Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP2: 210-218

T Suthin Raj
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

A Muthukumar
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

N Muthukumaran
Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

GB Sudhagar Rao
Department of Agronomy, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

H Ann Suji
Centre for Advance Studies in Marine Biology, Annamalai University, Chidambaram, Tamil Nadu, India

Correspondence
T Suthin Raj
Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India

Induction of defence enzymes activities in rice plant treated by seaweed algae against *Rhizoctonia solani* Kuhn causing sheath blight of rice

T Suthin Raj, A Muthukumar, N Muthukumaran, GB Sudhagar Rao and H Ann Suji

Abstract
Rice Sheath Blight is caused by *Rhizoctonia solani* which becomes a major problem. The use of natural products such as seaweed provides a rich source of structurally diverse and biologically active secondary metabolites, and is the ultimate way of combating these diseases. In this context, seaweed algae such as *Dictyota dichotoma, Chondrococcus hornemannii, Jania rubens and Caulerpa scalpelliformis* were used to control rice sheath blight. Enzymatic studies were carried out on peroxidase, polyphenol oxidase, phenylalanine ammonia lyase and β-1,3- glucanase. The rice plants were treated with *Dictyota dichotoma* along with soil application of *Bacillus subtilis*, the four enzymes raised the glucose concentration to 60.34 µg, 3.73 µg, 5.21 µg and 232 µg respectively, and then the plants were treated with the chemical called Hexaconazole and the four enzymes raised 59.20 µg, 3.57 µg, 5.17 µg and 229.3 µg respectively. While comparing these enzymes, β-1,3- glucanase release glucose content in large amount both in seaweed treated plant and also in chemical treated plant. This present study was undertaken to evaluate the various extracts of seaweeds algae such as brown, red and green seaweed along with the enzyme studied, would increase the resistance of grapes to sheath blight of rice.

Keywords: Rice, sheath blight, seaweed algae, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, β-1,3- glucanase

1. Introduction
Rice serves as the staple food of Indian people and it is grown as a monsoon based cereal crop in India (Hossain and Pingali, 1998) [15]. It is an important food crop, being the staple of more than half of the world's population. It is grown in tropical and sub tropical regions of the world. In the world, it occupies an area of 161.29 m ha with a total production of 480.02 mt with a productivity of 4.44t/ha, and in India, it occupies an area of 44.50 m ha with a total productivity of 3.59t/ha during January 2017 (Foreign Agriculture Services USDA, January 2017). In India, during 2016-17 rice is grown in an area of 433.88 lakh hectares with a production of 104.32 million tones and the productivity is 2.4 t/ha (Annual Report, 2016-17). In Tamil Nadu, during 2014-15 rice is grown predominantly in an area of 1.83 million hectares with a production of 5.84 million tones and the productivity is 3.19 kg/ha (Maps of India.com; Top rice producing states of India). In Cuddalore district during 2014-2015, rice is cultivated in a total area of 1,17, 000 hectares with a production of 5,13,406 metric tones and the productivity is 4.13 t/ha (Cuddalore District, 2014-15).
Rice cultivation is often subjected to several biotic stresses of which diseases like blast, sheath blight, stem rot and bacterial blight are the important ones (Ou, 1985) [30]. Sheath blight is one of the serious diseases of rice caused by *Rhizoctonia solani* Kuhn. No resistant cultivar is available for practical use and the present intensive rice cultivation practices offer favourable conditions for disease development. Pathogenic variability has a great concern in *R. solani* (Ogoshi, 1987; Sneh *et al.*, 1991) [29, 34]. Among these, sheath blight of rice is a destructive disease in all crop-growing areas of the world and the disease is caused by a soil-borne fungal pathogens, *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris* (A.B. Frank Donk) anastomosis group 1 and subgroup 1A. Rice sheath blight occurs throughout temperate and tropical production areas and is most prominent where rice is grown under intense production systems (Savary and Mew, 1996; Suthin Raj *et al.*, 2016a) [34, 43].
First reported in Japan that *Rhizoctonia solani* is an universal soil saprotrophic and facultative plant parasite and the pathogen has limited movement due to lack of spores and survives in unfavorable condition by forming sclerotia or dormant mycelia (Anees *et al.*, 2010) [2].

The symptom of the disease include greenish grey, elliptical or oval shaped spots with yellow margin mostly found on the leaf sheath and primary leaf blades (Damicone *et al.*,1993) [6]. Growing sheath blight resistant cultivar is the most economical and environment-friendly option to manage this disease. However, commercial rice cultivars or wild related species with complete resistance to sheath blight have not yet been found (Bonmann *et al.,* 1992; Han *et al.,* 2003; Srinivasachary *et al.,* 2011) [3, 13, 38]. *R. solani* can infect seed to fully mature plant, causing moderate to significant yield loss depending on the plant part affected. Visible plant disease symptoms include formation of lesions, plant lodging, and presence of empty grains. Large lesions formed on infected sheaths of lower rice leaves may lead to softness of the stem thereby initiating stem lodging (Wu *et al.,* 2012) [48]. However, loss due to rice sheath blight disease generally vary from 30 to 40 per cent and may be even 100 per cent in endemic areas, when the disease spreads to upper parts of the plant and panicles a total crop loss was observed (Srinivas *et al.,* 2013) [37].

During recent decades, attention has been paid to this group of fungi and subsequently they have been appied successfully as biocontrol agents against several plant diseases in commercial agriculture (Howell, 2003; Tewari and Mukhopadhyay, 2001) [16, 41]. Fungal and bacterial biocontrol agents have different mechanisms of disease suppression. The mechanism by which antagonistic organism act include antibiotic production, competitive ability, direct parasitism and lysis (Harman *et al.,* 2004) [14]. In general, fungal antagonist depend mainly on physical contact with their pathogen while, bacteria mainly use antibiotics as weapon for killing of the pathogens (Mohiddin *et al.,* 2010) [25].

