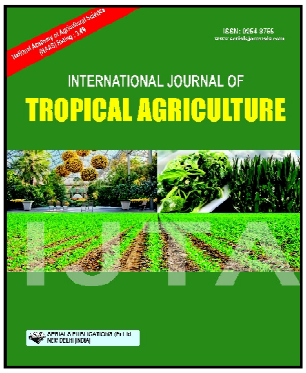

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Screening the efficacy of Seaweed extracts against sheath blight of rice caused by *Rhizoctonia solani* (Kuhn)

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Abstract: *Rhizoctonia solani* is the causative agent of rice sheath blight, which has become a major problem in rice production. Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolite and is proved to be better to decrease the foliar fungal diseases which ultimately increase its fertility and help the growth of plants. The use of natural products becomes the ultimate way of combating this disease. In this context, 15 different seaweeds were used against sheath blight disease. Evaluation of seaweed against

R. solani was carried out by spore germination assay, paper disc assay and agar well method. Among the sixteen seaweeds extracts tested, extracts of *Sargassum nightii* [brown seaweed algae] at a high concentration (20%) was found to be the best the in the reduction of spore germination (18.12 per cent). The leaf extracts of *Sargassum nightii* [brown seaweed algae] at highest concentration of (20%) showed a maximum reduction in both paper disc method and agar well method with 46.15 and 45.12 per cent zone of inhibition respectively.

Key words: Seaweeds, *Rhizoctonia solani,* Antifungal Compounds, Rice sheath blight

INTRODUCTION

Rhizoctonia solani Kuhn is the causal agent of rice sheath blight, which has become a major constraint to rice production during the last two decades (Kobayashi *et al.,* 1997). The intensification of rice cropping systems with the development of new short stature, high tillering, high yielding varieties, high plant density and an increase in nitrogen fertilization (Gangopadyay and Chakrabarthi, 1982; Ou, 1985) has seen the "emergence of *R. solani* as an economically important rice pathogen".

This pathogen can survive in soil for many years by producing small (1-3 mm diameter) irregular shaped, brown to black sclerotia in soil and on plant tissues. The ability of *R. solani* to produce sclerotia with a thick outer layer allows them to float and survive in water. *R. solani* also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Ghaffar, 1988). The sclerotia present in the soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fibre crops.

Presently, sheath blight disease management is mainly achieved through systemic fungicides (Pal *et al.,* 2005 and Suthin raj *et al.,* 2016a) and the bacterial bio-control agents like plant growth-promoting rhizobacteria (PGPR) offer a promising means of controlling plant diseases growth and yield in rice (Mew and Rosales, 1992). Brown seaweeds contain bio-control properties and contain many organic compounds and growth regulators such as auxins, gibberellins and precursor of ethylene and betaine which affect plant growth. Seaweed extracts have been reported to increase plant resistance to diseases, plant growth, yield and quality (Jolivet *et al.,* 1991). Thus seaweeds are bestowed with varied sources of bioactive natural products that exhibit biomedical and antimicrobial properties (Arunkumar *et al.,* 2005 and Suthin raj *et al.,* 2018). Peres (2012) were the first to observe antifungal substances in seaweeds. The seaweed is commercially available and some reports have indicated enhanced plant yield and health in different crops following application, although the mechanisms of action have not been determined (Norrie *et al.,* 2002, Colapietra and Alexander, 2006 and Suthin raj *et al.,* 2016b).

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). Arunkumar *et al.,* (2005) evaluated the bioactive potential of seaweeds against plant pathogenic bacterium *Xanthomonas oryzae* pv. oryzae. Kumar *et al.,* (2008) tested crude seaweeds extracts against the phytopathogenic bacterium *Pseudomonas syringae* causing leaf spot disease of the medicinal plant *Gymnemasyl vestris*. The use of anti-microbial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produce.

Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defence mechanism against herbivores, fouling organisms and pathogens for example; grazer- induced mechanical damage triggers the production of chemicals that act as feeding detergents or toxins in seaweeds (Ammirato, 1986). They contain all major and minor plant nutrients as well as biocontrol properties and many organic compounds such as auxins, gibberellins and precursors of ethylene and betaine which affect plant growth (Wu *et al.,* 1997).

Seaweeds are benthic marine macroalgae mainly used for the production of agar, alginate, liquid fertilizers and manures (Sivakumar, 2014). Most of the secondary metabolites are the bactericidal or the antimicrobial compounds derived from seaweeds which consist of diverse groups of bacteriostatic properties such as brominates phenols, oxygen heterocyclic; Terpenols, Sterols, Polysaccharides, dibutenolides peptides and proteins. Although most of the antibiotics found from terrestrial sources are used as therapeutic agents to treat various diseases, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value (Anderson *et al.,* 2006).In this context, the present study was carried out to evaluate the various marine products against *Rhizoctonia solani* under *in vitro* condition.

