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EFFECT OF *SARGASSUM TENERRIMUM* ON CONTROLLING SHEATH BLIGHT OF RICE CAUSED BY *RHIZOCTONIA SOLANI* KUHN

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Abstract

Rice (*Oryza sativa* L.) is an important staple food crop for majority of the world. Many biotic stresses hamper rice production and specifically, fungal diseases cause huge economic losses. Among different fungal diseases of rice, sheath blight caused by *Rhizoctonia solani* Kuhn (*Thanetoporous cucumeris* (Frank) Donk) is emerging as a very destructive disease and it is an important one responsible for losses in grain yield. The present study focused on the effect of various seaweed extracts on controlling sheath blight in rice caused by *R. solani* under *in vitro* condition. Five seaweed extracts viz. *Sargassum tenerrimum, Dictyota dichotoma, Padina gymospora, Sargassum wightii* and *Sargassum muticum* were tested against *R. solani*. Extracts of *S. tenerrimum* at a high concentration (20%) was found to be the best in the reduction of spore germination (28.80 per cent). Various red seaweed extracts were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The leaf extracts of *S. tenerrimum* a highest concentration (20%) was found to be the best in both paper disc method and agar well methods (30.20 and 29.50 per cent inhibition zone respectively). The GC-MS analysis of seaweed extract was done to determine the chemical compound responsible for the antimicrobial activity. The results revealed that 12 compounds were present in *S. tenerrimum*. The molecular weights, name of the compound, chemical formula, retention time and peak area percentage were given in figure 1. Among these, n-Hexadecanoic acid may be responsible for the inhibition of the growth of *R. solani*. Various treatments were given to the plant for further study. In the present study, it was proved that the *S. tenerrimum* extracts may be useful for the control of sheath blight diseases in rice plant.

***Keywords*:** Seaweeds, *Rhizoctonia solani*, Antifungal Compounds, Rice.

Introduction

Rice (*Oryza sativa* L.) is an important staple food crop for majority of the world. Many biotic stresses hamper rice production and specifically, fungal diseases cause huge economic losses. Rice is cultivated in about 4.19 Million Hectares with the production of 89.09 Million tonnes with the productivity of 2125 kg/ha. Among the rice producing states of India, Tamil Nadu ranks sixth in production (5.67 Million tonnes) and second in productivity of 3070 kg/ha and area 44 Million hectares production is 106.19 million tonnes (Anonymous, 2010). Among different fungal diseases of rice, sheath blight caused by *Rhizoctonia solani* Kuhn (*Thanetoporous cucumeris* (Frank) Donk) is emerging as a very destructive disease and it is an important one responsible for losses in grain yield. Many methods of plant disease control are presently being used to control the rice sheath blight disease, such as physical, chemical and cultural methods. The organic control of soil borne plant pathogens is a potential alternative to the use of chemical pesticide (Rathore *et al*., 2009). Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites (Jeffrey Norrie *et al*., 2014; Suthin raj *et al.,* 2016). The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens (Ammirato, 1986; Suthin Raj *et al.,* 2018). Application of seaweed extracts is proved to be better to decrease the foliar fungal diseases which ultimately increase its fertility and help the growth of plants (Jayaraj *et al*., 2008; Stirk and Van Staden, 1997; Suthin Raj *et al.,* 2018b).

Materials and Methods

Preparation of crude seaweeds extracts (Vallianayagam *et al.,* 2009)

1 kg of live, healthy and matured samples of Brown seaweeds was collected from the coastal areas of Rameshwaram. The samples are thoroughly washed with seawater and then washed with tap water to remove all the extraneous sunder shade and chopped and pulverized after drying. Each 50 g powdered sample was separately extracted for 7 days for thrice in 500 ml of 1:1(v/v) chloroform: methanol using 1 litre Erlenmeyer conical flask under dark condition. The extractants were pooled and concentrated by using vaccum flask evaporator under reduced pressure at 45°C and weighed stored at 0°C.

