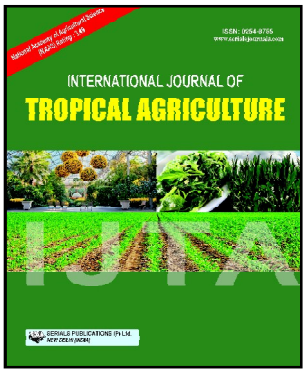

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Morphogenetic variability in *Rhizoctonia solani* and biochemical characterization of brown seaweed algae and its efficacy on management of rice sheath blight

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Abstract: Sheath blight disease of rice is one of the vital issues which exist in rice production which is caused by *Rhizoctonia solani*. The ultimate way of controlling this disease is done using natural means and products. There are twenty isolates of *Rhizoctonia solani* found and collected from various rice growing regions in Tamilnadu, which indicates the existence of various virulences. The morphogenetic relationship between twenty isolates is seen as *R.solani* which was tested using random amplified polymorphic DNA (RAPD) analysis. Molecular polymorphism generated by RAPD which established the variation in virulences of *R.solani* and different isolates, is grouped into two large clusters. While doing the pathological study of isolates and RAPD grouping, these don't have any correlation among the test isolates. In this situation five different seaweeds such as *Sargassum wightii, Sargassum muticum, Dictyota bartyrensiana, Padina gymospora,* and *Chnoospora implexa* were analysed with the aim of controlling sheath blight disease in rice. Spore germination assay, Paper disc assay and Agar well method were carried out for the evaluation of seaweed extracts against *R.solani*. Among the five seaweed extracts tested, extracts of *Sargassum wightii* [brown seaweed algae] was found with a higher concentration and (20%) was found to be the most excellent in the reduction of spore germination (19.60%). The extract of *Sargassum wightii* [brown seaweed] showed an utmost reduction in both paper disc method and agar well method with 44.65 and 45.90 per cent zone of inhibition respectively. In this present study, it is portrayed that the efficacy of seaweed extracts against sheath blight of rice may be due to early gathering of phenolics and phytoalexins and the field studies proved that the sheath blight disease can be managed by the application of brown seaweed.

Key words: Antifungal Compounds, Seaweeds, *Rhizoctonia solani,* RAPD, GCeMS

1. INTRODUCTION

A paramount issue in rice cultivation is the existence of sheath blight disease in rice caused by *Rhizoctonia solani* Kuhn now a day (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 (Gangopadhaya and Chakrabarti, 1982). Ou, 1985 has seen the "emergence of *R. solani* as an important rice pathogen".Sclerotia may be irregular to spherical shape and measure 4-5 mm in diameter, basidia and basidiospores are formed under natural conditions and measure 10-15 x 7-9 nm and 8-11 x 6.5 nm respectively. According to Wang *et al* (2009), the sclerotia which is present in the soil and/or on plant tissue, germinate to produce vegetative threads (hyphae) of the fungus that can get affected by a wide range of food and fibre crops.

Sheath blight disease have been efficiently controlled by the systemic fungicides as found in various studies (Pal *et al*., 2005, Mercier *et al.,* 2013). The bacterial bio-control agents like plant growth-enhancing rhizobacteria (PGPR) reduces the plant diseases and improves the growth and yield in rice (Mew *et al.,* 1986). The organic compounds and growth regulators such as auxins, gibberellins and precursor of ethylene and betaine and bio-control properties of brown seaweed enhances the growth of the plant. Seaweed extracts have been introduced to promote plant resistance, plant growth, yield and quality (Jolivet *et al.,* 1991 and Hane graff and Suthin Raj, 2018a). It is also bestowed with different sources of bioactive natural products that show biomedical and antimicrobial qualities (Arunkumar *et al*., 2005 and Suthin raj *et al.,* 2018c). Peers *et al.,* during 2012 experienced some antifungal substances in seaweeds. The seaweed are commercially obtainable and some reports have mentioned, enhanced plant yield and health in various 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).

Extracts of seaweeds application is proved to be improved to decrease the foliar fungal diseases which ultimately augment to its fertility and help the growth of plants (Jayaraj *et* al., 2008). The use of anti-microbial drugs has certain limits due to changing patterns of resistance in pathogens and side effects they create. Seaweeds which are benthic marine macroalgae and are mainly used for the manufacturing of agar, alginate, liquid fertilizers and manures (Sivakumar, 2014). Most of the secondary metabolites are bactericidal or 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 (Anderson *et al*., 2006). Eventhough most of the antibiotics found from earthly sources are used as healing agents to treat various diseases, the oceans have huge biodiversity and potential to provide new compounds with business value (Balboa *et al.,* 2013 and Hane graff and Suthin Raj, 2018b).In this context, the present study was carried out to assess the various marine products against *Rhizoctonia solani* under *in vitro* condition.

