

## POTENTIAL USE OF BROWN ALGAE AS AN IMMUNOSTIMULANT MATERIAL IN THE AQUACULTURE FIELD TO INCREASE NON-SPECIFIC IMMUNITY AND FIGHT DISEASE

Nuri Muahiddah<sup>1\*</sup>, Wastu Ayu Diamahesa<sup>1</sup>

<sup>1</sup> Aquaculture Study Program, Faculty of Agriculture, University of Mataram,  
Education Street No. 37 Mataram, West Nusa Tenggara.

\*Correspondence:  
nurimuahiddah@unram.ac.id

### ABSTRACT

Received : 2022-05-23  
Accepted : 2022-12-29

Keywords :  
Brown algae, Aquaculture,  
Immunostimulant, Non-specific  
immunity, Disease

The aquaculture field continues to grow from year to year. This is because aquaculture fulfills the world's food needs. Aquaculture intensification continues. As a result of this intensification, the aquaculture environment experienced a decrease in water quality. This led to the rapid growth of bacteria, fungi, parasites, and viruses, resulting in a high incidence of disease outbreaks. An alternative solution that has been extensively researched recently is using immunostimulants to increase the non-specific immunity of fish and shrimp, which will help fish and insects prevent disease outbreaks. One of the natural ingredients that have the potential to become an immunostimulant is from the brown algae group. Immunostimulants from brown algae such as Hot-water Extract of Tropical Brown Seaweed, *Sargassum cristaeifolium*, *Sargassum glaucescens*, *Sargassum duplicatum*, *Sargassum wightii*, *Sargassum* sp. Polysaccharides from seaweed, such as fucoidan extracts from brown algae, can enhance the non-specific immune system in shrimp *Litopenaneus vannamei*, *Penaeus monodon*, *Fenneropenaeus indicus*, and tilapia *Oreochromis niloticus*. These immunostimulants increase non-specific immunity, such as total hemocyte count, phagocytosis activity, phenoloxidase, phagocytic, respiratory burst, superoxide dismutase, and total plasma protein. Immunostimulants from brown algae can also fight bacterial disease attacks *Vibrio parahaemolyticus*, *Streptococciasis*, *Streptococcus iniae*, and White spot syndrome virus (WSSV) by increasing survival rate and can increase the growth and feed efficiency of cultivated commodities that are not given immunostimulants.

### INTRODUCTION

The aquaculture sector continues to overgrow in terms of the value and volume of fishery production (Rokhmah et al., 2014). This is because the aquaculture sector fulfills the world's food needs. The high demand for fishery products has resulted in the aquaculture

sector increasing production through the intensification of cultivation. However, along with the intensification of cultivation resulting in increased disease attacks. Intensification of cultivation results in a decrease in aquaculture water quality, which triggers the high development of bacteria, parasitic fungi, and viruses (Puteri & Haditomo, 2016). The high level of bacteria, fungi, parasites, and viruses in the cultivation environment causes cultivated commodities to be susceptible to disease outbreaks that cause death and cause losses. Apart from the importance of managing water quality and implementing good fish and shrimp farming methods as well as biosecurity, what has recently been frequently researched is the use of immunostimulants to increase non-specific immunity in aquaculture commodities to prevent disease attacks. Immunostimulants are components of herbs, seaweed, fungi, and bacteria that can increase non-specific immunity, thereby increasing the ability of cultivated commodities to fight disease attacks (Muahiddah & Diamahesa, 2022).

One of the potential ingredients as an immunostimulant from natural ingredients is brown seaweed (Yanuarti et al ., 2017). Brown seaweed has abundant production in nature, so it can be a potential ingredient for producing immunostimulants (Jabar & Natasia, 2021). Immunostimulants from brown seaweed have been extensively studied for their use in aquaculture and have shown promising results. Immunostimulants from brown seaweed are considered environmentally friendly and effective in increasing the non-specific immunity of cultivated commodities. Immunostimulants from seaweed are made through hot water extraction and acid extraction. There are many potential types of brown seaweed, including *Sargassum wightii*, *Sargassum glaucescens*, *Sargassum cristaefolium*, *Sargassum duplicatum*, *Sargassum sp*, *Undaria pinnatifida*, *Laminaria japonica*. This immunostimulant can be administered by injection, orally, or immersion ( Kurniawati et al ., 2017). Components of brown seaweed can also be used as immunostimulants, namely sodium alginate and fucoidan. Sodium Alginate and Fucoidan are polysaccharide groups that stimulate non-specific immune enhancement in fish and shrimp. Fish and shrimp have non-specific immunity that functions as a resistance to foreign bodies and diseases.

