Natural Resources for Human Health

visaga publishing house

Original Research

View Article Online

Check for updates

Received 12 September 2021
Revised 03 October 2021
Accepted 07 October 2021
Available online 10 October 2021

Edited by Carlos L. Cespedes Acuña

KEYWORDS:
Chlorella vulgaris
Euglena viridis
Spirulina plantases
Cyprinus carpio
Aeromonas hydrophila

Natr Resour Human Health 2022; 2 (2): 107-113
https://doi.org/10.53365/nrfhh/142934
eISSN: 2583-1194

Impacts on the immune system of *Cyprinus carpio* exposure with a mixed algal extract against *Aeromonas hydrophila*

Sattanathan Govindharajan [1], Tamizhazhagan Vairakannu [2,*]

[1]Department of Zoology, Sacred Heart Arts and Science College, Perani-605652, Tamil Nadu, India
[2]Department of Zoology, Syed Ammal Arts and Science College, Ramanathapuram 623513, India

ABSTRACT: This study evaluates the influence of mixed algal extract (*Chlorellavulgaris*, *Euglenaviridis* and *Spirulinaplatensis*) on common carp *CyprinusCarpio,* which infected infect with bacterial pathogen *Aeromonas hydrophila*. *C. carpio* was administered intraperitoneally with various doses such as methanol extract (0, 0,1, 1, 10 and 100 mg/kg). The immunological parameters of fish blood and serum samples (Neutrophil activity, Lysozyme activity, Serum myeloperoxidase intensity, Serum bactericidal activity, and Serum antiprotease activity) were investigated at 7, 14, 21, and 28 days of post-immunization. Fish had been tested by virulent *A. hydrophila* for 30 days after treatment and 14 days after infection were identified with mortalities. The findings showed that neutrophil levels, lysozyme activity, serum bactericidal activity, myeloperoxidase activity, and serum antiprotease activity significantly enhanced ($p<0.05$) compared to untreated control. Mixed dietary algae at 1 and 10 mg/kg levels demonstrated slightly ($p<0.05$) higher relative percentage survival (90 percent) than control against *A. hydrophila* disease infection. Results indicated that mixed algal extract in *C. carpio* positively impacts non-specific immune parameters and boosts disease tolerance to *A. hydrophila* infections.

1. INTRODUCTION

The compounds that modulate the by-host immune system called immunostimulants are naturally present and commonly used in aquaculture (Robertsen, 1999). One of the most capable approaches in aquaculture for managing diseases is developing the fish defence system during prophylactic immunostimulant administration (Ringo et al., 2010; Selvaraj et al., 2009). Due to the emergence of bacterial resistance and environmental and habitat safety issues, conventional disease prevention methods using Chemical disinfectants and antibiotics will be no longer required. While vaccination is an efficient prophylactic approach for preventing infection in fish, there are few clinical illnesses associated with high costs and stress (Ellis, 1999). With remarkable results, the use of immunomodulators as an even more eco-friendly method of deterring disease control has already been achieved (Peddie et al., 2002). Immunostimulants improve the adaptive immune system and therefore eliminate infectious diseases (Sakai, 1999).

Microalgae contain many bioactive compounds that can satisfy people's food and energy needs, encourage health conditions, and avoid chronic illness disease (Morais et al., 2014). The capacity of these bacteria and fungi in feeding and foodstuffs, cosmetics manufacturing and die industry and additive production have been defined by various processes. The ability of these micro-organisms in the food, animal feed, cosmetics, and die and additive manufacturing industries has been defined in various processes (Morais et al., 2014). These nutrients contribute to the development of these microorganisms by different enzymatic reactions for the biosynthesis of specific compounds (Brennan & Owende, 2010; Karemore et al., 2013; Wang et al., 2008).

Microalgae cultivation provides an economic outlet for humans and considerably helps decrease the greenhouse effect appropriate to carbon dioxide (CO_2) fixation potential of microalgae (Brennan & Owende, 2010; Wang et al., 2008). Microalgae have been recognized as bioactive substances, such as Botryococcus, Chlorella, Spirulina, Dunaliella, Haematococcus, and Nostoc. The natural bioactive compound is essential for algae metabolism and has many advantageous biological activities, including certain features many favourable biological activities such as anti-carcinogenic, anti-obesity, anti-angiogenic, antioxidant, anti-inflammatory and neuroprotective activities (Guedes et al., 2011; Pangestuti & Kim, 2011). Microalgae, including carotenoids and phycobiliproteins, were present at the primary pigments. Carotenoids are commonly used mainly for dietary supplement diets for animals and human beings, food colouring dyes, animal feed, fortified

* Corresponding author.
E-mail address: tamilzoon@gmail.com (Tamizhazhagan Vairakannu)

foods and pharmaceuticals, and few cosmetic goods productions products (Vílchez et al., 2011).

