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Effect of Various Green Seaweed Extracts on Controlling Sheath Blight in Rice Caused by *Rhizoctonia Solani* Under *Invitro* Condition

T. Suthin Raj[1], K. Hane Graff[1], A. Arumukapravin[1] and H. Ann Suji[2]*

Abstract: *Rhizoctoniasolaniis the causative agent of rice sheath blight, which has become a major problem in rice production. The use of natural products such as seaweeds provide a rich source of structurally diverse and biologically active secondary metabolite and is the ultimate way of combating this disease. In this context, five different seaweeds such as Ulva reticulate, Ulva rigida, Ulva lactuca, Caulerpa compressa and Caulerpa racemosawere used to control the sheath blight disease in rice. Evaluation of marine products against R. Solani was carried out by Spore germination assay, Paper disc assay; Agar well method and Mycelial dry weight analysis. Among the five marine extracts tested, extracts of Ulva reticulate [green seaweed algae] at a high concentration (20%) was found to be the best in reducing of spore germination. The leaf extracts of Ulva reticulate[green seaweed algae] at highest concentration (20%) showed a maximum reduction in both paper disc method and agar well method. The present study reveals that,the efficacy of seaweed extracts against fungal pathogens may be due to higher levels and early accumulation of phenolics and phytoalexins and the pot study proved that R. solani can be controlled by the application of green seaweed.*

Keywords: *Red Seaweeds, Rhizoctoniasolani, 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 (*Thanetoporouscucumeris* (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. Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens (Ammirato, 1986). 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).

1 Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram.
2 Centre for Advance Studies in Marine Biology, Annamalai University, Chidambaram. (T.N.) India.
* *E-mail: suthinagri@gmail.com*

MATERIALS AND METHODS

Symptom of the Disease

The symptom of the disease include greenish grey, elliptical or oval shaped spots with yellow margins mostly found on the leaf sheaths and primary leaf blades (Damicone *et al.*,1993).

Collection of Seed Materials

Fresh rice seed samples (var- ADT 36) were collected from seed farm, Department of Agronomy, Annamalai University, Chidambaram, Tamil Nadu.

Isolation of Pathogen

The diseased rice plants showing the typical symptoms of sheath blight disease were collected from 20 conventional ricegrowing areas of Nagappattinam districts. The pathogens were isolated on potato dextrose agar (Peeled potato–250g, dextrose–20g, agar–15 g, distilled water–1000 ml and pH–6.0/6.5) medium (Ainsworth, 1961) from the diseased specimen showing the typical symptoms. The infected portion of the sheath was cut into small bits, surface sterilized in 0.1 per cent mercuric chloride solution for 30 sec., washed in repeated changes of sterile distilled water and plated onto PDA medium in sterilized Petri dishes. The plates were incubated for room temperature (28 ± 2ºC) for five days and were observed the fungal growth. Totally 20 isolates were maintained and they were designated as Rs 1 to Rs 20.The per cent disease index (PDI) was calculated as given by McKinney (1923).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of tillers observed}} \times \frac{100}{\text{Maximum category value}}$$

Methods of Inoculation of Pathogen

Four methods were attempted on rice variety ADT-36 under pot culture. Each pot was filled with FYM and fertilizer. Thirty days old seedlings were transplanted in pots.Three replications were maintained for each treatment. The methods are as follows

1. Grain inoculation method

Here the infected seeds were kept in between the flag leaf sheath and in emerged sheath.

2. Sheath inoculum method

Rice sheath were collected, cut into small pieces (4 cm), transferred to open mouthed bottles and closed with a cotton wool plug. The desired quantity of water was added. The bottles were sterilized at 15 psi for 2 hr for three successive days. The medium was used to grow *Rhizoctonia solani* pathogen. From 20 days old culture of the pathogen grown in PDA, six discs of nine mm were taken and inoculated into each bottle. The bottles were then incubated at room temperature (28º±2°c) for 14 days and the inoculum thus prepared was used for subsequent studies.

In this method, infected sheath were cut into small pieces and then kept in between the flag leaf sheath and in emerged sheath.

3. Agar method

The grown up fungus on potato dextrose agar at room temperature were taken out in small bits with the help of a sterilized inoculum needle and inserted in a small hole in each tiller.

4. Spore suspension method

R. solani grown for ten days at room temperature on potato dextrose agar media was scraped off from the surface and mixed in sterilized distilled water to obtain spore suspension. One drop of spore suspension was placed by sterilized plastic dropping bottle inside the flag leaf sheath enclosing the unemerged panicle.

