Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 11(2016) 69-74

Environment & We
An International
Journal of Science
& Technology

Antimicrobial Effects of Seaweed Extracts against Silkworm (*Bombyx mori* L.) Fungal and Bacterial Pathogens

R. Ramamoorthy*, P. Priyadharshini and P. Mohanraj
Department of Sericulture, Forest College and Research Institute,
Tamil Nadu Agricultural University, Mettupalayam – 641301, Tamil Nadu.
*Email: rrrmoorthy@gmail.com

Article history:
Received 16 June 2016
Received in revised form
27 August 2016
Accepted 10 September 2016
Available online
30 September 2016

Keywords:
Seaweeds,
Silkworm,
Beauveria bassiana,
Metarhizium anisopliae,
Staphylococcus

Abstract

Antimicrobial activity of eight different species of seaweeds extracts *viz*., *Padina tetrastromatica, Sargassum wightii, Sargassum ilicifolium, Turbinaria conoides, Kappaphygus alvarezii, Gelidiella acrosa, Ulva lactuca* and *Chaetomorpha litorea* were evaluated against silkworm fungal and bacterial pathogens *viz., Beauveria bassiana, Metarhizium anisopliae* and *Staphylococcus aureus* . Among the eight seaweed extracts, 20 per cent concentration of *T. conoides* showed highest inhibition and lowest mycelial growth of 90.6% and 8.4 mm followed by *S. wightii* (88.2% and 10.6 mm), *S. ilicifolium* (83.8% and 14.5 mm) and *U. lactuca* (81.2% and 16.9 mm) against *B.bassiana*. Similarly, 20 per cent extract of *T. conoides* showed highest percentage inhibition and lowest mycelial growth of 89.2% and 9.7 mm followed by *S. wightii* (86.3% and 12.6 mm), *S. ilicifolium* (83.3% and 15 mm) and *U. lactuca* (82.3% and 15.9 mm) respectively against *M.anisopliae*. *T. conoides, S. wightii, S. ilicifolium and U. lactuca* showed complete inhibition of *S.aureus* by streak plate method. The results suggested that the seaweeds extracts could be a good alternative in developing a potent plant based disinfectant which can be used in eco-friendly management of silkworm fungal and bacterial pathogens.

Introduction

Silkworm (*Bombyx mori* L.) is an important economic lepidopteran insect and utilized for the commercial production of natural glamour silk fibre, “Queen of Textiles”. Silkworm also serves as a powerful laboratory model for the basic and applied research in insect biology (Ramesha *et al*., 2010). Silkworms are susceptible to a number of diseases caused by different infectious agents such as fungal, bacterial, viral and protozoan diseases. It is the main factor seriously affecting the cocoon production

(Doreswamy *et al*., 2004). Major constraints in silk cocoon production is the occurrence of silkworm diseases *viz*., Flacharie (bacterial), Muscardine (fungal), Grasserie (viral) and Pebrine (protozoan) to silkworm larvae which accounts for cocoon crop loss upto 30 to 40 per cent (Manimegalai *et al*., 2010). Fungal muscardine diseases can be caused by several fungi *viz*., *Beauveria bassiana*, *Metarhizium anisopliae*, *Aspergillus flavus* and *A. niger*. Fungal pathogen causes cocoon crop loss of about 5 to 50 per cent reported by Jhansi Lakshmi (2003). Use of chemicals for preventing diseases causes toxicity to silkworms. Hence, searching of natural, cheaply available and eco friendly product is the need of the hour.

India has a vast coastline of 6100 km supporting a rich flora of marine plants such as seaweeds, mangroves and sea grasses. Seaweeds (marine macroalgae) a specialized group of phytoplankton is available in plenty, throughout the year. Seaweeds were the specialized group of plants which are rich source of structurally diverse bioactive compounds with valuable pharmaceutical potential (Ravikumar *et al*., 2002). Extracts from many marine algae show antibacterial, antifungal, antiviral, antioxidant and antibiotic activity (Ravikumar *et al*., 2009). Developing “eco-friendly” seaweeds (marine macroalgae) based products that could prevent or cure silkworm diseases can be an alternative treatment process. Hence, the present study was focused on the screening of eight different seaweeds for antifungal and antibacterial activity.