Enzymes are ideal sources of proteins for human nutrition since they are cheaply further processed by the gastrointestinal tract relative to intact amino acids (Lisboa et al., 2014). Hydrolysates are an ideal source of protein for human nutrition while they are quickly processed additionally by the gastrointestinal tract relative to intact amino acids (Hoseini et al., 2013). Spirulina's high 50-70 percent weight basis protein content provides all the required amino acids, particularly valine, leucine and isoleucine and others (83-90 percent) (E.W. Becker, 2007). Studies in nutrition and toxicology have already shown that microalgal biomass is helpful as a replacement food or substitute for conventional rich sources of protein (Christaki et al., 2011; Spolaore et al., 2006). They comprise water-soluble vitamins and can be used to supplement nutrients and diets. Microalgae are considered a pool of essential vitamins, including ascorbic acid, tocopherols and several B vitamins complexes. These microbes comprise water-soluble vitamins it can be used as food supplements and diets. Microalgae have the primary source of essential vitamins such as ascorbic acid and multi-complex B tocopherols (Ambrosi et al., 2008). Spirulina is a concentrated supplement to vitamin A (β-carotene) and B12 (Ambrosi et al., 2008; Raa, 1996). The level of immune tolerance may be improved by adding immune stimulants by enriched feed or vaccines while preventing the use of antibiotics and chemotherapy agents (Raa, 1996). Many products of seaweed materials such as *Chaetomorpha aerea* (Sattanathan et al., 2020), *Eugena viridis* (Das et al., 2005), *Euglena gracilis* (Kondo et al., 1992) fish immunity has been reported to be improved the immune mechanism of fish. There is no known study of mixed algae against *A. hydrophila* on innate immune functions in *C. carpio*. Therefore, the present studies aimed to assess the effectiveness of the mixed algal extract against *A. hydrophila* on the immunity point of *C. carpio*.

2. MATERIAL AND METHODS

2.1. Experimental fish and their maintenance

C. carpio wet weight: 8.50 ± 0.50 g; length: 11 ± 0.50 cm was bought from a nearby fish farm, Kumbakonam, Tamil Nadu, India, and cultivated with dechlorinated water. Before treatment, all experimental fish were acclimatized at pH 7.0 ± 0.2 at a steady temperature of 22 ± 1 °C and commercial fish food was fed once a day.

2.2. Collection and culture techniques

2.2.1 *Spirulina platensis*

S. platensis was grown in a modified Zarrouk medium and sterilized at 121 °C for 20 min with 100 ml of the ideal combination. Each conical flask was inserted with a 10 ml culture usually containing a minimum level of 10^7–10^8 colony forming units/ml incubated for seven days and transferred to a 20 L white, clear polyethylene vessel incubated for 21 days. *S. platensis* samples were cultivated under laboratory conditions of 30 ± 2 °C, and continuous illumination was 5500-6500 Lux. During incubation, the sterilised air was used to spread and mix the culture. The culture mixture was filtered with a sterile cotton cloth and washed with distilled water. The cells were air dried for 24 hours at room temperature before being baked at 70°C (Ravelonandro et al., 2008).

2.2.2 *Euglena viridis*

The algal blooms were gathered from a local fish farm (0.2 to 2.5 ha) at Kumbakonam, Tamilnadu. Under the circumstances, the water samples were observed under a microscope. The water samples were examined under the microscope, under the circumstances. The *E. viridis* organisms have been flexibly enlarged, spindle-shaped, single-celled mobile, and greenish colored, and were observed (Das et al., 2005). For triplet methods in sterilized water to remove the suspended particles, the collected water samples were washed in bolting and silk cloth and then centrifuged at 1000 g using a macro rotor (Sorvall CE, UK). The mixed pellet, which can be prepared, was harvested at room temperature and dried for 2 or 3 days.

2.2.3 *Chlorella vulgaris*

C. vulgaris was cultivated in a 20 L tank of glass procured from marine water. Seawater 30 ppt filtered from the pollution-free zone (0, 5 lm pore-size Millipore filters), sterilized for 30 min at 120 °C, and accompanied by the Erd-Schreiber medium (Vijayavel et al., 2007). The cultivations of *C. vulgaris* were maintained at 28 ± 1 °C with sufficient aeration, and continuous flow centrifugation (10 l / h) harvested the algae for 40 min at 2,000 g at 4 °C. Weighed and air-dried the ensuing whole-cell pellet to remove moisture and refrigerated until it was used.

2.3. Preparation of mixed algal extract

Different shade-dried from the three different microalgae with seven days until weight constancy was achieved. The *E. viridis*, *C. vulgaris* and *S. platensis* algae powders were gently mixed with a ratio of 1:1:1 for preparation of the mixture, and the algae sample was lightly crushed using an electric blender. The mixed algal extract was obtained by dissolving 100 g of the mixture with 1000 ml (sterile) distilled water and transferred to 2000 ml conical flasks densely covered with aluminium foil coating, kept at room temperature agitated daily for seven days. Mixed algae methanol solvent extract was prepared and applied using the standard methods followed by slight adjustments (Harikrishnan et al., 2009). Using a cold maceration process previously reported, coarse powdered dry algae were gradually extracted using solvents such as petroleum ether, CH_3Cl_3, EtOAc, MeOH and 0.25 percent CH_3Cl_3:H_2O (v/v). The extracts were then dried and stored at -20 °C until utilised using a rotating vacuum evaporator (Buchi, Switcherl).