MATERIALS AND METHODS

2.1. Evaluation of marine products against *R.solani in vitro*

The efficacy of the marine products listed in table 1 was tested against *R. solani*

Table 1
List of seaweeds and its use of active compounds present

Sl. No.	*Scientific name*	*Anti-microbial property*	*Common name*	*Collected from*
1.	*Sargassum muticum*	Phenol	Brown seaweed	Kanyakumari
2.	*Dictyota bartyrensiana*	Ethyl acetate	Brown seaweed	Pamban
3.	*Padina gymospora*	Methanol	Brown seaweed	Pamban
4.	*Chnoospora implexa*	Chloroform	Brown seaweed	Kanyakumari
5.	*Sargasssum wightii*	Acetone	Brown seaweed	Pamban
6.	*Hydroclathrus hornemanii*	Phospholipase	Brown seaweed	Pondicherry
7.	*Gelidium pusillum*	Elatase	Red seaweed	Pondicherry
8.	*Liagora ceranoides*	Hexane	Red seaweed	Velankanni
9.	*Gracilaria corticata*	Protein	Red seaweed	Pamban
10.	*Hynea panosa*	Hexane	Red seaweed	Kanyakumari
11.	*Jania rubens*	Acetone	Red seaweed	Velankanni
12.	*Ulva reticulate*	Ethanolic	Green seaweed	Velankanni
13.	*Ulva rigida*	Methanolic	Green seaweed	Pondicherry
14.	*Ulva lactuca*	Acetone	Green seaweed	Kanyakumari

2.2. Preparation of crude seaweeds extracts (Suthin raj *et al.,* 2018)

Each 1 Kg of live, healthy and matured samples (Brown seaweeds, Red seaweeds and Green seaweeds) of each seaweed collected along the Coast of Pamban (Rameswaram (9°14'N; 79°14'E), Gulf of Mannar, Tamil Nadu, India) were washed thoroughly in seawater followed by tap water to remove extraneous particles and epiphytes. Then they were air dried under shade in laboratory for 3 days. The shade-dried samples were chopped and pulverized. 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 flask evaporator under reduced pressure at 45°C and weighed stored at 0°C.

2.3. Evaluation of seaweed extracts against *R. solani*

2.3.1. Spore germination assay (Macko et al., 1977)

One drop of 5, 10, 15 and 20 per cent Seaweed extracts was individually were placed in cavity slides and were allowed to air dry. A drop of the spore suspension (1×10^6spores ml^{-1}) of *R. solani* prepared in sterile distilled water was added to each of the dried marine products and was thoroughly mixed. The prepared cavity slides were incubated in a moist chamber. Three replications were maintained for each treatment. The spore germination was observed and recorded after 48 h and the per cent germination was calculated. The spore suspension prepared in sterile distilled water served as the control.

2.3.2. Paper disc assay (Saha et al., 1995)

Spore suspension of the fungi was prepared from a ten day old culture with sterile distilled water. Various concentrations like 5, 10, 15and 20 per cent of Seaweed extracts was were made.Twenty ml of PDA medium was seeded with three ml of sclerotial suspension ($1x10^6$sclerotia/ml) of the fungus and solidified. Sterile filter paper discs (10mm) were dipped separately in known concentration of treatments 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.

2.3.3. Agar well method (Thongson et al., 2004)

Seaweed extracts like 5, 10, 15 and 20 per cent individually (10ml) 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. Three replications were maintained. The radial growth of the colony was measured. The percent inhibition of the growth was calculated.

RESULTS

3.1 *In vitro* evaluation of marine products against *R.solani*

3.1.1. Spore germination

Among the fourteen seaweeds extracts tested against *R.solani,* extracts of *Sargassum wightii* [brown seaweed algae] at the high concentration (20%) was found to be the best in the reduction of spore germination (18.12 per cent). It was followed by the high concentration (20%) of *Hydroclathrus hornemanii* (brown seaweed) (17.13 per cent). The rate of reduction was corborated with its concentration in case of all the tested marine extracts. *Sargassum wightii* [brown seaweed algae] and *Hydroclathrus hornemanii* (brown seaweed) significantly reduced the spore germination than other marine products in all the concentrations. The *Hynea panosa* [Red seaweed algae] at different concentration (5, 10, 15 and 20%) were recorded as 55.60, 51.15, 47.12, and 44.40 per cent reduction in spore germination respectively (Table 2).

3.1.2. Paper disc method and well method

Various seaweeds extract were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The leaf extracts of *Sargassum wightii* [brown seaweed algae] at the highest concentration (20%) was found to be the maximum reduction in both paper disc method and agar well method recorded 46.15 and 45.12 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Hydroclathrus hornemanii* (brown seaweed) which recorded 44.41 and 43.77 per cent inhibition zone in paper disc method and agar well method respectively. All the concentrations of *Hynea panosa* [Red seaweed algae] recorded the minimum per cent inhibition zone than all other extracts (Table 2).