Application of seaweeds for the control of soil borne plant diseases has increased in recent years due to their environment friendly role (Suthin Raj *et al.* 2016b) [44]. Macro algae are an attractive and natural source of bioactive molecules. Such natural products may have potential for the management of fungal diseases in sustainable agriculture such as organic farming. In recent years, there have been many reports of macro algae derived compounds that have a broad range of biological activities, such as antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic and antimitotic activities (Demirel *et al.,* 2009) [7].

The seaweed extract bring about induced systemic resistance (ISR) fortifying the physical and mechanical strength of cell wall as well as chemical, physiological and biochemical reaction of host leading to synthesis of defense chemicals against pathogens. Defense reaction occurs due to accumulation of peroxidase, phenylalanine ammonia lyase and PR-protein like β-1, 3-glucanase etc. Several authors have reported the induction of defense enzymes in crop plants treated with organic products and challenged with the pathogen (Nakkeeran *et al.,* 2006; Suthin raj, 2008; Jayaraj *et al.,* 2008; Flora and Maria Victorial rani, 2012) [26, 42, 17, 8].

Increased PO activity has been correlated with resistance in many plants including barley, cucurbits, cotton, tobacco, wheat and rice and this enzyme is involved in the polymerization of proteins and lignin or suberin precursor into plant cell wall, thus constructing a physical barrier that could prevent pathogen penetration of cell walls or movement through vessels. Increase in PO activity associated with induced systemic resistance was observed in cucumber (Hammerschmidt *et al.,* 1982; Jayaraj *et al.,* 2008; Flora and Maria Victoria rani, 2012; Venkatesh *et al.,* 2013; Nandi *et al.,* 2013) [12, 17, 27].

Polyphenol oxidase (PPO) usually accumulates upon wounding in plants. The increased activation of PPO could be detected in the cucumber leaf in the vicinity of lesions caused by some foliar pathogens. Moreover PPO can be induced by octadecanoid pathway (Constabel *et al.,* 1995) [5]. Phenylalanine ammonia-lyase (PAL) is a defense gene activated in the incompatible interaction and it is the key enzyme in inducing synthesis of salicylic acid (SA) which induces systemic resistance in many plants. The gene was cloned and transgenic rice plants expressing PAL showed systemic resistance against rice pathogens (Lamb *et al.,* 1997) [20].

Evidence of β-1, 3-glucanases in disease resistance was first reported by Kauffimann *et al.,* (1987) [19]. In dicots, β-1, 3-glucanase genes are considered to constitute a part of the general array of defense genes induced during pathogenesis (Mauch *et al.,* 1988) [23]. β-1, 3-glucanases especially in conjunction with chitinase are capable of hydrolyzing fungal cell walls *in vitro* (Mauch *et al.,* 1988) [23].

2. Materials and Methods

2.1 Survey on the occurrence of sheath blight of rice in Cuddalore District

A field survey was conducted to assess the extent of sheath blight occurrence of rice in Cuddalore district. The villages where rice is traditionally grown are selected for assessing the prevalence of sheath blight disease caused by *R. solani.* Twenty locations (Fixed plot survey) representing rainfed (8 places) and irrigated (12 places) situations were selected for the survey. During a survey, plants affected due to sheath blight disease were found and also the total number of plants observed were counted and recorded. The per cent disease incidence was worked out as per phytopathometry (Sriram *et al.,* 2000) [39].

Also, the infected plants showing typical symptoms of sheath blight due to infection with *R. solani* were collected for isolation of the pathogen from the respective places.

2.2 Names and sites of collection of seaweeds algae

The names and cities of collection of the various seaweeds algae tested for inducing resistance against *R. solani* are shown.

2.3 Preparation of crude seaweeds extracts (Vallinayagam *et al.,* 2009) [46].

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 and Gulf of Mannar, Tamil Nadu, India) were washed thoroughly in sea water 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, thrice in 500 ml of 1:1(v/v) chloroform: methanol using a 1 litre Erlenmeyer conical flask under dark condition. The extractants were pooled and concentrated by using a flask evaporator under reduced pressure at 45 °C, weighed and stored at 0 °C.

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

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

Sl. No.	Scientific name	Anti microbial property	Common name	Collected from
1.	*Dictyota dichotoma*	Ethyl acetate	Brown seaweed	Pamban
2.	*Caulerpa sealpelliformis*	Hexane	Green seaweed	Velankanni
3.	*Chondrococcus hornemanii*	Acetone	Red seaweed	Kanyakumari
4.	*Jania rubens*	Benzene	Red seaweed	Pondicherry

2.5 Isolation of bacteria from seawater (Sutha *et al*., 2011) [41].
For the isolation of epibiotics, fresh seaweed thallus weighing 1.0g was swabbed aseptically with sterile cotton in 10ml 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 10ml of sterile water. Sediment sample weighing 1.0g was extracted in sterile water using an orbital shaker for 30 min and the volume was made up to 10ml. Different serial dilutions such as, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were prepared from the 10ml of made-up samples (seaweeds and sediments) as well as from the 10ml of seawater samples. For each dilution, 100µl swab was spread on to petriplates containing approximately 15 ml of 1.5% ZoBell marine agar. The plates were then incubated at 25±2 °C and bacterial colonies with different morphology were picked up every 6h up to 4 days and streaked on the fresh plates containing ZoBell marine agar. Pure culture of each isolates were confirmed by subsequent restreaking. Then, they were designated with unique codes and stored in glycerol suspension (glycerol/bacterial broth of 1:1 v/v) in Eppendorf tubes at -80 °C for further investigation.