2. MATERIALS AND METHODS

2.1. Isolation, Maintenance and Identification of Pathogen

In this paper, the rice plants affected by the sheath blight disease are gathered from twenty conventional rice growing areas of Nagappattinam district. The pathogens are secluded from the diseased specimen and placed on potato dextrose agar (PDA) medium (Ainsworth, 1961). The infected portion was chopped into tiny bits, surface cleaned in 0.1 per cent mercuric chloride solution for 30 sec. The pieces were washed continually by germ-free distilled water and

plated onto PDA medium in sterilized Petri dishes. The plates were incubated for room temperature (28 ± 2°C) for five consecutive days and there by observing the fungal development. The fungus was purified by single spore isolation technique (Rangaswamy, 1972). Identification of the isolate was established (Reddy *et al.*, 2008) by comparing with the culture obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants for further studies. Totally 20 isolates were maintained and they were selected as Rs 1 to Rs 20.

2.2. RAPD analysis of *R.solani*

2.2.1. Genomic DNA isolation and PCR amplification of DNA

The whole genomic DNA of *R.solani* was isolated from mycelia. Isolates were incubated at 28°C for 4 consecutive days in tubes with 20 ml of potato dextrose broth, agitated at 180 rpm. Mycelia were harvested by filtration through filter paper, dried between two layers of filter paper and stored at 80°C for further use. Using liquid nitrogen the dried mycelium was ground to fine powder with pestle and mortar and send to 1.5 ml Eppendorf tube and 600 ml Cetyltrimetylammonium bromide (CTAB) was supplemented and incubated at 65°C for 30 min, tubes were vortexed every 10 min. After cooling at room temperature equal volume (600 ml) of chloroform: isoamyl alcohol (24:1, v/v) was supplemented in fume hood cabinet, gently mixed for 20-30 min and centrifuged at 7000 rpm for 5 min at 4°C. This CIA removal is repeated after transferring the aqueous stage to new tubes. After the second CIA wash, the DNA was precipitated by adding up 300 ml isopropanol and then the tubes were gently mixed and incubated at room temperature for 30 min. Tubes were centrifuged at 12, 000 rpm for 10 min and supernatant was decanted. The DNA pellet was dissolved in 50 ml of ddH_2O.

At first, 20 random 10-mer primers (Genei, Bangalore) were screened to select primer exhibiting highest polymorphism, of these 5 primers (Table A) which formed easily scorable and reliable banding patterns were used for RAPD analysis of test isolates and reliable producing 4 to 8 bands of 0.3 to 2.5 Kb and the dendrogram collected from the RAPD patterns using UPGMA (Unweighed Pair Group Method with Arithmetic Mean).

Table A
Nucleotide sequence of primers generating amplification of *R.solani*

Primer code	*Base sequence (5'-3')*
OPK20	5'-GTGTCGCGAG-3'
OPD12	5'CACCGTATCC-3'
OPW05	5'-CCCGCTACAC-3'
OPS19	5'GAGTCAGCAG-3'
OPX13	5'ACGGGAGCAA-3'

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) procedure explained by (Williams *et al.* 1990) was used by the following of a response mixture of 25 ml volume which consisting of 2.5 ml of 10X PCR buffer, 2.5 ml of 25 mM $MgCl_2$, 0.5 ml dNTPs mix (10 mM each of dATP, dCTP, dGTP, dtp), 0.4 ml Taq DNA polymerase, 2.0 ml of primer, 1.0 ml of genomic DNA and 16.1 ml of fresh double-distilled water. The reaction mixture was vortexed and centrifuged at 12000 rpm for 2 min. Strengthening was carried out in a thermal cycle by using three temperature profiles, programmed for early DNA denaturation at 94°C for 3 min, followed by 35 cycles having DNA denaturation for 30 sec at 94°C, primer annealing at 35°C for 30 sec and polymerization for 1 min at 72°C with a final addition period of 10 min at 72°C. Amplification products were seggregated on 1.5% agarose gel in 1X TAE buffer at 110 V for about 3 h 100 bp + 1 kb DNA ladder mix (Fermentas) (0.5 mg/ml) was run for weight size comparison. Gels were stained with ethidium bromide for 30 min; they were seen with UV light and photographed.

2.2.2. Analysis of RAPD profiles

Visually we also assess the differences in fingerprinting patterns between the isolates. Polymorphisms including faint bands that could be scored clearly were included in the analyses. Presumed homologous bands were scored as present (1) or absent (0) to produce a binary matrix. A resemblance matrix was created from the binary data by DICE similarities coefficient in SIMQUAL program of NTSYS-PC Package (Rohlf, 2000). Cluster analysis was made with the Unweighed Pair Group Method with Arithmetic Mean (UPGMA) in the SAHN program of NTSYS-PC Package and a dendrogram was developed based on genetic distances.

2.3. Evaluation of seaweeds against *R. solani in vitro*

The effectiveness of the various seaweeds listed in table was tested against *R. solani*

Sl. No.	*Scientific name*	*Common name*	*Collected from*
1.	*Dictyota bartyrensiana*	Brown seaweed	Pamban
2.	*Sargassum wightii*	Brown seaweed	Pamban
3.	*Sargassum muticum*	Brown seaweed	Kanyakumari
4.	*Padina gymospora*	Brown seaweed	Pamban
5.	*Chnoospora implexa*	Brown seaweed	Pondicherry

