AQUAFOCUS

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ZOOCA in India

- Overcoming Viral Infection in Shrimp
- Medical Herbs as Natural Antibiotics in Aquaculture
- Bio Pigments in Aquaculture Industry
- Shrimp Production with Zooca
- Aqua - Cal+, a Water Conditioner for Shrimp

Calanus finmarchicus, a small but mighty amazing creature

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AQUAFOCUS

Dedicated to:
Aquaculture Research & Development

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"A pond full of fish is better than a river full of stones."

- Canadian Philosopher Matshona Dhliwayo

(Dr. Jesu Arockiaraj, a highly acclaimed Eminent Scientist with Stanford University World Ranking in Top 2%. He has also received many awards including "Young Scientist Award" by Government of India and Government of Tamil Nadu)

Greetings !

In consonance with the aforesaid adage, it is of quintessential importance to ensure a healthy balance of nutrients and immunity building feed in fish ponds, in juxtaposition with **sustainable aquaculture** practices in the vast ocean of aquaculture operations!

At FAITT, being a triple ISO certified company in ISO 9001, 14001 & 45001, we are strongly committed to sustainability, quality, safe and environmental friendly practices and operations in our aquaculture ponds and research labs. FAITT endeavors to spread awareness and enrich cognizance on the various facets of aquaculture through workshops, seminars, publications, special events, etc.

In this issue, FAITT brings you well-curated, discerning and insightful articles in the aquaculture trade and operations, by eminent academics and research scientists.

Of late, various types of easily available medicinal herbs are gaining traction in aquaculture use. They are highly useful in controlling diseases in aquaculture ponds. They also act as a safe alternative to harmful antibiotics. The "bioactive compounds" in these herbs play a great role in prevention of disease and maintenance of fish and shrimp health.

Zooplankton *Calanus finmarchicus*, a tiny marine crustacean, offers sustainable and highly nutritious alternatives to traditional shrimp feed sources. innovative zooca products are a true paradigm of ensuring sustainability in aquaculture practices.

In the modern context, natural bio-pigments are finding a lot of application in the aquaculture industry. As a paradigm, phycocyanins from spirulina and anthocyanins from fruits and vegetables have a vast degree of antioxidant, anti-carcinogenic and immune response-enhancing properties.

We bring you highly insightful articles in this issue on these and other aspects.

Rainbow Trout is a well adaptable species that grows fast and can easily be cultivated in captive environments. The trout is gaining a lot of traction in tropical countries and the US and we present you with an insight on the treatment of viral hemorrhagic septicemia virus (VHSV) in the trout.

In Tamilnadu, Aquaculture insurance products launched on World Fisheries Day, November 2, for freshwater fish farming and shrimp farming by the Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam. In August, Honourble Tamilnadu Chief Minister, Shri M K Stalin inaugurated various facilities constructed at a cost of Rs 43 crore on behalf of the State Fisheries and Fishermen Welfare Department.

Finally, it is great to look forward for a robust year 2023 ahead, as the scintillating year 2022 - a UN designated " International Year of Artisanal Fisheries and Aquaculture " come to a close.

Time to begin to change the future of Aquaculture now!

With Regards,
Dr. Jesu Arockia Raj. A
Editor-in-Chief
AQUAFOCUS

Overcoming viral Infection in shrimp culture by RNA Inteference (RNAI)

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Shrimp farming and infections

Shrimp farming is an economically important industry, particularly in developing countries sustaining the livelihood of many coastal communities.Shrimp and shrimp products make up a large portion of the global fish market, with an annual trade value exceeding $11 billion or 15% of the total fish exports in 2018 (FAO, 2022). However, as shrimp farming becomes more intensive, the shrimp are exposed to greater stress, making them more vulnerable to diseases.

Viral infections have caused significant damage to the shrimp industry, leading to significant economic losses for farmers and the industry as a whole(Flegel, 2006).Since the early 1990s, viruses such as White Spot Syndrome Virus (WSSV), Tura Syndrome Virus (TSV), Hepatopancreatic Parvovirus (HPV), Monodon Baculovirus (MBV),Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), and Yellowhead Disease Virus (YHV) inflicted both wild and cultured penaeid shrimps worldwide, due to their ability to adapt and infect a wide range of hosts(Gong & Zhang, 2021). Among these viruses, WSSV infection (Fig 1) has been the most lethal, widespread, and still prevalent to present.

Despite the existence of several techniques that have been effective against shrimp viruses under laboratory conditions, they have not been successful in field trials(Dang et al., 2010). One of the reasons for this is that laboratory conditions do not fully replicate the complex environmental factors present in the shrimp farming industry, which can impact the effectiveness of potential treatments.

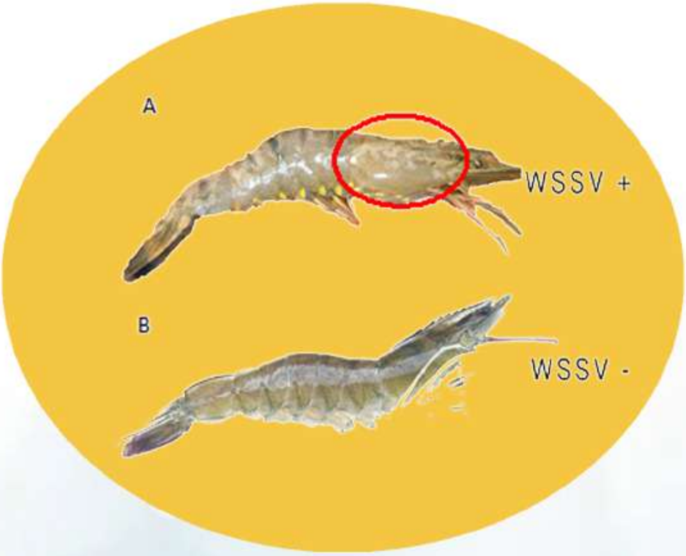


Fig. 1. WSSV-infected shrimp (A) and normal shrimp (B) collected from the Southern part of the Philippines

Moreover, unlike vertebrates, shrimps do not have an acquired immunity system that is required to respond to a specific infection and solely rely on their innate non-specific immunity against infections. The shrimp's innate immune response is a complex process involving various components that work together to recognize and eliminate invading pathogens, however, since it is non-specific, it may not provide long-lasting defense against specific pathogens, which is why it can be difficult to develop effective treatments for viral infections in shrimp. To address this challenge, it is crucial to further elucidate the host-pathogen interaction by investigating the functional genes involved in the viral infection.

RNA interference (RNAi)

Among the developed tool in gene functional studies, RNA interference (RNAi) was commonly utilized due to its reliability in gene silencing functions. In RNAi, small RNA molecules are used to target and degrade specific messenger RNA molecules, preventing the translation of genetic information into proteins (Nguyen et al., 2018). In aquaculture, RNAi can be used to inhibit the expression of genes that are responsible for certain traits or diseases in fish and Shellfish by introducing gene-specific double-stranded RNA (dsRNA) into a target organism (Nguyen et al., 2018).RNAi mechanism was first discovered in plants and nematodes and has since been found to be conserved across various organisms, including animals and humans(Wang & He, 2019). In shrimp, major proteins involved in the RNAi pathway, such as dicer and argonaut, have been identified for further applications. RNAi has been demonstrated to have a significant role in antiviral immunity in shrimp, and several studies have shown that injecting dsRNAs or siRNAs specific to viral genes can successfully inhibit viral infections. The successful activation and suppression of various viral proteins and immune-related genes confirms the critical role of RNAi in the antiviral immunity of shrimp and shed information on the therapeutic potential of this technique (Gong & Zhang, 2021).

RNA interference (RNAi) has been recognized for its high degree of specificity and efficacy, making it a promising platform for developing therapeutics. However, the development of RNAi-based agents has been hindered by several challenges. For instance, small interfering RNAs (siRNAs) are unstable in serum and are rapidly degraded, limiting their therapeutic potential (Nguyen et al., 2008). Additionally, delivering siRNAs across the cell membrane has proven to be highly inefficient. These challenges have prompted extensive research efforts aimed at developing effective delivery strategies for RNAi-based therapeutics (Dong et al., 2019; Nguyen et al., 2008).Several studies have explored RNAi delivery methods in shrimps for controlling viral diseases. Charoonnart et al. (2021) showed the effectiveness of dsRNA-encapsulated liposomes in delivering RNAi against white spot syndrome virus (WSSV). Chen et al. (2020) investigated exosome-mediated delivery of RNAi against WSSV and observed a significant reduction in viral replication. Liu et al. (2018) developed a chitosan-based nanoparticle system for oral delivery of RNAi against WSSV, resulting in a significant reduction in viral load and Zhang et al. (2017) reported the use of microRNA-mediated RNAi delivery in shrimp against WSSV, which effectively inhibited viral replication and improved shrimp survival.

Fig. 2. dsRNA injection of shrimp in SEAFDEC Tigbaoan station

The most effective way to induce RNAi in crustaceans is through dsRNA injections (Fig 2). Although this injection-based approach was successful on the laboratory scale, new delivery methods have been continuously developed to allow for quick, large-scale RNAi research (Sagi et al., 2013). A more practical delivery method would be oral administration (feeding), which is also feasible for larvae and post-larvae in shrimp hatcheries (Itsathitphaisarn et al., 2017). A comparative study on the effect of in vivo and in vitro produced dsRNA in *Penaeus vannamei* indicated that both are effective in mitigating viral infection. However, prolonged feeding might have sustained a more beneficial protective effect during the challenge test (Fig 3) (Gumatay et al, 2017 (unpublished)).

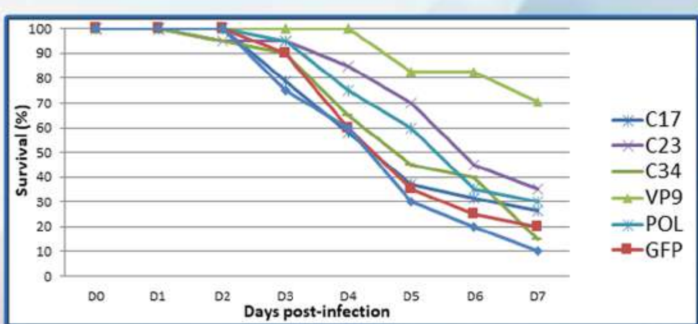


Fig. 3. Cummulative survival percentage

The dsRNA production through this system is also more cost-effective compared to in vivo production (Fig 4). Other dsRNA oral delivery methods reported to be effective in controlling shrimp viral diseases include the use of dsRNA-expressing bacteria and microalgae mixed with shrimp feeds, the use of living brine shrimp pre-fed with dsRNA or dsRNA enclosed in nanocontainers such as chitosan and liposomes and added in shrimp pellets (Itsathitphaisarn et al., 2017; Dekham et al., 2022; Charoonnart et al., 2021; Theerawanitchpan et al., 2012).

These methods have been used to control viral diseases in shrimp. However, continuous optimization of the delivery methods is essential for large-scale use and to further understand the potential risks and unintended effects of RNAi-based therapeutics on non-target organisms and the environment.

In conclusion, RNAi delivery has made significant promising progress in recent years in aquaculture, particularly in controlling viral diseases in shrimp and other aquaculture species. Several studies have demonstrated the efficacy of various RNAi delivery methods, including dsRNA injection, feeding with dsRNA-producing bacteria or microalgae, and oral administration of dsRNA-encapsulated liposomes, and chitosan-based nanoparticles

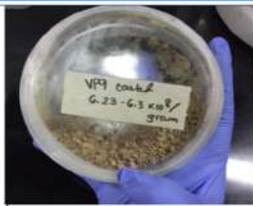
MODE OF PRODUCTION		
YIELD	2L culture of recombinant bacteria = 4 mg of dsRNA	1 kit = 4 mg of dsRNA
COST	**USD 270**	**USD 997**

Fig. 4. The cost of producing dsRNA using a bacterial system compared to in vitro production

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Introduction

The demand for fish, shrimp and algae is growing at a very high rate in both developed and developing nations and is estimated to grow much further in the coming years. This would invariably cause a great demand and place a humungous stress on the aquatic life in the Oceans. When overfishing occurs, the entire natural ecosystem in the oceans gets skewed and it further leads to unfavourable effects in the aquatic life food chain and has the potential to cause other detrimental environmental related effects.

As a paradigm, Coral Reefs are well known to support local tourism and the commercial fishing industry. They also assist in mitigating storms, sedimentations, and protect coastlines from flooding during extreme weather conditions such as cyclonic storms.

But, overfishing in oceans causes great anthropogenic stress on coral reef systems, causing them to expel the symbiotic algae living in their tissues, which in turn leads them to turn completely white(coral bleaching). These may further have a deleterious impact on marine ecology and ripple effects based on its inter-connection with allied aspects pertaining to Earth's Environment, climate change , etc.

In that aspect, land based Aquaculture operations plays a pivotal and positive role in saving the marine ecosystems and other consequent ecological disasters.

The Aquaculture saves Marine life

Aquaculture operations circumscribing the captive breeding, rearing, and harvesting of marine plants and animals in water bodies on specifically chosen or allocated land parcels in a controlled environment, vastly ameliorates the conservation of marine life forms. It thus improves the safety and resilience of marine ecosystems.

Aquaculture activities such as Fish and Shrimp farming make significant socio-economic contributions. It can lead to voluminous and nutritive food production by increasing the amount of seafood available for people to eat , thus improving food security and nutrition aspects. It can also give employment to several people including artisanal labour in both the mainland and hinterland.

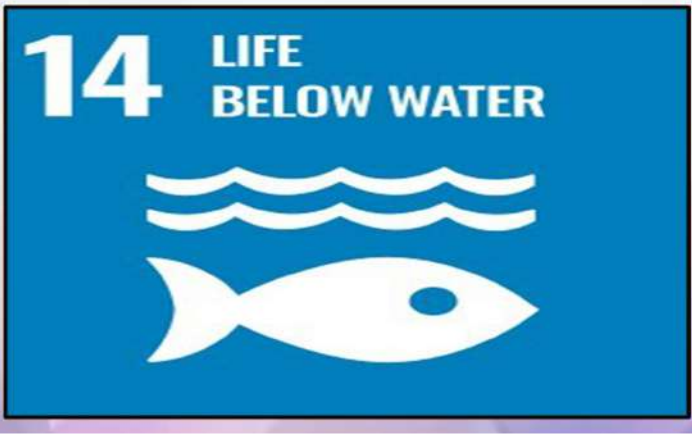
FAITT Supports the Sustainable Development Goals

Aquaculture fulfils UN SDGs

In juxtaposition with this, **aquaculture operations** also fulfills multifarious aspects relating to **UN SDGs, especially UN SDG 14 (Life below Water),** by reducing over-exploitation of marine resources and enhancing the conservation and sustainable use of oceans, seas and marine resources.

As a paradigm, **Aquaculture** food production process fulfils **5 IMPORTANT UN SDGs :**

- **UN SDG 1** - Reduce Poverty by generating and providing artisanal employment
- **UN SDG 2** - Reduce hunger via Vast Captive Nutritive sea-food production
- **UN SDG 3** - Good Health & Well-being via Nutritive sea-food production
- **UN SDG 8** - Promote Sustained, Inclusive and Sustainable Economic growth
- **UN SDG 14** - Life below Water



Aquaculture would thus be a robust and viable business proposition for various stakeholders including small and marginal farmers even in the rural segments in India as well as in other Nations.