2.4. Experimental design

Fifteen tanks were distributed to the *C. carpio* 180 fish, with each tank containing twelve fish being maintained with triplicate. The fish were injected intraperitoneally with 0.2 ml of reconstituted methanol extract at tenfold increases in body weight of 0; T1, 0.1; T2, 1; T3, 10; T4-100 mg/kg. It received 0.2 ml of sterile water from the control groups. Seven days before treatment, the fish were bled, and 7, 14, 21 and 28 days after treatment

2.5. Collecting blood and separating serums

The blood samples are taken using an approximately 1ml tuberculin syringe, placed with a 24 gauge needle (Michael et al., 1994). The serum was prepared within 1 min after collection of blood by collecting 200μl of blood. The blood in the serological tubes was store overnight in the refrigerator. On the clot, the centrifugation was performed at 400 g for 10 min. (Michael et al., 1994). For the use of assays, the obtained serum was placed in the storage vials with the highest sterility at -20 °C.

2.6. Immunological assays

2.6.1 Nitroblue tetrazolium assay

The neutrophil activity was assayed using Nitro blue tetrazolium (NBT, Sigma) assay using the Chung and Secombes (1988) method with modifications previously described (Sahu et al., 2007). Then at 620 nm was observed the OD value for the microplate reader (Systronics, India). They used KOH / DMSO as blank.

2.6.2 Serum lysozyme activity

Serum lysozymes were measured using the technique described by Parry et al. (1965), slightly modified to (Hutchinson & Manning, 1996) . One unit of lysozyme activity was defined as the total amount of enzyme that produces a decrease of 0.001 min^{-1} and mL in serum absorbance.

2.6.3 Bactericidal activity

According to standard serum bactericidal activity procedure Kajita et al. (1990). An adequate amount of 100 ml serum and bacterial suspension (105 CFU / ml) at room temperature was already mixed and incubated for one h. They prepared the blank control, too, by replacing serum with sterile PBS. The solution was diluted again with Buffered saline phosphate (PBS) at a ratio of 1:10. The diluted 100 mL suspension was poured on nutrient agar plates at 37 °C with 24 h incubation. The number of bacterial cells was determined by the new colonies cultivated in agar plates counted with nutrients.

2.6.4 Myeloperoxidase activity

The total amount of myeloperoxidase activity was determined using the described method (Quade & Roth, 1997). In brief, 10 mL of serum has been diluted with 90 mL of HBSS, adding 35 mL of 20 mM 3, 3, 5, 5 tetramethyl benzidine hydrochloride (TMB) (Genei, India) and 5 mM of H_2O_2 at 1:20. The reaction was stopped by adding 35 mL of 4 M sulfuric acid (H_2SO_4) to it after 2 minutes of exposure, and the optical density was examined at 450 nm using a micro plate reader.

2.6.5 Serum antiprotease activity

The antiprotease activity was determined by following the previous method Bowden et al. (1997), and 2 mN BAPNA was used as the substrate. 10mL of serum was incubated with 20 ml of trypsin solution, then 0.1 M Tris HCL pH 8.2 was placed and incubated at 22 ° C for 25 min from the 500 ml volume substratum. The reaction was halted in a plate reader with 150 ml 30% acetic acid, and the OD was read at 415 nm.

Percentage of trypsin inhibition (%) = (Trypsin blank OD-Sample OD)/(Trypsin blank OD) X 100

2.7. Culture of *A. hydrophila* and the Challenge study

A. hydrophila microbial strain (MTCC 1739) was obtained from IMTECH, Chandigarh, India, where it was grown at 37 ° C in (TSB) tryptone soy broth (HiMedia, India) for 24 hours; TSB broth culture was centrifuged at 3000 g for 10 min. The supernatant was filtered, and the phosphate-buffered saline pellets (PBS, pH 7.4) were suspended while the solution's OD values were adjusted to 0.5 at 456 nm, equivalent to 1 x 10^7cfu / ml. Defined by intraperitoneal injection of *A. hydrophila* evaluated doses (10^6, 10^7, 10^8 and 10^9 CFU per fish), the seven-day lethal dose 50 (LD_{50}) in 20 fish was 10^7cfu / ml. Nine fish from each group completely at random treated with selected fish have injected i.p at the end of the feeding trial. *A. hydrophila* with 1 x 107 live, containing 0.2 ml PBS. Fourteen days after infection, all groups recorded total mortality. Furthermore, the relative survival percentage (RPS) was illustrated according to the formula below after the challenge study.