2.6 Induced systemic resistance
2.6.1 Sample Collection
A glasshouse experiment was laid out in completely randomized design using the ADT-36 variety to assess the induction of defense enzymes by seaweeds formulation against challenge inoculation of *R. solani* with following treatments.

T_1	Application of *Chondrococcus hornemanii* (Red seaweed algae) (Seed treatment (10g/kg) prophylactic spray (10%) at 20, 35 and 50 DAT)
T_2	Application of *Caulerpa sealpelliformis* (Green 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 *Jania rubens* (Red seaweed algae) (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 20, 35 and 50 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}	Inoculated control
T_{11}	Healthy control

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.

2.6.2 Enzyme extraction
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 of peroxidase (PO), polyphenol oxidase (PPO) and L-phenylalanine ammonia-lyase (PAL).

2.6.3 Spectrophotometric assay
2.6.3.1 Peroxidase (PO) (Hammerschmidt *et al*., 1982) [12].
Peroxidase activity was assayed spectrophotometrically (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 ηm was recorded at 30 sec. interval for 3 min and the boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture min^{-1} g^{-1} on fresh weight basis.

2.6.3.2 Polyphenol oxidase (PPO) (Mayer *et al*., 1965) [24]
The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl 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.

2.6.3.3 Phenylalanine ammonia-lyase (PAL) (Ross and Sederoff, 1992) [33].
The assay mixture containing 100 µl of enzyme, 500 µl of 50 mm Tris HCl (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 ηm 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 η moles of cinnamic acid min^{-1} g fresh $tissue^{-1}$.

2.6.3.4 β-1, 3-glucanase (Pan *et al*., 1991) [31].
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 dinitro salicylic 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 µm. The crude extract preparation mixed with laminar in at zero time incubation served as blank. The enzyme activity was expressed as µg equivalents of glucose min^{-1} g fresh $weight^{-1}$.

3. Results

3.1 Survey of Sheath blight disease incidence

The data presented in Table 1 on the survey in different locations in Cuddalore revealed the prevalence of sheath blight disease in all the villages. Among the different locations of Cuddalore surveyed for sheath blight disease incidence, Kulakudi (Rs5) registered a maximum incidence of the disease (18%) followed by Maruthur (Rs10) with (16%). The other locations *viz.,* Boothangudi (6%), Kannangudi (6%), Kiliyanur (4%) and Rayanallur (4%) had lesser diseases incidence.

Table 1: Survey of disease incidence of rice sheath blight in different locality of Cuddalore

S. No.	Locality	Crop stage	Variety	Disease incidence (%)
1.	Rs1 – Bhuvanagiri	Panicle initiation	ADT-36	13[c]
2.	Rs 2 –Sathapadi	Panicle initiation	ADT-36	9[f]
3.	Rs 3 – Manaveli	Grain filling	ADT-43	8[g]
4.	Rs 4 – Kundiyamallur	Panicle initiation	ADT-36	11[d]
5.	Rs 5 – kulakudi	Panicle initiation	ADT-36	18[a]
6.	Rs 6 –Puthur	Panicle initiation	ADT-36	13[c]
7.	Rs 7 – Boothangudi	Grain filling	ADT-36	6[h]
8.	Rs 8 – Rayanallur	Grain filling	ADT-43	4[i]
9.	Rs 9 – Vazhakollai	Grain filling	ADT-36	10[c]
10.	Rs 10 – Maruthur	Panicle initiation	ADT-43	16[b]
11.	Rs 11 – kalkunam	Panicle initiation	ADT-43	13[c]
12.	Rs 12 – Orathur	Grain filling	ADT-43	7[g]
13.	Rs 13 – Kumudimoolai	Panicle initiation	ADT-43	9[f]
14.	Rs 14 – Kiliyanur	Panicle initiation	ADT-36	4[i]
15.	Rs 15 – Vilakam	Panicle initiation	ADT-36	15[b]
16.	Rs 16 – Pannapattu	Grain filling	ADT-43	6[h]
17.	Rs 17 – Sakkangudi	Panicle initiation	ADT-43	9[f]
18.	Rs 18 – Kurinjipadi	Grain filling	ADT-43	12[d]
19.	Rs 19 – Adoor	Panicle initiation	ADT-36	8[g]
20.	Rs 20 – Kannangudi	Grain filling	ADT-43	6[h]

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

3.2 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.

3.2.1 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 *Dictyota dichotoma* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and soil application of *Bacillus subtilis* followed by challenge inoculated with *R. solani* (T_8) recorded a maximum induction of β-1,3-glucanase activity 232 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 Hexaconazole (seed treatment, prophylactic spray at 20, 35 and 50 DAT) (T_9) recorded 229.3µg of Glucose released/ min/g of fresh tissue on 5th day after pathogen inoculation. The enzyme activity significantly increased up to 5th day from the pathogen inoculation and then declined slowly in all the treatments (Fig.1).