This would assist in significantly and affirmatively contributing to a robust Blue Revolution and to a Sustainable Blue economy.

Using innovative and sustainable Aquaculture practices like efficient aquaculture water quality and resources management, health management and disease control in Aqua ponds, and using innovative food and technology based solutions in improving the net yield and profits, the aquaculture farmers can ensure Sustainable Aquaculture Practices and economics for all round, long-term benefits.

Medical herbs - a safe alternative to antibiotics in aquaculture

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Aquaculture growth and diseases

Aquaculture is one of the fastest-growing food production sectors in the world with an annual growth rate of 7.5%. In 2020, the world fisheries and aquaculture production has attained 177.8 million tones, with a contribution of 90.3 million tonnes from inland and marine capture fisheries and 87.5 million tonnes from the inland and marine aquaculture(FAO, 2022). The development and expansion of aquaculture is negatively affected due to the outbreak of several diseases which not only affect the production but also serious economic losses in aquaculture operations. Increasing disease incidence in aquaculture has resulted in the adoption of Chemotherapy for the control and prevention of diseases(Murray and Peeler, 2005). The use of antibiotics and chemicals in aquaculture is widely criticized because it is often very expensive and leads to the selection of antibiotic-resistant bacterial strains, immunosuppression, environmental pollution and the accumulation of residues in edible fish which can be potentially harmful to the health of consumers. On the other hand, though vaccination is the most effective and safe method of protection against diseases, the production of effective formulations for a number of pathogens is often hindered by high production costs and the antigenic heterogeneity of the microbial strains (Toranzoet al., 2009). In recent days, herbal medicines have attracted much attention as a promising alternative to chemical drugs for aquaculture disease management as they are considered safe, easily available and cost-effective.

Herbal plants for fish diseases

According to the World Health Organization (WHO, 2005), about 80% of the people in the worldrely on traditional medicine for their primary health care in developing countries. About 85% of such medicines involve the use of phytochemical extracts from medicinal plants which are a cheap source of antimicrobial agents without any side effects. Phytochemicals are bioactive molecules regarded as secondary metabolites like tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoidswith numerous pharmacological benefits(Wang et al., 2018). Any part of the plant body like stem, root etc. may contain active components which make them rich sources of different types of phytochemicals.

These bioactive compounds play an important role in disease control because they exhibit antioxidant, antimicrobial, antistress, growth promotion, appetite stimulation, immunostimulation and aphrodisiac properties. The various identified advantages of these phytochemicals are they are available in plenty, cheap with no adverse effects on the natural ecosystem. They could be used for the control of fish diseases by administering through feed or the rearing medium. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects in vitro on all types of microorganisms like bacteria, viruses, fungi and parasites (Cowan, 1999).

Some of the traditional herbal plants that could be used for the control and treatment of various fish disease are listed below.

Scientific Name	Common Name	Identified activity
Acalypa indica	Indian copperleaf	Anthelminthic, anti-inflammatory, anti-bacterial, anti-cancer, anti-diabetes, anti-hyperlipidemic, anti-obesity, anti-venom, hepatoprotective, hypoxia and wound healing properties
Azadirachta indica	Neem	Immunomodulatory,antiulcer, anti-inflammatory, antihyperglycaemic, antimalarial, antimicrobial, antioxidant, antimutagenic and anticarcinogenic properties.
Cantheranthus roseus	Periwinkle	Antioxidant, antimicrobial, antidiabetic and anticancer properties.
Clitoria ternatea	Butterfly pea	Memory enhancer, antistress, anxiolytic, antidepressant, anticonvulsant, tranquilizing and sedative properties
Curculigo orchiodes	Golden eye-grass	Adaptive, immunostimulatory, antioxidant, antihistaminic, antiasthmatic, hepatoprotective and neuroprotective activity.
Curcuma longa	Turmeric	Anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, antimicrobial, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities
Cynodondactylon sp	Bermuda grass	Anti-inflammatory, antidiabetic, anticonvulsive, anticancer, diuretic, immunomodulatory, antiviral and antimicrobial activity
Enicoste maaxillare	Indian whitehead	Hypolipidemic, antioxidant,hepato-protective, anti-nociceptive and antimicrobial properties
Euphorbia hirta	Asthma plant	Antimalarial, antifertility, antispasmodic, sedative, antiasthmatic, anthelmintic, anticancer and antimicrobial properties
Gloriosa superba	Glory lily	Antioxidant, antitumor, anti-inflammatory, antiviral, antiallergic, anticancer activities
Justicia adhathoda	Malabar nut	Antibacterial, antifungal, anti-asthmatic, antihistaminic, anti-inflammatory, anti-ulcer, antioxidative, antitubercular, antitussive, larvicidal, anti-Alzheimer, and hepatoprotective effects
Mimosa pudica	Touch me not	Antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac activities.
Oxalis corniculata	Creeping woodsorrel	Anti-inflammatory, anxiolytic, anticonvulsant, antifungal, antiulcer, antinociceptive, anticancer, antidiabetic, hepatoprotective, hypolipidemic, abortifacient, antimicrobial and wound healing properties.
Pyllanthus niruri	Gale of the wind	Hepatoprotective, antimicrobial,analgesic,hypolipidaemic, hypoglycaemic, anti-inflammatory, cardioprotective, anti-urolithiaticand antihyperuricaemic properties
Senna auriculata	Tanner's Cassia	Antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing activity
Tridax procumbens	Coat buttons	Anti-hyperglycemic, hepatoprotective activities, anti-inflammatory, antioxidant, anticoagulant, anti-hepertic, antibacterial activities






The various parts of the herbs, methods followed for the extraction of phytochemicals and their concentrations contribute to the varied and significant effects on the health, growth and reproductive performance of fishes.

Several studies have reported the versatile functions of phytochemical extracts from medicinal herbs such as improved fish immunity, antimicrobial activity against the pathogens, growth stimulation and increased feed conversion in fish, which therefore makes it a promising potential alternative to replace the antibiotics used in fish health management. phytochemicals belonging to several chemical classes have been shown to have inhibitory effects in vitro on all types of microorganisms like bacteria, viruses, fungi and parasites (Cowan, 1999).

Biopigments and their application in the aquaculture industry

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Introduction

Numerous synthetic colourants have been created in recent centuries. They are utilised for a wide variety of purposes in the cosmetics, food, pharmaceutical, and textile sectors, due to their lower costs, easier manufacture, desirable flavours and better colouring qualities.

However, due to their toxicity and the production of harmful effluents while manufacturing, their market prominence subsequently declined (Manikprabhu and Lingappa, 2013).

Contrary to this, biopigments, which are made naturally by living organisms, are non-toxic and have increasing market demand than synthetic colourants. According to Sen et al. (2019), consumer demand for natural food colours will progressively rise by 7 percent yearly. As a result, natural colourants are predicted to generate $1620 million in revenue by 2023, representing a 5.4 percent CAGR from $1180 million in 2017. (https://www. marketresearchfuture.com/reports/).

Moreover, the international market for riboflavin is anticipated to grow by 4.8 percent over the next five years, with estimates ranging from $7790 to $10,300 million during 2019-2024 (https://www.fiormarkets .com/report/global-vitamin-b2-riboflavin-market-growth-2019-2024-374891.html). The global carotenoid market is anticipated to reach $2000 million by 2026.

Natural pigments

Natural colours are primarily derived from living organisms. E.g. Anthocyanins from fruits and vegetables (such as the skins of black grapes, black carrots, and red cabbage), annatto from achiote seeds, betanins from red beets, carmines from the blood of cochineal beetles, capsanthin from red peppers, and lycopene from tomatoes, blue pigment phycocyanins from algae Spirulina, Chlorophylls from green plants such as nettles, asparagus, avocado, broccoli, brussels sprouts, cabbage, celery, cucumber, green beans, green onion, kiwi fruit, lettuce, okra, spinach, zucchini, spinach, grass, parsley, Curcumin from turmeric, carotenoids from palm trees, and yellow pigment xanthones from plant families like Bonnetiaceae, Clusiaceae, and Podostemaceae (Henry, 1996; Park et al, 2018; Simpson and Klomklao, 2012).

Nonetheless, animal/plant pigments have limitations due to unsteady/seasonal availability, deforestation concerns, and the production of unstable and insoluble compounds. Microalgae, on the other hand, is considered an important source of pigments, and a source of natural nutrients in the aquaculture industry (Natrah et al, 2007; Park et al, 2018; Koller et al 2014). Microalgae are used as live feeds, supplements for water quality, bioremediation, growth promoters, antioxidants and animal colour enhancers (Khatoon et al, 2007; Begum et al, 2016). Microalgae are a source of several polyunsaturated fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and omega-3 fatty acids, and also do have several pigments such as chlorophylls, carotenoids, phycobiliproteins and phenolic compounds which enhances the functional, nutritional, therapeutic values (Natrah et al, 2007; Park et al, 2018; Koller et al 2014).

Microalgae are used as live feeds, supplements for water quality, bioremediation, growth promoters, antioxidants and animal colour enhancers (Khatoon et al, 2007; Begum et al, 2016). Microalgae are a source of several polyunsaturated fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and omega-3 fatty acids, and also do have several pigments such as chlorophylls, carotenoids, phycobiliproteins and phenolic compounds which enhances the functional, nutritional, therapeutic values (Natrah et al, 2007; Park et al, 2018; Koller et al 2014).

The cultivation of microalgae is becoming more and more essential due to its sustainable use in fish and shellfish aquaculture and other industries.

Natural Pigments such as astaxanthin and β-carotene are products used in aquaculture for their immune-enhancing functions such as antioxidants, immune enhancers, anti-inflammatory agents and vitamin precursors. Hence, the cultivation of natural microalgae is gaining an advantage globally because of its sustainability and eco-friendly production. The antioxidants astaxanthin, β carotene, lutein, lycopene, and canthaxanthin are the most significant carotenoids obtained from microalgae (Gong & Bassi 2016). Only a few numbers of microalgae have been used in industries. Genera like Chlorella, Spirulina, Dunaliella, Muriellopsis and *Haematococcus* is widely used (Sanchez et al. 2008; Lin et al. 2015; Wang et al. 2018a).

Haematococcus pluvialis is rich in xanthophylls and is regarded as the best natural source of astaxanthin. *Dunaliella salina* is used as an industrial source of β-carotene (Levi 2001; Sanchez et al. 2008; Jeffrey & Egeland 2019).Although microalgae production is eco-friendly, the extraction process will be costlier for biomass or bio-compound production (Yusoff et al. 2019) and needs large-scale harvesting methods like centrifugation, fractionation, flocculation and coagulation, filtration and ultrasonic separation.

Microbes such as fungi and bacteria are also reliable alternate sources of biopigments due to their rapid growth, and production of stable and high product yields through cost-effective strategies (Narsing et al, 2017).

Several microbes are used for the production of pigments in various industries. Many vibrantly coloured pigments, including anthraquinones, carotenoids, dihydroxy naphthalenes, flavins, indigo pigments, naphthoquinones, phenazines, melanins, monascins, violaceins, etc., are produced by microorganisms from different biological niches (Fig 1).

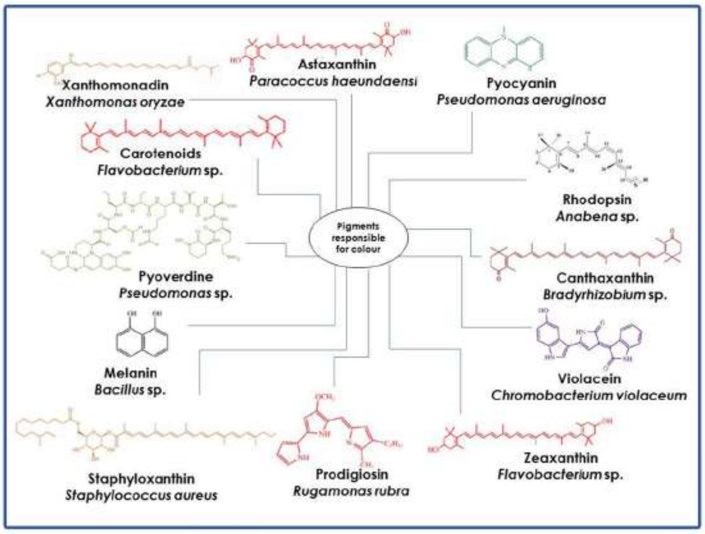

Fig. 1. Biopigments and their bacterial source.

Benefits of biopigments

It has been noted that biopigments made by microbes in reaction to environmental factors frequently have positive effects on human health. *Serratia* and other gram-negative bacteria produce the versatile red bio pigment prodigiosin. According to reports, prodigiosin has cytotoxic, anti-inflammatory, antibacterial, antiprotozoal, and antifungal activities. The pharmacological characteristics of Monascus pigments, such as their anti-cancer, anti-mutagenic, anti-obesity, and antibacterial activities, are also well documented. Additionally, biopigments made by yeasts have been described as having antioxidant, anti-carcinogenic, and immune response-enhancing properties (Demain and Martens, 2017; Panesar et al., 2015). Melanin is another biopigment that is typically present in microbes, plants, and animals and has qualities that include immunogenic, antibacterial, anti-inflammatory, and anti-oxidant (El-Naggar and El-Ewasy, 2017; Rosas et al., 2000).

Pigment Name	Properties
Astaxanthin	Anti-stress
Canthaxanthin	
Carotenoids	Antibiotic, Antioxidant, Cytotoxic activity
Melanin	Photoprotection, Antioxidant, Anticancer
Zeaxanthin	
Indigoidine	Antioxidant, Signaling, Antibiotic
Prodigiosin	Biocontrol, Antibiotic, Algaecidal, Anti-inflammatory, Anticancer, Antimalarial, Antidiabetic, Immune system modulator
Staphyloxanthin, Zeaxanthin	
Violacein	Antibiotic, Antiparasitic, Antiviral, Antitumoral Anticancer
Pyocyanin	Virulence factor, Iron uptake, Cytotoxic activity Antibacterial activity
Pyoverdine	Bioluminescence, Virulence factor, Iron uptake
Rhodopsins	Active transport
Xanthomonadin	

Table 1: Various functions and properties of biopigments

After that, strains can be cultured in a fermenter/bioreactor to obtain pigments at the commercial scale. Photobioreactors are often used for the cultivation of algae for the production of algal pigments.

Genetic engineering approaches have been used to improve pigment production in native strains such as site-directed mutagenesis, or random mutation. Besides, heterologous hosts such as model bacteria or yeast have also been tried to produce pigment after transporting the genes or operons from pigmented strains. This method resolves the issues such as variable yield, safety, or difficulty to cultivate strains (Orlandi et al., 2020). Efforts have been taken, in particular for the biosynthesis of astaxanthin, owing to its high commercial demand in various industries including livestock, fish feed, cosmetics and nutraceuticals (Gong et al., 2020). Genes involved in the manufacture of carotenoids have been characterised in *Hematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlamydomonas reinhardtii* (Gao, Honzatko, and Peters 2012; Kathiresan et al. 2015).