The inoculated plants were incubated in a humid chamber for 48 h and subsequently moved to a greenhouse maintained at 28°C, 70-90% relative humidity under a light intensity of 85 µmol m^{-1} S^{-1} and 12 h photoperiod. The incidence was recorded after 30, 50 and 70 days after transplanting.

Preparation of Crude Seaweeds Extracts (Vallianayagam *et al.*, 2009)

I kg of live, healthy and matured samples of green seaweeds was collected from the coastal areas of

Isolation of the pathogen from different locality of Nagapattinam district and the per cent disease incidence

S. No.	*Locality*	*Crop stage*	*Variety*	*Disease incidence (%)*
1.	Rs 1 – Papakovil	Panicle initiation	ADT-36	14[c]
2.	Rs 2 – Orathur	Panicle initiation	ADT-36	14 [c]
3.	Rs 3 – Kudineyveli	Panicle initiation	ADT-43	11[e]
4.	Rs 4 – Nariyankudi	Panicle initiation	ADT-36	18[a]
5.	Rs 5 – Sikalpattu	Panicle initiation	ADT-36	15[b]
6.	Rs 6 – Agalamkannu	Panicle initiation	ADT-36	13[d]
7.	Rs 7 – Aalankudi	Panicle initiation	ADT-36	10[e]
8.	Rs 8 – Pirinchumulai	Grain filling	ADT-43	8[g]
9.	Rs 9 – Karuveli	Grain filling	ADT-36	6[h]
10.	Rs 10 – Sikkal	Panicle initiation	ADT-43	8[g]
11.	Rs 11 – Poravacharry	Panicle initiation	ADT-43	8[g]
12.	Rs 12 – Thanilapaddi	Panicle initiation	ADT-43	4[i]
13.	Rs 13 – Aaimalai	Grain filling	ADT-43	6[h]
14.	Rs 14 – Valivalam	Panicle initiation	ADT-36	12[d]
15.	Rs 15 – Thrukkuvali	Panicle initiation	ADT-36	10[e]
16.	Rs 16 – Thavur	Grain filling	ADT-43	4[i]
17.	Rs 17 – Thirukadaiyur	Grain filling	ADT-43	4[i]
18.	Rs 18 – Paalayur	Grain filling	ADT-43	7[g]
19.	Rs 19 – Kizhavenmani	Panicle initiation	ADT-36	9[f]
20.	Rs 20 – Keelaiyur	Grain filling	ADT-43	4[i]

Evaluation of greenseaweeds against *R. solaniin vitro*

The efficacy of the various greenseaweeds listed in tablewas tested against *R. solani*

Sl. No.	*Scientific name*	*Anti-microbial property*	*Common name*	*Collected from*
1.	*Ulva reticulate*	Ethanolic	Green seaweed	Kanyakumari
2.	*Ulva rigida*	Methanolic	Green seaweed	Pamban
3.	*Ulva lactuca*	Acetone	Green seaweed	Pamban
4.	*Caulerpa compressa*	Benzene	Green seaweed	Kanyakumari
5.	*Caulerpa racemosa*	Benzene	Green seaweed	Pamban

Pamban and Kanyakumari. The samples are thoroughly washed with seawater and then washed with tap water to remove allt the extraneous particles and epiphytes. The samples are air dried under 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 (1×10^6 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 oldculture with sterile distilled water. Various concentrations like 5, 10, 15and 20 per cent of Seaweed extracts were made and twenty ml of PDA medium was seeded with three ml of sclerotial suspension (1×10^6sclerotia/ml) of the fungus and solidified. Sterile filter paper discs (10mm) were dipped separately in known concentration of sea weeds and placed equidistantly over the seeded medium. Three replications were maintained. The plates were incubated at 28±2°C 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±2°C 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.

Efficacy of Green Seaweed Algae Against Sheath Blight Disease of Rice in Pot Culture

The pot culture study was conducted with 8 treatment s and three replications at the Department of Plant Pathology, Annamalai University, Annamalainagar at kuruvai (June to September) (Trial-I) and late Samba (November to April) (Trial-II) seasons of the year 2014. The green sea weeds (5 per cent concentration) and chemical fungicide hexaconozole 5 SC (0.2 percent) were tested against sheath blight disease. *R. solani*was inoculated thoroughly over the plant canopy by one gram rice hull/rice grain, placed on basal leaves and covered with polythene bags on the 20th day after transplanting. The inoculated plants were incubated in a humid chamber for 48h and subsequently moved to a green house maintained at 22-28°C, 70-90% relative humidity,under a light intensity of 85 μmol $m^{-1}S^{-1}$,12 h photoperiod and subsequently transferred to pot culture yard. The cultivar ADT 36 was raised and the packages of practices were followed as per the Crop Production Guide (2014).