2.3.1. Preparation of crude seaweeds extracts

Each 1 Kg of live, healthy and matured samples (Brown seaweeds, Green seaweeds and Red seaweeds) of each seaweed collected along the Coast of Pamban (Rameswaram (9°14'N; 79°14'E), Kanyakumari, Pondicherry, Velankanni, Gulf of Mannar, Tamil Nadu, India) were cleaned thoroughly in sea water followed by tap water to take out extraneous particles and epiphytes. Then they were air dehydrated under shade in laboratory for 3 days. The shade-dried samples were chopped and crushed. 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 extracts were mixed and concentrated by using flask evaporator under condensed 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 independently were placed in cavity slides and were permitted 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. The spore germination was seen 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 deferment of the fungi was prepared from a ten days old culture with sterile distilled water. Various concentration like 5, 10, 15and 20 per cent of Seaweed extracts were prepared. Twenty ml of PDA medium was seeded with three ml of sclerotial suspension (1 x 10^6 sclerotia / ml) of the fungus and solidified. Sterile filter paper discs (10mm) were dipped disjointedly in known concentration of treatments and placed equidistantly over the seeded medium. Three replications were maintained. The plates were incubated at 28±2°C for 48 hr. The reserve zone of the fungal growth around the treated

paper discs was measured and recorded. The paper disc dipped in sterile distilled water acted as control.

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

Seaweed extracts like 5, 10, 15 and 20 per cent independently (10ml) were included to the sterilized potato dextrose agar medium and meticulously mixed just before plating. Twenty ml of these mixtures separately were immediately poured into sterilized Petri plates and were allowed to solidify. A 9 mm of PDA disc was detached by using cork borer to form wells; 1 ml of spore suspension was poured into the well. All these were carried out under aseptic situations. The plates were incubated at 28±2°C 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 calculated and the percent inhibition of the growth was calculated.

2.4. Identification of antifungal compounds

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

Based on the growth inhibition studies, Seaweed extract was collected and chemical constituents were resoluted 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 irecognised with the aid of a computer-driven algorithm and then by matching the mass spectrum of the investigation with that of a library (NIST Version. 2.0, year 2005). Turbo mass-5.1 Software was used for gas chromatography mass spectroscopy (GCeMS).

2.5. Preparation of trash fish compost (Ann Suji and Suthin Raj, 2015)

Rectangular shaped, fifteen piles (03 Treatments and 01 Control each with triplicates) of dimension 1.5 m × 1.5 m × 1.0 m (Length × Width × Height) were developed using pre-weighted and Trash fish and shell fish processing wastes were added as raw materials for composting. The aeration procedures were calculated using perforated Poly Vinyl Chloride (PVC) pipes with 4" diameter and 2m length. The wetness content of piles was maintained between 55% and 60% during the composting process. All piles were subjected to turn once a week with a spade. After five weeks period of the experiment, all piles were transferred under a roof cover. The unstable solid and total nitrogen contents were analyzed once a week while temperature was calculated daily using standard procedures given by Tendon, 1993. Colour, odour, moisture content, particle size, sand content, pH, total nitrogen percent by mass, phosphorous content as P_2O_5 percent by mass, potassium content as K_2O percent by mass, C: N ratio and viable weed seed of resultant compost shaped from each pile were analyzed and evaluated according to Tendon, 1993 for compost production from fish waste.

2.6. Efficacy of seaweed extracts and fungicide against sheath blight disease of rice in field condition

The field trail was conducted with 11 treatments and three replications at the Department of Plant Pathology, Annamalai University, Annamalainagar at kuruvai (June to September) (Trial-I) season of the year 2017. The thirty days old rice seedlings of var. ADT 36 were transplanted in the field. The seaweeds components *viz., Sargassum wightii, Sargassum muticum, Dictyota bartyrensiana, Padina gymospora,* and *Chnoospora implexa,* Trash fish manure and Hexaconazole were tested against sheath blight disease with the following suggestion in a field experiment. Seaweeds were used @ 10 per cent concentration. Hexaconazole 5 SC

was sprayed @ 0.2 per cent concentration. *R. Solani* was inoculated scrupulously over the plant cover by one gram rice hull/rice grain, placed on basal leaves and covered with polythene bags on the 20th day after transplanting. Three replications were maintained for each treatment. The below given treatment schedule were designed on the basis of the above phenomenon. The cultivar ADT 36 was raised and the packages of practices were followed as per the Crop Production Guide (2016).

2.7. Treatment Details

T_1 - Application of *Sargassum wightii* (Seed treatment (10g/kg) prophylactic spray (10%) at 20, 35 and 50 DAT)

T_2 - Application of *Sargassum muticum* (Seed treatment (10g/kg) + prophylactic spray (10 %) at 20, 35 and 50 DAT)

T_3 - Application of *Padina gymospora,* (Seed treatment (10g/kg) + prophylactic spray (10 %) at 20, 35 and 50 DAT)

T_4 - Application of *Chnoospora implexa* (Seed treatment (10g/kg) + prophylactic spray (10 %) at 20, 35 and 50 DAT)

T_5 - Soil application of trash fish compost (12.5ton/ha)

T_6 - $T_1 + T_5$

T_7 - $T_2 + T_5$

T_8 - $T_3 + T_5$

T_9 - Seed treatment with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)

T_{10} - Inoculated control

T_{11} - Healthy control

2.8. Disease incidence

The sheath blight incidence was analysed for the plants during their 30th, 50th and 70thdays after transplanting. The strength of sheath blight was considered as per cent disease index (PDI) grade chart proposed by (Mckinney *et al.*, 1923) and using the formula proposed by (Mckinney *et al.*, 1923) was described as earlier.