Materials and Methods

Collection of seaweeds

Eight seaweeds were collected from the low intertidal pools at Bay of Bengal from Gulf of Mannar Biosphere reserve of $9^{o}15'$N latitude to $79^{o}12'$E longitude at 2-10 m depth from Mandapam Coastal Area, Tamil Nadu, India. All the collected halophytes were washed once with tap water and distilled water and shade dried under room temperature (28±2ºC) for further use.

Preparation of extracts

Seaweed material was mixed with acetone solvent separately (1:50, w/ v) and placed into the soxhlet apparatus. Each extraction was carried out in a soxhlet apparatus for 24 h and after evaporation in vacuum the extracts were stored at -20°C until use for antimicrobial sensitivity assay against silkworm pathogens.

Antifungal assay

Food poison technique was employed to screen the antifungal efficacy of seaweed extracts (Grover and Moore, 1962). Sabourand dextrose agar media was mixed with seaweed extracts was autoclaved and poured into sterile petriplates. Fungal discs of 9 mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of *B. bassiana* and *M.anisopliae* and the discs were transferred aseptically on SDA plates poisoned with seaweed extracts. A plate only with SDA and fungal disc was considered as control for the calculation of per cent inhibition of test fungi (Ambika and Sujatha, 2014).

Antibacterial assay

Seaweeds extract @ 20 per cent concentration was prepared and added to the nutrient agar (NA) medium. A loop of bacterial culture of *S. aureus* was drawn and streaked on the plates and kept for incubation. Observations were made on the growth after 24 hours.

Result and Discussion

With increasing resistance of pathogens to antibiotics, there is a health priority for exploring and developing cheaper and effective natural antimicrobial agents with better potential, lesser side effects than antibiotics, good bioavailability, and minimal toxicity. Seaweed or macroalgae provide a great variety of metabolites and natural bioactive compounds with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins and other phenolic compounds, and carotenoids (Paul and Puglisi, 2004; Bhadury and Wright, 2004).

Antifungal activity of acetone extracts of seaweeds were screened against *B. bassiana* and *M. anisopliae*. The results showed that the 20 per cent concentration of *T. conoides* showed highest inhibition and lowest mycelial growth of 90.6% and 8.4 mm followed by *S. wightii* (88.2% and 10.6 mm), *S. ilicifolium* (83.8% and 14.5 mm) and *U. lactuca* (81.2% and 16.9 mm) (Table 1) against *B.bassiana.* Sugnankumari *et al*. (2011) studied the antifungal activity of *T.conoides* against *B.bassiana*, an entomopathogenic fungus of silkworm. 20 per cent acetone extract of *T. conoides* showed highest percentage inhibition and lowest mycelial growth of 89.2% and 9.7 mm followed by *S. wightii* (86.3% and 12.6 mm), *S. ilicifolium* (83.3% and 15 mm) and *U. lactuca* (82.3% and 15.9 mm) respectively(Table 2) against *M.anisopliae*. Ameer Junaithal Begum *et al.* (2016) studied the antifungal activity of *T.conoides* against plant fungal pathogens. Seaweed contains biogenic compounds (Cox *et al.* 2010) which are characterized by the presence of one or more aromatic hydroxyl groups and the antimicrobial activity is due to the alteration of cell permeability and the loss of internal molecules or by the interference with the membrane function and the loss of cellular integrity and eventual cell death. Shibu and Dhanam (2016) studied the antifungal activity of *T.conoides* collected from Mandapam coasts of Tamil Nadu.

Eight different seaweed extracts *viz., P. tertastromatica, S. wightii, S. ilicifolium, T. conoides, K. alvarezii, G. acrosa, U. lactuca* and *C. littorea* were tested for their anti-bacterial activity against gram positive bacteria, *Staphylococcus aureus* by streak plate method. The result of antibacterial activity against tested pathogen was tabulated (Table 3). *T. conoides, S. wightii, S. ilicifolium and U. lactuca* showed complete inhibition of pathogen. *K. alvarezii, G. acrosa, U. lactuca* and *P. tertastromatica* showed partial inhibition of tested *S.aureus* compared to control showed no inhibition.