The result of the experiment revealed the superiority of *Sargassum wightii*. Hence the same was used for further studies.

DISCUSSION

The seaweeds and the prepared seaweed extracts has significant role in the control of the *R. solani* in *in-vitro* condition. Generally all seaweeds extract inhibited the mycelial growth of pathogen in the present study of which, *Sargassum wightii* [Brown

Table 2
Evaluation of various seaweed extracts against *R. Solani under in vitro* condition

S. No.	*Marine products*	*Inhibition zone (mm)*														
		Spore germination (%)					*Paper disc method*					*Agar well method*				
		5%	*10%*	*15%*	*20%*	*Mean*	*5%*	*10%*	*15%*	*20%*	*Mean*	*5%*	*10%*	*15%*	*20%*	*Mean*
1.	*Sargasssum wightii*	37.50	35.11	28.81	18.12	29.88[a]	39.50	14.15	43.31	46.15	42.50[a]	35.10	37.15	40.12	45.12	39.37[a]
2.	*Hydroclathrus hornemanii*	37.88	36.11	29.31	17.13	30.10[b]	36.61	40.11	41.31	44.41	40.61[b]	33.12	35.31	38.19	43.77	37.59[b]
3.	*Jania rubens*	38.50	37.15	31.30	26.19	33.28[c]	35.18	38.12	40.12	41.12	38.63[c]	31.10	33.31	35.13	39.11	34.66[c]
4.	*Gelidium pusillum*	39.63	39.01	32.31	29.13	35.02[d]	33.19	36.12	38.15	40.12	36.89[cd]	30.16	31.62	34.15	36.12	33.01[d]
5.	*Ulva reticulate*	40.15	40.90	33.11	29.96	36.03[de]	28.11	35.11	37.11	37.00	34.33[e]	28.17	30.19	32.11	34.12	31.12[e]
6.	*Caulerpa compressa*	41.93	41.98	34.61	30.11	37.15[f]	28.00	34.91	35.17	36.12	33.55[f]	27.11	29.11	31.11	33.21	30.13[f]
7.	*Sargassum muticam*	42.08	42.00	35.16	33.22	38.11[g]	27.93	32.17	34.13	35.11	32.33[g]	25.22	28.18	30.12	32.22	28.93[g]
8.	*Dictyota bartyrensiana*	44.15	43.11	36.19	35.15	39.65[h]	27.01	30.19	33.18	34.11	31.12[h]	24.31	26.17	28.17	30.15	27.20[h]
9.	*Liagora ceranoides*	45.62	44.12	37.71	36.12	40.89[i]	26.81	30.01	31.40	32.93	30.28[i]	22.18	25.19	27.12	29.60	26.02[i]
10.	*Gracilaria corticata*	47.63	46.11	38.18	37.95	42.46[j]	24.11	28.68	30.92	31.11	28.70[j]	21.71	23.33	25.90	27.41	24.58[j]
11.	*Ulva rigida*	48.13	47.15	40.12	39.12	43.63[jk]	23.80	26.15	30.17	30.93	27.76[k]	19.69	22.42	25.11	26.16	23.34[k]
12.	*Ulva lactuca*	49.15	48.21	42.15	40.18	44.92[l]	23.18	25.12	27.12	29.15	26.14[l]	17.61	21.17	23.23	25.18	21.79[l]
13.	*Padina gymospora*	51.31	49.13	44.11	41.12	46.41[m]	21.73	23.17	25.31	28.17	24.59[m]	15.16	20.30	21.16	24.01	20.15[m]
14.	*Chnoospora implexa*	53.44	52.33	45.16	43.19	48.53[n]	20.68	22.61	54.11	26.12	23.38[n]	14.21	17.37	20.15	22.17	18.47[n]
15.	Control	55.60	51.15	47.12	44.40	49.56[o]	20.01	22.05	23.11	25.35	22.63[o]	12.31	15.40	18.31	20.15	16.54[o]

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

Sargassum wightii

Dictyota bartyrensiana

Ulva reticulate

Ulva lactuca

Chnoospora implexa

Hydroclathrus hornemanii

Caulerpa compressa

Caulerpa racemosa

Gelidium pusillum

Liagora ceranoides

Gracilaria corticata

Jania rubens

List of seaweeds which were collected from different parts of Tamilnadu

seaweed algae] @ 20% exhibited the highest level of inhibition of *R. solani*. This statement has been confirmed by several workers. Sultana *et al.*, (2007), 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 (Norrie *et al.*, 2002, Jayaraj *et al.,* 2008 and Suthin raj *et al.,* 2016c). This may be due to higher levels and early accumulation of phenolics and phytoalexins (Garcia-Mina *et al.,* 2004). 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.

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