2.9. Statistical analysis

All the experiments were of completely randomized design (CRD) and repeated twice. Data were subjected to analyses of variance, and treatment means were compared by an appropriate Duncan's multiple-range test ($P < 0.05$). The IRRISTAT package version 92-1, prepared by the IRRI Biometrics Unit, Philippines, was used for analysis

3. RESULTS

3.1. RAPD analysis of *R. solani*

Twenty isolates of *R. solani* collected from different rice growing areas of Tamil Nadu (Table. 1) were tested for their relatedness. The Dendrogram results exposed that these twenty isolates portrayed 60 per cent to 80 per cent similarity coefficient. The twenty isolates were divided into two clusters viz., A (3.39) and B (5.19). Again cluster A is divided into two clusters viz., A1 (4.58) and A2 (7.39). The cluster A1 represented isolates of Kizhavenmani, Kelaiyur, Pirinchumali, Sikkal, Aaimalai and Nariyankudi and A2 represented isolates of Thrukkuvali, Valivalam, Kudineyveli, Orathur, Karuveli, Papakovil and Poravacharry. These two show 72.5 per cent similarity coefficient. The Cluster B then is alienated into two clusters viz., B1 (16.94) and B2 (35.46). The cluster B1 represented isolates of Thirukadaiyur, Agalamkannu, Alankudi and Thavur. This exhibited similarity coefficient of 80 percent. The cluster B2 represented Paalayur isolate which differed from other isolates. The isolates of Thanilapaddi and Sikalpattu which are exhibited more variation percent when compared to all other isolates of the pathogen (Fig. 1 and 3).

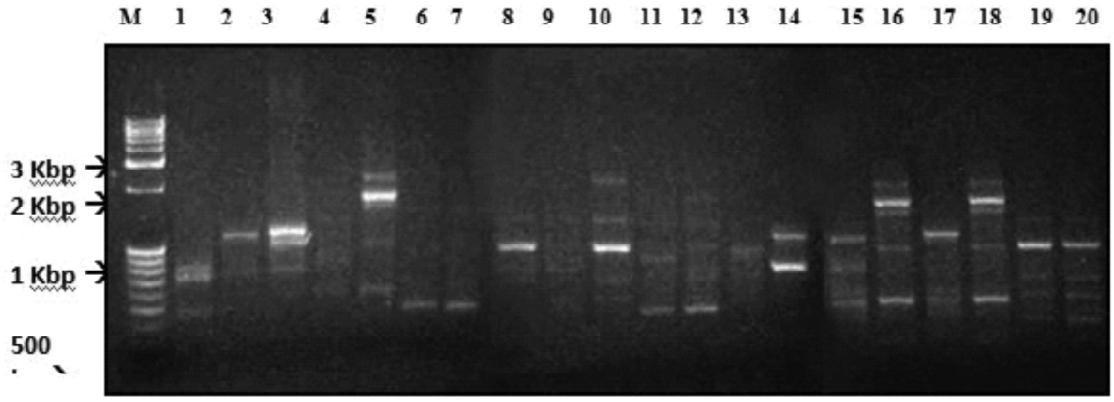


Figure 1: Random amplified polymorphic DNA profile of isolates of *Rhizoctonia solani* generated using PCR analysis

