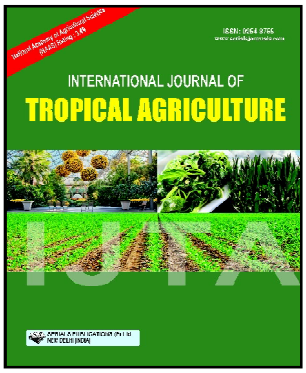

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Bio chemical characterization of brown seaweed, Identification of antifungal activity associated with mangrove leaf and its efficacy on management of rice sheath blight caused by *Rhizoctonia solani* (Kuhn)

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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, five different seaweeds such as *Dictyota dichotoma, Sargassssum wightii, Padina gymospora, Avicenia mariena* and *Bacillus subtilis* were used in control of sheath blight disease in rice were studied. Evaluation of marine products against *R. solani* was carried out by Paper disc assay; Agar well method and Mycelial dry weight. Among the five marine extracts tested, extracts of *Dictyota dichotoma* [brown seaweed algae] at a high concentration (20%) was found to be the best the in the reduction of spore germination 18.60 (per cent). The leaf extracts of *Dictyota dichotoma* [brown seaweed algae] at highest concentration of (20%) showed a maximum reduction in both paper disc method and agar well method with 43.65 and 46.90 per cent zone of inhibition respectively. The antifungal compounds were identified through Gas Chromatography Mass Spectroscopy. The results revealed that, 6 compounds were present in *Dictyota dichotoma* and among that 10, 13- Eicosadienoic acid which was closely related to n- Hexadecanoic acid may be responsible for the inhibition of the growth of *R. solani*. In the present study it revealed that the efficacy of seaweed extracts against fungal pathogens may be due to higher levels and early accumulation of phenolics and phytoalexins and the field study proved that *R. solani* can be controlled by the application of marine products.

Key words: Seaweeds, mangrove leaf extracts, GC-MS, *Rhizoctonia solani,* Antifungal Compounds, Rice

1. 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 (Acharya *et al.,* 2004). 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 (Basu *et al.,* 2004).

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 (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; Suthinraj *et al.,* 2016a). Thus seaweeds are bestowed with varied sources of bioactive natural products that exhibit biomedical and antimicrobial properties (Arun kumar *et al.*, 2005; Karthikeyan and Shanmugam 2016). Peres (2012) was 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; Suthinraj *et al.*, 2018). Arun kumar *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 *Gymnema sylvestris*. The use of anti-microbial drugs (Tonyarioli *et al.*, 2015) 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 macro_algae 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; Suthinraj *et al*., 2016b).In this context, the present study was carried out to evaluate the various marine products against *Rhizoctonia solani* under *in vitro* and field condition.

2. MATERIALS AND METHODS

2.1. Collection of seed materials

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

2.2. Isolation, maintenance and identification of pathogen

The diseased rice plants showing the typical symptoms of sheath blight disease were collected from 20 conventional rice growing areas of Nagappattinam. The pathogens were isolated on potato dextrose agar (PDA) 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. The fungus was purified by single spore isolation technique (Rangaswami, 1958). Identification of the isolate was confirmed (Reddy *et al*., 2012) by comparing with the culture obtained from ITCC, IARI, New Delhi and the purified isolates were maintained on PDA slants for further studies.

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 through Whatman 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.

2.4. Isolation of Bacteria from Seaweeds

For the isolation of epibiotic bacteria, fresh seaweed thallus weighing 1.0 g was swabbed aseptically with sterile cotton in 10 ml sterile water and left for 30 min. For the isolation of endobiotic bacteria, the sample after swabbing the epibiotic bacteria was homogenized under aseptic conditions using 10 ml of sterile water.

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

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

Sl. No.	*Scientific name*	*Common name*	*Collected from*
1.	*Dictyota dichotoma*	Brown seaweed	Gulf of Mannar Coast
2.	*Sargassssum wightii*	Brown seaweed	Gulf of Mannar Coast
3.	*Padina gymospora*	Brown seaweed	Gulf of Mannar Coast
4.	*Sargassum muticum*	Brown seaweed	Kanyakumari
5.	*Chnoospora implexa*	Brown seaweed	Pondicherry

2.5.1. Preparation of crude seaweeds extracts (Suthinraj et al., 2018)

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

2.6. Evaluation of marine products against *R. solani*

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

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.

2.6.2. 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.

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

One drop of 5, 10, 15 and 20 per cent Seaweed extracts were placed in cavity slides and were allowed to air dry. A drop of the spore suspension (1 × 10^6 spores 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.