Fig. 2. Outdoor photobioreactor for the cultivation of *Haematococcus pluvialis* for the production of astaxanthin (source: https://www.scientistlive.com/content/high-quality-microalgae-products)functions and properties of biopigments

Strategies to obtain biopigments

Microbial biopigment production has two basic approaches: either, discovers new pigmented microbes, and optimizes the productivity; or, enhances the production of pigment from already proven strain by strain improvement and process development (Venil et al., 2020). As mentioned earlier, numerous microbes have been isolated producing diverse pigments, shown to have diverse applications. However, the yield of the pigment depends on environmental conditions. Hence, optimization is required to increase the production of pigment in the native-producing strains in laboratory settings.

Carotenoid biosynthesis involves multi-copy genes, which makes the route specialised and challenging but also adaptive. Evolutionary studies showed that the majority of the important genes involved in carotenogenesis in algae were derived from cyanobacteria (Shanshan et al. 2018). The genes involved in carotenogenesis are coordinated to create a specific type of carotenoid depending on environmental conditions. PSY enzyme which is a crucial regulatory enzyme in carotenoid biosynthesis is encoded by the PSY gene in algae, cyanobacteria, and plants and the crtB gene in bacteria. These enzymes' potential genes, once identified, can be inserted into the right hosts to produce particular bio-pigments.

The pigments produced by organisms are mostly intracellular, e.g. carotenoids by yeast. Hence, cell disruption and pigment extraction are the two main processes in recovering intracellular bio pigments. The effectiveness of this process depends on the processing method including the protection of pigment from high temperatures, and organic solvents (Nigam and Luke, 2016). Cell disruption can be done mechanically or through a chemical process. Chemical extraction has been proven to yield higher concentrations (Park et al., 2007). Different extraction methods are known to produce biopigments with unique profiles (Mapari et al., 2005). It is important to note that intracellular biopigment extraction remains difficult because there aren't any standardised, environmentally friendly methods for doing so (Park et al., 2007). The usage of optimum solvent also depends on the information on pigment molecular properties. For E.g. carotenoids are solubilized in various non-polar solvents because they are held in lipid vesicles (Park et al., 2007). Differently, prodigiosin is a pigment that has a higher hydrophobicity than several carotenoids and may be easily removed using acetone (Sun et al., 2015).

If the pigments are produced extracellularly, cell disintegration is not required, e.g. Monascus pigments. The supernatant is used and generally processed using ethanol (Nimnoi and Lumyong, 2011).

Potential applications in aquaculture

Under some rearing circumstances, aquatic species' vibrant and natural colouration may fade (Pavlidis et al., 2006). The vibrant reds and yellows found in fish skin, as well as the orange and green hues of the eggs and muscle, are all a result of fat-soluble bio-pigments called carotenoids (Kop & Durmaz, 2008). These substances are not necessary nutrients, but they can help organisms develop (Dall, 1995; Petit et al., 1997), survive (Wyban et al., 1997), maintain a healthy immune system, reproduce (Linan Cabello et al., 2003), have antioxidant action (Meyers & Latscha, 1997), and withstand stress (Chien et al., 2003; Guerin et al., 2003).

Salmonids consume astaxanthin, a crucial micronutrient with important biological properties. These include improving the biological status of the organism as a whole and the stability of cell membranes, as well as enhancing fish health and immunity by reducing free radicals and boosting resistance to environmental stressors (Latscha, 1989). Astaxanthin also can produce vitamin A and shield the body from the harmful effects of UV light (Higuera-Ciapara et al., 2006). The pigment also inhibits the oxidation of unsaturated fatty acids; astaxanthin antioxidant activity has been compared to beta-carotene and alpha-tocopherol and is 10 and 100 times more potent, respectively (Hussein et al., 2006; Rao et al., 2014).

In reality, astaxanthin is what gives rainbow trout their natural colour, but in a fish-rearing setting, astaxanthin or canthaxanthin must be added to the fish diet (Craik & Harvey, 1986). The pigmentation of fish depends on the storage and retention of pigment in muscle, which varies from 3 to 18 percent for astaxanthin, in addition to the dietary component (Choubert et al., 2009). To increase the protection of astaxanthin in the feed, several techniques have been developed, including nanoencapsulation using emulsions, liposomes, and polymer nanoparticles (Rao et al., 2014; Raposo et al., 2015).

According to the findings of a study by Besharat et al. (2021), adding nanoliposome-coated astaxanthin to rainbow trout diets at doses up to 75 mg/kg can improve growth metrics. Fish growth was positively impacted by the presence of nanoliposome-coated astaxanthin, as shown by the fact that the mean total length and weight of the fish rose significantly with this dose and differed significantly from the control diet.

Microalgae are one of the most chosen microorganisms for biotechnology and applied processes due to their high photosynthetic efficiency, rapid growth rate, and capacity to collect a wide variety of bioactive chemicals (Guedes et al. 2011; Hayes et al. 2018). The extraction of these compounds from microalgae has resulted in biotechnological innovations that have helped the food, pharmaceutical, cosmetic, nutraceutical, and aquaculture industries (Pulz & Gross 2004; Richmond 2004; Shah et al. 2016). More than 30 000 species have been reported, which means that many unique products could potentially be commercially exploited from a huge number of existing species (Lorenz & Cysewski 2000; Leon et al. 2003; Ip et al. 2004; Sanchez et al. 2008; Hayes et al. 2018). More than 7000 of these involve green algae, which are found in many habitats (Shah et al. 2016).

These microalgae are being used as additives for poultry, crustacean, and fish feeds because they provide vibrant colours in egg yolks, skin, and fatty tissues due to their pigmenting properties (Levi 2001; Sanchez et al. 2008), free radical scavenging capacity (Shah et al. 2016), and also,they have shown to improve growth and reduce mortality in larval development (Muller-Feil). So that, these pigments are in strong demand (Ignacio et al, 2019).

Other uses

Apart from aquaculture use, bio-pigments are used as colourants in the food, textile, cosmetic, and pharma-ceutical industries, and also used in different functional applications like antioxidant, anticancer, and antibacterial characteristics. (Gilda Mariano-Silva, Salvador Sa'nchez-Mun oza 2020).

Aqua - commercial products

There are several bio pigment products esp. astaxanthin which is used in the hatchery sectors of shrimp larval development. Astaxanthin is derived from either synthesis or natural production or krill extract oil. The products are formulated in different methods such as conjugated with starch or any other carbon source or in the form of oils or microencapsulated powder. Several companies are coming up with different formulations as feed additives or direct feed supplements. The pigmentation and animal immunity are enhanced by the use of astaxanthin. Few countries are adding astaxanthin to their feed formulations itself to enhance the immunity of the animal.

Amaze asta

Amaze Asta is one such product which was derived naturally from *H. pluvialis* and is 10% pigment conjugated with corn starch which is 100% water soluble. The product can be used as a feed supplement and also can be used for post-larvae packing. It acts as a perfect stress reliever and antioxidant.

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Revolutionize shrimp farming and feed production with Zooca

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The shrimp farming industry has experienced significant transformations over the past few decades, becoming a dynamic and global practice in over 50 countries. However, it faces various challenges, such as viral, bacterial, fungal, and other diseases, the demand for innovative ingredients to support aquafeed production, and environmental impact. Zooca,the Calanus Company provides products designed to address these challenges and mitigate their effects.

Fig. 1. Testing & QC at Zooca

Zooca®, the Calanus® company

Zooca – The Calanus Companyis a Norwegian company with over two decades of experience promoting the sustainable use of the zooplankton Calanus finmarchicus zooplankton. They focus on creating unique and nutritious products for humans and animals.

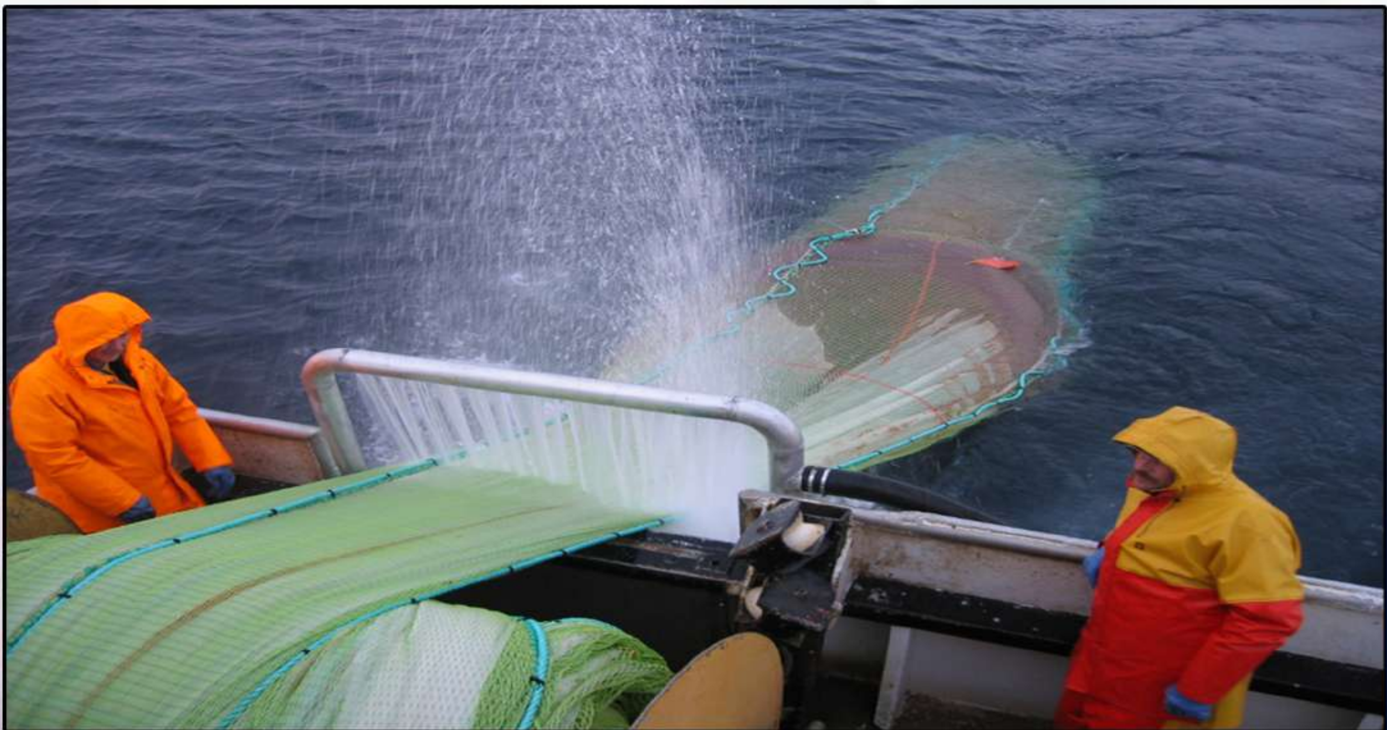

Fig. 2. Zooca Harvesting

Zooca products

The company has four main products intended for aquaculture use in its portfolio:

Zooca® Powder is a highly nutritious and versatile feed ingredient explicitly developed for aquaculture. It is derived from sustainable and traceable sources, making it an eco-friendly choice for shrimp and fish farming. Zooca® Powder contains amino acids, chitin, astaxanthin, minerals, and lipids, providing aquatic species with a complete and balanced diet. It promotes optimal growth, improved feed conversion, and enhanced disease resistance in aquaculture species.

Zooca® Hydrolysate is a hydrolyzed protein concentrate designed to enhance aquafeeds' palatability, digestibility, and nutritional value. It is produced through a carefully controlled enzymatic process, breaking down proteins into smaller peptides and amino acids. This process increases the availability of nutrients, improves feed intake, and optimizes growth performance in shrimp and fish.

Zooca® Hydrolysate is an effective ingredient for formulating high-quality and highly digestible aquafeeds.

Zooca® Lipids is a premium lipid supplement tailored to the specific needs of aquatic species in aquaculture with an extremely high concentration of astaxanthin. Zooca® Lipids support optimal growth, immunity, and reproductive performance in shrimp and fish. They play a crucial role in enhancing feed conversion efficiency, providing energy,enhancing pigmentation, and improving the overall health of aquaculture animals.

Zooca®SeaFrozen contains essential nutrients, including proteins, lipids, vitamins, minerals, and omega-3 fatty acids. These nutrients are vital for shrimp's growth, development, and health.The product is delivered in ready-to-use packages for easy administration in the late PL, nursery, or broodstock stages.

Zooca® products are designed to meet the nutritional needs of aquaculture animals while addressing the industry's sustainability and quality concerns. With innovative formulations and production techniques, these products contribute to the advancement of aquaculture practices, supporting the growth and success of shrimp and fish farming operations.

Fig. 3. The Zooca Process Plant Tromsoe Norway

Fig. 4. The Zooca Powder.

Fig. 5. The Zooca Powder.

Zooca solutions for shrimp farms

The two most critical challenges the shrimp industry faces are diseases and the need for new aquafeed ingredients. Zooca suggests introducing products derived from C. finmarchicus to mitigate these issues to a large extent. All Zooca products are derived from C. finmarchicus, one of the largest renewable resources on the planet. Withan annual production of almost 300 million metric tons in Norwegian waters alone,C. finmarchicus is one of the few marine resources that can impact the makeup of future aquafeeds.

To ensure the quality and safety of Zooca products for shrimp farming, the harvesting is carefully conducted in cold Norwegian waters with an harmful pathogens and adherence to strict regulatory guidelines.Furthermore, rigorous quality control measures, including testing and certification processes, ensure that the harvested C. finmarchicus meets the necessary safety standards. These measures ensure the product is free from harmful pathogens and antibiotics and can be safely used in shrimp farming without posing a risk to the shrimp stock.

Copepods, such as C. finmarchicus, play a crucial role in the marine ecosystem as a primary food source for various aquatic species, including shrimp. Zooca has found a method to take care of the natural and valuable nutrients of the copepods, giving the aquaculture industry access to this sustainable source through the Zooca™ brand.

In Norway, Zooca, in cooperation with Skretting and salmon producer Andfjord Salmon has developed a new salmon feed to address the challenging transfer phase in Salmon production and has achieved an almost non-existent mortality rate, surpassing industry standards. These findings suggest that the Zooca-fed fish have the potential to be more robust and grow at a faster rate than those that have been fed with conventional feed.

Zooca advantage

Zooca maintains that Zooca-based feed will promote enhanced nutrition beyond the nutritional values alone. Specific functional properties linked to the suitability of the Zooca ingredients promote the growth, development, and overall health of farmed shrimp, as these ingr edients contain bioactive compounds that have been shown to have immunostimulatory effects. Incorporating Zooca ingredients into shrimp diets is believed to strengthen the immune system of the shrimp, making them more resistant to diseases and infections. Other studies have shown homogenous growth in juvenile shrimp populations, which provides a lower risk of cannibalism and low handling costs.

Another major challenge the Zooca-based feed can cover is the global impact of the rapidly changing environment. Asian and South Asian, including Indian shrimp farmers, have been experiencing large production losses due to unpredictable and changing weather conditions.

Here shrimp produced with a Zooca-based diet is expected to be more resilientand with a more robust immune system that can tackle such conditions better than their peers.

Lastly, Zooca products are sustainable, traceable, and eco-friendly feed ingredients. Zooca is harvested from the wild in a manner that does not deplete fish stocks or harm the marine environment. By incorporating Zooca into shrimp farming practices, we can adopt an environmentally conscious alternative to traditional feed ingredients. This can help reduce the dependence on fish-derived ingredients and promote a more sustainable approach to aquaculture.