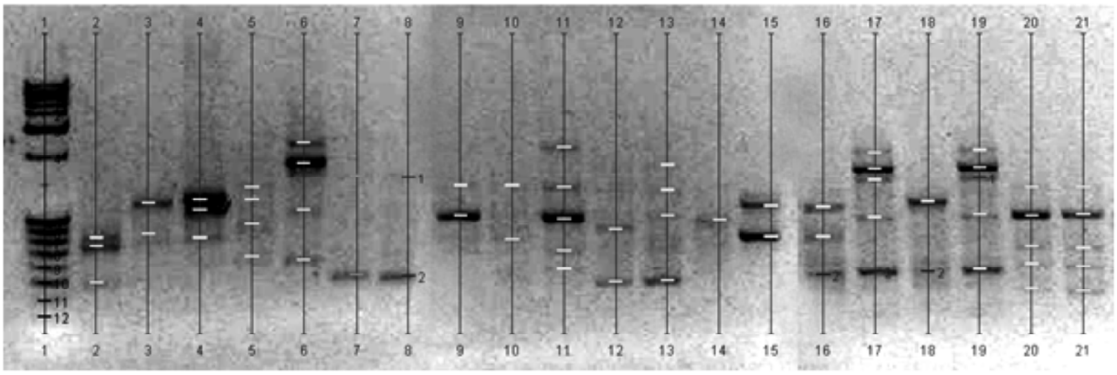


Figure 2: RAPD Pattern Analysis

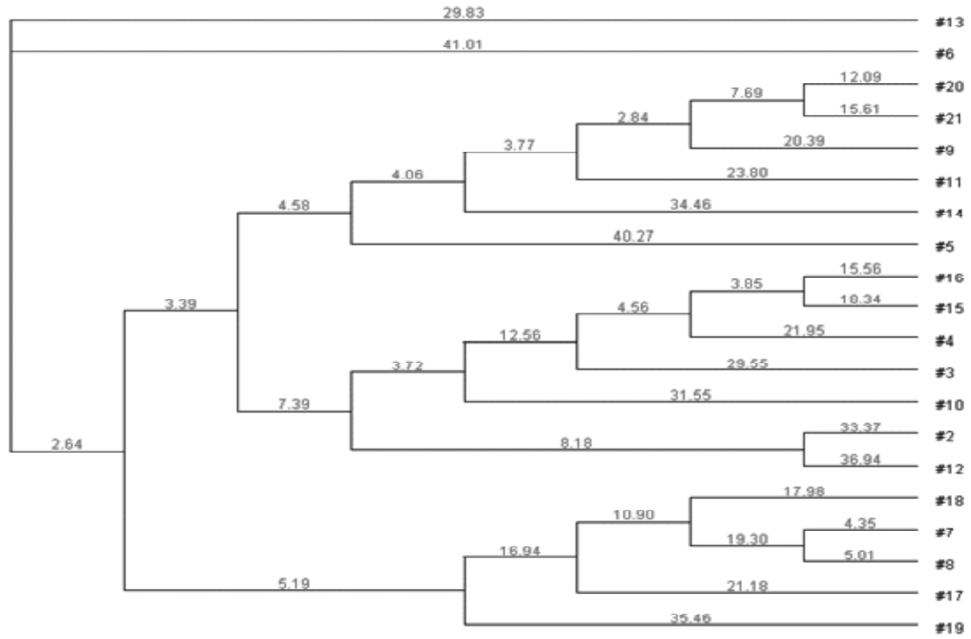


Figure 3: Dendrogram of twenty isolates of *Rhizoctonia solani* generated by unweighted pair group method arithmetic mean (UPGMA)

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

3.2.1. Spore germination

Among the six brown seaweed extracts tested against *R.solani,* extracts of *Sargassum wightii* at a towering concentration (20%) was found to be the most excellent in the reduction of spore germination (17.50 per cent). It was continued by a high concentration (20%) of *Sargassum muticum* (19.00 per cent). The rate of reduction was corborated with its concentration in case of all the tested brown seaweed extracts. *Sargassum wightii* and *Sargassum muticum* considerably reduced spore germination than other brown seaweed products in all the concentrations (Table 1).

3.2.2. Paper disc method and Agar well method

Various brown seaweed extracts were collected and evaluated for the antimicrobial activity by two ways, such as paper disc and agar well method. The leaf extracts of *Sargassum wightii* at a highest concentration (20%) was found to be the maximally reduced in both paper disc method and agar well methods and recorded 49.60 and 51.50 per cent reserve zone respectively. It was continued by a highest concentration (20%) of *Sargassum muticum* which recorded 48.50 and 49.50 per cent inhibition zone in paper disc method and agar well method respectively (Table 1).

3.2. 3. Gas Chromatography Mass Spectroscopy (GC/MS) analysis

On the basis of presentation of marine products in the previous *in vitro* studies, *S. wightii* (Brown seaweed) was experienced to determine the nature of chemical compound (s) present in the seaweed extract. The results exposed that 15 compounds were present in *S.wightii*. The molecular weights, name of the compound, chemical formula, preservation time and peak area percentage were given in Figure 4. Among these, heptane which was closely related to 2, 6, 6 – trimethyl may be accountable for the inhibition of the growth of *R. solani*. (Fig 4 & 5).

The result of the experiments has given away the superiority of *Sargassum wightii*. Hence the same was used for further studies.

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 muticum*	39.60	38.00	29.60	19.00	31.55[b]	37.50	38.90	44.60	48.50	42.37[b]	33.70	35.60	40.50	49.50	39.82[b]
2	*Dictyota bartyrensiana*	42.50	38.60	31.30	25.30	34.42[c]	35.00	37.50	42.80	46.00	40.32[c]	31.50	33.15	37.41	45.66	36.93[c]
3	*Padina gymospora*	45.30	39.50	33.20	28.00	36.50[d]	31.50	33.50	40.00	43.60	37.15[d]	30.50	31.16	33.14	41.30	34.02[d]
4	*Chnoospora implexa*	48.70	41.30	39.100	33.50	40.65[e]	28.10	30.50	36.50	40.20	33.82[e]	28.60	29.15	31.12	37.11	31.49[e]
5	*Sargassum wightii*	37.50	35.40	28.00	17.50	29.60[a]	39.50	41.00	45.50	49.60	43.90[a]	35.00	36.80	41.80	51.50	41.27[a]
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[e]