Table 1. Growth inhibitory effect of seaweeds under against *B. bassiana*

Seaweeds	Mycelial colony growth inhibition (mm)	Percentage of colony inhibition over control
Padina tetrastromatica	27.3[e]	**69.6**[e]
Sargassum wightii	10.6[b]	**88.2**[b]
Sargassum ilicifolium	14.5[d]	**83.8**[d]
Turbinaria conoides	8.4[a]	**90.6**[a]
Kappaphygus alvarezii	29.1[f]	**67.6**[f]
Gelidiella acrosa	20.0[g]	**77.7**[g]
Ulva lactuca	16.9[c]	**81.2**[c]
Chaetomorpha litorea	34.5[h]	**61.6**[h]
Control	90.0	**0.0**

In a column by a common letter are not significantly different by DMRT (P = 0.05)

Table. 2. Growth inhibitory effect of seaweeds under against *M. anisopliae*

Seaweeds	Mycelial colony growth inhibition (mm)	Percentage of colony inhibition over control
Padina tetrastromatica	24.5[e]	**72.7**[e]
Sargassum wightii	12.6[b]	**86.3**[b]
Sargassum ilicifolium	15.0[d]	**83.3**[d]
Turbinaria conoides	9.7[a]	**89.2**[a]
Kappaphygus alvarezii	30.1[f]	**66.6**[f]
Gelidiella acrosa	20.2[g]	**77.5**[g]
Ulva lactuca	15.9[c]	**82.3**[c]
Chaetomorpha litorea	37.3[h]	**58.5**[h]
Control	90.0	**0.0**

In a column by a common letter are not significantly different by DMRT (P = 0.05)

The presence of phenolic compounds in sea weed extracts may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Alberto *et al.,* 2001 and Reguant *et al.,* 2000). Vanitha *et al*. (2003) reported the antibacterial action of nine seaweeds collected from Kanyakumari coast against gram positive organisms. Vijayabhaskar and Shiyamala (2011) reported that the brown seaweeds, *Turbinaria ornata* and *Sargassum wightii* extracts were active against nine pathogens such as *Aeromonas hydrophila, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Shigella flexneri* and *Staphylococcus aureus* which may be due to masking of antibacterial activity by the presence of some inhibitory compounds in seaweed extracts (Sastry and Rao, 1994). Gram-positive bacteria were more effectively controlled by the *Turbinaria conoides* extracts than gram-negative bacteria. Similar observations indicating that the more susceptibility of gram-positive bacteria to the algal extracts is due to the differences in their cell wall structure and the composition of the cell wall1 (Rao and Parekh, 1981; Paz *et al.*, 1995).

Table 3. Growth inhibitory effect seaweed extracts against *S. aureus*

Seaweeds	Inhibition of bacterial colony growth
Padina tetrastromatica	±
Sargassum wightii	-
Sargassum ilicifolium	-
Turbinaria conoides	-
Kappaphygus alvarezii	±
Gelidiella acrosa	±
Ulva lactuca	-
Chaetomorpha litorea	±
Control	+

+ No inhibition ; ± Partial inhibition ; - Complete inhibition

The present study concluded that the seaweeds exhibited strong antifungal and antibacterial activities. The seaweeds extracts could be a good alternative in developing a potent plant based disinfectant which can be used in eco-friendly management of silkworm fungal and bacterial pathogens.

Authors' contributions: Dr. Ramamoorthy performed the research work and is the corresponding author of the manuscript. Dr.P.Priyadharshini (Asssitant Professor) assisted in writing and interpretation of data Dr. P. Mohanraj (Teaching Assistant) also helped in editing the article.

.

References

Alberto, M.R., Farýas, M.E., Manca de Nadra, M.C., 2001. Effect of gallic acid and catechin on *Lactobacillus hilgardii* growth and metabolism of organics compounds. *J. Agric. Food Chem*., 49, 4359-4363.

Ambika, S., Sujatha, K., Balakrishnan, K., 2014. Antifungal activity of seaweed extract against *Alternaria porri* in onion. In: National Seminar on Algae for sustainable agricultural production, Madurai, Tamil Nadu, India, Pp: 87.