3.2.2 Peroxidase (PO)

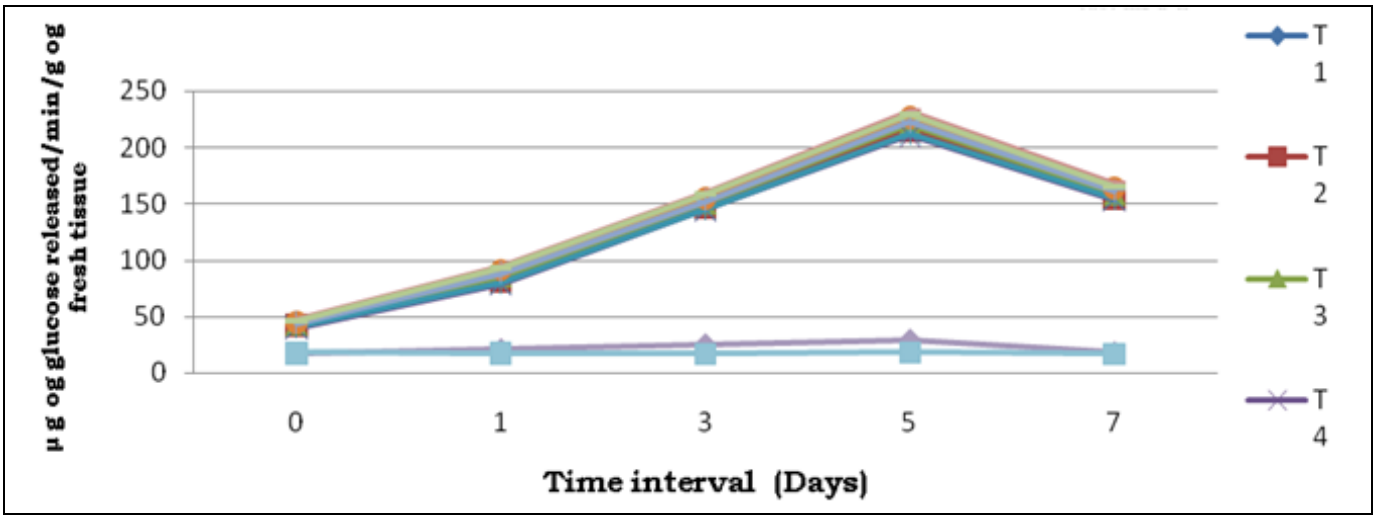
The activity of PO was observed in leaf sample of rice at different days interval. Among the various treatment, the plants treated with *Dictyota dichotoma* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and soil application of *Bacillus subtilis* followed by challenge inoculated with *R. solani* (T_8) recorded maximum induction of Peroxidase activity (60.34 changes in absorbance/min/g of fresh tissue) at 7th day after pathogen inoculation. It was followed by the plants treated with Hexaconazole (seed treatment, prophylactic spray at 20, 35 and 50 DAT) (T_9) recorded a maximum induction of 59.20 changes in absorbance/ min/g of fresh tissue respectively at the 7th day after pathogen inoculation. The enzyme activity significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Fig. 2).

3.2.3 Phenylalanine ammonia lyase (PAL)

PAL activity was found to increase significantly in plants treated with *Dictyota dichotoma* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and soil application of *Bacillus subtilis* followed by challenge inoculated with *R. solani* (T_8). Maximum induction of PAL activity (5.21 changes in absorbance/min/g of fresh tissue) at 7th day there after it decreased. It was followed by the application of Hexaconazole (seed treatment, prophylactic spray at 20, 35 and 50 DAT) (T_9) recorded a maximum induction at the 7th day of 5.17 changes in absorbance/min/g of fresh tissue respectively. The enzyme activity significantly increased up to 7th day from the pathogen inoculation and then declined slowly in all the treatments (Fig.3).

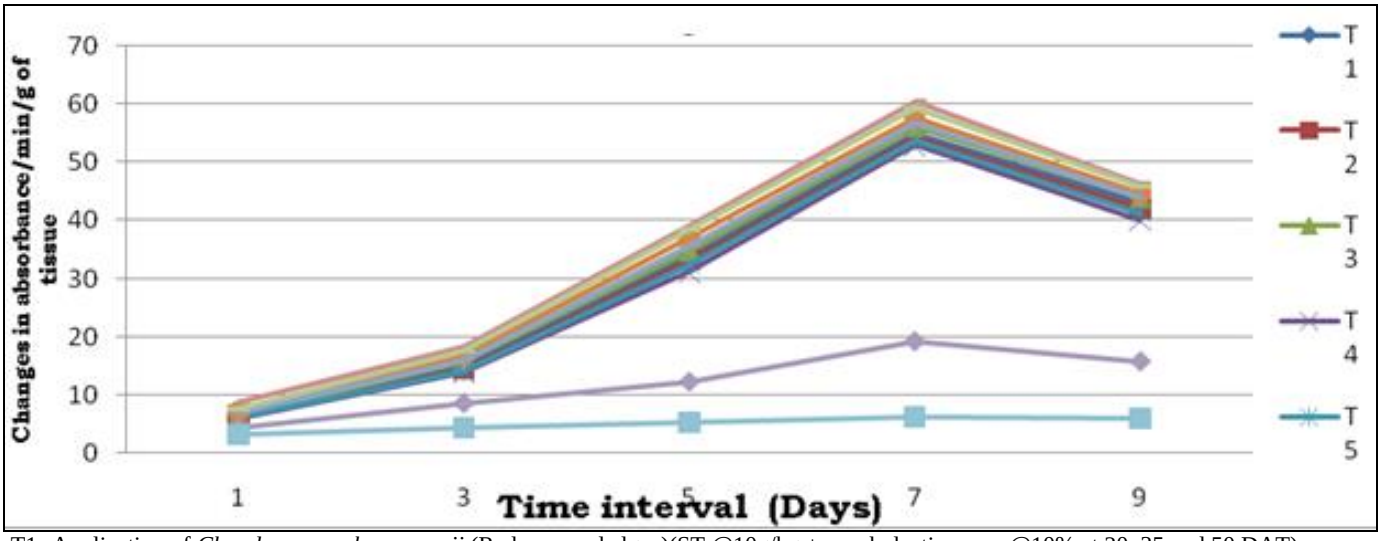
3.2.4 Polyphenol oxidase (PPO)

Application of *Dictyota dichotoma* (seed treatment, prophylactic spraying at 20, 35 and 50 DAT) and *Bacillus subtilis* followed by challenge inoculated with *R. solani* (T_8) recorded maximum induction of PPO activity (3.73 changes in absorbance/min/g of fresh tissue) at 7th day, which decreased further. Without inoculation of pathogen and IDM 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 (Fig. 4).