Evaluation of seaweed extracts against *R. solani*

Spore germination assay (Macko *et al.,* 1977) : A drop of different concentration (5, 10, 15 and 20 per cent) of Seaweed extracts were individualy placed in a cavity slide and the drop of spore suspension of

R. solani (1 × 106 spore ml^{-1}) is also added to the marine products and mixed thoroughly. The prepared cavity slides were incubated in a moist chamber. The spore germination was observed and recorded after 48 h and the per cent germination was calculated.

Paper disc assay (Saha *et al.,* 1995) : Spore suspension of the fungi was prepared from a ten days old culture with sterile distilled water. Various concentrations like 5, 10, 15 and 20 per cent of Seaweed extracts were made and twenty ml of PDA medium was seeded with three ml of sclerotial suspension (1 × 106sclerotia/ml) of the fungus and solidified. Sterile filter paper discs (10 mm) were dipped separately in known concentration of seaweeds and placed equidistantly over the seeded medium. Three replications were maintained. The plates were incubated at 28±2ºC for 48 hr. The inhibition zone of the fungal growth around the treated paper discs was measured and recorded. The paper disc dipped in sterile distilled water served as control.

Agar well method (Thongson *et al.,* 2004) : Seaweed extracts like 5, 10, 15 and 20 per cent individually (10 ml) were added to the sterilized potato dextrose agar medium and thoroughly mixed just before plating. Twenty ml of these mixtures individually were immediately poured into sterilized Petri plates and were allowed to solidify. A 9 mm of PDA disc was removed by using cork borer to form wells; 1 ml of spore suspension was poured into the well. All these were carried out under aseptic conditions. The plates were incubated at 28±2ºC for 10 days. Potato dextrose agar medium without natural product served as the control. The radial growth of the colony was measured. The percent inhibition of the growth was calculated.

Analysis of antifungal compound through gas chromatography mass spectroscopy (GCeMS) (NIST Version. 2.0, 2005)

Based on the growth inhibition studies, *Sargassum tenerrimum* extract was selected and chemical constituents were determined with a GC Clarus 500 Perkin Elmer Gas chromatography equipped with a mass detector. Turbo mass gold containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID employed were the following: Carrier gas, helium (1 mL/min); oven temperature program 110°C (2 min) to 280°C (9 min); injector temperature (250°C); total GC time (36 min). The water extract was injected into the chromatograph in 2.0 Ml aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then by matching the mass spectrum of the analysis with that of a library (NIST Version. 2.0, year 2005). Software used for gas chromatography mass spectroscopy (GCeMS) was Turbo mass-5.1.

Statistical Analysis

Data were analyzed using ANOVA and significance at 5% level was tested with Duncans multiple range test (DMRT), using SAS/STAT software.

Results and Discussion

In vitro* evaluation of various red seaweed algae against *R. solani

Spore Germination : Among the five brown seaweed extracts tested against *R. solani,* extracts of *Sargassum tenerrimum* at a high concentration (20%) was found to be the best in the reduction of spore germination (28.80 per cent). It was followed by a high concentration (20%) of *Sargassum wightii* (26.61 per cent). The rate of reduction was corborated with its concentration in case of all the tested red seaweed extracts. *S. tenerrimum* and *S. wightii* significantly reduced spore germination than other red seaweed products in all the concentrations (Table 1).

Paper disc method and agar well method : Various brown seaweed extracts were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The leaf extracts of *Sargassum tenerrimum* at a highest concentration (20%) was found to be the maximally reduced in both paper disc method and agar well methods and recorded 30.20 and 29.50 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Sargassum wightii,* which recorded 32.72 and 33.33 per cent inhibition zone in paper disc method and agar well method respectively. The result of the experiments revealed the superiority of *S. tenerrimum*. Hence the same was used for further studies.

Gas Chromatography Mass Spectroscopy (GCeMS) Analysis

On the basis of performance of marine products in the preceding *in vitro* studies, *Sargassum tenerrimum* was tested to determine the nature of chemical compound (s) present in the seaweed extract. The results revealed that 12 compounds were present in *Sargassum tenerrimum*. The molecular weights, name of the compound, chemical formula, retention time and peak area percentage were given in figure 1. Among these, n-Hexadecanoic acid may be responsible for the inhibition of the growth of *R. solani*.