Research is continuing to be carried out to find out the right way of administering immunostimulants to cultivated commodities and the best dosage that should be given to increase non-specific immunity, as well as the ability of cultivated commodities to fight disease attacks.

## DISCUSSION

The use of natural ingredients as immunostimulants in aquaculture has been extensively studied recently. They were using natural ingredients as immunostimulants are considered because they are environmentally friendly and can potentially increase non-specific immunity. Natural ingredients that are widely used come from the seaweed group (Muahiddah & Sulystyaningsih, 2021). Many groups of seaweed are used as immunostimulants from red seaweed, namely *Gracilaria*, brown seaweed, *Sargassum*, and laminarin, as immunostimulants in improving health conditions, quality of life of fish, growth and increasing immunity and enabling fish to overcome stress caused by artificial infections ( Aly et al ., 2008).

Brown algae is a source of immunostimulants because they contain bioactive compounds that produce secondary metabolites with a broad spectrum of activity and can enhance shrimp's non-specific immunity (Febriani et al ., 2013). The cell walls of brown algae are rich in polysaccharides which can be helpful as immunostimulants, anticoagulants, antioxidants, antibacterial, antiviral, anti-cancer, and immune modulating activation

(Wijesekara et al ., 2011). Polysaccharides from brown algae that are often used are fucoidan and sodium alginate (brown seaweed). Crude extracts and hot water extracts can also be used as immunostimulants to increase aquaculture commodities' non-specific immunity.

Giving immunostimulants from brown algae can be done orally, by immersing, or by injection. Research on the dose and frequency is still being carried out to determine the most effective way to use immunostimulants. The use of immunostimulants from fish and shrimp brown algae in the following references:

Seaweed species	Aquaculture commodity	Method	Results	Resistant to disease	Reference
Hot-water Extract of Tropical Brown Seaweed, <i>Sargassum cristaefolium</i>	<i>Litopenaeus vannamei</i>	Oral	Total hemocyte count (THC), Differential Hemocyte Count (granular and hyaline cells), and Phagocytic Activity	<i>Vibrio parahaemolyticus</i>	Sudaryono et al ., (2018)
<i>Sagassum</i> sp. extract .	Tilapia ( <i>Oreochromis niloticus</i> )	Injection	Leukocyte Differential (Lymphocyte, Monocyte, Neutrophil) Survival Rate (SR) value of feed consumption rate (TKP)	Streptococciasis	Zahra et al ., 2017
Brown Seaweed Flour ( <i>Sargassum</i> sp.)	Tiger prawns ( <i>Penaeus monodon</i> )	Oral	relative growth rate (RGR) Feed Utilization Efficiency (EPP) Survival Rate (SR)	-	Widyantoko, and Herawati, 2015
Fucoidan from the brown alga <i>Sargassum wightii</i>	<i>Penaeus monodon</i>	Oral	Total hemocyte count (THC) Pro Phenoloxidase Respiratory burst (RB) Superoxide dismutase Activity and Phagocytic activity	<i>White Spot Syndrome Virus</i>	Immanuel et al ., 2012
<i>Sagassum</i> sp. extract .	Tilapia ( <i>Oreochromis niloticus</i> )	Injection	Diferensial Leukosit (Limfosit, Monosit, Neutrofil) Survival Rate (SR) Total hemosit count (THC)	<i>Streptococcus iniae</i>	Rustikawati, 2012
Hot-water extract dari Alga Coklat <i>Sargassum glaucescens</i>	<i>Fenneropenaeus indicus</i>	Immersion	differential haemocyte count (DHC), total plasma protein (TPP), Phagocytic activity (PA), bacterial clearance efficiency (BCE) and bactericidal activity (BE)	-	Ghaednia et al., 2011
Hot-water extract of Brown seaweed <i>Sargassum duplicatum</i>	<i>Litopenaeus vannamei</i>	Immersion Injection	Phagocytic Activity and Clearance Efficiency	<i>Vibrio alginolyticus</i>	Yeh et al ., (2006)