Treatment Details

T1 - *Ulva reticulate*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT)

T2 –*Ulva rigida*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT)

T3-*Ulva lactuca*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT)

T4 – *Caulerpa compressa*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT)

T5 –*Caulerpa racemosa* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT)

T6 – Healthy control

T7 – Inoculated control.

Induced Systemic Resistance

Twenty days after transplanting, the plants were challenge inoculated with a conidial suspension of *R. solani* with a spore load of 1×10^6 ml^{-1}. The samples of the above treated plants were collected at different time intervals (1, 3, 5, 7 and 9 days) after pathogen inoculation. Three replications were maintained in each treatment. Fresh plant samples were used for analysis.

The plant tissues collected from plants were immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 4°C. The homogenate was centrifuged for 20 min at 10,000 rpm. Plighting extract prepared from leaves was used for the estimation ofperoxidase (PO), polyphenol oxidase (PPO) and L-phenylalanine ammonia-lyase (PAL).

Peroxidase (PO) (Hammerschmidt et al., 1982)

Peroxidase activity was assayed spectrophoto-metrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1 per cent H_2O_2 which was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 hm was recorded at 30 sec. interval for 3 min and the boiled enzyme preparation served as blank. The enzyme activity wasexpressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} on fresh weight basis.

Polyphenol oxidase (PPO) (Mayer et al., 1965)

The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 200 ml of 0.01 M catechol was added and the activity was expressed as changes in absorbance at 495 μm min^{-1} g^{-1} fresh weight of tissue.

Phenylalanine ammonia-lyase (PAL) (Ross and Sederoff, 1992)

The assay mixture containing 100 μl of enzyme, 500 μl of 50 mMTrisHCl (pH 8.8) and 600 μl of 1mML-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later, 1.5 ml of toluene was added and vortexed for 30 sec. The centrifuged (1000 rpm, 5 min) toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured at 290 hm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as hmoles of cinnamic acidmin^{-1} g fresh $tissue^{-1}$.

β-1, 3-glucanase (Pan et al., 1991)

Crude enzyme extract of 62.5 μl was added to 62.5 μl of 4 per cent laminar in and incubated at 40°C for 10 min. The reaction was stopped by adding 375 μl of dinitrosalicylic acid (DNS) and heated for 5 min on boiling water bath (DNS prepared by adding 300 ml of 4.5 per cent NaOH to 880 ml containing 8.8 g of DNS and 22.5 g potassium sodium tartarate).

The resulting coloured solutions were diluted with distilled water, vortexed and the absorbance was read at 500 mm. The crude extract preparation mixed with laminar in at zero time incubation served as blank. The enzyme activity was expressed as mg equivalents of glucose min^{-1} g fresh $weight^{-1}$.

RESULT AND DISCUSSION

Methods of Inoculation

Among the five methods of artificial inoculation, grain inoculation and covered with polythene bags was found to be the best in plant infection. Its recorded mean per cent infection was 61.4 per cent and followed by the grain inoculation method which recorded 42.4 per cent. Grain inoculation and covered with polythene bags method of inoculation was applied for artificial inoculation in the present study (Table 1).

In Vitro Evaluation of Various Green Seaweed Algae Against *R.solani*

Spore germination

Among the five green seaweed extracts tested against *R.solani*, extracts of*Ulva reticulate*at a high concentration (20%) was found to be the best in the reduction of spore germination (16.80 per cent). It was followed by a high concentration (20%) of*Ulva*

Table 1
Infection of rice plants by different methods of inoculation with *R. solani*

		Days after inoculation (per cent infected leaf sheaths)					
S. No.	*Methods*	*5*	*7*	*9*	*11*	*13*	*Mean*
1.	Grain inoculation method	0.00	15.00	45.00	70.0	82.0	42.4[b]
2.	Sheath inoculum method	0.00	0.00	8.00	32.00	62.00	20.4[c]
3.	Agar method	0.00	5.00	10.00	19.0	25.0	11.8[d]
4.	Spore suspension method	0.00	5.00	18.00	30.0	42.0	19.0[c]
5.	Grain inoculation + covered with polythene bags	0.00	32.00	75.00	100.0	100.0	61.4[a]

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

rigida (19.20 per cent). The rate of reduction was corborated with its concentration in case of all the tested green seaweed extracts. *Ulva reticulateandUlva rigida*significantly reduced spore germination than other green seaweed products in all the concentrations (Table 2).