The use of Zooca-based feed in shrimp farming can alsoprovide market advantages. With consumers' growing interest in sustainable and high-quality seafood, shrimp farmers who adopt Zooca™ products can cater to this demand, allowing them to sell their premium products at higher prices.

All the information presented in this article is primarily derived from internal and external testing and is based on data yet to be published. During the next few months, we will issue several peer-reviewed articles to strengthen the scientific evidence of the functional properties of the Zooca products.

We are eager to bring the transformative potential of the Zooca product line to Asian shrimp farmers and feed producers. Our initial efforts will be focused on India, the leading producer in this region.By leveraging these innovative products, Indian farmers can significantly advance shrimp health, productivity, and profitability while promoting sustainable practices and meeting evolving market demands.

Zooca believes that the solution to global challenges begins with the individual choices of feed producers and shrimp farmers and that the tiniest things can have the biggest impact.

Fig. 6. The Zooca Plant Tromsoe Norway.

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President of India appreciates ICAR- NBFGR model for livelihood

SPOT NEWS

During the visit to Lakshadweep by the Hon'ble President of India Smt. Droupadi Murmuon on 18 March, 2023, she appreciated the efforts of ICAR National Bureau of Fish Genetic Resources (NBFGR) in the livelihood development of island women.

During her address, the President added that this initiative is a unique venture in which science and societal development are taken up hand in hand with local bioresources.

Following her address, there was an interactive session (19 March) with the women beneficiaries attached with the community aquaculture units for marine ornamentals and the president was appraised of the mode of operation of the community aquaculture units and their role in enhancing the income of the women islanders.

The beneficiaries attached with the NBFGR's community aquaculture units, viz., Ms. Haslamathbi, T., Mrs. Sabeena Beegum, U., Mrs. Nisa Soorath, S. M., Mrs. Nafeesath, M. P., Mrs. Amminabi, K. T. P., attended the interaction with the President and requested to expand the activities, so that they can get more income.

A germplasm resource centre for marine ornamentals was established at Agatti, Lakshadweep by the institute, and they have discovered four new marine ornamental shrimps and developed captive propagation technology.

Following this, the institute has given training to the self-help group clusters who have adopted simplified technology and are successfully running the community aquaculture units and rearing ornamental shrimps and fishes.

Fig. 1. Beneficaries with President of India

This has resulted in creation of sustainable livelihood among the local population with subsistence income. The success of the developed model by the institute, leads to the possibility of expanding the same to other islands of Lakshadweep for livelihood development in the coming days, with the funding support of CMLRE, Ministry of Earth Sciences, Govt. of India.

Fig. 2. Dr. U. K. Sarkar, Director, NBFGR along with Dr. T.T. Ajith Kumar, Scientist In-Charge, The Peninsular and Marine Fish Genetic Resources (PMFGR), Cochin is distributing seeds and culture devices to beneficiaries.

Fig. 3. Seeds of Thor hainanensis.

ICAR scientists name new fish species after Tamil Nadu

SPOT NEWS

ICAR-National Bureau of Fish Genetic Resources (NBFGR) functioning under the aegis of Indian Council of Agricultural Research (ICAR), have discovered a new fish species, Moray eel fish of the genus Gymnothorax, from the Cuddalore coast.

The newly discovered fish species has been named after state "Tamil Nadu", as Gymnothoraxtamilnaduensis with a common name as "Tamil Nadu brown moray".

The species has been described based on specimens / samples collected through exploration -survey conducted along the coastal waters of Cuddalore district,Tamil Nadu. This was particularly undertaken at fish landing centres of Parangi pettai and Mudasalodai by the Ph.D. Scholar Mr. P. Kodeeswaran and Scientist, Dr.G. Kantharajan.

The researchers conducted extensive morphological analysis, skeleton radiography, and advanced molecular markers combined with species delimitation computational techniques

To conclude that this Moray eel specimens from Mudasaloodai is distinct from other species of the genus Gymnothorax. Further, few specimens were sent to Dr. Anil Mohapatra, Scientist, Zoological Survey of India, who is an expert in the field, for final confirmation.

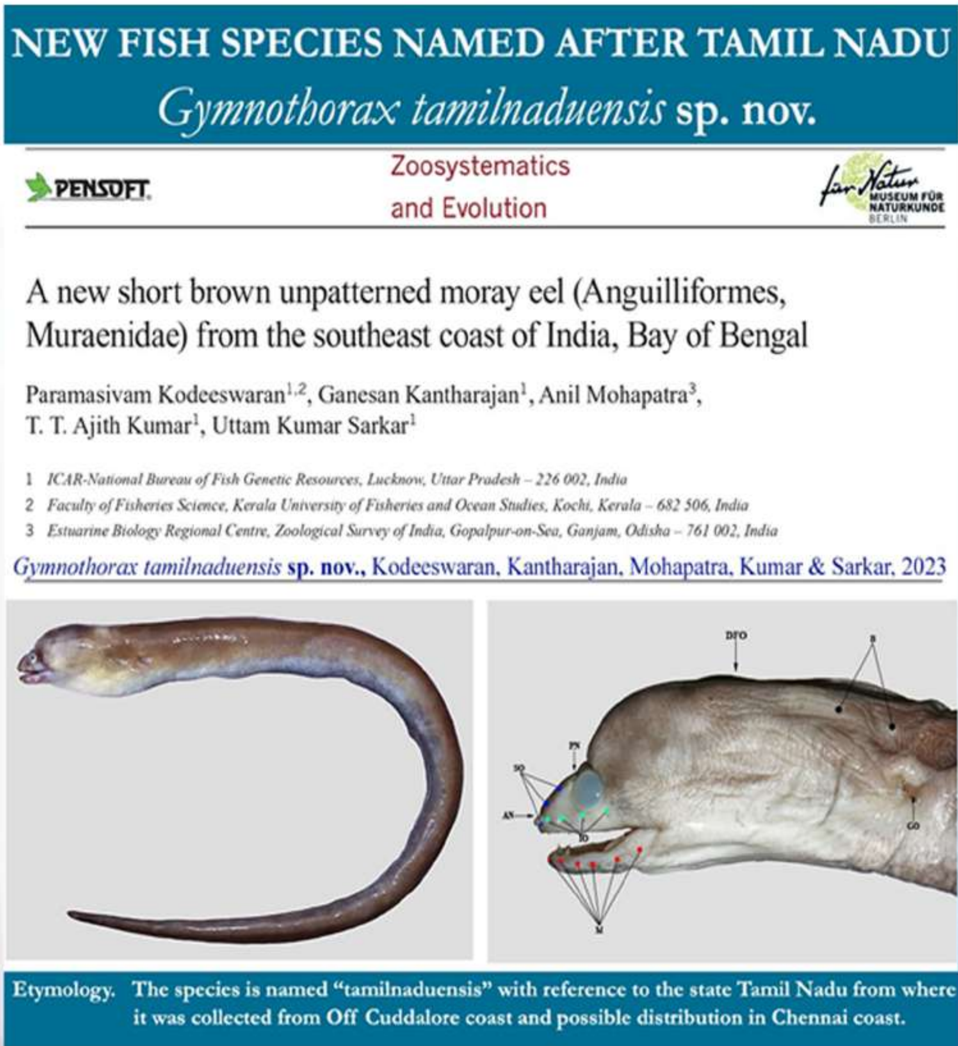

Fig. 1. New Fish species named after Tamil Nadu.

In Indian waters, hitherto 28 species of Gymnothorax have been documented so far. The species described herein represents India and increases the total amount of species of Gymnothorax to 29. The present description is also the first new species of the genus Gymnothorax from the south-eastern coast of India, Bay of Bengal.

The holotype of this new species is registered at the National Fish Museum and Repository of the ICAR-NBFGR, Lucknow. The name of the species is registered in ZooBank, the online registration system for the International Commission on Zoological Nomenclature (ICZN). The discovery was accepted by the international scientific community and the findings published in the international peer-reviewed journal "Zoosystematics and Evolution".

Fig. 2. The short brown unpatterned moray eel

A field trial study of AQUA-Cal+ on shrimp in earthen ponds

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Introduction

Shrimp farming has become a crucial part of aquaculture in many locations, considerably contributing to global seafood output and economic growth (B et al., 2022; Páez-Osuna, 2001). Shrimp farmers, on the other hand, frequently confront various obstacles in maintaining ideal circumstances for shrimp development, health, and survival (Jamal et al., 2019).

The quality of the aquaculture water in which shrimp are grown is a critical aspect that considerably impacts their output(Páez-Osuna, 2001).Water quality becomes undesirable when farmers opt for overstocking, overfeeding, and usingpolluted water. Poor water quality promotes ailments, mortality, poor growth, and low shrimp yield(Venkateswarlu et al., 2019).Another activity connected with environmental degradation in receiving waters is the discharge of pond water effluent(Venkateswarlu et al., 2019).Temperature, dissolved oxygen, pH, salinity, and nutrient levels, among other water quality characteristics, must be carefully regulated to provide an atmosphere suitable for practical shrimp farming. Alkalinity measures a water's ability to resist changes in pH, which is critical for aquatic organisms to maintain a stable environment. Managing alkalinity levels, however, can be difficult, especially in closed systems, where natural buffering systems are limited.

Earthen ponds are frequently employed in Indian shrimp farming because of their low cost and ease of building(Saraswathy et al., 2022). Bottled soil silt's composition greatly influences pond water's physical and chemical properties. Bottom soil sediment delivers both nourishment and shelter to shrimp in culture pond environments, as well as acting as a nutrient reservoir for the growth of microalgae, which are natural food for aquatic animals(Boyd & Green, 2002; Boyd & Tucker, 2012; Venkateswarlu et al., 2019). However, these ponds bring intrinsic water quality management difficulties, such as changes in pH, dissolved oxygen levels, and nutrient dynamics(Venkateswarlu et al., 2019). As a result, there is an increasing demand for practical solutions that may optimize water quality parameters in earthen ponds and increase shrimp farm productivity.

In recent years, the aquaculture industry has witnessed the emergence of various innovative products and approaches aimed at improving shrimp farm productivity. One such product is AQUA-Cal+, a mineral supplement for enhancing shrimp growth, survival, and overall pond water quality. AQUA-Cal+ is designed to address the unique challenges shrimp farmers face, particularly those cultivating shrimp in earthen ponds. AQUA-Cal+ is a product intended to maintain alkalinity levels in aquatic systems. AQUA-Cal+ offers versatility as it comes in different forms, such as powder, granules, and tablets, allowing aqua farmers to choose the most convenient application method.

It is user-friendly, as it dissolves rapidly, making it an ideal choice for aqua farmers seeking simplicity and efficiency in their operations. The product assists in pH stabilization, avoiding drastic shifts that can harm fish and other aquatic creatures. AQUA-Cal+ also promotes aquatic plant growth and development by supplying vital nutrients such as calcium and carbonate ions. Furthermore, the product aids in the reduction of harmful toxins such as ammonia and nitrite, which can be fatal to fish and other aquatic organisms.

A small shrimp farm in the West Godavari district of Andhra Pradesh, India, has encountered various difficulties. The farm has been dealing with unexpected fluctuations in magnesium, calcium, and alkalinity levels and an increased presence of bacteria. It has been further compounded by frequent sludge drainage, leading to a decline in the overall quality of the shrimp produced. A trial experiment was conducted in this shrimp pond, comparing a control pond that followed regular practices instead of dosing AQUA-Cal+ in other ponds. The water quality and shrimp yield in the two ponds were compared in this field study.

This article serves as a comprehensive introduction to the field trial study, setting the stage for the subsequent sections that delve into the methodology, results, and discussion surrounding the effectiveness of AQUA-Cal+ on shrimp in earthen ponds.

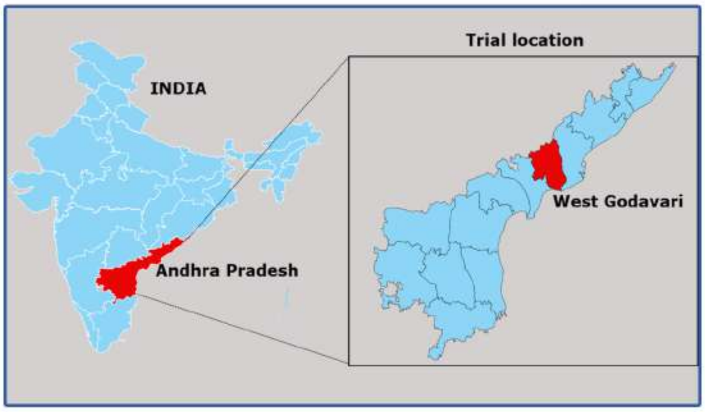

Fig. 1. Map of West Godavari district located in Andhra Pradesh state.

Pond	Size	PL age (days)	Stocking density (per m2)	Number of shrimp stocked	Duration of trail (days)
Control	0.64 HA	60	28	180000	30
AQUA-Cal+	0.8 HA	60	25	200000	30

Table 1. Earther ponddetails of the trail

Pond	Pre-treatment rate (ppm)	Pre-treatment rate (kg)
Control	0	-
AQUA-Cal+	4	40/week until 9th week, 50/week until harvest

Table 2. AQUA-Cal+ dosing details of the trail

2. Methodology

2.1 Trial Settings

West Godavari district in Andhra Pradesh state, India was the Trial Location (Fig 1). The trial farm consists of 10 active ponds, each constructed using earthen materials. On average, these ponds cover an area of half a hectare and have a depth of one meter. To optimize space, the farm typically stocks 25 shrimp per square meter. It takes approximately 10-12 weeks for these shrimp to reach the desired size for harvesting. For the trail, ponds were named Control Pond, which does not receive AQUA-Cal+ treatment and AQUA-Cal+ pond,which received proper AQUA-Cal+ treatment. The stocking density and pond details of the trial ponds are given in the Table. 1. Dosing of AQUA-Cal+ pretreatment was four ppm, and it is suggested to dose 40 kg/pond till the 9th week of culture and 50 kg/pond from the 10th week to harvest (Table. 2).

2.2 Water quality parameter analysis

Water from each pond was sampled at the subsurface (0.3 m depth) by a water sampler. Samples were always taken in the morning (0600 to 0800) to minimize errors. Using commercially available kits, physical and chemical parameters of pond water, such as alkalinity, hardness, and ammonia levels, were measured by titration method as previously stated (APHA, 1992). Total vibrio was counted in Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) culture medium. Parameters in this field trial study were analyzed at Angel Aqua Clinic, Bhimavaram, following standard methods described by American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF) (APHA et al., 1998).

3. Results and Discussion

NH_3

3.1 AQUA-Cal+ lowers alkalinity level

Alkalinity is critical in shrimp farming because it affects the molting process. When alkalinity levels are low, pH levels fluctuate significantly, hampering shrimp growth and possibly resulting in mortality. Excessive alkalinity, conversely, can interrupt the molting process by causing the shrimp to lose excess salt (Boyd et al., 2016). The AQUA-Cal+ procedure easily controls the alkalinity of the pond. AQUA-Cal+ stabilizes the alkalinity throughout the culture period and significantly reduces the alkalinity level by 10.4 % compared to the control pond (Fig 2). Increased alkalinity in untreated ponds might have resulted in undesirable pH and decreased the molting process.