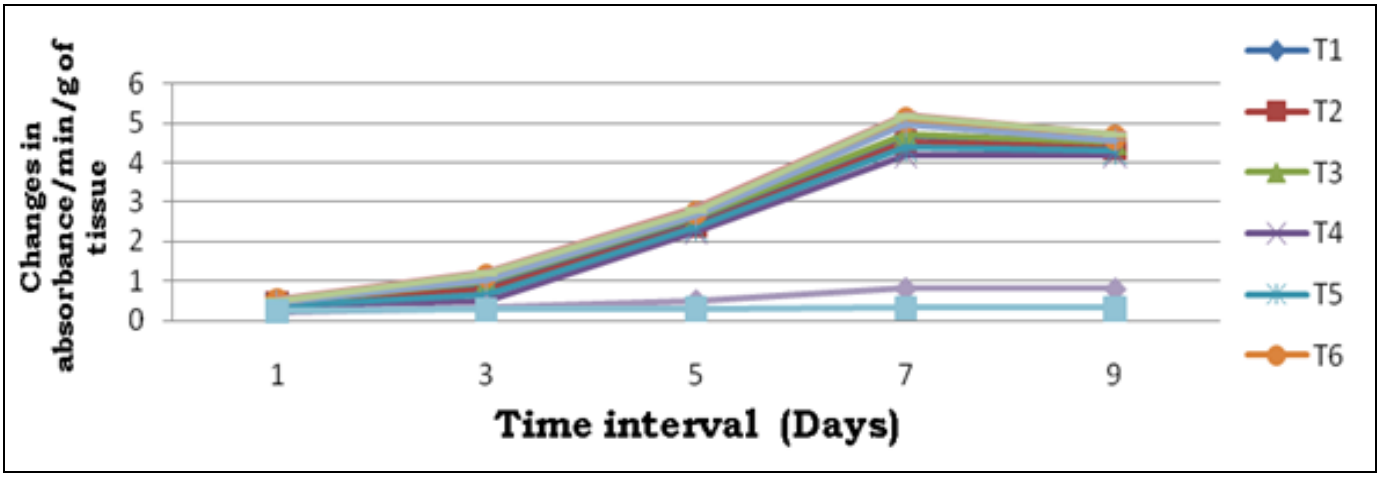
T_1- Application of *Chondrococcus hornemanii* (Red seaweed algae)(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)
T_2- Application of *Caulerpa sealpelliformis* (Green seaweed algae)(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)
T_3- Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_4- Application of *Avicenia mariena* (Marine leaf)(ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_5- Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_6 -T_1 + T_5
T_7-T_2 + T_5
T_8- T_3 + T_5
T_9- ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)
T_{10}-Inoculated control T_{11} -Healthy control

Fig 1: B-1, 3 Glucanase activity* in rice plates treated with different IDM formulation under greenhouse condition



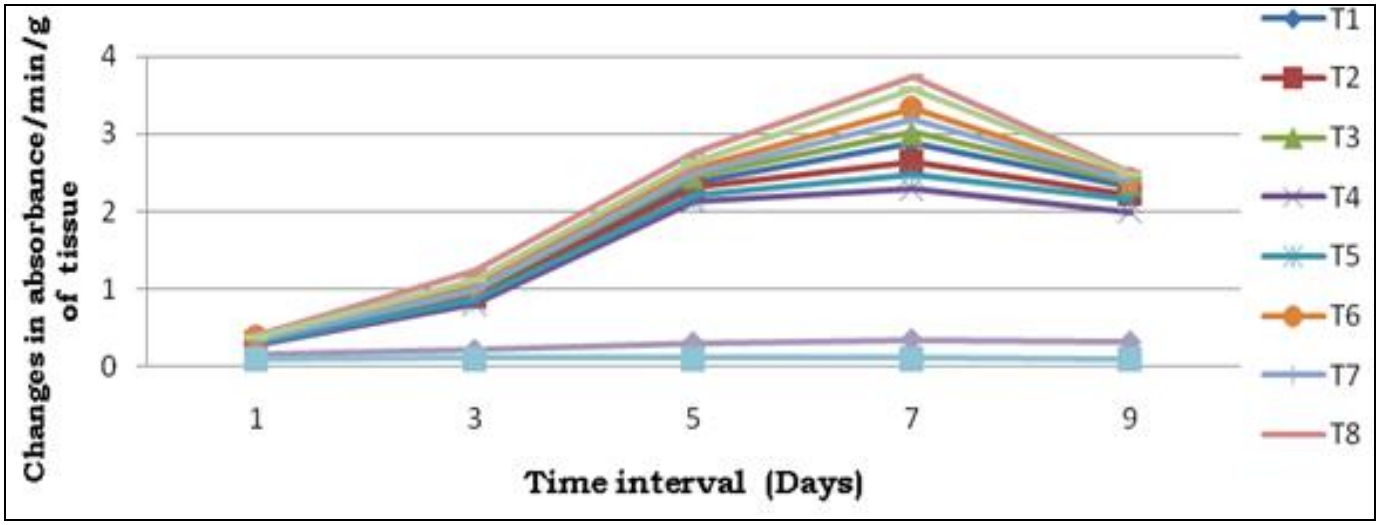
T1- Application of *Chondrococcus hornemanii* (Red seaweed algae)(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)
T2- Application of *Caulerpa sealpelliformis* (Green seaweed algae)(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)
T3- Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T4- Application of *Avicenia mariena* (Marine leaf)(ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T5- Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T6 -T1 + T5
T7-T2 + T5
T8- T3 + T5
T9- ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)
T10-Inoculated control T11 -Healthy control