Relative percentage survival (RPS) = (Number of surviving fishes after challenge)/ (Number of fishes infected with bacteria) $\times 100$

2.8. Statistical analysis

The quantitative data was evaluated using one-way variance analysis (ANOVA). All data were analyzed statistically using the SPSS (version 21) software. The mean level was $p<0.05$, and the results are analyzed as mean (S.E.M) values.

3. RESULTS AND DISCUSSION

Figure 1 to Figure 6 shows the effect of mixed algae extract i.p (intraperitoneal) injection at different doses like (0, 0, 1, 10 and 100 mg/kg) on immunity response at weekly intervals of 28 days, respectively. The *C. carpio* neutrophils' experimental group respiratory burst activities (NBT reduction) are shown in Figure 1.

In comparison with the control group, respiratory burst activity increased statistically significantly during the experiment after treatment with mixed algae extract. The highest value of respiratory burst activity was found in the T2 group, and the least amount of respiratory burst activity in the control group was found. The lysozyme activity revealed an increasing trend

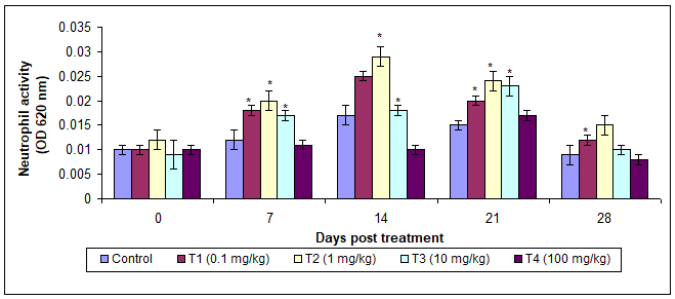

Figure 1. Neutrophil activity in C. carpio after varied treatments with mixed algal extract (results are mean±SE).

in exposure to mixed algal groups during the 7-day to 28-day sampling period and differed significantly ($p<0.05$) between treatment groups. Furthermore, the highest lysozyme activity was found in group T2, followed by control of sampling on T3, T4 and 14 days (Figure 2).

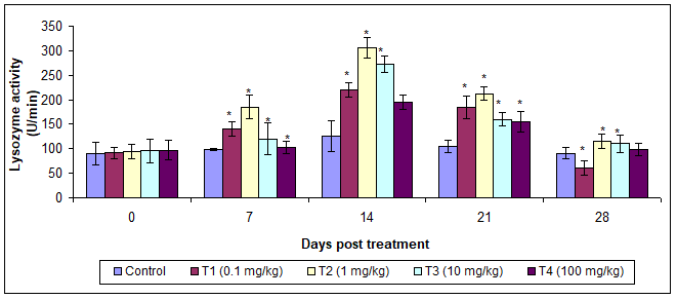

Figure 2. Various doses of mixed algal extract were used to treat C. carpio serum lysozyme activity (values are mean±SE).

Due to the administration of different dosages of a mixed algal diet during the 28-day sampling period, serum myeloperoxidase activity increased significantly ($p<0.05$) compared to control (Figure 3).

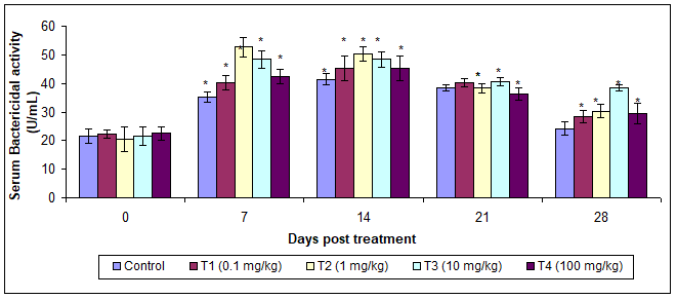

Figure 3. During post-treatment days, C. carpio serum bactericidal activity was supplied with varied amounts of mixed algal extract (results are mean±SE).

During all sampling days, serum bactericidal activity improved significantly ($p<0.05$) during the experimental period (Figure 4).

Statistically significant ($p<0.05$) increases in serum antiprotease activity were noted up to 28 days after sampling in fish from different treatment groups (Figure 5).

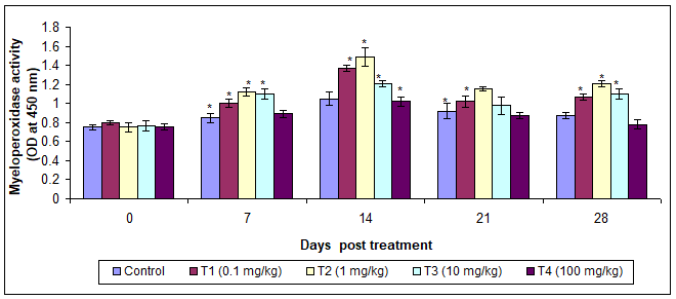

Figure 4. During post-treatment days, serum myeloperoxidase activity was measured in C. carpio with varying amounts of mixed algal extract (results are mean±SE).