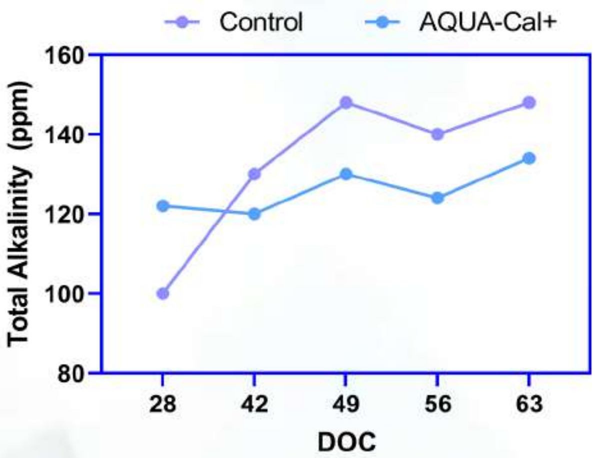

Fig. 2. Total Alkalinity in ppm of control and AQUA -Cal+ treated ponds. Alkalinity was more stable in the AQUA-Cal+ pond than in the control pond.

3.2 AQUA-Cal+ suppresses ammonia level

Ammonia is the primary byproduct of the breakdown of proteins in crustaceans, accounting for 40% to 90% of nitrogen excretion(Parry, 1960). The total ammonia level of the control pond is unstable throughout the culture period. AQUA-Cal+ treated pond showed a 16% reduction in ammonia level at the end of the culture period (Fig 3). Shrimp farmers take extra pond care to reduce ammonia levels in their ponds. As ammonia is excreted extensively by shrimps due to their metabolism, overstocked pond tends to have higher ammonia levels. Unionized ammonia (NH_3) is hazardous to shrimps due to its capacity to pass through cell membranes and cause undesirable effectson shrimps.

The amount of NH_3 greatly depends on pH and temperature, fluctuation in pH may directly raise ammonia levels and cause ammonia toxicity(Bower & Bidwell, 1978). Therefore, controlling ammonia (NH_3) in shrimp ponds is crucial for shrimp health.

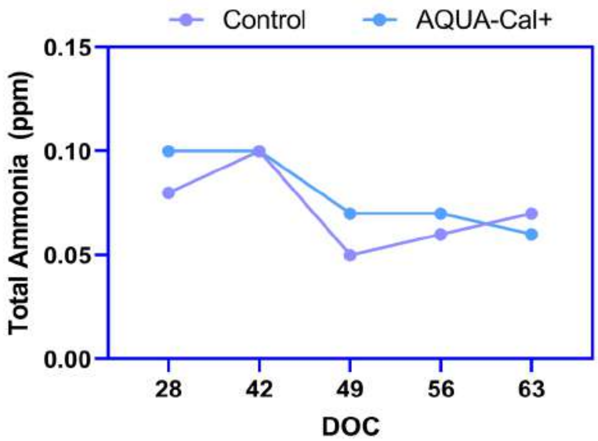

Fig. 3. Total Ammonia in ppm of control and AQUA-Cal+ treated ponds. AQUA-Cal+ suppresses ammonia levels when compared with untreated control ponds.

3.3 AQUA-Cal+ reduces hardness

Maintaining appropriate hardness levels in a shrimp farm is crucial for the overall health and well-being of the shrimp. By maintaining optimal hardness levels, shrimp farmers can create an environment that promotes healthy shell development, proper osmoregulation, stable pH, nutrient availability, and reduced risks of metal toxicity, ultimately leading to improved shrimp growth, survival, and overall farm productivity(Boyd et al., 2016).Hardness was higher in the control pond than AQUA-Cal+ treated pond throughout the culture period (Fig 4). AQUA-Cal+ significantly reduced the hardness by 23.9% than untreated control ponds—AQUA-Cal+ aids in maintaining optimal hardness for the growth vannameiin Indian culture.

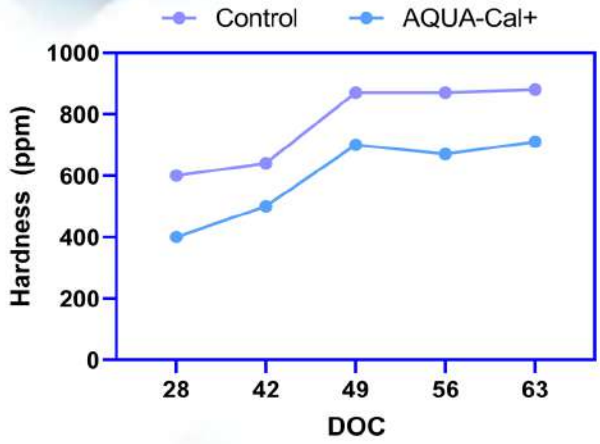

Fig. 4. Hardness of water in ppm of control and AQUA-Cal+ treated ponds.

3.4 AQUA-Cal+ controls vibriosis

Vibrio outgrowth is a common factor in shrimp culture and drastically affects cultured species leading to economic loss. Vibrio bacteria thrive in poor water quality conditions, particularly in ponds with high organic matter content, low dissolved oxygen levels, and inadequate water exchange(Chandrakala & Priya, 2017). Vibrio is introduced mainly through contaminated water;water pretreatment is crucial in controlling vibriosis.AQUA-Cal+ treated pond showed a significantly reduced vibriosis (yellow colonies) than untreated control ponds (Fig 5). Antibiotic use will be reduced if the farmer opts for AQUA-Cal+. It will significantly help reducethe environmental impact and promote environmentally-friendly farming methods.

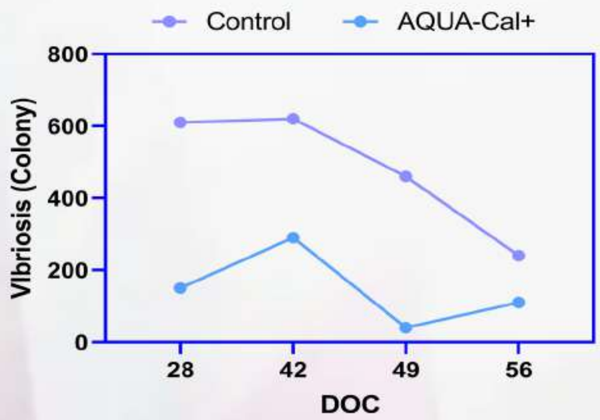

Fig. 5. Vibrio outgrowth in control and AQUA-Cal+ treated ponds. Vibrio growth was much lesser in AQUA-Cal+ pretreated ponds.

3.5 AQUA-Cal+ increased the chance of shrimp survival

Shrimp survival depends on upholding suitable water quality. Poor water quality can cause stress, impair the shrimp's immune system, and make them more vulnerable to diseases and infections.Regular pond maintenance, including toxic sludge removal, is essential for maintaining good water quality and reducing the risk of disease outbreaks. Proper pond preparation contributes to a healthier and more conducive environment for survival for shrimp.The estimated survival rate (E.SVR %) was much higher in AQUA-Cal+ treated ponds than in untreated control ponds (Fig 6). AQUA-Cal+ increased the survival by about 5% to control ponds.This result shows the potential of AQUA-Cal+ in increasing the revenue and economy of shrimp culture in India.

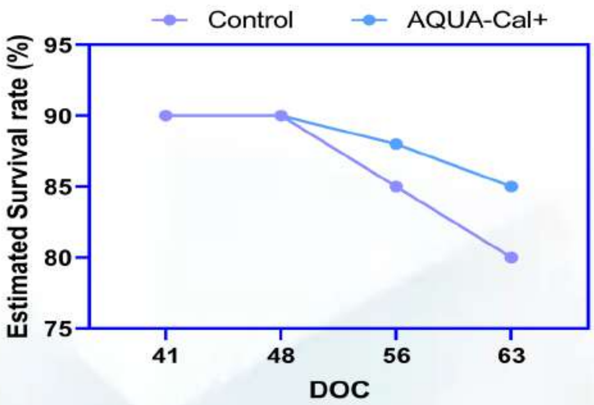

Fig. 6. Estimated survival rate in control and AQUA-Cal+ treated ponds. Due to water quality improvement, AQUA-Cal+ treated pond showed a higher survival rate.

3.6 Increase in Average daily growth in AQUA-Cal+ pond

The average daily growth of shrimps was 5.2% higher in the AQUA-Cal+ pond than control pond (Fig 7A). It implies that AQUA-Cal+ treatment Could increase the daily growth of shrimps. Shrimp's average growth rate depends on water quality, feed, stocking density, and farm management. As stated earlier, water quality was improved in AQUA-Cal+ ponds. In return, the shrimp growth performance was also improved. The feed conversion ratio was low in AQUA-Cal+ ponds (Fig 7B). Shrimps in AQUA-Cal+ ponds showed 4% less FCR than control shrimps. It helps in generating higher revenue by low feed input.

3.7 Increase of biomass in AQUA-Cal+ pond

Increasing biomass in a shrimp farm is a goal for many shrimp farmers as it directly translates to higher productivity and profitability.The estimated Biomass of AQUA-Cal+ ponded shrimp was significantly higher by 19.5% than the untreated control pond (Fig 8A). Average body weight of the shrimp in the AQUA-Cal+ pond was also increased by 13.1 g,which is 5.7% higher than the control shrimps (Fig 8B). This shows that AQUA-Cal+ promotes the growth of the shrimps. AQUA-Cal+ has improved the water quality, resulting in improved survival and biomass (Fig 9). Low vibrio growth also indirectly contributes to biomass increase, as vibrio may hinder shrimp growth and productivity.

Fig. 9. Harvested shrimps of control (without AQUA-Cal+) and AQUA-Cal+ treated ponds.

Pond	Avg. body weight (g)	Survival (%)	FCR	Harvest weight (kg)	Difference against Control (%)			
					Avg. body weight (g)	Survival (%)	FCR	Harvest weight (kg)
Control	12.4	79	1.3	1686	+5.7 ↑	+ 7 ↑	-4 ↓	+19.5 ↑
AQUA-Cal+	13.11	86	1.25	2097				

Table 3. Growth parameters at the end of the trail.

Objectives	Properties
Alkalinity stability	Maintained between 110 – 150 ppm
Nitrite control	Maintained at 0 ppm
Hardness	Maintained between 700 - 730
Water turbidity	Minimal turbid against control with better water clarity
ADG	Around 16% higher against control
ABW	Around 23% higher against control
FCR	Around 10% lower against control
Vibriosis control	Controlled yellow colonies and no growth of green colonies

Table 4. Measurement observed in AQUA-Cal+ treated ponds.

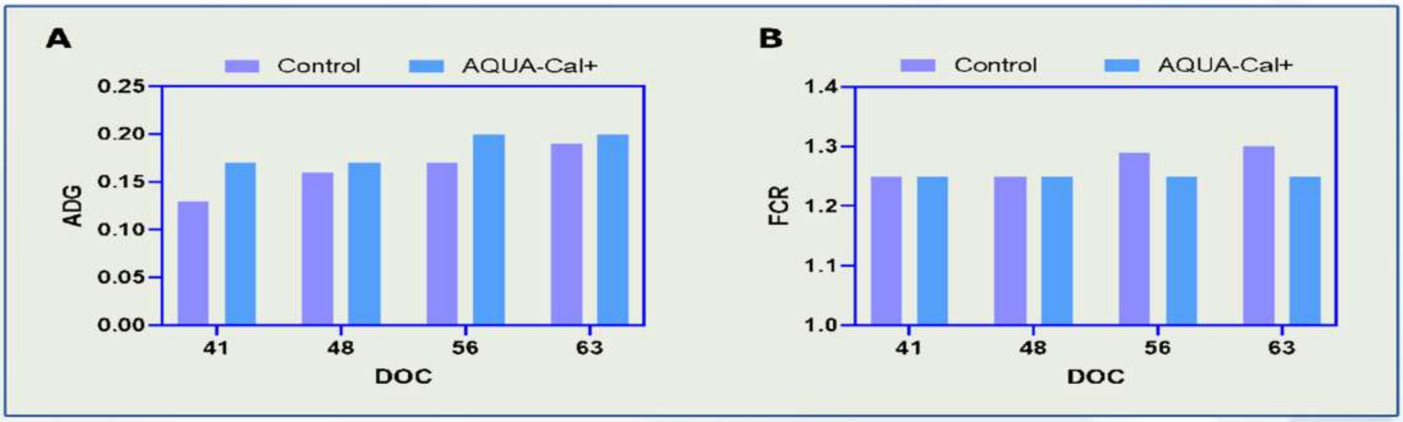

Fig. 7. (A) Average daily growth (ADG) and (B) feed conversion ratio (FCR) of control and AQUA-Cal+ treated ponds. ADG was improved, and FCR was reduced in AQUA-Cal+ n AQUA-Cal+ treated ponds.

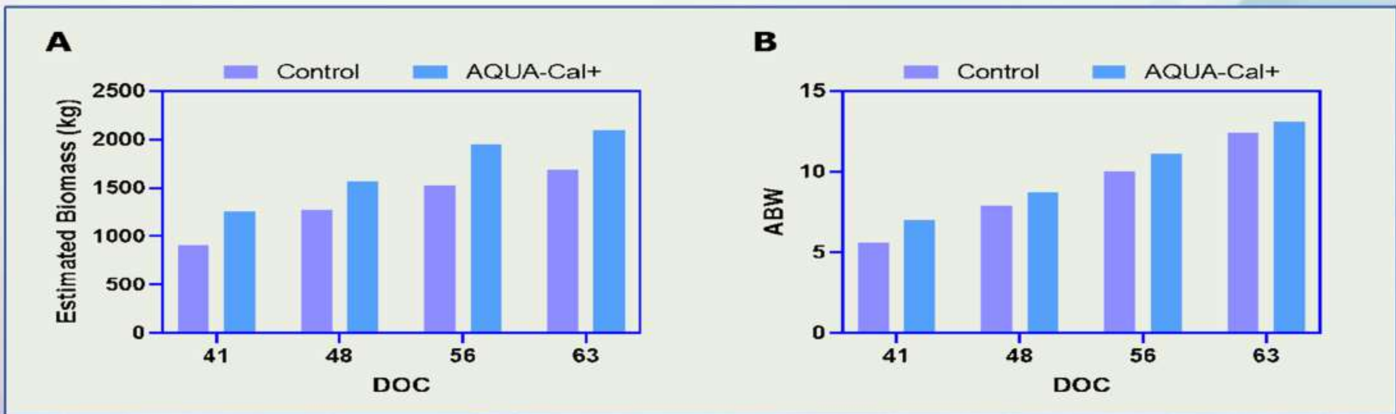

Fig. 8. (A) Estimated Biomass in kg and (B) average body weight (ABW) of control and AQUA-Cal+ treated ponds. Both biomass and ABW were improved in the AQUA-Cal+ pond compared to the control pond.