Administration of Fucoidan from the brown algae *Sargassum wightii* given orally

increases Total Hemocyte count (THC), Pro Phenoloxidase, Respiratory burst (RB), Superoxide dismutase Activity, and Phagocytic activity and fights against the White Spot Syndrome Virus attack (Immanuel et al ., 2012). *Sargassum* sp. extract . given by injection to *Tilapia* (*Oreochromis niloticus* ) can increase the survival of tilapia from *Streptococcus iniae* bacterial attack through increasing Leukocyte Differential (Lymphocytes, Monocytes, Neutrophils) (Rustikawati, 2012 ). Hot-water extract of brown algae *Sargassum glaucescens* by immersion increased the total hemocyte count (THC), differential hemocyte count (DHC), total plasma protein (TPP), phagocytic activity (PA), bacterial clearance efficiency (BCE), and bactericidal activity (BE). ) in *Fenneropenaeus indicus* Ghaednia et al ., 2011. Use of Hot-water extract of Brown seaweed *Sargassum* duplicated,

Fucoidan is a polysaccharide from brown seaweed that can stimulate non-specific immunity in fish and shrimp (Purbomartono et al ., 2020). The stimulation is in the form of a respiratory burst mechanism through the receptor surface interaction mechanism. This immunostimulant from brown algae polysaccharides increases phagocytic activity, which indicates an increase in the cell's ability to fight or destroy foreign bodies (Siswati, 2021). The fucoidan and brown algae extracts increased the total hemocyte count, indicating the non-specific defense of fish and shrimp from cellular and humoral. Total hemocytes are closely related to proPO stimulation to induce phenoloxidase activity. Phenoloxidase activity shows the ability of vaname shrimp to recognize foreign objects that enter the body (Garcia-Carreño et al ., 2008) and the activity of the shrimp's body defense.

In fish and shrimp, Hemocytes play an essential role in the immune system. Hemocytes can remove foreign particles or disease in the hemocoel by phagocytosis, encapsulation, and nodular aggregation mechanisms. Haemocytes in shrimp and fish play a role in wound healing by cellular clumping and initiation of the coagulation process through the release of factors needed for plasma gelation and the release of the prophenoloxidase proPO system (Arifin & Supriyono, 2014).

Phagocytosis is the most common response in cell protection. During phagocytosis, particles or microorganisms enter the cell and form digestive vacuoles called phagosomes. The mechanism of particle removal by phagocytic cells called a respiratory burst involves the release of degradative enzymes into the phagosome (an oxygen-dependent killing mechanism) and the production of ROI (reactive oxygen intermediates) (Martín et al ., 2012). An increase in a respiratory burst is correlated with an increase in phagocytosis activity and vice versa (Jasmanindar, 2009; Sirirustananun et al ., 2011). During phagocytosis, viral particles are recognized by receptors on the cell surface and are engulfed by cells that re-prepare the cytoskeleton for phagosome formation. In the first stage, phagosomes undergo maturation through cleavage and fusion with lysosomes and become mature  $\alpha$  phage-lysosomes. Viruses in phage-lysosomes will be destroyed by low pH conditions, hydrolysis processes, and radicals (Xu et al ., 2014).

Melanization is essential to the shrimp's immune defense system against pathogens. Phenoloxidase is an enzyme that plays a role in the process of melanization. This enzyme is produced through the proPO (prophenoloxidase) system, which is activated in the presence of an immunostimulant. ProPO plays an essential role in introducing foreign bodies, including phagocytosis, melanization, production of cytotoxic reactants, particle encapsulation, and formation of nodules and capsules (Amparyup et al ., 2013). Activation of the proPO system induces the production of melanin, a brown pigment responsible for various processes, such as activation of foreign particles, protecting their distribution in the host's body, and repairing cuticle damage.

Immunostimulants from seaweed have been shown to increase the non-specific immunity of shrimp and fish. Hot-water Extract of Tropical Brown Seaweed, *Sargassum cristaefolium*, can increase Total Hemocyte Count (THC), Differential Hemocyte Count (granular and hyaline cells), and Phagocytic Activity in *Litopenaeus vannamei* and fight against *Vibrio parahaemolyticus* disease (Sudaryono et al., 2018). According to Zahra et al., 2017 *Sargassum* sp. given by injection to tilapia increases leukocyte differential (lymphocytes, monocytes, neutrophils), Survival Rate (SR) to prevent Streptococcosis. Even Brown Seaweed Flour (*Sargassum* sp.) given orally to Tiger Shrimp (*Penaeus monodon*) can increase the value of feed consumption level (TKP), relative growth rate (RGR), Feed Utilization Efficiency (EPP) (Widyantoko, and Herawati, 2015).

In addition to increasing the non-specific immunity of fish and shrimp, immunostimulants from seaweed also increase the relative growth rate of shrimp that are not given immunostimulants. The shrimp feed also increases its efficiency if it is given an immunostimulant. This shows that immunostimulants from brown algae increase non-specific immunity to fight disease attacks and increase the growth of shrimp given immunostimulants (Widyantoko & Herawati, 2015).