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

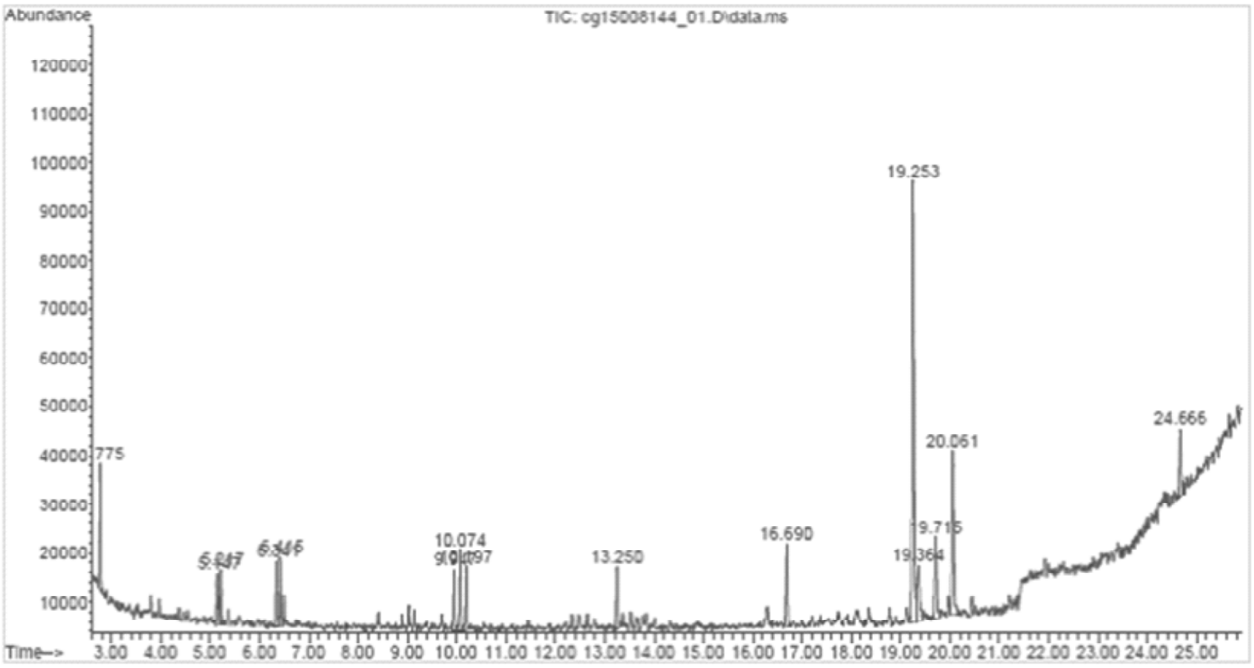
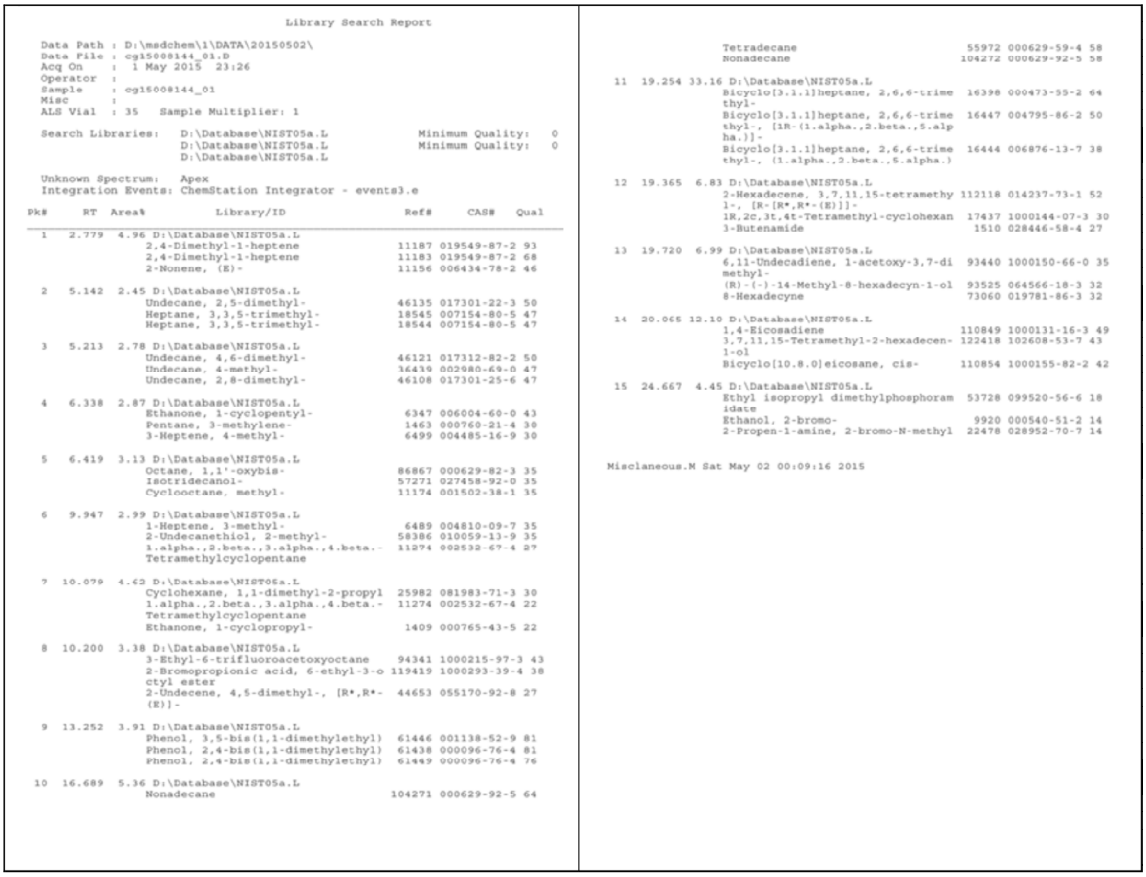