Paper Disc Method and Agar Well Method

Various green 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 at a highest concentration *Ulva reticulate* (20%) was found to be the maximally reduced in both paper disc method and agar well methods and recorded 44.20 and 45.50 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Ulva rigida*which recorded 40.12 and 41.15 per cent inhibition zone in paper disc method and agar well method respectively (Table 2).

The result of the experiments revealed the superiority of*Ulva reticulate*. Hence the same was used for further studies.

Effect of Green Seaweed Algae on Sheath Blight Incidence Under Greenhouse Condition

Among the treatments, application of *Ulva reticulate*, (seed treatment, prophylactic spraying at 30, 50 and 70 DAT) (T_1) recorded significantly less sheath blight incidence of 82, 80 and 86 per cent increase over control at 30, 50 and 70 days after transplanting than other treatments. It was followed by T_6 which recorded 79, 76 and 83 per cent increase over control at 30, 50 and 70 days respectively (Table 3).

Effect of Green Seaweed Algae on Growth and Yield Attributes Under Greenhouse Condition

The rice plants were treated with different green seaweed algae and the biometric observations and yield parameters were also recorded on 70 DAT. All the treatments were effective to promote the growth of the plant. Especially, among them the application of *Ulva reticulate* (seed treatment, prophylactic spraying at 30, 50 and 70 DAT) (T_1) was found to significantly increase the mean plant height (110 cm) mean number of productive tillers (10 nos.), mean 1000g weight (23g), straw yield (7.26 ton/ha) and grain yield (30 g/plant) as compared to all the other treatments.

This was followed by treatment T_6 which gave good biometric observations and yield parameters recording mean plant height (109 cm) mean number of productive tillers (9 nos.), mean 1000 g weight (22 g), straw yield (6.91 ton/ha) and grain yield (29 g/plant) respectively, which were statically on par with each other (Table 4).

Induction of Defense Enzymes

Green house study was conducted to test the induction of defense enzyme on rice plants with different application of IDM formulation.

Table 2
Evaluation of various green seaweed algae against *R. solani* under *in vitro* condition

							Inhibition zone (mm)									
		Spore germination (%)					*Paper disc method*					*Agar well method*				
S. No.	*Seaweed*	*5%*	*10%*	*15%*	*20%*	*Mean*	*5%*	*10%*	*15%*	*20%*	*Mean*	*5%*	*10%*	*15%*	*20%*	*Mean*
1.	*Ulva reticulate*	36.20	31.80	25.20	16.80	27.50^a	36.20	38.50	41.20	44.20	40.02^a	30.60	33.90	39.80	45.50	37.45^a
2.	*Ulvarigida*	37.90	33.80	28.50	19.20	29.85^b	34.80	36.33	39.11	40.12	37.59^b	28.50	30.13	36.66	41.15	34.11^b
3.	*Ulvalactuca*	39.80	35.23	30.11	22.10	31.81^c	30.00	33.12	36.33	37.41	34.21^c	24.50	27.30	32.16	39.90	30.96^c
4.	*Caulerpacompressa*	42.50	38.41	33.77	25.61	35.07^e	24.80	28.63	31.47	32.72	29.40^e	20.90	23.15	26.32	35.33	26.42^e
5.	*Caulerparacemosa*	46.80	41.71	36.86	28.30	38.41^d	19.80	24.44	27.13	28.33	24.92^d	18.00	20.16	23.15	30.20	22.87^d
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).

Table 3
Effect of green seaweed algae on Sheath blight incidence under green housecondition

Treatments	*Sheath blight incidence on 30th DAT*	*% Increase over control*	*Sheath blight incidence on 50th DAT*	*% Increase over control*	*Sheath blight incidence on 70th DAT*	*% Increase over control*
T_1– Application of *Ulva reticulate* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	3.5[a]	82	7.8[a]	80	9.2[a]	86
T_2– Application of *Ulvarigida* (ST @ 10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	4.2[b]	78	10.5[b]	73	13.8[b]	80
T_3– Application of *Ulvalactuca* (ST @ 10g/kg + prophylactic spray @ 10% at 20, 35 and 50 DAT)	4.8[c]	75	10.8[d]	73	14.5[c]	79
T_4 – Application of *Caulerpacompressa* (ST @ 10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	4.9[d]	74	11.5[d]	71	16.8[d]	75
T_5– Application of *Caulerparacemosa* (ST @ 10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)compost	5.2[e]	73	12.4[e]	69	18.0[e]	74
T_6– ST withHexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	3.6 [a]	79	9.6[b]	76	11.5[a]	83
T_7– Inoculated control	19.5[g]		40.20[g]		69.5[g]	
T_8–Healthy control	7.50[j]		8.70[j]		9.20[j]	