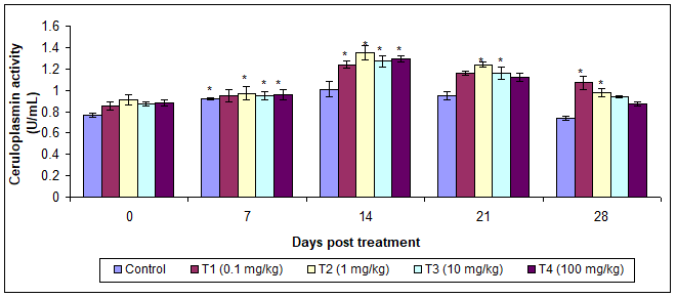

Figure 5. Ceruloplasmin activity was measured in C. carpio after several treatments with mixed algal extract (results are mean±SE).

The relative percentage (percent) survival of *C. carpio* in mixed algal treatment conditions is illustrated in Figure 6 .

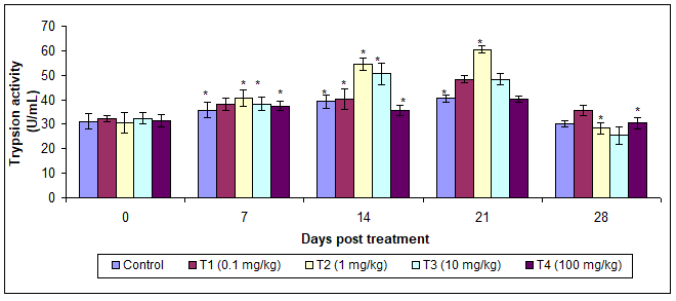

Figure 6. Post-treatment serum antiprotease activity in C. carpio treated with varied doses of mixed algal extract (results are mean±SE).

The highest post-challenge survival rate ($p<0.05$) was observed in the fish group with 1 and 10 mg/kg mixed algal exposure (95 and 90 per cent). The control group was shown at 50 per cent. Microalgae are microscopic unicellular organisms that can describe food and food metabolites, including certain carbohydrates, proteins, vitamins, fat, and organic minerals. Environmental pollution occurs when environmental degradation crosses limits and leas lethal to living organisms (Usha et al., 2017). Adversely human activities are directly or indirectly affect the environment (Tamizhazhagan et al., 2017). Mainly, the indiscriminate use of pesticides resulted in the contamination of the aquatic system has become a global problem and is being extensively researched worldwide (Tamizhazhaganv et al., 2016). Microalgae are

microscopic unicellular that can make a wide range of metabolites for food and feeding stuffs, such as carbohydrates , proteins, vitamins, lipids, and organic and inorganic minerals (E. Becker, 1994) .

The present study shows that mixed algal extract enhanced concentrate upgraded the safe reaction in *C. carpio* against *A. hydrophila*. In Figure 1 to Figure 6 , respectively, the impact of mixed algal growth removes i.p (intraperitoneal) infusion at different portions (0, 0.1, 1, 10 and 100 mg/kg) on non-specific immune mechanisms parameters at weekly intervals for 28 days 1 month. The experimental group's respiratory burst activities (NBT decrease) of *C. carpio* neutrophils appear in Figure 1. In the research analysis, various agents, including bacteria and bacterial products, have found the increase in NBT levels beneficial for fish in protecting them from invading pathogens (Khan et al., 2005).

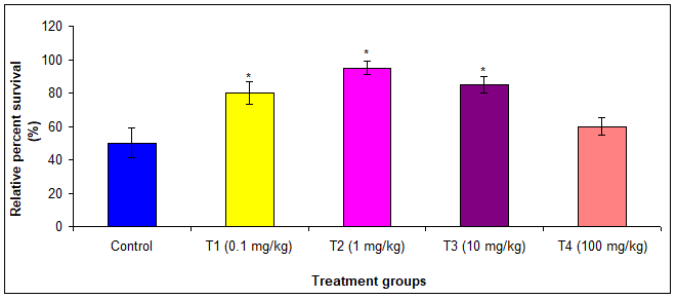

Figure 7. Effect of mixed algal extract on C. carpio survival when challenged with virulent A. hydrophila (mean±SE).

In this current study, the maximum higher neutrophil activity was noted compared to control in all treatment groups. In this study, higher respiratory burst activity was recorded as compared to control in all treatment groups. Similar results were reported with varying levels in *E. viridis* fed by Labeo rohita. Similar results have been reported in *Labeo rohita* fed *E. viridis* with varying levels (Lamas & Ellis, 1994; Solem et al., 1995), levamisole (Wijendra & Pathiratne, 2007) . T1 and T2 were found to have a high maximum value of respiratory burst activity, and decreased respiratory burst activity was defined in the un-treated control group. During sampling periods from 7 to 28 days, lysozyme activity (Figure 2) showed an increasing trend in exposed mixed algal groups and significantly increased ($p<0.05$) between treated groups. Additionally, the highest lysozyme activity was found in group T2, followed on the 14th day by T3, T4 and untreated control. In addition, the maximum activity of lysozyme was recorded in group T2, followed by T3, T4 and untreated control on the 14th day.

