ANTIFUNGAL ACTIVITY OF MARINE PRODUCTS AGAINST *RHIZOCTONIA SOLANI* UNDER *IN VITRO* CONDITION

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ABSTRACT- *Rhizoctoniasolani*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, five different seaweeds such as *Sargassumwightii, Dictyotabartyrensiana, Ulva reticulate, Gelidiellaacerosa*and *Odonusniger*were used along with the fish powder extract in control of sheath blight disease in rice were studied. Evaluation of marine products against *R. Solani*was carried out by Spore germination assay, Paper disc assayand Agar well method. Among the five marine extracts tested, extracts of *Sargassumwightii*[brown seaweed algae] at a high concentration (20%) was found to be the best the in the reduction of spore germination (19.60 %). The leaf extracts of *Sargassumwightii*[brown seaweed algae] at highest concentration of (20%) showed a maximum reduction in both paper disc method and agar well method with 44.65 and 45.90 per cent zone of inhibition respectively. In the present study it revealed that the efficacy of seaweed extracts and against fungal pathogens is may be due to higher levels and early accumulation of phenolics and phytoalexins and the pot study proved that the *R. solani* can be controlled by the application of brown seaweed.

***Key words:*Seaweeds, *Rhizoctoniasolani,* Antifungal Compounds, Rice**

INTRODUCTION

*Rhizoctoniasolani*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 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). 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).

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

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 *Rhizoctoniasolani*under *in vitro* condition.

MATERIALS AND METHODS

2.1. Evaluation of marine products against *R.solaniin 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	Active ingredient	Common name	Collected from
1.	*Sargassumwightii*	Fucoidan	Brown seaweed	Gulf of Mannar Coast
2.	*Dictyotabartyrensiana*	----	Brown seaweed	Gulf of Mannar Coast
3.	*Ulva reticulate*	Caccamese and Azzolina	Green seaweed	Gulf of Mannar Coast
4.	*Gelidiellaacerosa*	Dimethicone	Red seaweed	Gulf of Mannar Coast
5.	*Odonusniger*	----	Trash fish	Paliyaru (Nagappattinam dist.)

2.2. Preparation of marine products

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

Each 1 Kg of live, healthy and matured samples (Brown seaweeds and Red 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.

2.2.2. Preparation of fish powder extracts (Ann Suji,2004)

Two marine fish species (Trash fish and Edible fish) were processed at a local processing plant, using 3.5% sucrose and 0.15% phosphate as cryoprotectants. The frozen blocks were transported to the laboratory and stored at 18 °C until drying. A 500 gm block of each fish was dried using a Labconco Freeze Dry System at a temperature of 40°C until the moisture content reached 5%. The samples were milled and sieved using a 40 mm screen mesh. The resulting powder was vacuum packed and stored at 4°C. Powdered samples were soaked in chloroform (1:4 w/v) and extracted for 2 days at room temperature and the extracts were collected and concentrated.

2.2.3. Preparation of mangrove leaves extracts

For preparation of aqueous extract, the mangrove leaves were shade-dried and powdered. Fifty grams of leaf powder of each species was soaked separately in a conical flask containing minimum quantity of double distilled water and frozen to –20°, thawed and frozen repeatedly for three times and blended and the extract was filtered through a fine meshed (100 µm) cloth, centrifuged at 10,000 g for 20 min and the supernatant was maintained at 4° till used. To prepare the ethanol extract, 50 g of leaf powder of each species was soaked in 200 ml of 80% ethanol for 72 h. The mixture was stirred at 24 h interval using a sterile glass rod. Then the extract was filtered throughWhatman No. 1 filter paper (Whatman, England) and the filtrate obtained was concentrated up to 20 ml in vacuum using rotary evaporator. The extract was stored at 4°C until used.

2.3. Evaluation of marine products 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 and Fish powder extracts 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 oldculture with sterile distilled water. Various concentrations like 5, 10, 15and 20 per cent of Seaweed extracts and Fish powder extracts 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 (Plate 9).