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

Table 4
Effect of green seaweed algae on growth and yield attributes under greenhouse condition

Treatments	*Mean plant height (cm)*	*Mean no. of productive tillers*	*Mean 1000 g weight*	*Straw yield (ton/ha.)*	*Grain yield (g/plant)*
T_1– Application of *Ulva reticulate* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	110[b]	10[a]	23[c]	7.26[b]	30[b]
T_2– Application of*Ulvarigida* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	107[c]	7[c]	21[d]	6.07[c]	28[d]
T_3– Application of*Ulvalactuca* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	106[c]	6[d]	18[d]	5.82[c]	26[d]
T_4 – Application of*Caulerpacompressa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	105[d]	6[d]	17[e]	4.58[c]	24[d]
T_5– Application of*Caulerparacemosa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT) compost	103[c]	5[e]	15[e]	4.21[c]	22[e]
T_6– ST withHexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	109[c]	9[b]	22[d]	6.91[c]	29[c]
T_7– Inoculated control	79[e]	4[g]	10[f]	2.03[g]	16[f]
T_8–Healthy control	94[d]	5[f]	14[e]	4.55[f]	18[e]

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

β-1, 3-glucanase

β-1,3-glucanase activity was observed in the leaf samples of rice at different day intervals. Among the various treatment, the plants treated with *Ulva reticulate*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_1) recorded a maximum induction of β-1,3-glucanase activity 220.7 μg of Glucose released/min/g of fresh tissue on 5th day after pathogen inoculation. It was followed by the plants treated with application of Seed treatment with Hexaconazole (2g/kg) + spraying (0.2percent) 20,35 and 50 DAT (T_6) recorded 218.3μg of Glucose released/min/g of fresh tissue on 5th day after pathogen inoculation. The enzyme activity was significantly increased up to 5th day from the pathogen inoculation and then declined slowly in all the treatments (Table 5).

Peroxidase (PO)

The activity of PO was observed in leaf sample of rice at different days interval. Among the various treatment, the plants treated with *Ulva reticulate* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_1) recorded maximum induction of Peroxidase activity (58.89 changes in absorbance/ min/g of fresh tissue) at 7th day after pathogen inoculation. It was followed by the plants treated withSeed treatment with Hexaconazole(2g/kg) + spraying (0.2 percent) 20, 35 and 50 DAT (T_6) 57.49 changes in absorbance/min/g of fresh tissue respectively at the 7th day after pathogen inoculation. The enzyme activity was significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 6).

Polyphenol oxidase (PPO)

Application of *Ulva reticulate*(seed treatment, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_1) recorded maximum induction of PPO activity (3.10 changes in absorbance/min/g of fresh tissue) at 7th day, which decreased further. Without inoculation of pathogen and green seaweed algae combination, a minimum poly phenol activity was recorded when compared to all other treatments. In all the treatments, enzyme activity increased up to 7th day and there after declined (Table 7).

Table 5
β-1,3 glucanase activity* in rice plants treated with different green seaweed algae under greenhouse condition

	*β-1,3 glucanase activity * in plants Time interval (days)*				
Treatments	*0*	*1*	*3*	*5*	*7*
T_1– Application of*Ulva reticulate*(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	43.9	86.2	153.2	220.7	160.9
T_2– Application of*Ulvarigida* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	40.9	84.3	151.3	216.4	158.1
T_3– Application of*Ulvalactuca*((ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	40.7	82.9	149.2	212.3	156.3
T_4 – Application of *Caulerpacompressa*(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	40.1	80.3	147.3	210.5	155.4
T_5– Application of *Caulerparacemosa*(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT) compost	39.9	79.7	145.6	209.4	153.6
T_6– ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	41.2	85.8	152.7	218.3	158.7
T_7– Inoculated control	17.7	17.5	17.3	18.1	17.4
T_8–Healthy control	17.6	21.3	25.6	29.6	18.6

CD for Treatment: 0.06. CD for time interval (Day's): 0.08.

CD for interaction between Treatment × Time interval (Days): 0.17.