Figure 4: GC- MS analysis of brown seaweed (*Sargassum wightii*)



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			Undecane, 4-methyl-	36419	002980-69-0	47
			Undecane, 2,8-dimethyl-	46108	017301-25-6	47
4	6.338	2.87	D:\Database\NIST05a.L			
			Ethanone, 1-cyclopentyl-	6347	006004-60-0	43
			Pentane, 3-methylene-	1463	000760-21-4	30
			3-Heptene, 4-methyl-	6499	004485-16-9	30
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			Octane, 1,1'-oxybis-	86867	000629-82-3	35
			Isotridecanol-	57271	027458-92-0	35
			Cyclooctane, methyl-	11174	001502-38-1	35
6	9.947	2.99	D:\Database\NIST05a.L			
			1-Heptene, 3-methyl-	6489	004810-09-7	35
			2-Undecanethiol, 2-methyl-	58386	010059-13-9	35
			1.alpha.,2.beta.,3.alpha.,4.beta.- Tetramethylcyclopentane	11274	002532-67-4	27
7	10.079	4.62	D:\Database\NIST05a.L			
			Cyclohexane, 1,1-dimethyl-2-propyl	25982	081983-71-3	30
			1.alpha.,2.beta.,3.alpha.,4.beta.- Tetramethylcyclopentane	11274	002532-67-4	22
			Ethanone, 1-cyclopropyl-	1409	000765-43-5	22
8	10.200	3.38	D:\Database\NIST05a.L			
			3-Ethyl-6-trifluoroacetoxyoctane	94341	1000215-97-3	43
			2-Bromopropionic acid, 6-ethyl-3-octyl ester	119419	1000293-39-4	38
			2-Undecene, 4,5-dimethyl-, (R*,R*-(E)]-	44653	055170-92-8	27
9	13.252	3.91	D:\Database\NIST05a.L			
			Phenol, 3,5-bis(1,1-dimethylethyl)	61446	001138-52-9	81
			Phenol, 2,4-bis(1,1-dimethylethyl)	61438	000096-76-4	81
			Phenol, 2,4-bis(1,1-dimethylethyl)	61449	000096-76-4	76
10	16.689	5.36	D:\Database\NIST05a.L			
			Nonadecane	104271	000629-92-5	64
			Tetradecane	55972	000629-59-4	58
			Nonadecane	104272	000629-92-5	58
11	19.254	33.16	D:\Database\NIST05a.L			
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	16398	000473-55-2	64
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	16447	004795-86-2	50
			Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	16444	006876-13-7	38
12	19.365	6.83	D:\Database\NIST05a.L			
			2-Hexadecene, 3,7,11,15-tetramethyl-	112118	014237-73-1	52
			1-, [R-[R*,R*-(E)]]-			
			1H,2c,3t,4t-Tetramethyl-cyclohexan	17437	1000144-07-3	30
			1-Butenamide	1510	028446-58-4	27
13	19.720	6.99	D:\Database\NIST05a.L			
			6,11-Undecadiene, 1-acetoxy-3,7-dimethyl-	93440	1000150-66-0	35
			(R)-(-)-14-Methyl-8-hexadecyn-1-ol	93525	064566-18-3	32
			8-Hexadecyne	73060	019781-86-3	32
14	20.066	12.10	D:\Database\NIST05a.L			
			1,4-Eicosadiene	110849	1000131-16-3	49
			3,7,11,15-Tetramethyl-2-hexadecen-1-ol	122418	102608-53-7	43
			Bicyclo[10.8.0]eicosane, cis-	110854	1000155-82-2	42
15	24.667	4.45	D:\Database\NIST05a.L			
			Ethyl isopropyl dimethylphosphoramidate	53728	099520-56-6	18
			Ethanol, 2-bromo-	9920	000540-51-2	14
			2-Propen-1-amine, 2-bromo-N-methyl	22478	028952-70-7	14

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Figure 5: Components identified in the brown seaweed (*Sargassum wightii*)

3.4. Effect of IDM formulation on sheath blight incidence under field condition

The diverse IDM formulations were tested against sheath blight disease occurrence in rice under field state. The results showed that, all the seaweed extracts considerably abridged the sheath blight disease incidence than the control. Among the treatments, application of *S.wightii* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and soil application of fish compost (T_6) recorded significantly less sheath blight incidence of 87, 83 and 88 per cent boost over control 30, 50 and 70 days after planting than other treatments. It was continued by T_7 which recorded 85, 82 and 87 per cent augment over control, 30, 50 and 70 days respectively (Table 2).

3.4. Effect of IDM formulation on growth and yield attributes under field condition

The rice plants were treated with various IDM formulations and the biometric remarks and yield parameters were also recorded on 70 DAT. All the treatments were effective to endorse the growth of the plant. Especially among them, the application of *S. wightii* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and soil application of fish compost (T_6) was found to be considerably augmented to the mean plant height (116 cm) mean number of productive tillers (21 nos.), mean 1000g weight (25g), straw yield (8.55 ton/ha) and grain yield (33 g/plant), compared to all other treatments and continued by treatment T_7 which gave good biometric remarks and yield parameters recording mean plant height (114cm) mean number of productive tillers (17 nos), mean 1000g weight (22 g), straw yield (7.55 ton/ha) and grain yield (32 g/plant) respectively, which were statistically on par with each other (Table 3).