2.7. Identification of antifungal compounds

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

Based on the growth inhibition studies, Seaweed extract was selected and chemical constituents were determined with a GC Clarus 500 Perkin Elmer Gas chromatography equipped with a mass detector. Turbo mass gold containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID employed were the following: Carrier gas, helium (1 mL/min); oven temperature program 110ºC (2 min) to 280ºC (9 min); injector temperature (250ºC); total GC time (36 min). The water extract was injected into the chromatograph in 2.0 Ml aliquots. The major

constituents were identiûed with the aid of a computer-driven algorithm and then by matching the mass spectrum of the analysis with that of a library (NIST Version. 2.0, year 2005). Software used for gas chromatography mass spectroscopy (GCeMS) was Turbo mass-5.1. This work was carried out in Indian Institute of Crop Processing Technology (IICPT), Thanjavur (Fig 1 & 2).

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	8.20	Palmitoleic acid	$C_{16}H_{30}O_2$	254	3.82
2.	12.17	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$	272	5.60
3.	13.85	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	3.36
4.	15.40	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.58
5.	22.05	10,13-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	1.95
6.	28.21	Cholesta-4,6-dien-3-ol, (3β)-	$C_{27}H_{44}O$	384	80.68

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

Figure 1: Compound identified in the *Dictyota dichotoma* (Brown seaweed)

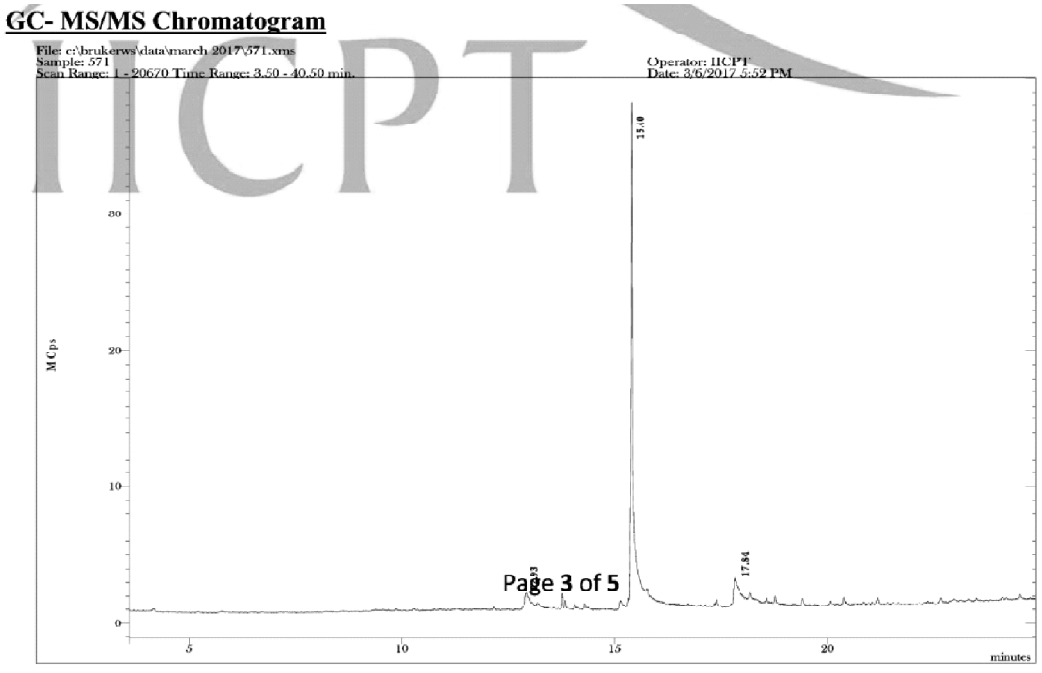

Figure 2: GC-MS analysis of *Dictyota dichotoma* (Brown seaweed)

2.8. Efficacy of marine products 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) season of the year 2016. The thirty days old rice seedlings of var. ADT 36 were transplanted in main field. The seaweeds *viz., Dictyota dichotoma, Sargasssum wightii, Padina gymospora* (brown seaweed algae), *Avicenia mariena* (mangrove leaf), *Bacillus subtilis* (marine bacteria) and Hexaconazole were tested against sheath blight disease with the following recommendation 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 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 below mentioned treatment schedule were designed on the basis of the above phenomena. The cultivar ADT 36 was raised and the packages of practices were followed as per the Crop Production Guide (2016).