### CONCLUSION

Immunostimulants from brown algae such as Hot-water Extract of Tropical Brown Seaweed, *Sargassum cristaefolium*, *Sargassum glaucescens*, *Sargassum duplicatum*, *Sargassum wightii*, *Sargassum* sp. Polysaccharides from seaweed, such as fucoidan extracts from brown algae, can enhance the non-specific immune system in shrimp *Litopenaeus vannamei*, *Penaeus monodon*, *Fenneropenaeus indicus*, and tilapia *Oreochromis niloticus*. These immunostimulants increase non-specific immunity, such as total hemocyte count, phagocytosis activity, phenoloxidase, phagocytic, respiratory burst, superoxide dismutase, and total plasma protein. Immunostimulants from brown algae can also fight against the bacterial disease *Vibrio parahaemolyticus*, Streptococcosis, *Streptococcus iniae*, and White spot syndrome virus (WSSV) by increasing the survival rate and can increase the growth and feed efficiency of cultivated commodities that are not given immunostimulants.

### ACKNOWLEDGMENT

Thanks to Wastu Ayu Diamahesa, who has helped in the preparation of this article.

### REFERENCES

- Aly, S. M., Ahmed, Y. A. G., Ghareeb, A. A. A., & Mohamed, M. F. (2008). Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. *Fish & shellfish immunology*, 25(1-2), 128-136.
- Amparyup, P., Charoensapsri, W., & Tassanakajon, A. (2013). Prophenoloxidase system and its role in shrimp immune responses against major pathogens. *Fish & Shellfish Immunology*, 34(4), 990-1001.
- Arifin, M. Y., & Supriyono, E. (2014). Total Hemosit, Glukosa Dan Survival Rate Udang Mantis (*Harpisquilla Raphidea*) Pasca Transportasi Dengan Dua Sistem Yang Berbeda. *Jurnal Kelautan Nasional*, 9(2), 111-119.

- Febriani, D., & Nuryati, S. (2013). Kappa-carrageenan as immunostimulant to control infectious myonecrosis (IMN) disease in white shrimp *Litopenaeus vannamei*. *Jurnal Akuakultur Indonesia*, 12(1), 70-78.
- García-Carreño, F. L., Cota, K., & Navarrete Del Toro, M. A. (2008). Phenoloxidase activity of hemocyanin in whiteleg shrimp *Penaeus vannamei*: conversion, characterization of catalytic properties, and role in postmortem melanosis. *Journal of Agricultural and Food Chemistry*, 56(15), 6454-6459.
- Ghaednia, B., Mehrabi, M. R., Mirbakhsh, M., Yeganeh, V., Hoseinkhezri, P., Garibi, G., & Ghaffar Jabbari, A. (2011). Effect of hot-water extract of brown seaweed *Sargassum glaucescens* via immersion route on immune responses of *Fenneropenaeus indicus*.
- Immanuel, G., Sivagnanavelmurugan, M., Marudhupandi, T., Radhakrishnan, S., & Palavesam, A. (2012). The effect of fucoidan from brown seaweed *Sargassum wightii* on WSSV resistance and immune activity in shrimp *Penaeus monodon* (Fab). *Fish & shellfish immunology*, 32(4), 551-564.
- Jabar, A. A., & Natasia, N. (2021). Potensi Alga Coklat (*Sargassum polycystum* c. agardh) sebagai Produk Teh untuk Meningkatkan Imunitas Tubuh. *Berkala Ilmiah Mahasiswa Farmasi Indonesia*, 8(1), 80-94.
- Jasmanindar, Y. (2009). Penggunaan ekstrak *Gracilaria verrucosa* untuk meningkatkan sistem ketahanan udang vaname *Litopenaeus vannamei*.
- Muahiddah, N., & Sulystyaningsih, N. D. (2021). Analisis Hasil Ekstraksi *Sargassum* Sp. Dari Teluk Ekas, Pemicu Peningkatan Produksi Rumput Laut, Lombok Timur, Nusa Tenggara Barat. *Jurnal Agroqua: Media Informasi Agronomi Dan Budidaya Perairan*, 19, 131-142.
- Rokhmah, N. A., Ammatillah, C. S., & Sastro, Y. (2014). Vertiminaponik, mini akuaponik untuk lahan sempit di perkotaan. *Buletin Pertanian Perkotaan*, 4(2), 14-22.
- Rustikawati, I. (2012). Efektivitas ekstrak *Sargassum* sp. terhadap diferensiasi leukosit ikan nila (*Oreochromis niloticus*) yang diinfeksi *Streptococcus iniae*. *Jurnal Akuatika*, 3(2).
- Rustikawati, I. (2011). Peningkatan Imunitas Ikan Nila (*Oreochromis niloticus*) terhadap Serangan *Streptococciosis* Menggunakan Ekstrak *Sargassum* sp. *Indonesian Journal of Applied Sciences*, 1(1).
- Sirirustananun, N., Chen, J. C., Lin, Y. C., Yeh, S. T., Liou, C. H., Chen, L. L., ... & Chiew, S. L. (2011). Dietary administration of a *Gracilaria tenuistipitata* extract enhances the immune response and resistance against *Vibrio alginolyticus* and white spot syndrome virus in the white shrimp *Litopenaeus vannamei*. *Fish & shellfish immunology*, 31(6), 848-855.
- Siswati, S. (2021). *Kinerja Immunostimulan Rumput Laut Codium Hubbsii Pada Dosis Yang Berbeda Terhadap Peningkatan Respon Kekebalan Tubuh Udang Windu (Penaeus Monodon Fabricus, 1978) Yang Diuji Tantang Dengan Bakteri Vibrio harveyi* (Doctoral dissertation, Universitas Hasanuddin).
- Sudaryono, A., Chilmawati, D., & Susilowati, T. (2018). Oral Administration of Hot-water Extract of Tropical Brown Seaweed, *Sargassum cristaefolium*, to Enhance Immune Response, Stress Tolerance, and Resistance of White Shrimp, *Litopenaeus vannamei*, to *Vibrio parahaemolyticus*. *Journal of the World Aquaculture Society*, 49(5), 877-888.
- Tapsell, L. C., Neale, E. P., Satija, A., & Hu, F. B. Foods, nutrients, and dietary patterns: interconnections and implications for dietary guidelines. *Adv Nutr*. 2016 Dec; 7 (3): 445–54. doi: 10.3945/an.115.011718.
- Permanti, Y. C., Julyantoro, P. G. S., & Pratiwi, M. A. (2018). Pengaruh penambahan *Bacillus* sp. terhadap kelulushidupan pasca larva udang vannamei (*Litopenaeus vannamei*) yang