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

Seaweed extracts and Fish powder 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 five marine extracts tested against *R.solani*, extracts of *Sargassumwightii*[brown seaweed algae]@ 20% concentration was found to be the best in reduction of the spore germination (19.60 per cent) of *R. solani*. It was followed by the high concentration (20%) of *Odonusniger*[Trash fish] (22.1 per cent). The rate of reduction was corborated with its concentration in case of all the tested marine extracts. *Sargassumwightii*[brown seaweed algae] and *Odonusniger*[Trash fish] significantly reduced the spore germination than other marine products in all the concentrations. The *Gelidiellaacerosa*[Red seaweed algae] at different concentration (5, 10, 15 and 20%) were recorded as 81, 74.65, 62.10 and 54.30 per cent reduction in spore germination respectively (Table 2).

3.1.2. Paper disc method and Agar well method

Various marine products were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The leaf extracts of *Sargassumwightii*[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 44.65 and 45.90 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Odonusniger*[Trash fish] which recorded 39.00 and 34.33 per cent inhibition zone in paper disc method and agar well method respectively. All the concentrations of *Gelidiellaacerosa*[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 *Sargassumwightii*. Hence the same was used for further studies.

Table 1- Evaluation of various marine products against R. Solaniunder in vitro condition

S. No.	Marine products	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	*Sargassumwightii*[Brown seaweed algae]	49.10	41.60	30.00	19.60	35.07 [a]	36.10	40.28	46.10	44.65	41.78 [a]	30.71	34.65	39.16	45.90	37.60 [a]
2	*Dictyotabartyrensiana*[Brown seaweed algae]	65.80	60.50	51.66	35.00	53.24 [c]	20.00	21.50	26.70	28.30	24.12 [c]	18.20	20.50	23.10	25.95	21.93 [c]
3	*Ulva reticulate* [Green seaweed algae]	79.20	66.20	50.38	44.50	60.07 [d]	12.25	14.50	18.80	27.10	1816 [d]	10.00	11.50	15.20	18.00	13.67 [d]
4	*Gelidiellaacerosa*[Red seaweed algae]	81.00	74.65	62.10	54.30	68.01 [e]	12.00	14.21	16.28	19.72	15.55 [e]	10.66	11.92	14.00	17.00	13.39 [d]
5	*Odonusniger* [Trash fish]	54.80	49.10	35.08	22.1	40.27 [b]	24.00	25.60	30.00	39.00	29.65 [b]	20.00	22.67	28.67	34.33	26.41 [b]
6	Control	96.00	95.00	94.00	92.00	94.25 [f]	0.00	0.00	0.00	0.00	0.00 [f]	0.00	0.00	0.00	0.00	0.00 [e]

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

Table 2- Evaluation of various mangrove leaves extract against R. Solaniunder in vitro condition

S. No.	Marine products	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	*Sargassumwightii*[Brown seaweed algae]	49.10	41.60	30.00	19.60	35.07 [a]	36.10	40.28	46.10	44.65	41.78 [a]	30.71	34.65	39.16	45.90	37.60 [a]
2	*Dictyotabartyrensiana*[Brown seaweed algae]	65.80	60.50	51.66	35.00	53.24 [c]	20.00	21.50	26.70	28.30	24.12 [c]	18.20	20.50	23.10	25.95	21.93 [c]
3	*Ulva reticulate* [Green seaweed algae]	79.20	66.20	50.38	44.50	60.07 [d]	12.25	14.50	18.80	27.10	1816 [d]	10.00	11.50	15.20	18.00	13.67 [d]
4	*Gelidiellaacerosa*[Red seaweed algae]	81.00	74.65	62.10	54.30	68.01 [e]	12.00	14.21	16.28	19.72	15.55 [e]	10.66	11.92	14.00	17.00	13.39 [d]
5	*Odonusniger* [Trash fish]	54.80	49.10	35.08	22.1	40.27 [b]	24.00	25.60	30.00	39.00	29.65 [b]	20.00	22.67	28.67	34.33	26.41 [b]
6	Control	96.00	95.00	94.00	92.00	94.25 [f]	0.00	0.00	0.00	0.00	0.00 [f]	0.00	0.00	0.00	0.00	0.00 [e]

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

DISCUSSION

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, *Sargassumwightii*[Brown 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. Solaniin 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; Jayara*et al.*, 2008). 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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