeutic agents and does not pollute the environment; however, its use at a commercial scale is still not a common practice.

Author contribution Marcel Martínez-Porchas conceived the review, structured and wrote the document, organized the information collected by himself and the authors, and supervised the development of the writing. Rocío Valenzuela-Gutiérrez participated in the organization and writing of the manuscript and collected and analyzed information. Asunción Lago-Lestón collected and analyzed information about specific sections of the manuscript and supervised the development of the writing. Francisco Vargas-Albores collected and analyzed information about specific sections of the manuscript. Francesco Cicala collected and analyzed information about specific sections of the manuscript.

Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Consent to participate All authors consent to participate in this manuscript.

Consent for publication All authors consent to participate in this publication.

Conflict of interest The authors declare no competing interests.

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