- terinfeksi vibriosis. *Aquatic Sci*, 1(1), 91-97.
- Prabowo, S. A. (2003). Asian Aquaculture Magazine. Buletin Biru Laut, Edisi I Maret 2003. Lampung, Indonesia: Unit Data & Informasi Departemen Laboratorium & Monitoring Research and Development PT. Biru Laut Khatulistiwa.
- Putri, S. M., & Haditomo, A. H. C. (2016). Infestasi monogenea pada ikan konsumsi air tawar di kolam budidaya Desa Ngrajek Magelang. *Journal of Aquaculture Management and Technology*, 5(1), 162-170.
- Purbomartono, C., Mulia, D. S., & Priyambodo, D. (2020). Respon Imun Non-spesifik Ikan Gurami (*Osphronemus gouramy*) yang Diberi Fucoidan dari Ekstrak Rumput Laut Cokelat *Padina* sp. *Sainteks*, 16(1).
- Muahiddah, N., Diamahesa, W. A. (2022). Pengaruh Immunostimulan dari bahan-bahan alami pada Ikan dalam Meningkatkan Imun non-spesifik untuk Melawan Penyakit. *Clarias: Jurnal Perikanan Air Tawar*, 3(2), 37-44.
- Widyantoko, W., & Herawati, V. E. (2015). Optimalisasi penambahan tepung rumput laut coklat (*Sargassum* sp.) Yang berbeda dalam pakan terhadap pertumbuhan dan kelulushidupan juvenil udang windu (*Penaeus monodon*). *Journal of Aquaculture Management and Technology*, 4(2), 9-17.
- Wijesekara, I., Pangestuti, R., & Kim, S. K. (2011). Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. *Carbohydrate polymers*, 84(1), 14-21.
- Xu, H., Yang, J., Gao, W., Li, L., Li, P., Zhang, L., ... & Shao, F. (2014). Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. *Nature*, 513(7517), 237-241.
- Yanuarti, R., Nurjanah, N., Anwar, E., & Pratama, G. (2017). Kandungan senyawa penangkal sinar ultra violet dari ekstrak rumput laut *Eucheuma cottonii* dan *Turbinaria conoides*. *Majalah Ilmiah Biologi BIOSFERA: A Scientific Journal*, 34(2), 51-58.
- Yeh, S. T., Lee, C. S., & Chen, J. C. (2006). Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. *Fish & Shellfish Immunology*, 20(3), 332-345.