Fig 2: Peroxidase activity* in rice plates treated with different IDM formulations under greenhouse condition



T_1- Application of *Chondrococcus hornemanii* (Red seaweed algae)(ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)
T_2- Application of *Caulerpa sealpelliformis* (Green seaweed algae)(ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)
T_3- Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_4- Application of *Avicenia mariena* (Marine leaf)(ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_5- Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_6 -T_1 + T_5
T_7-T_2 + T_5
T_8- T_3 + T_5
T_9- ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)
T_{10}-Inoculated control
T_{11} -Healthy control

Fig 3: Phenylalanine ammonia-lyase activity *in rice plates treated with different IDM formulations under greenhouse condition



T_1- Application of *Chondrococcus hornemanii* (Red seaweed algae) (ST @10g/kg + prophylactic spray @10% at 20, 35 and 50 DAT)
T_2- Application of *Caulerpa sealpelliformis* (Green seaweed algae) (ST @ 10g/kg + prophylactic spray @10 % at 20, 35 and 50 DAT)
T_3- Application of *Dictyota dichotoma* (Brown seaweed algae) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_4- Application of *Avicenia mariena* (Marine leaf) (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_5- Aplliction of *Bacillus subtilis* (ST @ 10g/kg + prophylactic spray @ 10 % at 20, 35 and 50 DAT)
T_6 -T_1 + T_5
T_7-T_2 + T_5
T_8- T_3 + T_5
T_9- ST with Hexaconazole (2g/kg) + spraying (0.2 per cent) 20, 35 and 50 DAT)
T_{10}-Inoculated control
T_{11} -Healthy control

Fig 4: polyphenol oxidase activity*in rice plates treated with different IDM formulation under greenhouse condition

4. Discussion

Sheath blight caused by *R. solani* is one of the most important fungal diseases affecting rice plant and assuming greater importance all over the rice growing areas. In India, the yield loss due to the disease is in a greater percentage.

4.1 Survey of the disease incidence

Variations in incidence and intensities of the disease occurring in the different conventional rice growing areas of Cuddalore were observed. Intensive rice cultivating areas like Kulakudi and Maruthur recorded a maximum disease intensity. Available literature (Jia *et al.*, 2012 and Prasanna Reddy *et al.*, 2012) [18, 32] revealed that the amount of crop and yield loss (5-60 per cent) by the disease varied from place to place because of the existence of different races, biotypes of strains of the pathogen. So, the incidence and severity of

sheath blight in rice differed in different locations of Cuddalore.

4.2 Evaluation of seaweed extract against *R. solani*

Generally, all marine products inhibited the mycelial growth of pathogen in the present study. Of which, *Dictyota dichotoma* [Brown seaweed algae], *Chondrococcus hornemanii* [Red seaweed algae], *Caulerpa sealpelliformis* [Green seaweed algae] @ 20% exhibited the highest level of inhibition of *R. solani*. This statement has been confirmed by several workers. Sultana *et al*., (2007) [40], reported that brown, green and red seaweeds were highly effective against *R. solani in vitro* and *in vivo* conditions. There are several workers which have been reported on the efficacy of seaweed extracts against fungal pathogens (Norrie *et al.*, 2002; Jayaraj *et al.*, 2008) [28, 17]. The brown seaweeds show high antifungal activity as compared to red and green algae. The brown seaweeds contain high amount of flavonoid and phenolic compounds which could be the reason for antifungal activity (Ambika and Sujatha, 2014) [1]. This may be due to higher levels and early accumulation of phenolics and phytoalexins (Garcia-Mina *et al.*, 2004) [9, 10]. The above results lends supports to the present findings.

4.3 Induction of defense mechanism

Plants are bestowed with various defense related genes and it is well known that the defense genes are sleeping genes and appropriate stimuli or signals are needed to activate them. Inducing the plants own defense mechanisms by prior application of biological inducer is thought to be a novel plant protection strategy. Inductions of systemic resistance by marine products have been reported earlier by several workers (Jayaraj *et al.*, 2008; Flora and Maria Victorial rani, 2012) [17, 8].

In the present study, rice plants pretreated with *Dictyota dichotoma* (seed, prophylactic spraying at 20, 35 and 59 DAT) and soil application of *Bacillus subtilis* (T_8) significantly induced the synthesis and accumulation of β-1,3-glucanase, PO, PPO and PAL against *R. solani* when compared to all the other treatments.

The enzyme activity significantly increased from 7th day after the pathogen inoculation and then declined in all the treatments. Similar results were shown by Jayaraj *et al.*, 2008 [17], who reported that, *Ascophyllum nodosum* + chlorothalonil treatment recorded a maximum induction of PO, PPO, PAL, β-1,3-glucanase, and total phenols than the application of *A. nodosum* alone in carrot against *Alternaria* leaf spot disease.

One application of extract at 0.8 or 1.6 l ha^{-1} stimulated PO activity and two applications caused an eight-fold increase in PO activity. The treated leaves accumulated highest capsidiol (a phytoalexin in peppers) concentrations when compared to the control (Lizzi *et al*., 1998) [21]. Incorporation of *A. nodosum* extract into the planting medium caused a delayed and reduced incidence of Verticillium wilt of pepper plants. These plants also contained higher levels and early accumulation of phenolics (Garcia-Mina *et al.*, 2004) [9, 10].

