Studies on anti-fungal activity of different macro algal extracts against soil borne pathogen *Sclerotium rolfsii* Sacc.

Abstract

Management of soil-borne plant pathogens is one of the single greatest challenges facing modern agriculture worldwide. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. The seaweeds derived compounds showing broad range of biological activities such as antiviral, antibacterial and antifungal activities. The antimicrobial activity of macro algal extracts is generally analysed using various organic solvents which always present higher efficiency in extracting bioactive compounds. Among the different the solvent extracted macro algal extracts tested, methanol extracts of *Sargassum wightii* recorded maximum mycelial inhibition (88.22 %) and on par with aqueous extracts recorded 66.44 % of mycelial inhibition @ 10% concentration compared to other macro algal extracts.. Among the fifteen macro algal extracts, maximum inhibition of mycelial growth was recorded the *Sargassum wightii* (Brown algae), *Ulva lactuca* (Green algae) and *Gracilaria salicornia* (red algae) at different concentrations. In this study, we have shown that *Sargassum wightii* has antimicrobial potential against the *S. rolfsii*. Methanol extract was found to be the best solvent for retention of the antimicrobial activity.

Keywords:Sclerotium rolfsii, Macro algae, Sargassum wightii, Anti-fungal activity, Methanol extracts, Chloroform extracts, Aqueous extracts

1. INTRODUCTION

*Sclerotium rolfsii*Sacc. (Teleomorph: *Athelia rolfsii*) is an omnivorous, necrotrophic fungal plant pathogen which has a wide host range of over 500 plant species in 100 families, throughout the globe. This pathogen produce various enzymes viz., phytotoxin (oxalic acid), cell wall degrading enzymes and polysaccharides degrading multiplex enzymes which are affects thehost plant system [2].The typical symptom of this pathogenis rapid wilting and sickly appearance of plants with brownish lesion at the stem base near the soil lane which later girdles the stem. The whitish mycelial growth forms over the infected tissue and often radiates over the soil surface [7].*S. rolfsii* typically produces abundant white mycelium and small, brown, round sclerotia on the diseased tissue under hot humid conditions [9]. Sclerotia are the primary source for the infection which survives in soil for the several years in dormant stage. These sclerotia infect suitable host and left over in plant residues [19].

Seaweeds or “Marine algae” or “Marine macro algae” are the excellent source for plant disease management. These macro algae categorized into three groups based on the presence of pigments such as Green algae (Chlorophyta), Red algae (Rhodophyta) and Brown algae (Phaeophyta) which comprise nearly 9,000 algal species all over the world [3]. Seaweeds contain elaborate secondary metabolites that play a significant role in the host defence against various plant pathogens and parasites. These bioactive compounds (40000 and above) as secondary metabolites like Polysaccharides, polyphenols, carotenoids, proteins, peptides, sterols, terphenes and fatty acids are the main components of macro algal species which induce disease resistance in crop plants [17, 15]. Different organic solvents of the algal extracts could provide a potential tool to explore the bioactive compounds responsible for positive effects on plant pathogens and mechanisms of their action [11]. The present investigation was carried out to analyse the efficiency of various macro algal extracts in the management of *S. rolfsii* under in vitro condition.

2. MATERIALS AND METHODS

2.1 Isolation of pathogen

The pathogen was isolated from the diseased plants showing typical symptoms of groundnut stem rot disease by tissue segment method [11]. The diseased portion of the stem was cut into small bits, surface sterilized with 0.1 per cent NaOCl2 solution for 30 seconds washed in repeated changes of sterile distilled water. Potato Dextrose Agar medium was prepared and poured @15ml into sterilized Petri dishes. The Petri dishes were incubated in room temperature @ 28±2°C for five days and were observed for the fungal growth. The isolated fungal culture was purified by single hyphal tip method [18]. The purified culture were identified as *Sclerotium rolfsii* based on morphological and colony characteristics such as mycelial growth, mycelial dispersion, sclerotial germination, sclerotial shape, colour, number and arrangement of sclerotia on surface media.