*mg of glucose released/min/g of fresh tissue

**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P = 0.05).

Table 6
Peroxidase activity* in rice plants treated with different green seaweed algae under greenhouse condition

	*PO activity * in plants Time interval (days)*				
Treatments	*1*	*3*	*5*	*7*	*9*
T_1– Application of *Ulva reticulate*(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	7.96	16.91	35.94	58.89	43.61
T_2– Application of*Ulvarigida*(ST @ 10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	6.88	15.37	33.80	56.61	41.73
T_3– Application of*Ulvalactuca*((ST @ 10g/kg + prophylactic spray @ 10% at 20, 35 and 50 DAT)	6.72	14.73	31.82	55.89	40.81
T_4 – Application of *Caulerpacompressa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	6.48	14.53	30.87	55.22	40.28
T_5– Application of *Caulerparacemosa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT) compost	6.12	14.18	30.18	54.27	40.02
T_6–ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	7.08	16.58	35.63	57.49	43.12
T_7– Inoculated control	3.21	7.34	9.31	12.19	9.86
T_8–Healthy control	4.32	9.62	28.31	39.26	30.81

CD for Treatment: 0.06. CD for time interval (Day's): 0.08.

CD for interaction between Treatment × Time interval (Days): 0.17.

*Changes in absorbance/min/g of fresh tissue

**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P = 0.05).

Table 7
Polyphenoloxidase activity*in rice plants treated with different green seaweed algae under greenhouse condition

	*PPO activity * in plants Time interval (days)*				
Treatments	*1*	*3*	*5*	*7*	*9*
T_1– Application of *Ulva reticulate*(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	0.27	0.91	2.71	3.10	2.32
T_2– Application of*Ulvarigida*(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	0.25	0.87	2.67	2.97	2.23
T_3– Application of*Ulvalactuca* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	0.24	0.85	2.63	2.92	2.20
T_4 – Application of *Caulerpacompressa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	0.23	0.83	2.40	2.87	2.17
T_5– Application of*Caulerparacemosa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT) compost	0.22	0.81	2.31	2.81	2.11
T_6– ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	0.26	0.88	2.69	3.03	2.27
T_7– Inoculated control	0.10	0.17	0.55	0.80	0.66
T_8–Healthy control	0.15	0.21	0.83	1.12	0.91

CD for Treatment: 0.05. CD for time interval (Day's): 0.07.

CD for interaction between Treatment × Time interval (Days): 0.16.

*Changes in absorbance/min/g of fresh tissue

**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT (P = 0.05).

Phenylalanine ammonialyase (PAL)

PAL activity was found to increase significantly in plants treated with *Ulva reticulate* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) followed by challenge inoculated with *R. solani* (T_1). Maximum induction of PAL activity (5.03 changes in absorbance/min/g of fresh tissue) at 7th day there after it decreased. It was followed by the application of Seed treatment with Hexaconazole (2g/kg) + spraying (0.2 percent) 20,35 and 50 DAT (T_6) recorded a maximum induction at the 7th day of 4.98 changes in absorbance/min/g of fresh tissue respectively. The enzyme activity was significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Table 7).

Table 8
Phenylalanine ammonia-lyase activity*in rice plants treated with different green seaweed algae under greenhouse condition

	*PAL activity * in plants Time interval (days)*				
Treatments	*1*	*3*	*5*	*7*	*9*
T_1– Application of *Ulva reticulate* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	0.47	1.01	2.43	5.03	4.44
T_2– Application of*Ulvarigida* (ST @ 10g/kg + prophylactic spray @ 10% at 20, 35 and 50 DAT)	0.45	0.95	2.35	4.92	4.32
T_3– Application of*Ulvalactuca* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	0.43	0.83	2.31	4.81	4.29
T_4 – Application of *Caulerpacompressa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	0.41	0.72	2.25	4.62	4.23
T_5– Application of *Caulerparacemosa* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT) compost	0.40	0.39	2.20	4.40	4.19
T_6– ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	0.46	0.98	2.38	4.98	4.37
T_7– Inoculated control	0.23	0.35	0.80	1.19	0.88
T_8–Healthy control	0.25	0.38	1.03	1.80	1.28

CD for Treatment: 0.05. CD for time interval (Day's): 0.06.

CD for interaction between Treatment × Time interval (Days): 0.15.

*Changes in absorbance/min/g of fresh tissue

**In a row under each treatment, value in the column followed by common letters do not differ significantly by DMRT ($P = 0.05$).

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