Activity of certain defense – related enzymes, including peroxidase, polyphenyl oxidase, phenylalanine ammonia-lyase, chitinase and ß- 1,3- glucanase were significantly increased in plants treated with seaweed (Jayaraj *et al.*, 2008, Manoj Kumar Solanki *et al*., 2012) [17, 22]

PAL contents confirming their resistance mode. Ghosal *et al*., (2004) [11] also reported that triggering of PAL and successive increase in phenol contents were considered to be the key enzymes associated with resistance mechanism in plants. These compounds act as barriers against pathogen invasion and hence constitute part of host resistance mechanisms. Both POD and PPO were considered to be the key enzymes in plant defense system. Increased activity of PAL, POD and PPO and also antioxidant enzyme CAT was observed (Srideepthi *et al.*, 2017) [36]. Increase in PO and PPO activity at a later stage may contribute to cross linking of hydroxyl proline rich glycoproteins (HRGPs), lignifications that will act as barriers against pathogen entry. PO-generated hydrogen peroxide may function as an anti-fungal agent in disease resistance. Hydrogen peroxide inhibits pathogens directly or it may generate other free radicals that are antimicrobial in nature (Chen *et al.*, 2000) [4]. The phenomenon of h free-proline accumulation in plants exposed to diverse environmental and biological stresses have considerable physiological significance.

5. References

1. Ambika S, Sujatha K. Comparative studies on brown, red and green alga seaweed extracts for their antifungal activity against *Fusarium oxysporum* f. sp. *udum* in Pigeon pea var. CO (Rg)7 (*Cajanus cajan* (L.) Mills.). Journal of Biopesticides. 2014; 7(2):167-176.
2. Anees M, Edel-Hermann V, Steinberg C. Buildup of patches caused by *Rhizoctonia solani*. Soil Biology and Biochemistry. 2010; 42:1661-1672.
3. Bonmann JM, Khush GS, Nelson RJ. Breeding rice for resistance to pests. Annual Review of Phytopathology. 1992; 30:507-517.
4. Chen C, Belanger RR, Benhamou N, Paulitz T. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiology and Molecular Plant Pathology. 2000; 56:13-23.
5. Constabel CP, Bergey DR, Ryan CA. System inactivates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadeconoid defence signaling pathway. Proceeding National Academy of Science, USA. 1995; 92:407-412.
6. Damicone JP, Patel MV, Moore WF. Density of sclerotia of *Rhizoctonia solani* and incidence of sheath blight in Mississpi. Plant Dis. 1993; 77:257-260.
7. Demirel Z, Yılmaz-Koz FF, Karabay-Yavasoglu NU, Ozdemir G, Sukatar A. Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. J Serb. Chem. Soc. 2009; 74:619-628.
8. Flora G, Maria Victoria Rani S. An approach towards control of blast by foliar application of seaweed concentrates. Science Research Reporter. 2012; 2(3):213-217.
9. Garcia-Mina JM, Antolin MC, Sanchez-Diaz M. Metal humic complexes and plant Micro-nutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant and Soil. 2004; 258:57-68.
10. Garcia-Mina JM, Goicoechea N, Aguirreolea J. Alleviation of *Verticillium* wilt in pepper (*Capsicum annuum* L.) by using the organic amendment COAH of natural origin. Sci. Hortic. 2004; 101:23-37.
11. Ghosal TK, Dutta S, Senapati SK, Deb DC. Role of phenol contents in legume seeds and its effect on the biology of *Callosobruchus chinensis.* Ann plant prot. Sci. 2004; 12:442-4.
12. Hammerschmidt R, Nuckles EM, Kuc J. Association of

enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium.* Physiological Plant Pathology. 1982; 20:73-80.
13. Han YP, Xing Y, Gu S, Chen Z, Pan X. Effect of morphological, traits on sheath blight resistance in rice. Acta. Botanica. Sinica. 2003; 45:825-831.
14. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M. *Trichoderma* species – opportunistic, avirulent plant symbionts. Nature Reviews Microbiology. 2004; 2:43-56.
15. Hossain M, Pingali PL. Rice research technological progress, and impact on productivity and poverty: an overview. In Pingali, P.L. and Hossain, M. eds. Impact of rice research. Los Baños, Philippines, IRRI, 1998.
16. Howell CR. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases. The History and evolution of current concepts. Plant Dis. 2003; 87:4-10.
17. Jayaraj J, Wan A, Rahman M, Punja ZK. Seaweed extract reduces foliar fungal disease on carrot. Crop Protection. 2008; 27:1360-1366.
18. Jia L, Yan W, Zhu C, Agrama HA, Jackson A. Allelic analysis of sheath blight resistance with association mapping in rice. Plos one. 2012; 7(3):e-32703.
19. Kauffimann S, Legrand M, Geoffroy P, Fritig B. Biological function of pathogenesis related proteins: four PR proteins of tobacco have β-1,3-glucanase activity. Emboj. 1987; 6:3209-3212.
20. Lamb C, Zhu Q, Dabi T, Zhong J, Potnis A. Emerging strategies for engineering enhanced resistance and yield in rice. In: Proc Rockefeller foundation international programme on rice Biotech. Malacca, Malaysia, 1997, 166-167.
21. Lizzi Y, Coulomb C, Polian C, Coulomb PJ, Coulomb PO. Seaweed and mildew: Laboratory tests have produced encouraging results. Phytoma. 1998; 508:29-30.
22. Manoj Kumar Solanki, Sudheer Kumar, Akhilesh Kumar Pandey, Supriya Srivastava, Rajesh Kumar Singh, Prem Kashyap L *et al*. Diversity and antagonistic potential of *Bacillus* spp. associated to the rhizosphere of tomato for the management of *Rhizoctonia solani*. Biocontrol Science and Technology. 2012; 22:203-217.
23. Mauch F, Mauch Mani B, Boller T. Anti-fungal hydrolases in pea tissue. Plant Physiology. 1988; 88:936-942.
24. Mayer AM, Harel E, Shaul RB. Assay of catechol oxidase, a critical comparison of methods. Phytochemistry. 1965; 5:783-789.
25. Mohiddin FA, Khan MR, Khan SM, Bhat BH. Why *Trichoderma* is considered super hero (super fungus) against the evil parasites? Plant Pathol. J. 2010; 9:92-102.
26. Nakkeeran S, Kavitha K, Chandrasekar G, Renukadevi P, Fernando WGD. Induction of plant defense compounds by *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSSBE4 in controlling damping-off of hot pepper caused by *Pythium aphanidermatum*. Biocontrol Sci. Technol. 2006; 16:403-416.
27. Nandi S, Adhikari A, Dutta S, Chatopadhaya A, Nath R. Potential effects of plant growth promoting rhizobacteria *(Pseudomonas fluorescens)* on cowpea seeding health and damping off disease control. Afr. J Biotechnol. 2013; 12(5):1853-1861.
28. Norrie J, Branson T, Kethley PE. Marine plants extract impact on grape yield and quality. Acta. Hort., (ISHS). 2002; 594:315-319.
29. Ogoshi A. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Ann. Rev. Phytopathol. 1987; 25:125-143.
30. Ou SH. Rice disease. Common wealth mycological institute, Kew, Surray, UK, 1985.
31. Pan S, Ye Q, Kuc J. Association of β – 1,3 glucanase activity and isoform pattern with systemic resistance to blue mold in tobacco induced by stem injection with *Perenospora tabacina* (or) leaf inoculation with tobacco mosaic virus. Physio. Mol. Pl. Pathol. 1991; 39:25-39.
32. Prasanna Reddy B, Jansi Rani, Reddy MS, Vijay Krishna Kumar K. *In vitro* antagonistic potential of *pseudomonas fluorescens* isolates and their metabolites against rice sheath blight pathogen, *Rhizoctonia solani*. International J of Applied Biology and Pharmaceutical Technol. 2012; 1(2):0976-4550.
33. Ross WW, Sederoff RR. Phenylalanine ammonia lyase from loblolly pine: purification of the enzyme and isolation of complementary DNA clone. Plant Physiol. 1992; 98:380-386.
34. Savary S, Mew TW. Analyzing crop losses due to *Rhizoctonia solani*: rice sheath blight, a case study. In: SnehB, Javaji-Hare S, Neate S, Dijst G (eds) *Rhizoctonia*. species: taxonomy, molecular biology, ecology, pathology and disease control, Kluwer, Dordrecht, 1996, 237-244.
35. Sneh B, Burpee L, Ogoshi A. Identification of *Rhizoctonia* species. APS Press, St. Paul, Minnesota, USA, 1991.
36. Srideepthi R, Krishna MSR, Suneetha P, Srupanika D, Bhavana SVL, Lakshmi Sahitya U *et al*. Antioxidant potential of chilli seedlings against anthracnose. International Journal of Green Pharmacy. 2017; 11(2):305-311.
37. Srinivas P, Ratan Prakash Patel, Bindu Madhavi G. Review on Banded leaf and sheath blight of rice caused by *Rhizoctonia solani* Kuhn. International Journal of Applied Biology and Pharmaceutical Technology. 2013; 61(2):80-97.
38. Srinivasachary, Willocquet L, Savary S. Resistance to rice sheath blight (*Rhizoctonia solani* Kuhn) [teleomorph: *Thanatophorus cucumeris* (A.B. Frank) Donk.] disease: current status and perspectives. Euphytica. 2011; 178:1-22.
39. Sriram S, Raguchander T, Babu S, Nandakumar R, Shanmugam V, Vidhyasekaran P *et al*. Inactivation of phytotoxin produced by the rice sheath blight pathogen *Rhizoctonia solani*. Canadian Journal of Microbiology. 2000; 46(6):520-524.
40. Sultana V, Ehteshamul-Haque S, Ara J. Management of root diseases of soybean and tomato with seaweed application. Phytopathol, 2007, 97:112.
41. Sutha SP, Venkatesan M, Arun Kumar K. Endobiotic bacteria in some seaweed to thondi costal region in palk bay Tamil Nadu India. J marinebiolo. ass. India. 2011; 53:251-256.
42. Suthin Raj T, John Christopher D, Usha Rani S. Effect of plant products on the *in vitro* production of cellulolytic and pectinolytic enzymes by *Colletotrichum capsici.* Pl. Dis. Res. 2008; 23(1):24-27.
43. Suthin Raj T, Hane Graff K, Ann Suji H. Bio Chemical Characterization of a Brown Seaweed Algae and its Efficacy on Control of Rice Sheath Blight Caused by *Rhizoctonia Solani* Kuhn. International Journal of Tropical Agriculture. 2016a; 34(2):429-439.
44. Suthin Raj T, Hane Graff K, Arumuka Pravin A, Ann Suji

H. Effect of various green seaweed extracts on controlling sheath blight in rice caused by *Rhizoctonia solani* under *in vitro* condition. International Journal of Tropical Agriculture. 2016b; 34(7):2253-2263.
45. Tewari AK, Mukhopadhyay AN. Testing of different formulation of *Gliocladium virens* against chickpea wilt-complex. Indian phytopathology. 2001; 54:64-71.
46. Vallinayagam K, Arumugam R, Kannan RR, Thirumaran G, Anantharaman P. Antibacterial activity of some selected seaweeds from pudumadam coastal region. Glob. J Pharmacol. 2009; 3:50-52.
47. Venkatesh, Jae-Woong Yu, Se Won Park. Genome-wide analysis and expression profiling of the *Solanum tuberosum aquaporins.* Physiology and Biochemistry, 2013, 392-404.
48. Wu W, Huang J, Cui K, Nie L, Wang Q. Sheath blight reduces stem breaking resistance and increases lodging susceptibility of rice plants. Field Crops Research. 2012; 128:101-108.