Serum bactericidal activity increased statistically during all sampling days ($p<0.05$) during the experimental period. Figure 6 shows significant increases ($p<0.05$) in serum antiprotease activity from the treatment group compared to the control groups up to a sampling day of 28 were noted in fish. The neutrophils are considered an indicator of lysozyme, and the enzyme seems to be much more bactericidal than the higher lysozyme of the vertebrate (Barsanti et al., 2001; Ellis, 1999). In this research, serum lysozyme activity increased considerably at different concentrations in treatment groups fed with a mixed algal diet. It was found that *E. viridis* is microalgae may improve the activity of antiprotease in fish as recognized in *L. rohita* (Amar et al., 2004; Das et al., 2005). Similar results with Chinese herbal medicine have been observed for lysozyme activity on 20 to 30 days after feeding with Pseudosciaenas crocea (Guzmán et al., 2001; Janczyk et al., 2009; Kotrbacek et al., 1994). The serum myeloperoxidase levels were increased significantly ($p<0.05$) compared with the control group (Figure 3) due to the administration during the 28-day sampling period of different dosages of mixed algal diet.

In the current study of this investigation, a maximum higher of myeloperoxidase activity was observed compared to the control group in the mixed algal dietary fed groups. The relative percentage of *C. carpio* survival (percentage) under mixed algal treatment conditions are shown in (Figure 6). The maximum relative percent survival was significantly ($p<0.05$) increased in experimental group exposure with 1 and 10 mg/kg mixed algal (95 and 90 percent), and the control group was observed at 50 percent. In *L. rohita*, a similar diet study supplemented *E. viridis* showed significantly higher relative percentage survival after challenge with A. hydrophila after immunization (Das et al., 2005). The results provide strong evidence that the mixed algal extract displayed a more robust immune response and sustainability in common carp, *C. carpio*. The promising effects of the challenge trial indicate that due to the ubiquitous presence and opportunistic pathogenesis of Aeromonas, a wide-spectrum disease resistance develops.

CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest.

ACKNOWLEDGMENTS

The authors thank the Principals of Sacred Heart Arts and Science College, Perani, Tamilnadu, India, and Syed Ammal Arts and Science College, Tamilnadu, India for their support.

ORCID

Sattanathan Govindharajan 0000-0002-9452-0888

Tamizhazhagan Vairakannu 0000-0003-0729-6572

AUTHOR CONTRIBUTIONS

TV - Research concept and design, SG - Collection and/or assembly of data, SG - Data analysis and interpretation, SG - Writing the article, TV - Critical revision of the article, TV - Final approval of the article .

REFERENCES

Amar, E.C., Kiron, V., Satoh, S., Watanabe, T., 2004. Enhancement of innate immunity in rainbow trout (Oncorhynchus mykiss Walbaum) associated with dietary intake of carotenoids from natural products. Fish & Shellfish Immunology. 16, 527–564. https://doi.org/10.1016/j.fsi.2003.09.004

Ambrosi, M.A., Reinehr, C.O., Bertolin, T.E., Costa, J.A., Colla, L.M., 2008. Propriedades de saúde de Spirulina spp. Rev. Ciências Farm. Básica e Apl. 29, 109–117.

Barsanti, L., Vismara, R., Passarelli, V., Gualtieri, P., 2001. Paramylon (b-1,3-glucan) content in wild type and WZSL mutant of Euglena gracilis. Effects of growth conditions. Journal of Applied Phycology. 13, 59–65. https://doi.org/10.1023/A:1008105416065

Becker, E., 1994. Microalgae : biotechnology and microbiology,. Cambridge university press, London, p. 304.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnology Advances. 25, 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002

Bowden, T.J., Butler, I.R., Bricknell, I.R., Ellis, A.E., 1997. Serum trypsin inhibitory activity in five species of farmed fish. Fish & Shellfish Immunology. 7, 377–385. https://doi.org/10.1006/fsim.1997.0092

Brennan, L., Owende, P., 2010. Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renewable and Sustainable Energy Reviews. 14, 557–577. https://doi.org/10.1016/j.rser.2009.10.009

Christaki, E., Florou-Paneri, P., Bonos, E., 2011. Microalgae: a novel ingredient in nutrition. International Journal of Food Sciences and Nutrition. 62, 794–799. https://doi.org/10.3109/09637486.2011.582460

Chung, S., Secombes, C.J., 1988. Analysis of events occuring within teleost macrophages during the respiratory burst. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 89, 539–583. https://doi.org/10.1016/0305-0491(88)90171-X