The seaweeds and the prepared marine products has significant role in the control of the *R. solani* in *in-vitro* condition. Generally all marine products inhibited the mycelial growth of pathogen in the present study of which, *Sargassum tenerrimum* @ 20% exhibited the highest level of inhibition of *R. solani*. This statement has been

confirmed by several workers. Sultana *et al.* (2005), reported that brown, green and red seaweeds were highly effective against *R. Solani in vitro* and *in vivo* conditions.There are several workers have been reported on the efficacy of seaweed extracts against fungal pathogens (Jayaraj *et al.,* 2008; Hane and Suthin Raj, 2018). This may be due to higher levels and early accumulation of phenolics and phytoalexins (Flora *et al.,* 2012; Suthin Raj *et al.,* 2018a). The above results lend supports to the present findings and helpful for the further study in the treatment of sheath blight caused by *R. solani* in rice plant.

Table 1 : Evaluation of various brown seaweed algae against *R. solani* under *in vitro* condition

S. No.	Seaweed	Spore Germination (%)					Inhibition zone (mm)									
							Paper disc method					Agar well method				
		5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
1	*Sargassum tenerrimum*	45.70	40.30	36.20	28.80	37.75[d]	18.15	23.55	28.20	30.20	25.03[d]	19.60	21.90	23.80	29.50	23.70[d]
2	*Dictyota dichotoma*	35.50	33.80	25.50	17.20	27.85[a]	35.80	37.30	40.10	42.12	38.84[b]	31.50	33.13	39.66	44.15	37.11[a]
3	*Padina gymospora*	37.80	34.23	32.11	20.10	31.06[c]	32.00	35.12	37.33	39.41	35.97[c]	27.50	30.30	37.16	41.90	34.22[b]
4	*Sargassum wightii*	42.50	39.41	28.77	26.61	34.32[e]	25.80	27.63	29.47	32.72	28.91[e]	21.90	24.15	28.32	33.33	26.93[e]
5	*Sargassum muticum*	39.80	36.71	30.86	24.30	32.92[c]	29.80	33.44	32.13	36.33	32.93[c]	25.00	27.16	33.15	39.20	31.13[c]
6	Control	96.00	96.00	96.00	96.00	96.00[f]	0.00	0.00	0.00	0.00	0.00[f]	0.00	0.00	0.00	0.00	0.00[f]

*Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

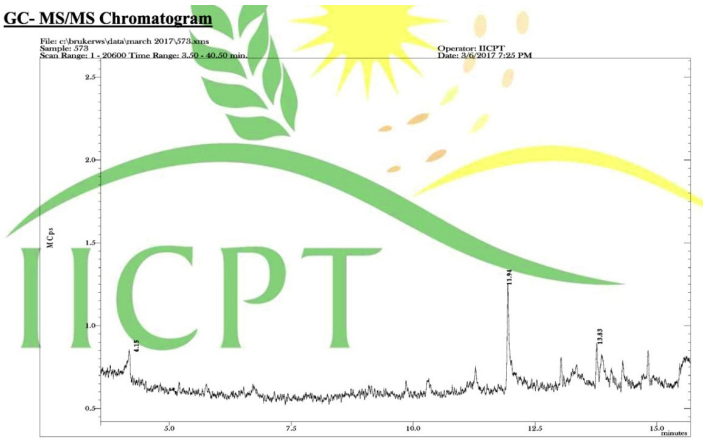

Fig. 1 : GC-MS analysis of *Sargassum tenerrimum* (brown seaweed) extract

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	12.16	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	0.34
2.	12.93	Oleic Acid	$C_{18}H_{34}O_2$	282	2.48
3.	13.83	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.25
4.	14.05	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	0.36
5.	15.40	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	89.22
6.	17.84	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	0.93
7.	18.18	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.79
8.	18.56	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	0.37
9.	18.77	Gibberellic acid	$C_{19}H_{22}O_6$	346	0.90
10.	19.42	Fenretinide	$C_{26}H_{33}NO_2$	391	0.95
11.	21.19	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	416	0.74
12.	20.38	Cholestan-3-ol, 2-methylene-, (3β,5α)-	$C_{28}H_{48}O$	400	1.67

*Parameters tested are not covered under the scope of NABL accreditation

Fig. 2 : Compound identified in the *Sargassum tenerrimum*

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