Amazing Biotech Pvt Ltd.,

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- **Physical Quality analysis**
- **Water and Soil Quality testing**
- **Plankton analysis**
- **Microbiology - Luminescence bacteria analysis**
  Vibro Plating
  General plating analysis
- **Algae Counting and Quality analysis**

- **WSSV**
- **EHP**
- **IHHNV**
- **AHPND**
- ***Vibrio parahaemolyticus***
- ***Vibrio harveyi***
- ***Vibrio alginolyticus***
- ***Vibrio vulnificus***
- Species specific Identification for Vibrios

MPEDA

NFDB

ISO 9001:2015

DSIR RECOGNIZED

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Identification of natural bioactive compounds against rainbow trout viral hemorrhagic septicemia virus (VHSV) by targeting NV protein (R116S) : A computational drug design approach

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Abstract

Fish of all ages are prone to infection, with fry and juveniles being the most sensitive. The viral hemorrhagic septicemia virus (VHSV) is one of the most fatal infectious fish infections, causing severe mortality in a variety of marine fish species including rainbow trout, with a death rate of up to 100% in young fish. This fatality is caused by a virus from the Rhabdoviridae family. However, there is no obvious way to control the spread of this virus due to a lack of effective therapeutic drugs or vaccines. VHSV has been discovered to employ NV protein (R116S) to enter the host cell and cause disease in Rainbow trout.

The NV inhibition may prevent virus budding and virion maturation and might be used to build an antiviral therapeutic candidate. Therefore, the research sought to find prospective natural antiviral drug candidates from the four different marine algae, viz., Halymenia dilatata zanardini, Chlorococcum humicola, Caulerpa peltata, and P. gymnospora that would be able to inhibit the virus's budding and virion maturation process by blocking the virus's NV protein activity. Following a homology modeling technique, the protein's 3D structure was identified and refined, and then validated. The improved protein structure was then used to simulate molecular docking. The binding affinity of all the compounds was estimated using the docking technique, and the top three inhibitory compounds (PubChem CID: 124120485, 33934, and 610132) were chosen for further ADME and toxicity properties. The ADME and toxicity analyses demonstrated that the compounds were effective and nontoxic with the targeted protein. The data suggest that the selected three phytochemicals from H. dilatata zanardini, C. humicola, C. peltata, and P. gymnspora may be important VHSV inhibitors in rainbow trout, which could be studied further in the lab.

Introduction

Rainbow trout (Oncorhynchus mykiss) is produced commercially in many nations across the world because to its fast growth and high market value due to the high quality of its flesh(Jamal et al., 2020; Sumon et al., 2022). However, rainbow trout can be affected by a variety of viral pathogens, which can have profound economic effects on farms and the industry through reduced growth and mortality. Viral hemorrhagic septicemia virus (VHSV) is a most deadly infectious fish pathogens leading to high mortality in a large panel of marine fish species including Rainbow troutwith a mortality rate as high as 100% in juvenile fish (Baillon et al., 2020; Kesterson et al., 2020).

Several studies have found that the Non virion (NV) protein plays an important biological role in either viral replication or the pathogenicity of the VHSV virus. (Baillon et al., 2017; Baillon et al., 2020; Biacchesi et al., 2017; Thoulouze et al., 2004). VHSV or Piscine novi rhabdo virus is a bullet-shaped, encapsulated virion that contains a non-segmented, negative sense, single stranded RNA molecule that belongs to the Rhabdoviridae family of the Mononegavirales order(Schütze et al., 1996).Rhabdoviruses encode five structural proteins with conserved functions in the order 3′-N-P-M-G-NV-L-5′ (Dietzgen et al., 2017).Viral RNA is tightly encapsidated with a nucleoprotein (N), a polymerase-associated phosphoprotein (P), and a large RNA-dependent RNA polymerase (L), forming the helical ribonucleoprotein complex (RNP). A matrix protein (M) participates in budding and interacts with the RNP and viral envelope (Ke et al., 2017). As a last step, the viral surface glycoprotein (G) is involved in the entry process, making it a distinct target for neutralizing and protective antibodies. The VHSV genome, on the other hand, has an additional gene located between the G and L genes that codes for a small non-structural NV (Non-Virion) protein. It was dem llon et al.(Baillon et al., 2020) revealed 38 single amino acid polymorphisms (SAPs) dispersed over the rainbow trout genome, which may be modulating the pathogenicity of the virus and serving as a molecular marker for the virulence of the virus.

Marine organisms, particularly algae, produce a wide range of compounds having pharmacological activity, such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory, and others, and are potential sources of novel therapeutic agents (Pérez et al., 2016). Metabolites of green, brown, and red marine algae may be beneficial in suppressing bacteria, viruses, and fungus.

Algal fractions or purified algae components, for instance, have anticoagulant (Wijesinghe et al., 2011), antiviral (Damonte et al., 2004), antioxidant (Cox et al., 2010), anticancer (Namvar et al., 2013) and antiinflammatory (Kazłowska et al., 2010)properties.

Marine algae, Halymenia dilatata zanardini (Jainab et al., 2019), Chlorococcum humicola(Kavitha & Palani, 2016), Caulerpa peltata, and P. gymnospora(Murugan & Iyer, 2014)have been utilized for medicinal and other purposes (fish feed, food additives etc.). Several studies confirmed that these algae have rich of bioactive compounds. Algae, sponges, fungus, seaweed, corals, and other marine flora are plentiful and have pharmacologically active novel chemical signatures that are capable of providing new antimicrobial compounds(Wali et al., 2019). Functional bioactive compounds derived from marine plants have proven to be a valuable source of many therapeutic drugs, and they have benefited mankind in treating numerous diseases (Egbuna et al., 2019).

The selection of small bioactive molecules and investigation of their interactions with the targeted protein are required for the development of targeted therapeutics for the fish industry (Aljahdali et al., 2021). Computer aided drug design (CADD) or in-silico methodologies are increasingly important in drug discovery, particularly in the cost-effective identification of promising drug candidates (Brogi et al., 2020).

In-silico methods, such as molecular dynamics, homology modeling, and molecular docking have been used to find a compound with the best properties for a specific target. With molecular docking-based scoring functions, compounds that are most effective against specific targets can be identified, and their interactions can be documented (Torres et al., 2019).

By integrating early ADMET (absorption, distribution, metabolism, excretion and toxicity) profiling of compound, their efficacy and toxicity can be easily predicted, and MD simulations confirm a compound's affinity to the targeted protein (Tareq Hassan Khan, 2010).Developing new drugs can be challenging, but there is a need to advance investigations to identify bioactive compounds by targeting novel protein classes. Therefore, we intend to apply an in-silico approach to test the new drug candidates against the VHSV virus that targets non-virion (NV) protein.

2. Materials and methods

2.1. Protein structure prediction through homology modeling

The amino acid (aa) sequence of the NV protein found in Nervous necrosis virus (NNV) was retrieved from the NCBI database and downloaded in FASTA format. This was done in order to study the structure of the NV protein. The retrieved sequence was sent on 30 April 2022 to the prominent online web portal Iterative Threading Assembly Refinement (https://zhanglab.dcmb.med.umich.edu/ITASSER/) in order to ensure or anticipate the three-dimensional (3D) structure of the intended protein(Roy et al., 2010). Top five protein structure models were developed by I-TASSER, which also offered the C-score, TM-score value as well as the root mean square deviation (RMSD) of protein structure. The best protein 3D structure was selected based on the C-score and downloaded as a PDB file. A higher C-score implies that the protein model had a wide range of confidence from positive to negative values.

2.2. Protein structure refinement and validation

The 3D structure of proteins was refined via the GalaxyWeb server.The structural validity is a vital step in homology modeling, which relies on empirically confirmed 3D protein structures.PyMol v2.3.4 software is used to visualize the improved structure. The Ramachandran plot score function was utilized to validate the improved model. Furthermore, the 3D structure is evaluated using the Ramachandran plot score (drug design) and z-score value, which determines the standard deviation via the primary value (Ho et al., 2003).

2.3. Protein and ligand preparation

To construct and develop the protein's 3D structure, the following criteria were applied: water, metal ions, and cofactors were eliminated; polar hydrogen atoms were inserted; nonpolar hydrogen was combined; and gasteiger charges were calculated using AutoDockTools.IMPPAT database was utilized to identify the compounds of the selected plants(Mohanraj et al., 2018). A total of 40natural phytochemical compoundswas retrievedfrom Halymenia dilatata zanardini, Chlorococcum humicola, Caulerpa peltata, and P. gymnospora. To configure and reduce energy for molecules chosen from the database, the Universal Force Field (UFF) designed for each ligand was employed.

2.4. Binding site identification and grid box generation

The NV protein structure has been uploaded to the CASTp 3.0 server (http://sts.bioe.uic.edu/) and examined on 2022 in order to estimate the active site residues. Different active pockets of the protein were detected by the server, and the first active pocket was chosen based on its surface area (SA) and volume.

To visualize the binding pocket of the protein, the active pocket and their corresponding amino acid residues were extracted using BIOVA Discovery Studio Visualization Tool 16.1.0.The binding site residues generated by the server were then utilized to select the grid box for molecular docking simulations.

2.5. Molecular docking simulation

PyRx is an open-source tool for virtual screening that is largely utilized in CADD procedures. It is capable of screening libraries of compounds to determine how well they perform against a specified therapeutic target(Dallakyan & Olson, 2015).

PyRx includes AutoDock 4 and AutoDock Vina docking wizards with an intuitive user interface, making it a more reliable CADD tool. In this experiment, the AutoDock Vina molecular docking wizard was used to find the best way for the protein and ligand to bind.

2.6. ADME analysis

The selected compounds were also subjected to ADME analysis.SwissADME software was used to predict the absorption, distribution, metabolism, and excretion (ADME) characteristics of all of the selected substances(Daina et al., 2017).Based on Lipinski's criteria, this software can validate the drug-likeness of ligands.

2.7. Toxicity test

To discover the harmful effects of chemicals on humans, animals, plants, and the environment, their toxicity must be assessed. In silico toxicology analyzes, simulates, visualizes, or predicts chemical toxicity using computational methods(Raies & Bajic, 2016).ProTox-II was utilized in this research to investigate acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity of the substances.

3. Results

3.1. Protein 3D structure, refinement, and validation

In this study, homology modelling was used to generate NV protein structure. A best 3D protein structure of NV protein was selected from top five models of protein provided by the I-TASSER server. Theselected best protein structure has lowest C-score is -4.03.The NV protein structure after refinement has a GDTHA score of 0.8934, an RMSD value of 0.618, and MolProbityvalue of 2.417.Prior to refinement, Using the Ramachandran plot, the NV protein had 83.7 percent, 11.3 percent, and 4.7 percent residues in the favorable, allowed, and disallowed regions, whereas the refined the NV protein model had 83.962%, 11.321%, and 4.717%residues in the favorable, allowed, and disallowed regions, respectively. (Fig 1A). Similarly, the crude Exportin1 model has a Z-score value of4.2, which has improved to -4.66 after refinement (Fig 1B).

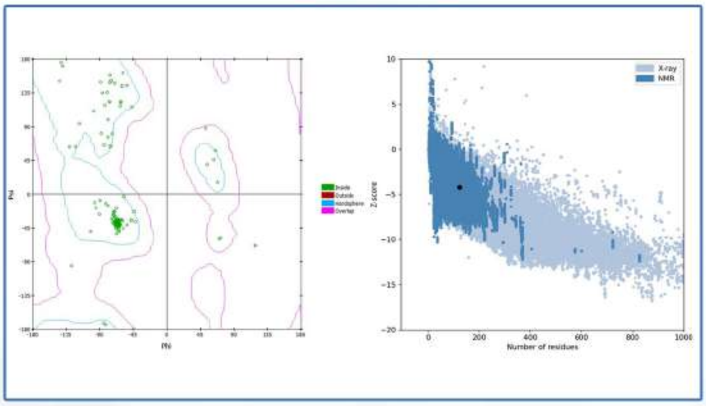

Fig. 1. Validation of the 3D structure of the NV protein. (A) The Ramachandran plot statistics represent the most favorable, accepted, a disallowed region with a percentage of 83.962%, 11.321%, and 4.717% respectively, and (B) the Z-score of refine NV protein -4.66.

3.2. Protein phytochemical and protein preparation

A total of forty phytochemical compounds of C. candolleana and H. fomes plants were retrieved from the popular database IMPPAT and stored in a 2D (SDF) file format. Compounds were prepared, optimized, and then converted into pdbqt files for further analysis during the ligand preparation process. The protein was optimized and prepared for docking using the AutoDock tool, and then saved in the pdbqt format.

3.3. Binding site active site identification and receptor grid generation

Enzyme active sites (AS) possess a specific shape that permits them to bind with a specific substrate and undergo a chemical reaction.The study first identified AS of the NV protein peptide from CASTpi server then the combined binding position of the active site was retrieved (Fig 2). Analysis of the active pocket of the protein helped to retrieve the binding site residue of the protein (Fig 2). Active site pocket analysis revealed binding site position at ILE3, GLN4, LEU71, ARG74, PRO101,GLY102, PHE104, SER107 residual positions that have been represented in ball shape with different colors red, pink, light and deep green, yellow and blue colors as shown in Figure structure has been uploaded to the CASTp 3.0 server (http://sts.bioe.uic.edu/) and examined on 2022 in order to estimate the active site residues.

Different active pockets of the protein were detected by the server, and the first active pocket was chosen based on its surface area (SA) and volume. To visualize the binding pocket of the protein, the active pocket and their corresponding amino acid residues were extracted using BIOVA Discovery Studio Visualization Tool 16.1.0.The binding site residues generated by the server were then utilized to select the grid box for molecular docking simulations.

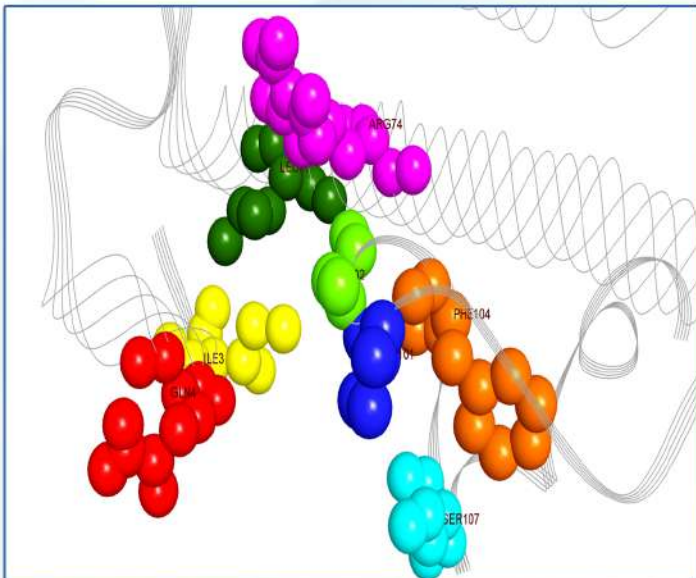

Fig. 2. Showing the active site and correspondence binding site of the NV protein. Ball shapes with red, pink, green, yellow, and blue colors, respectively, with their binding site position of the NV protein.