Das, B.K., Pradhan, J., Pattnaik, P., Samantaray, B.R., Samal, S.K., 2005. Production of antibacterials from the freshwater alga Euglena viridis (Ehren). World Journal of Microbiology and Biotechnology. 21, 45–50. https://doi.org/10.1007/s11274-004-1555-3

Ellis, A.E., 1999. Immunity to bacteria in fish. Fish & Shellfish Immunology. 9, 291–308. https://doi.org/10.1006/fsim.1998.0192

Guedes, A.C., Barbosa, C.R., Amaro, H.M., Pereira, C.I., Malcata, F.X., 2011. Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. International Journal of Food Science & Technology. 46, 862–870. https://doi.org/10.1111/j.1365-2621.2011.02567.x

Guzmán, S., Gato, A., Calleja, J.M., 2001. Antiinflammatory, analgesic and free radical scavenging activities of the marine microalgae Chlorella stigmatophora and Phaeodactylum tricornutum. Phytotherapy Research. 15, 224–230. https://doi.org/10.1002/ptr.715

Harikrishnan, R., Balasundaram, C., Kim, M.C., Kim, J.S., Han, Y.J., Heo, M.S., 2009. Innate immune response and disease resistance in Carassius auratus by mixed herbal solvent extracts. Fish and Shellfish Immunology. 27, 508–523. https://doi.org/10.1016/j.fsi.2009.07.004

Hoseini, S.M., Khosravi-Darani, K., Mozafari, M.R., 2013. Nutritional and medical applications of Spirulina microalgae. Mini-Reviews in Medicinal Chemistry. 13, 1231–1238. https://doi.org/10.2174/1389557511313080009

Hutchinson, T.H., Manning, M.J., 1996. Seasonal trends in serum lysozyme activity and total protein concentration in dab (Limanda limandaL.) sampled from Lyme Bay, U.K. Fish & Shellfish Immunology. 6, 473–482. https://doi.org/10.1006/fsim.1996.0045

Janczyk, P., Halle, B., Souffrant, W.B., 2009. Microbial community composition of the crop and ceca contents of laying hens fed diets supplemented with Chlorella vulgaris. Poultry Science. 88, 2324–2332. https://doi.org/10.3382/ps.2009-00250

Kajita, Y., Sakai, M., Atsuta, S., Kobayash, M., 1990. The immunomodulatory effects of levamisole on rainbow trout, Oncorhynchus Mykiss. Fish Pathol. 25, 93–101.

Karemore, A., Pal, R., Sen, R., 2013. Strategic enhancement of algal biomass and lipid in Chlorococcum infusionum as bioenergy feedstock. Algal Research. 2, 113–121. https://doi.org/10.1016/j.algal.2013.01.005

Khan, Z., Bhadouria, P., Bisen, P.S., 2005. Nutritional and therapeutic potential of Spirulina. Current Pharmaceutical Biotechnology. 6, 373–379. https://doi.org/10.2174/138920105774370607

Kondo, Y., Kato, A., Hajo, H., Nozoe, S., Takeuchi, M., Ochi, K., 1992. Cytokine-related immunopotentiating activities of paramylon, a albha 1,3 D-glucan fro, Euglena gracilis. Journal of Pharmacobio-Dynamics. 5, 617–638. https://doi.org/10.1248/bpb1978.15.617

Kotrbacek, V., Halouzka, R., Jurajda, V., Knotkova, Z., Filka, J., 1994. Increased immune response in broilers after administration of natural food supplements. Vet Med (Praha). 39, 321–328. 8053120.

Lamas, J., Ellis, A.E., 1994. Atlantic salmon (Salmo salar) neutrophil responses to Aeromonas salmonicida. Fish & Shellfish Immunology. 4, 201–220. https://doi.org/10.1006/fsim.1994.1019

Lisboa, C.R., Pereira, A.M., Ferreira, S.P., Jorge, A.V., 2014. Utilisation of Spirulina sp. and Chlorella pyrenoidosa Biomass For The Productionof Enzymatic Protein Hydrolysates. Int. J. Eng. Res. Appl. 4, 29–38.

Michael, R.D., Srinivas, S.D., Sailendri, K., 1994. A rapid method for repetitive bleeding in fish. Indian. J. Exp. Biol. 32, 838–847.

Morais, M.G., Vaz, B.S., Morais, E.G., Costa, J.A.V., 2014. Biological Effects of Spirulina (Arthrospira) Biopolymers and Biomass in the Development of Nanostructured Scaffolds. BioMed Research International, 1–9. https://doi.org/10.1155/2014/762705

Pangestuti, R., Kim, S.K., 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. Journal of Functional Foods. 3, 255–266. https://doi.org/10.1016/j.jff.2011.07.001

Peddie, S., Zou, J., Secombes, J., 2002. Immunostimulation in the rainbow trout (Oncorhynchus mykiss) following intraperitoneal administration of Ergosan. Veterinary Immunology and Immunopathology. 86, 101–113. https://doi.org/10.1016/s0165-2427(02)00019-3