3.9. Treatment details

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

T_2 - Application of *Padina gymospora* (Brown seaweed algae) (Seed treatment (10g/kg) + prophylactic spray (10 %) at 20, 35 and 50 DAT)

T_3 - Application of *Dictyota dichotoma* (Brown seaweed algae) (Seed treatment (10g/kg) + prophylactic spray (10 %) at 20, 35 and 50 DAT)

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

T_5 - Application of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 30, 45 and 60 DAT)

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} - Control

3. RESULTS

3.1. *In vitro* evaluation of various Mangroves leaf extract against *R. solani*

3.1.1. Spore germination

Among the five mangroves leaf extracts tested against *R.solani,* powder of *Avicenia mariena* at a high concentration (20%) was found to be the best in the reduction of spore germination (18.60 per cent). It was followed by a high concentration (20%) of *Rhizophora stylosa* (22.1 per cent). The rate of reduction was corborated with its concentration in case of all the tested mangroves leaf extracts (Table 1).

3.1.2. Paper disc method and Agar well method

Various mangroves leaf extracts were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The mangroves leaf extracts of *Avicenia mariena* at a highest concentration (20%) showed a maximum reduction in both paper disc method and agar well method and recorded 44.65 and 45.90 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Rhizophora stylosa* which recorded 39.00 and 34.33 per cent inhibition zone in paper disc method and agar well method respectively. All the concentrations of *Kandelia candel* recorded a minimum per cent inhibition zone than all other powders (Table 1).

Table 1
Evaluation of various mangrove leaves extract against *R. Solani* under *in vitro* condition

S. No.	*mangrove leaves*	*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	*Avicenia mariena*	48.10	40.60	29.00	18.60	34.07 [a]	36.10	40.28	46.10	44.65	41.78 [a]	30.71	34.65	39.16	45.90	37.60 [a]
2	*Avicenia germinans*	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	*Rhizophora mangle*	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	*Kandelia candel*	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	*Rhizophora stylosa*	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 isolates of *B. spp.* against *R.solani* by dual culture technique

S. No.	Isolates	Linear growth (mm)		% Growth inhibition	Mycelial dry weight (mg/50 m/broth)				
		Antagonist	C. capsici		10%	20%	30%	40%	Mean
1.	*Bacillus subtilis* (B. I_1)	63.00	27.00	70.00* [a] (4.26)**	214 (5.37)	168 (5.12)	80 (4.38)	28 (3.35)	4.55[a]
2.	*Bacillus cereus* (B. I_2)	61.80	28.20	68.66[a] (4.24)	231 (5.44)	204 (5.32)	109 (4.69)	38 (3.66)	4.78[b]
3.	*Bacillus coagulens* (B. I_3)	57.33	32.67	63.70[ab] (4.16)	276 (5.62)	214 (5.37)	111 (4.71)	42 (3.72)	4.86[c]
4.	*Bacillus alvei* (B. I_4)	53.60	36.40	59.55[ab] (4.10)	288 (5.66)	219 (5.38)	128 (4.84)	48 (3.87)	4.94[c]
5.	*Bacillus megaterium* (B. I_5)	50.72	39.28	56.35[c] (4.04)	306 (5.72)	232 (5.45)	145 (4.98)	49 (3.91)	5.01[d]
6.	Control	-	90.00	(0.04) -[d]	540 (6.29)	540 (6.29)	540 (6.29)	540 (6.29)	6.29[e]
	Mean				5.68[d]	5.49[c]	4.98[b]	4.14[a]	

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05), Figures with in parenthesis are arcsin transformation

Table 3
Evaluation of various seaweed extracts against *R. solani* under *in vitro* condition

S. No.	Marine products	Agar well method					Paper disc method				
		5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
1	*Dictyota dichotoma*	35.10	39.28	45.10	43.65	40.78[a]	31.71	35.65	40.16	46.90	38.60[a]
2	*Sargasssum wightii*	25.00	26.60	31.00	40.00	30.65[b]	20.00	22.67	28.67	34.33	26.41[b]
3	*Padina gymospora*	20.00	21.50	26.70	28.30	24.12[c]	18.20	20.50	23.10	25.95	21.93[c]
4	*Sargassum muticum*	12.00	14.21	16.28	19.72	15.55[e]	10.66	11.92	14.00	17.00	13.39[d]
5	*Chnoospora implexa*	12.25	14.50	18.80	27.10	1816[d]	10.00	11.50	15.20	18.00	13.67[d]
6	Control	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).