3.4. Protein molecular docking simulation

In this study, homology modelling was used to generate NV protein structure. Forty phytochemical compounds were utilized for molecular docking process by using PyRx tools AutoDock Vina wizard. The binding affinity showed a distributed range from −3.2 and −8 after molecular docking of phytochemicals compound. The top ten percentage phytochemical compounds have been chosen from the 40 compounds based on the capacity of top binding affinity. The best three compounds, which are namely 3−methoxy−(3.beta)−cholest−5−ene(CID124120485), Diisooctyl phthalate (CID33934), and Anthracene, 9,10-diethyl-9,10-dihydro- (CID610132) have been selected based on their docking score −8 kcal/mol, −7.3 kcal/mol, and −6.9 kcal/moland further evaluated through different screening methods. The best three compounds selected based on molecular docking score are listed in Table 1 and docking scores for all comA best 3D protein structure of NV protein was selected from top five models of protein provided by the I-TASSER server. Theselected best protein structure has lowest C-score is -4.03.The NV protein structure after refinement has a GDTHA score of 0.8934, an RMSD value of 0.618, and MolProbityvalue of 2.417.Prior to refinement, Using the Ramachandran plot, the NV protein had 83.7 percent, 11.3 percent, and 4.7 percent residues in the favorable, allowed, and disallowed regions, whereas the refined the NV protein model had 83.962%, 11.321%, and 4.717%residues in the favorable, allowed, and disallowed regions, respectively (Fig 3). Similarly, the crude Exportin1 model has a Z-score value of 4.2, which has improved to -4.66 after refinement (Fig 4).

Pubchem ID	Compound Name	Docking Score	Molecular Formula	Molecular Weight
124120485	(3R,8R,9R,10S,13S,14R,17S)-3-methoxy-10,13-dimethyl-17-[(2S)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopentaphenanthrene	-8	$C_{28}H_{48}O$	400.7
33934	Diisooctyl phthalate	-7.3	$C_{24}H_{38}O_4$	390.6
610132	Anthracene, 9,10-diethyl-9,10-dihydro-	-6.9	$C_{18}H_{20}$	236.4

Table 1. List of selected three compounds identified based on molecular docking score (kcal/mol) and their chemical name, formula, and correspondence PubChem CID

3.5. Protein-Ligand interaction analysis

The NV protein with the highest binding score generating compounds was chosen and retrieved to study their interaction.Using the BIOVIA Discovery Studio Visualizer tool, the interaction created between the three selected ligands and the target protein was evaluated.According to the findings, the molecule CID 124120485 establishes a number of hydrogen and hydrophobic bonds with the targeted NV protein.The hydrogen bonds found to formed at SER8position, where the hydrophobic bonds from at the position LEU71, ILE106, ILE75, ARG74, LEU78, and ILE3 position is shown in Fig 3 and the bond types are listed in Table 2.

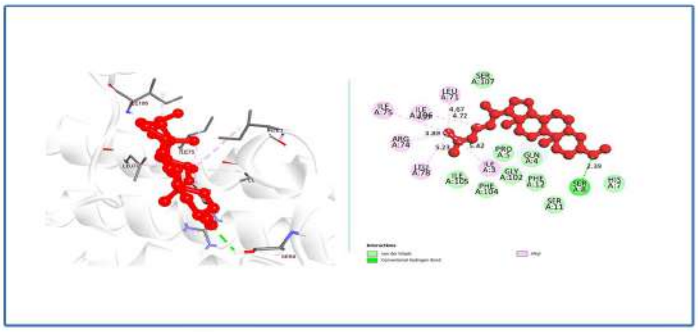

Fig. 3. Shown the interaction between the compound CID124120485 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein ligands complex.

PubChem CID	Residue	Distance	CategoryD	Type
CID 124120485	SER8	2.39	Hydrogen Bond	Conv-H-Bond
	LEU71	4.67	Hydrophobic	Pi-Alkyl
	ILE106	4.97	Hydrophobic	Pi-Alkyl
	ILE75	4.72	Hydrophobic	Pi-Alkyl
	ARG74	3.89	Hydrophobic	Pi-Alkyl
	LEU78	5.23	Hydrophobic	Pi-Alkyl
	ILE3	5.42	Hydrophobic	Pi-Alkyl
CID 33934	THR115	3.05	Hydrogen Bond	Conv-H-Bond
	PHE48	4.96	Hydrophobic	Pi-Pi Tshaped
	PHE48	4.51	Hydrophobic	Pi-Pi Tshaped
	PHE48	5.11	Hydrophobic	Pi-Pi Tshaped
	ALA44	3.70	Hydrophobic	Pi-Pi Tshaped
	PHE47	5.22	Hydrophobic	Pi-Pi Tshaped
CID 600132	ILE3	3.89	Hydrophobic	Alkyl
	PRO5	4.91	Hydrophobic	Pi-Alkyl
	PRO5	4.14	Hydrophobic	Pi-Alkyl
	PHE12	4.67	Hydrophobic	Pi-Alkyl
	ILE106	4.48	Hydrophobic	Pi-Alkyl

Table 2. List of bonding interactions between selected four phytochemical with the NV protein.

In the case of compound CID33934,it has been observed to form several hydrophobic bonds in the PHE48, ALA44, PHE47, and ILE3residual position. One conventional hydrogen bond was found to form at the position of THR115 AA position as shown in Figure 4 and are listed in the Table 2.

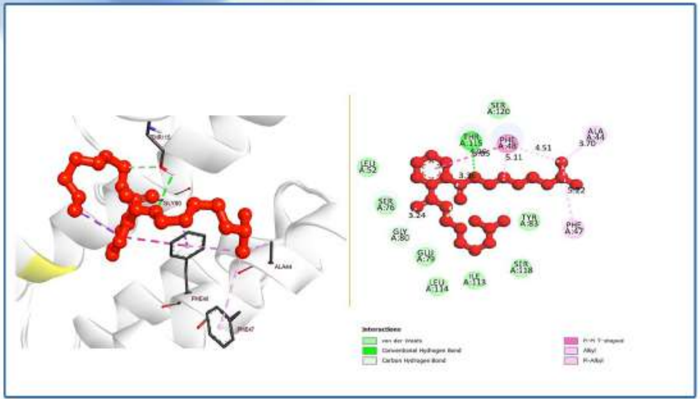

Fig. 4. Shown the interaction between the compound CID33934 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein -ligands complex.

A total of 14 hydrophobic bonds were also found to form with the compounds CID600132, including alkyl bonds in the ILE3, PRO5, PRO5, PHE12, and ILE106 residual positions as depicted in Figure 5 and listed in Table 2.

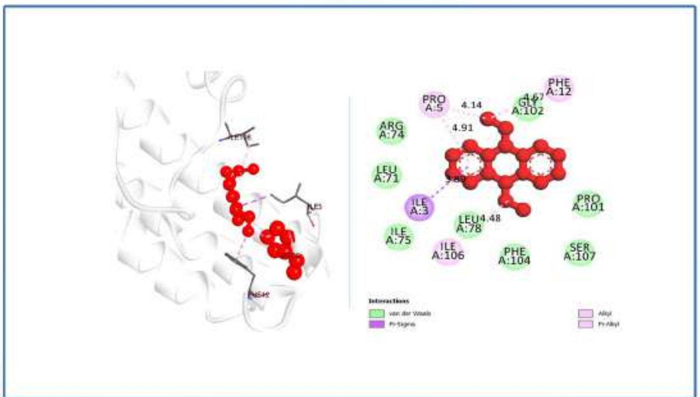

Fig. 5. Shown the interaction between the compound CID600132 and the NV protein. Left side indicate 3D interaction and the right portion indicates 2D interaction of the protein -ligands complex.

3.6. ADME analysis

The SwissADME server was used to analyze the ADME properties (hydrophilic nature, solubility, pharmacokinetics, medicinal chemistry, and drug-likeness feature) of three compounds (CID124120485, CID33934, and CID 610132). The lipophilicity of the druglike compounds allows them to dissolve in fats, oils, and nonpolar solvents. Pharmacophore characteristics have demonstrated that the molecule can be employed as an effective and druggable in the study.All of the compounds have retained optimal pharmacokinetics properties as summarized in Table 3.

Properties	Parameters	CID 124120485	CID 33934	CID 610132
	MW (g/mol)	400.68 g/mol	390.6g/mol	236.35
	Heavy atoms	29	28	18
	Arom. heavy atoms	0	6	12
	Rotatable bonds	6	16	2
	H-bond acceptors	1	4	0
	H-bond donors	0	0	0
	Molar Refractivity	128.35	116.30	78.13
Lipophiliciqy	Log Po/w	8.04	6.43	5.08
Water solubility	Log S (ESOL)	-7.65	-6.66	-5.18
Pharmacokinetics	GI absorption	Low	High	Low
Drug likeness	Lipinski, Violation	Yes	Yes	Yes
Medi. chemistry	Synth. accessibility	6.03	3.41	3.22

Table 3. List of pharmacokinetics includes ADME properties of the selected three compounds. The lists also present different physicochemical properties of the three compounds.

3.7. Toxicity prediction

The study employed the ProTox-II website to evaluate the chemical's toxicity because it's quick, cheap, and doesn't require any ethical concerns. The three compounds selected previously through different screening processes, PubChem CID: 124120485, 33934, and 610132, have been submitted to the ProTox-II web server, which determines the oral toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, and mutagenicity of the compounds listed in Table 4. There was no evidence of oral toxicity or organ toxicity in any of the compounds.

Endpoint	Target	CID 124120485	CID 33934	CID 610132
Organ toxicity	Hepatotoxicity	Inactive	Inactive	Inactive
Toxicity endpoints	Carcinogenicity	Inactive	Light active	Inactive
	Immunotoxicity	Active	Inactive	Inactive
	Mutagenicity	Inactive	Inactive	Inactive
	Cytotoxicity	Inactive	Inactive	Inactive
	LD50 (mg/kg)			
	Toxicity class	4	3	5
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	Inactive	Inactive	Inactive
Tox21-Stress response pathway	Aryl hydrocarbon Receptor (AhR)	Inactive	Inactive	Inactive
	Heat shock factor response element	Inactive	Inactive	Inactive
Fathead minnow LC50 (96 h)	-Log10(mol/L)	6.49	5.96	5.73
48-h Daphnia magna LC50	-Log10(mol/L)	6.07	5.22	5.61
Developmental toxicity	value	0.85	0.75	0.89
Oral rat LD50	mg/kg	841.35	959.71	27861.66
Bioaccumulation factor	Log10	2.9	0.87	1.40

Table 4. List of the drug-induced toxicity profile includes hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity of selected three compounds.

4.Discussion

The viral hemorrhagic septicemia virus (VHSV) is a prominent viral disease agent for farmed rainbow trout that has resulted in substantial economic losses in the aquaculture industry around the world. The NV protein (i.e., NV116) demonstrated a strong role in the virulence of VHSV in rainbow trout. The importance of position 116 at the C-terminal of the NV protein in virulence was validated by Baillon et al. (2020). The NV protein occupies this position within an intrinsically disordered region. Multifunctional viral proteins contain intrinsically disordered regions that interact with several binding partners for signaling, regulation, and control functions in the infected cell. This research focused on inhibiting the virus's NV protein in attempt to find a novel and effective antiviral drug candidate that can be utilized to treat VHSV infections. Recently, marine scientists have become interested in the commercial potential of marine algae, the effects of climate change on the marine environment, the decline of water quality in some marine habitats, the spread of diseases among marine organisms, and biodiversity studies of algal species. (Fantonalgo, 2018). Several studies have found that Halymenia dilatata zanardini, Chlorococcum humicola, Caulerpa peltata, and P. gymnospora are among the most beneficial traditional medicinal algae (Jainab et al., 2019; Kavitha & Palani, 2016; Murugan & Iyer, 2014). These are a source of unique natural ingredients for immunostimulants to treat a range of diseases.

Computational approaches are increasingly becoming more popular, acknowledged, and applied in the drug discovery and development process (Kapetanovic, 2008). We used Computer-Aided Drug Design (CADD) which is one of the most promising techniques for finding novel compounds that target a specific protein since it incorporates so many advanced characteristics and methodologies (Sastry et al., 2013). CADD saves time; it is quick and cost-effective in the overall drug discovery process, which includes molecular docking, molecular dynamic simulation, ADMET, and other as vital aspects of drug designing. CADD can identify the specific target molecule based on its behavior and the mechanism of ligand binding. The molecular docking method can be used to describe the atomic interaction between a small molecule and a protein, allowing us to define small molecule behavior in target protein binding sites and elucidate crucial biochemical processes (McConkey et al., 2002). MD simulations, on the other hand, disclose the mechanics of protein-ligand interaction. As a result, small molecule candidates can be identified as potential therapeutic candidates for the treatment of a certain disease.

In this study, protein structure was initially predicted using Homology Modeling, and then revised and validated using a popular online web service (including, I-TESSER, GalaxyRefine). The best protein 3D structure with the lowest c-score was used as the best model generated from I-TESSER. After refining from the Galaxy Refine server, the model quality was enhanced, and the final refined model exhibited 95.2% in the most favored region of the Ramachandran plot and 0% in the disallowed region, indicating excellent model quality (Rani & Pooja, 2018). While the protein model's Z-score was -4.2 prior to refinement, it increased to -4.66 after refinement. We identified potential drug-like compounds from Halymenia dilatata zanardini, Chlorococcum humicola, Caulerpa peltata, and P. gymnospora using molecular docking and an in-silico techniques. The 40 natural phytochemical compounds of Halymenia dilatata zanardini, Chlorococcum humicola, Caulerpa peltata, and P. gymnospora from the The Indian Medicinal Plants, Phytochemistry, and Therapeutics (IMPPAT) database were primarily screened using the molecular docking method. The top three compounds, PubChem CID: 124120485, 33934, and 610132, were chosen for further validation because they had the highest binding affinities, which were -8, -7.9 and -6.7 kcal/mol, respectively.

The top three selected compounds, PubChem CID: 124120485, 33934, and 610132 were chosen for further validation because they had the highest binding affinities of -8, -7.9, and -6.7 kcal/mol, respectively. The selected compounds' drug-like qualities were demonstrated by Lipinski's rule of five (RO5) (Lipinski, 2004). Three selected compounds were found to follow the five Lipinski guidelines of drug-likeness attributes. The substance that has good ADME capabilities has been subjected to additional toxicity testing in order to determine the potentially hazardous impacts it may have on both humans and animals (Aljahdali et al., 2021).

After conducting toxicity tests, we verified that the three selected compounds are non-toxic or low-toxic.

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Switzerland

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FAITT Participation in CATWA 2023 – Bengal Aqua Expo

SPOT NEWS

An interesting Aquaculture Expo, **"CATWA 2023 Aqua Expo",** was held in West Bengal , on 12th & 13th February, 2023.

Shri Biplab Roy Chowdhury, Minister of Fisheries, Govt. of West Bengal as part of his visit to the Expo, came to and appreciated Foundation for Aquaculture Innovations and Technology Transfer (FAITT), at its Stall .

A very unique and interesting Presentation was made at the Expo by **FAITT Director, Dr. M. Dhanaraj,** on the theme : **"Benefit to Microalgae for Aqua farmers".**

In juxtaposition with this, many youth and students visited FAITT stall and acquired knowledge on various Aquaculture aspects.

Entrepreneurs also visited FAITT stall in order to enlighten themselves on the multifaceted activities of FAITT in Aquaculture. Staff members lucidly elucidated on FAITT's Aquaculture Innovations.

In addition, **Organic Spirulina juice was served to about 4300 visitors by FAITT.** This was well appreciated by all the recipients. Organic Spirulina helps to considerably improve immunity aspects for human wellbeing.

FAITT also conducted a Quiz program and presented mementos to Aqua professionals. FAITT conducted the Lucky-Draw and presented the gifts to Aqua Farmers.