2.2 Collection of Marine macro algae

Fifteen Marine macro algae were collected from east coastal areas of Tamil Nadu (Table 1). The collected materials were washed in fresh water to remove the debris, sand and extraneous matter. After draining off the water, the macro algae were wiped with a blotting sheet and air-dried under shade for two weeks, then cut into small pieces and dried in an oven at 45°C for 24 hours. The completely dried material was weighed and ground finely in a mechanical grinder. The powdered sample was stored in freezer for further study [1].

Table 1 Macro algae collected from various seashore areas

Sl. No	Marine Macro algae(Seaweeds)	Common name orGroupofMacroAlgae	Placeofcollection
1	*Dictyotadichotoma*	Brownalgae	Kanyakumari
2	*Sargassumwightii*	Brownalgae	Pamban
3	*Padina gymnospora*	Brownalgae	Pamban
4	*Hydroclathrushornemanii*	Brownalgae	Pamban
5	*Turbinariaconiodes*	Brownalgae	Rameshwaram
6	*Caulerpascalpelliformis*	Greenalgae	Pamban
7	*Chaetomorphaantennina*	Greenalgae	Mandapam
8	*Enteromorphaintestinalis*	Greenalgae	Velankanni
9	*Ulvalactuca*	Greenalgae	Rameshwaram
10	*Halimedagracilis*	Greenalgae	Puducherry
11	*Acanthophoraspicifera*	Red algae	Mandapam
12	*Gracilariasalicornia*	Red algae	Pamban
13	*Janiarubens*	Red algae	Puducherry
14	*Kappaphycusalvarezii*	Red algae	Mandapam
15	*Hypneamusciformis*	Red algae	Mandapam

2.3 Preparation of Aqueous extracts of Marine macro algae

100 grams of powdered macro algae was mixed with 100ml of distilled water and autoclaved at 150 lbs pressure for 1 h. The mixture extracts were filtered immediately through a muslin cloth. All the extracts were labelled and stored in separate bottles which were kept in a refrigerator. The extracts thus obtained were crude macro algal extracts. Crude extract of different concentration was prepared with distilled water for further studies.

2.4 Preparation of Solvent extracts of Marine macro algae

Solvent extracts of marine macro algae was prepared by using soxhlet extractor. 20 grams of powdered macro algae was poured in the thimble and extracted successively with each 100 ml of two different solvents viz., Methanol and Chloroform. The extracts were filtered using a Millipore filter unit 0.45 µm pore size, kept in -20^{o} C in airtight brown bottles for further studies [8].

2.5 Poison food technique (Groover and Moore, 1962)

Potato dextrose agar media amended with macro algal extracts at different concentrations viz., 2.5%, 5% and 10% were autoclaved and poured into sterile Petri dishes. Then the plates were inoculated with five days old culture disc of Sclerotium rolfsii isolate (SrALR). Three replications were maintained in each treatment and incubated at laboratory temperature 28 ± 2^0C. Potato dextrose agar media without macro algal extract served as control. The diameter of the mycelial growth was measured in 5 days after incubation (DAI). The per cent inhibition of the test fungi was calculated by the formula of Vincent (17)

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Percentinhibition offungalgrowth

C = Growth in Control

T = Growthintreatment

3. RESULTS

3.1 Antifungal activity of macro algal extracts against *Sclerotium rolfsii* (SrALR)

Fifteen different brown, green and red macro algal extracts were selected and evaluate the antifungal activity against *S. rolfsii* by Poison food technique. Among the different macro algal extracts were tested, *Sargassum wightii, Ulva lactuca* and *Gracilaria salicornia* showed effective antifungal activity against the *S. rolfsii*.The brown algae Sargassum wightii significantly reduced the mycelial growth (1.12 cm) of *S. rolfsii* and recorded highest mycelial inhibition 87.56% at 10% concentration (Table 2& Plate 1). The green algae Ulva lactuca showed maximum reduction in mycelial growth 1.39 cm with highest mycelial inhibition (84.56%) of test pathogen at 10% concentration (Table 3& Plate 2). Likewise, the red algae Gracilaria salicornia effectively inhibit the radial growth (2.08 cm) of *S. rolfsii* with 76.89% of mycelial inhibition at 10% concentration (Table 4&Plate 3).