Quade, M.J., Roth, J.A., 1997. A rapid, direct assay to measure degran- ulation of bovine neutrophil primary granules. Veterinary Immunology and Immunopathology. 58, 239–287. https://doi.org/10.1016/s0165-2427(97)00048-2

Raa, J., 1996. The use of immunostimulatory substances in fish and shellfish farming. Reviews in Fisheries Science. 4, 229–288. https://doi.org/10.1080/10641269609388587

Ravelonandro, P.H., Ratianarivo, D.H., Joannis-Cassan, C., Isambert, A., Raherimandim, M., 2008. By Influence of light quality and intensity in the cultivation of Spirulina platensis from Toliara (Madagascar) in a closed system. Journal of Chemical Technology & Biotechnology. 83, 842–848. https://doi.org/10.1002/jctb.1878

Ringo, E., Olsen, R.E., Gifstad, T.O., Dalmo, R.A., Amlund, H., Hemre, G.I., Bakke, A.M., 2010. Prebiotics in aquaculture: a reviews. Aquaculture Nutrition. 16, 117–136. https://doi.org/10.1111/j.1365-2095.2009.00731.x

Robertsen, B., 1999. Modulation of the non-specific defence of fish by structurally conserved microbial polymers. Fish and Shellfish Immunology. 9, 269–290. https://doi.org/10.1006/fsim.1998.0186

Sahu, S., Das, B.K., Mishra, B.K., Pradhan, J., Sarangi, N., 2007. Effect of Allium sativum on the immunity of Labeo rohita infected with Aeromonas hydrophila. Journal of Applied Ichthyology. 23, 80–86. https://doi.org/10.1111/j.1439-0426.2006.00785.x

Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture. 172, 63–92. https://doi.org/10.1016/S0044-8486(98)00436-0

Sattanathan, G., Thanapal, G., Swaminathan, S., Kim, Vijaya, .R.,

Kim, H., Balasubramanian, B., 2020. Influences of dietary inclusion of algae Chaetomporpha aerea enhanced growth performance, immunity, haematological response and disease resistance of Labeo rohita challenged with Aeromonas hydrophila. Aquaculture Reports, 100353. https://doi.org/10.1016/j.aqrep.2020.100353

Selvaraj, V., Sampath, K., Sekar, V., 2009. Administration of lipopolysaccharide increaseds specific and non-specific immune parameters and survival in carp infected with Aeromonas hydrophila. Aquaculture. 286, 176–183. https://doi.org/10.1016/j.aquaculture.2008.09.017

Solem, S.T., Jorgensen, J.B., Robertsen, B., 1995. Stimulation of respiratory burst and phagocytic activity in Atlantic salmon (Salmo salar L.) macrophages by lipo- polysaccharide. Fish & Shellfish Immunology. 5, 475–491. https://doi.org/10.1016/S1050-4648(95)80049-2

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering. 101, 87–96. https://doi.org/10.1263/jbb.101.87

Tamizhazhagan, V., Pugazhendy, K., Jayanthi, S.V., Sawicka, C.B., Gerlee, S., Ramarajan, K., Manikandan, P., 2017. The toxicity effect of pesticide Monocrotophos 36% E.C on the enzyme activity changes in liver and muscles of Labeo rohita (Hamilton, 1882). International Journal of Pharma Sciences and Research. 8(5), 60–67.

Tamizhazhaganv, Pugazhendy, K., Sakthidasan, J.V.C., 2016. The toxicity effect of Monocrotophos 36 % E.C on the Histological changes in gill of Labeo rohita. International Journal of Innovative Research in Multidisciplinary Field. 2(11), 435–439.

Usha, R., Pugazhendy, K., Tamizhazhagan, V., Sakthidasan, V., Jayanthi, C., 2017. Potential efficacy of Tribulu sterrtri against toxic impact of chlorpyrifos on enzymological alteration in the fresh water fish Oriochrommis mossambicus. International Journal of Pharmacy and Biological Sciences. 7(3), 168–184.

Vijayavel, K., Anbuselvam, C., Balasubramanian, M.P., 2007. Antioxidant effect of the marine algae Chlorella vulgaris against naphthalene-indcued oxidative stress in the albino rats. Molecular and Cellular Biochemistry. 303, 39–44. https://doi.org/10.1007/s11010-007-9453-2

Vílchez, C., Forján, E., Cuaresma, M., Bédmar, F., Garbayo, I., Vega, J.M., 2011. Marine carotenoids: Biological functions and commercial applications. Marine Drugs. 9, 319–333. https://doi.org/10.3390/md9030319

Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology. 79, 707–718. https://doi.org/10.1007/s00253-008-1518-y

Wijendra, G.D.N.P., Pathiratne, A., 2007. Evaluation of immune responses in an Indian carp, L. rohita (Ham.) fed with levamisole incorporated diet. Journal of Science of the University of Kelaniya Sri Lanka. 3, 17–28. http://doi.org/10.4038/josuk.v3i0.2735