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

3.2. *In vitro* evaluation of *B. spp.* against *R. solani*

The results of the screening of five isolates of *B. spp.* against *R. solani* on PDA plates are presented in Table 2. Among the *B. subtilis* isolates B.I-1was appear to be most effective against the test pathogen showing 70.00 per cent inhibition of colony growth and minimum mean mycelial growth of pathogen (4.26 cm). It was followed by isolate B.I-2 showing 68.66 per cent inhibition and minimum mean mycelial growth (4.24). Which where statistical on par each other. The isolate B.I-5 gave minimum growth inhibition (56.35 cm) and maximum mycelial growth of pathogen. All the

Table 4
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 *Sargasssum wightii* (Brown seaweed algae) (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	4.6[d]	76	9.7[d]	75	13.3[d]	80
T_2 - Application of *Padina gymospora* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	4.9[c]	74	10.6[d]	73	15.2[d]	78
T_3 - Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	4.1[d]	78	8.8[b]	78	12.6[d]	81
T_4 - Application of *Avicenia mariena* (Marine leaf) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	5.3[e]	72	12.3[e]	69	18.1[c]	73
T_5 - Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 30, 45 and 60 DAT)	5.1[e]	73	11.9[c]	70	16.7c	75
T_6 - T_1 + T_5	3.1[b]	84	7.3[b]	81	10.9[d]	84
T_7 - T_2 + T_5	3.7[b]	81	8.1[b]	79	11.7[d]	83
T_8 - T_3 + T_5	2.3[a]	88	6.6[a]	83	8.3[a]	88
T_9 - ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	2.6[a]	86	7.0[a]	82	10.2[b]	85
T_{10} - Control	7.5[e]		8.7[e]		9.2[c]	

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

Table 5
Effect of IDM formulation on growth and yield attributes under field condition

Treatments	*Mean plant height (cm)*	*Mean no. of productive tillers*	*Mean1000 g weight*	*Straw yield (g/plant)*	*Grain yield (g/plant)*
T_1 - Application of *Sargasssum wightii* (Brown seaweed algae) (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)	86.28^{c}	14^{d}	19^{d}	6.83^{d}	28^{d}
T_2 - Application of *Padina gymospora* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)	85.34^{b}	13^{d}	18^{d}	6.11^{d}	27^{d}
T_3 - Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	87.92^{b}	15^{d}	22^{c}	7.24^{b}	30^{c}
T_4 - Application of *Avicenia mariena* (Marine leaf) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)	81.98^{d}	10^{e}	16^{e}	4.97^{e}	24^{e}
T_5 - Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 30, 45 and 60 DAT)	84.37^{c}	11^{e}	17^{e}	5.92^{e}	26^{d}
T_6 - T_1 + T_5	92.64^{a}	17^{c}	24^{b}	8.10^{a}	33^{b}
T_7 - T_2 + T_5	89.4^{b}	16^{c}	23^{b}	7.78^{a}	31^{b}
T_8 - T_3 + T_5	97.31^{a}	21^{a}	27^{a}	8.81^{a}	36^{a}
T_9 - ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)	95.16^{b}	19^{b}	26^{a}	8.40^{a}	35^{a}
T_{10} - Control	89.00^{d}	11^{e}	15^{e}	4.15^{f}	16^{e}

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

isolates significantly reduce the mycelial growth of the pathogen over the control.

3.3. *In vitro* evaluation of seaweeds against *R.solani*

3.3.1. Paper disc method and 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 *Dictyota dichotoma* [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 43.65 and 46.90 per cent inhibition zone respectively. It was followed by a highest concentration (20%) of *Sargasssum wightii* which recorded 39.00 and 34.33 per cent inhibition zone in paper disc method and agar well method respectively (Table 3).

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

3.5. Gas Chromatography Mass Spectroscopy (GCeMS) analysis

On the basis of performance of marine products in the preceding *in vitro* studies, *Dictyota dichotoma* (Brown seaweed) was tested to determine the nature of chemical compound (s) present in the seaweed extract. The results revealed that 6 compounds were present in *Dictyota dichotoma*. The molecular weights, name of the compound, chemical formula, retention time and peak area percentage were given in Figure 1.

Among these, 10, 13- Eicosadienoic acid which was closely related to n- Hexadecanoic acid may be responsible for the inhibition of the growth of *R. Solani* (Figure 2).

4. DISCUSSION

The seaweeds and the prepared marine products has significant role in the control of the *R. solani* in *in-vitro* and *in vivo* condition. Generally all marine products inhibited the mycelial growth of pathogen in the present study of which, *Dictyota dichotoma* (brown seaweed algae) (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT) + Soil application with *Bacillus subtilis* (T_8). The same treatment significantly recorded a maximum plant growth and yield parameters of rice. Further, the same treatment significantly decreased disease incidence and increased growth and yield parameters than the standard check chemical, hexaconazole. 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; Suthinraj *et al.*, 2018). 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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