Ameer Junaithal Begum M., Selvaraju, P., Vijayakumar, A., 2016. Evaluation of antifungal activity of seaweed extract (*Turbinaria conoides*) against *Fusarium oxysporum. Journal of Applied and Natural Science* 8 (1), 60 – 62.

Bhadury, P. , Wright, C.P., 2004. Exploitation of marine algae: biogenic compounds for potential antifouling application. *In Planta*, 219, 561–578.

Cox, S., Abu Ghannam, N., Gupta, S., 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int Food Res J, 2010; 17: 205-220.

Doreswamy, C., Govindan, R., Devaiah, M.C., Muniswamappa, M.V., 2004. Deterioration of cocoon traits of silkworm, *Bombyx mori* L. by the synergistic infection with late larval flacherie pathogens. *Karnataka J Agric. Sci*., 17, 345-348.

Grover, R.K., Moore, J.D., 1962. Toximetric studies of fungicides against brown spot organism *Sclerotia fructicola* and *S. laxa*. *Phytopathology,* 52, 876-880.

Jhansi Lakshmi, V. N. S., 2003. Ultrastructural studies on tissue of the silkworm, *Bombyx mori* L. infected with *Beauveria bassiana* (Balsmo) Vuillemin Ph.D. Thesis submitted to Sri Padmavathi Vishvavidyalayam, Tirupati.p 90

Manimegalai, S., Rajeswari, T., Shanmugam, R., Rajalakshmi.G., 2010. Botanicals against Nuclear Polyhedrosis Virus infecting three breeds of mulberry silkworm, *Bombyx mori* L. *Journal of Biopesticides*, 3, 242 – 245.

Paul, V.J., Puglisi, M.P., 2004. Chemical mediation of interactions among marine organisms. *Nat. Prod. Rep.***,** 21, 189-209.

Paz, E.A., Lacy, R.N., Bakhtian, M., 1995. The β-Lactum antibiotics penicillin and cephalosporin in perspective, Hodder strong, London. P.324.

Ramesha, C., Anuradha, M., Lakshmi, H., Kumari, S.S., Goel.,A.K., 2010. Nutrigenetic traits analysis for identification of nutritionally efficient silkworm germplasm breeds. *Biotechnology*, 9, 131-140.

Rao, P.S., Parekh, K.S., 1981. Antibacterial activity of Indian seaweed extracts, *Botanika Marina*, 24, 577-582.

Ravikumar, S., Anburajan, L., Ramanathan, G., Kaliaperumal, N., 2002. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens. *Seaweed. Res. Utln. Assoc*., 24(1), 95-99.

Ravikumar, S., Ramanathan, G., Subhakaran, M., Inbaneson, S.J., 2009. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *International Journal of Medicine and Medical Sciences*, 1(5), 184-191.

Reguant, C., Bordons, A., Arola, L., Roze, N., 2000. Influence of phenolic compounds on the physiology of *Oenococcus oeni*. *J. Appl. Microbiol*., 88, 1065-1071.

Sastry and Rao, 1994. Antibacterial substances from marine algae: Successive extraction using Benzene, Chloroform and Methanol. *Botanica Marina,* 37, 335-360.

Shibu, Dhanam. 2016. *In vitro* antifungal activity of *Turbinaria conoides* collected from Mandapam coast, Tamil Nadu, India. *Journal of Experimental Sciences*. 7, 27-30.

Sugnana Kumari, S., Rao, S.V.S., Misra, S., Murty, U.S., 2011. Antifungal activity of *Turbinaria conoides* and evaluation for the effective concentration against the infection of *Beauveria bassiana* in silkworm larvae. *J. Microbiology Res*. 6(2), 115-123.

Vanitha, J., Prakash, S., Bhimba, Lazarus, B.V., 2003. Antibacterial action of Seaweeds against human upper respiratory tract pathogens. *Seaweed Res.Utiln.,* 25(1&2), 181-187.

Vijayabaskar, P., Shiyamala, V., 2011. Antibacterial Activities of Brown Marine Algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. *Advances in Biological Research,* 5 (2), 99-102.