4. DISCUSSION

In the current study, application of *S. wightii* (seed, prophylactic spraying at 20, 35 and 50 DAT) and soil purpose of fish manure on rice plants significantly abridged the incidence of *R. solani*. Collective applications of IDM formulation efficiently prohibited disease incidence than the individual application of above mechanism. Similarly, Sulthana *et al*., 2011 reported the minimum root disease incidence was with seaweed extract + urea + topsin -M application on soybean under greenhouse and field environment. Minimum root rot disease occurrence was found by spraying *Stokeyia indica* (brown seaweed) in okra plants (Sulthana *et al.,* 2007). Similar findings were made by several workers by using diversed seaweed extracts or through various formulations (Flora and Victoria Rani, 2012 and Suthin raj *et al.,* 2018a).

Spraying with seaweed *Ascophyllum nodosum* and fungicide chlorothalonil were effectual against foliar diseases on carrot in pot culture experiments (Jayaraj *et al.,* 2008). Seaweed extracts have several carbohydrate molecules mostly in the form of oligosaccharides as well as oligogalacturonides and some polysaccharides (Norrie *et al*., 2002). Oligosaccharides are known to act as elicitor and signal transduction molecules in plants (Vidhyasekaran, 1997) Most probably, the existence of easily degradable organic matter of seaweed provided the food base for the development of the antagonistic bacteria, which increased their population in the rhizosphere or the alginate of seaweed directly concealed the pathogens (Jacob and Veluthambi, 2003). Application of brown seaweed is often performed by foliar spraying and it has been reported that they diminish *R. solani* infection (Rathore *et* al., 2009 and Suthin raj *et al.,* 2018b). This may be due to higher levels and early gathering of phenolics and phytoalexins (Garcia-Mina *et al.,* 2004 and Suthin raj *et al.,* 2016a).However, the supplementary nutrients such as minerals, amino acids, alginic acid, simple and complex carbohydrates, growth stimulators in seaweed extract also played a obvious role in the improvement of growth that also would have added confrontation. This present study

Table 2
Effect of IDM formulation on Sheath blight incidence under field condition

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 *S.nightii* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	4.0[d]	79	9.6[d]	76	11.5[d]	83
T_2– Application of *S. muticum*(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	4.2[d]	78	10.5[d]	73	13.8[d]	80
T_3– Application of *D. bartyrensiana* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	4.8[d]	75	10.8[d]	73	14.5[d]	79
T_4 –Application of *P. gymospora* (ST @10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	4.9[c]	74	11.5[c]	71	16.8[c]	75
T_5– Soil application of fish compost	5.2[e]	73	12.4[e]	69	18.0[e]	74
T_6–T_1 + T_5	2.5[a]	87	6.5[a]	83	8.2[a]	88
T_7–T_2 + T_5	2.8[a]	85	6.9[a]	82	8.8[a]	87
T_8–T_3 + T_5	3.8[b]	80	8.5[b]	78	9.8[b]	85
T_9– ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	3.5[b]	82	7.8[b]	80	9.2[b]	86
T_{10}– Inoculated control	19.5f		40.20[f]		69.5[f]	
T_{11} –Healthy control	7.50[e]		8.70[e]		9.20[e]	

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

Table 3
Effect of IDM formulation on growth and yield attributes under greenhouse condition

Treatments	*Mean plant height (cm)*	*Mean no. of productive tillers*	*Mean1000 g weight*	*Straw yield (ton/ha.)*	*Grain yield (g/plant)*
T_1– Application of *S.wightii* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	110[c]	15[c]	20[d]	6.88[c]	29[c]
T_2– Application of *S. muticum* (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	108[c]	13[e]	19[d]	5.71[c]	27[d]
T_3– Application of *D. Bartyrensiana* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	107[c]	12[e]	17[d]	5.51[c]	26[d]
T_4 –Application of *P. gymospora* (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	106[d]	11[f]	16[e]	4.78[c]	25[d]
T_5– Soil application of fish compost	105[c]	10[f]	15[e]	4.15[c]	23[e]
T_6–T_1 + T_5	116[a]	21[a]	25[a]	8.55[a]	33[a]
T_7–T_2 + T_5	114	17[b]	22[d]	7.55[a]	32a
T_8–T_3 + T_5	111[b]	15[c]	20[d]	7.10[b]	30[b]
T_9– ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	112[b]	16[c]	21[c]	7.30[b]	31[b]
T_{10}– Inoculated control	79[e]	7[g]	10[f]	2.03[g]	16[f]
T_{11} –Healthy control	94[d]	10[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).

confirms earlier works. This may be due to the increased fusion of hormones like gibberellin, which trigger the activity of specific enzymes that promoted early germination, such as á-amylase, which have brought an increase in accessibility of starch assimilation.

5. CONCLUSION

To conclude, prior treatment of combined application of *S. wightii* (seed, prophylactic spraying at 20, 35 and 50 DAT) and soil application of fish manure (T_6) followed by challenge inoculation with *R. solani* triggered the plant mediated protection mechanism that in turn minimize the sheath blight occurrence in rice.

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