Overall, it was a well organised and wonderful experience for all the Expo visitors and FAITT and other stakeholders look forward to more such Expos in India in future.

Prospects of breeding and seed production of Stinging Catfish, Heteropneustes fossilis (Bloch)

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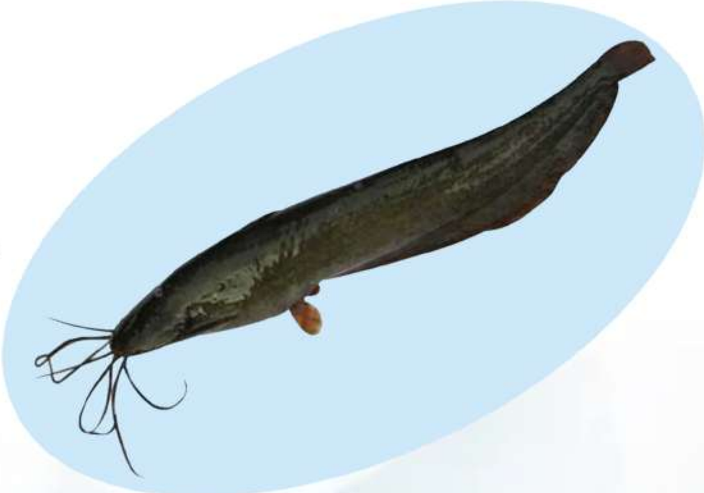
About the stinging catfish

The Indian catfish,Heteropneustes fossilis (Bloch), is commonly known as the stinging catfish or singhi. The singhi is considered as one of the most highly demanded freshwater fish species in the Indian subcontinent and Southeast Asian region. It belongs to the sub-order Siluroidei and the family of Sacchobranchidae. The range encompasses from India, Thailand, Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar, Indonesia, and Cambodia. It is also considered one of the very popular and highly prized freshwater food fish species due to its tender and delicious meat. It has good consumer preference due to its nutritional and medicinal.



Recently, the farming of singhi catfish and other air breathing fishes has gained much importance among the Indian fish farmers. Further inherently they are hardy fish, resistant to diseases, handling stress, ability to tolerate a wide range of environmental parameters and high stocking densities under culture conditions, fast growth rate, fewer intramuscular spines, and good quality. They possess accessory air-breathing organs which allow them to breathe atmospheric oxygen. Besides, the fish can thrive in harsh environmental conditions in muddy, turbid, and oxygen-depleted water bodies. Due to these adaptive abilities, they thrive in all kinds of shallow freshwater habitats such as marshes, paddy fields, swamps, streams, lakes, and irrigation canals. Generally, they are carnivorous and feed on insects, plankton, and snails in the natural water bodies. Hence, it is one of the ideal fish species available for successful low-cost farming for small-scale fish farmers.

Any successful small-scale fish farming requires simple breeding and seed production methods and culture practices. It is easy to breed and grow under captive conditions and does not require high technology. The main constraint in the expansion of catfish culture in India is the lack of an adequate and reliable supply of catfish fry and fingerlings for fish culture.They are highly cannibalistic when substantial differences in size occur. Hence it is apparent that simple protocols covering induction of egg production, egg hatching, and particularly larval rearing techniques that enhance fry and fingerling survival need to be further simplified to ensure a sufficient supply of catfish seed. This article presents a simple and low-cost seed production technology for Singhi catfish, Heteropneustes fossilis for small-scale fish farmers. The breeding and feeding techniques are very simple and they can be adopted and practiced by any fish farmers.

Reproductive biology

Catfish breed naturally during the southwest monsoon and northeast monsoon seasons in flooded rivers and ponds in southern parts of India but, the absence of the monsoons often limits seed production. It attains sexual maturity at one year of age when the male is generally 5.5 cm and the female is 12 cm in size in the natural habitat. It spawns one or two times a year in the natural environment in flood plains during the rainy seasons. The fry collection from the wild is unreliable and limited to the rainy season. Although stinging catfish reproductively mature in captive conditions, they rarely spawn naturally in captivity and hence to overcome these problems, induced spawning is thought to be the only alternative method for quality seed supply and production. Sexes are separate in catfish and visible and easy to identify male and female fish. Males have elongated urino-genital papillae near the anus region, whereas females have a simple round opening. Morphological features like a bulging abdomen, and slightly reddish and protruded genital papilla-like structures with blunt tip can help in determining the maturity of female fish. The fecundity of the species is higher than other freshwater fishes which ranges from 2000 to 5000 eggs per female with body weight ranging from 75 – 250 g respectively.

Broodstock management

The establishment of catfish farming requires a consistent supply of good quality seed, necessitating captive breeding; careful broodstock management, and suitable larval rearing techniques. Catfish broodstock can be obtained from natural water bodies, rivers, ponds, and fish farms. For best breeding performance, brood fish should be more than 1 year old at least and should be more than 75 g body weight for both males and females. Brood fish should be collected several weeks in advance of spawning to minimize stress and transportation injury. They can be stocked in circular or rectangular cement and plastic tanks at a stocking density of 10-20 fish square meters. A minimum water depth of 60 cmis recommended for catfish. A weekly water exchange of 50–75% can be made to maintain the water quality parameters within the favorable ranges.The quality and quantity of feed, as well as the feeding regime, are important for spawning as well as egg quality. They are carnivores and mainly feed on small bottom-dwelling animals in natural conditions, but animal bio wastes, trash fishes, and formulated feeds can be offered as feed. Broodstock can be fed with a commercial formulated diet with 35- 40% protein levels twice daily at ad libitum. Larger mature females produce more eggs than smaller females. Proper care and acclimation of bloodstock will improve egg quantity and quality.

Induced spawning and seed production

The two sexes can easily be distinguished by the shape of the belly and by the form of the genital papilla. Milt can not be squeezed from males as with other freshwater fish species like carp. Milt from 2 males with a body weight of 100 g can fertilize the eggs of ten females of similar ages and sizes. Final maturation of eggs and sperm and release of eggs from the female ovary are induced by injection of both males and females with appropriate dosage of spawning agents. Several inducing agents and dosages have been studied and reported in catfish. Based on our previous experience 0.4 ml of synthetic hormonal preparation (ovaprim or ovatide) per kg body weight of fish is sufficient to stimulate the final maturation of eggs and sperm. Fishes are removed from the holding tank and the body weight is measured immediately for determination of hormone dose. For hormone injection and handling the fishes can be immobilized by partial wrapping of the body in soft-textured cloth or tissue papers.

The selected fishes are administered intramuscularly. The administration of hormones to the fish must be injected in the muscular region just below the anterior part of the dorsal fin and just above the lateral line. Immediately after hormone administration, the male and female fishes are separately released into the cement tanks or circular plastic tanks until the recommended latency period of 10 to 12 hrs at 28–30 ºC. The broodfish holding tanks should be covered with plastic nets. After 10-12 hours of hormone injection male and female fishes arecarefully collected from the tank for eggs and sperm collection. The body moisture of fish is blotted with a paper towel or cotton cloth and the water should not drip into and mix with egg mass while striping of eggs. Eggs are striped manually from females by applying slight pressure on the abdomen toward the genital pore and then eggs are collected in a dry circular plastic container. The testes are surgically removed and placed into a petri dish.

Testes tissues are cut or chopped into small pieces using a scalpel or single-edged razor blade, mixed with about 5-10 ml tap water, and then macerated to obtain sperm suspension. The suspension is then added to the eggs immediately upon preparation and evenly mixed for fertilization. The egg mass with sperm is swirled gently to mix properly. After 10 – 15 seconds, water is added to cover the eggs to stimulate the motility of the sperm and fertilize the eggs. After five minutes the eggs were subsequently washed thoroughly with clean chlorine free aged tap water. During this time any testis debris and tissue particles need to be removed from the mixture. The eggs are transferred to a glass aquarium/cement tank for hatching, with constant aeration. The eggs began to hatch after 20 – 24 hrs hours at 28–30 ºC (Fig A-D).

Larval rearing

The Catfish larvae are very delicate and require utmost care for their growth and survival during hatchery rearing. The hatched larvae are separated from unfertilized and dead eggs. The newly hatched larvae are 2.0- 3.0 mm in length. They are released in rectangular glass aquariums or circular polythene pools or cement tanks for further rearing. The tanks are filled with chlorine free clean tap water with gentle aeration. During this time the larvae migrate to the corner of the rearing containers and aggregate in patches. Initially, the water level of containers needs to be maintained at 5 – 10 cm and gradually increased to 20 – 30 cm after one week. Water levels need to be adjusted at different stages of rearing to minimize the stress on larvae. On the third day, the yolk sac is fully absorbed and the mouth is well formed and readily accepts exogenous feed. From day three onwards, the larvae can be fed with small live plankton harvested from earthen ponds and/or Artemia nauplii and frozen moina until day 15. Boiled egg paste or artificial crumble feed can be used as a feed for larvae from the 10th day after exogenous feeding until day 20.The supply of live plankton can be withdrawn gradually during the rearing period of 10-15 days. This mixed feeding not only enhances growth but also ensures a higher survival rate. The early larvae are sensitive to light and hence few plastic pipes need to be provided in the rearing tanks to give shelter for the larva. Higher stocking density in the larval rearing phase will affect growth and survival due to overcrowding stress. From day 20 onwards until day 30, the fry can be fed ad libitum with commercial feed crumbles containing 30 – 35 % protein and 7 % lipid. The daily feed ratio should be best divided into three to four meals a day

Broodstock Inherent size variation and cannibalism are also one of the problems of the culture of this fish species. Frequent size grading is advisable to avoid cannibalism and increase the survival rate in the larval rearing stage. Fry can be further reared in cement for anothbe fed with a commercial formulated diet with 35- 40% protein levels twice daily at ad libitum. Larger mature females produce more eggs than smaller females. Proper care and acclimation of bloodstock will improve egg quantity and quality.

Cement tanks may be used for nursery rearing. Rearing of fry in earthen pond conditions may not give a good survival rate due to natural mortality or predation by predators. Small sized cement tanks of 10-20 m2 are preferred to monitor the survival rate and to avoid predation. These cement nursery tanks are provided with a 2-3 cm soil base and a water level of 10-20 cm. To promote the natural algal and plankton production a single dose of cow dung 2-3kg are applied for this size of tanks. The tanks are then inoculated with plankton collected from earthen ponds. After 4 days of preparation, the fry can be stocked. The fry should be stocked after the development of a plankton bloom in the nursery tanks. The sizes of 50- 60 mg fry are suitable for initial stocking in nursery tanks. They are reared at a density of 100-200 fry/m2. Increasing stocking density usually results in stress (aggressive behaviour, dominance) which leads to size variation in growth among the fingerlings and also reduces the survival rate at harvesting. During these periods the fry should be fed with pellet feed at the rate of 5% body weight twice daily. The feed ratio needs to be increased by a weekly sampling of fish biomass. Appropriate feeding is very important because an inadequate food supply has a direct impact on fish growth and production costs. The excess unconsumed feed and waste matter during nursery rearing pose problems for maintaining water quality. Therefore, once in two weeks water must be exchanged for good growth and survival. Growth heterogeneity has been associated with cannibalism and therefore with mortality. Hence size grading of fingerlings has to be routinely performed to minimize growth variation and reduce cannibalism. The nursery cement tanks should be covered with nets to prevent birds and other predators. The fingerlings thus produced can be harvested depending on the demand. Usually, the fish grow up to 2 - 5 g in size within 30 days of nursery rearing, with a survival rate of 50-80%.

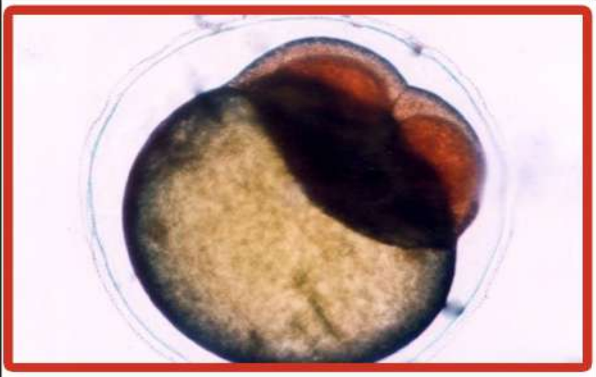
Water quality management during larval rearing

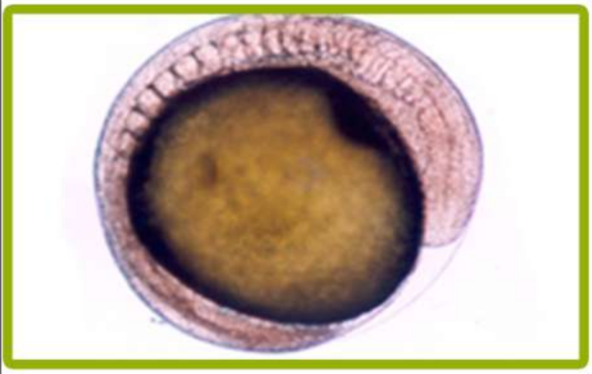
Larvae of catfish are small, sensitive, and delicate and they require a good aquatic environment for their survival. Water quality management is an important aspect during the rearing of catfish larvae. Hence good quality and appropriate water depth during the indoor rearing phases are maintained. Aerial respiration commences after 10-12 days and hence, aeration must be provided to the larval rearing tanks by aerators. Accumulation of metabolites and unconsumed feed in the rearing tanks pollute the environment and ultimately lead to oxygen depletion, disease incidence, and mortality.

Therefore, it is advisable to clean the bottom of the tank and renew 50-60% of the water daily to maintain a 20-30 cm depth. Care should be taken to minimize the stress on the delicate larvae while exchanging water from the tanks. The waste from fry and unconsumed feed in the rearing tanks under high-density rearing produces ammonia and hydrogen sulphide. Ammonia is toxic at low concentrations affecting the gills and accessory respiratory organs, whereas hydrogen sulfide causes stress to the fry. Aeration and frequent water exchange are required to get rid of the above problems.

Conclusion

In India, there is a huge demand for stinging catfish, which also commands a high market price. Numerous farmers have begun to cultivate catfish, and the demand for catfish seed has been steadily rising.The technology for breeding and seedproduction has been developed and the technique is very easy to be adopted by fish farmers. The growth and survival of larvae and fingerlings depend on the careful management of rearing tanks, feeding, and size grading. It has been felt that the production of catfish depends on the availability of high-quality seed and careful control throughout the larval rearing phase. For successful growing and lucrative catfish aquaculture, more concerted efforts and attention must be given throughout the early larvae, fry, and fingerling stages of rearing.


A. 2 cell stage


B. 20 hrs old embryo


C. Newly hatched embryo


D. 48 h old hatchlings

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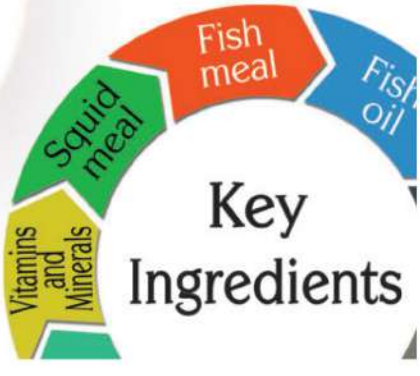

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