Table2*In vitro* EvaluationofBrown macro algalextract against*Sclerotiumrolfsii*(SrALR)

S.No	Treatment	Mycelial growth (cm) (5DAI)					
		2.5%	Percentinhibitionovercontrol	5%	Percentinhibitionovercontrol	10%	Percentinhibitionovercontrol
1	*Dictyotadichotoma*	4.53^{c}(12.29)	49.67	3.92^{d}(11.42)	56.45	3.46^{d}(10.71)	61.56
2	*Sargassumwightii*	2.86^{a}(9.74)	68.23	1.39^{a}(6.77)	84.56	1.12^{a}(6.08)	87.56
3	*Padina gymnospora*	3.19^{b}(10.28)	64.56	2.36^{b}(8.84)	73.78	1.43^{b}(6.87)	84.12
4	*Hydroclathrus hornemanii*	5.69^{d}(13.80)	36.78	5.02^{e}(12.94)	44.23	4.79^{e}(12.64)	46.78
5	*Turbinariaconiodes*	3.40^{b}(10.62)	62.22	2.96^{c}(9.91)	67.12	2.10^{c}(8.33)	76.67
6	Control		9.00				

Meanofthreereplications
Valuesinthecolumnfollowedby samesuperscriptletters donotdiffersignificantlyat5%levelbyDuncan'smultiplerangetest(DMRT)

Plate 1 *In vitro* Evaluation of *Sargassum wightii* (Brown macro algalextract)against*Sclerotium rolfsii*(SrALR)

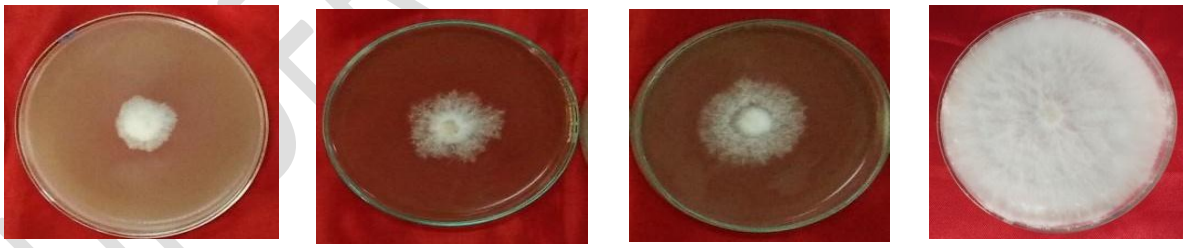
10%Conc. **5%Conc.** **2.5%Conc.** **Control**

Table3*In vitro* EvaluationofGreen macro algalextract against*Sclerotiumrolfsii*(SrALR)

S.No	Treatment	Mycelialgrowth(cm)(5DAI)					
		2.5%	Percent inhibitionover control	5%	Percentinhibitionovercontrol	10%	Percent inhibitionover control
1	*Caulerpascalpelliformis*	5.89^{e}(14.05)	34.56	5.63^{d}(13.72)	37.45	5.00^{e}(12.92)	44.44
2	*Chaetomorphaantennina*	5.26^{d}(13.26)	41.56	4.91^{c}(12.80)	45.67	4.53^{d}(12.29)	49.67
3	*Enteromorpha intestinalis*	4.10^{b}(11.67)	54.45	3.62^{b}(10.97)	59.78	2.86^{b}(9.73)	68.23
4	*Ulvalactuca*	3.39^{a}(10.61)	62.33	2.48^{a}(9.05)	72.45	1.39^{a}(6.77)	84.56
5	*Halimedagracilis*	4.47^{c}(12.21)	50.32	3.86^{b}(11.33)	57.12	3.13^{c}(10.19)	65.23
6	Control		9.00				

Meanofthreereplications
Valuesinthecolumnfollowedby samesuperscriptletters donotdiffersignificantlyat5%levelbyDuncan'smultiplerangetest(DMRT)

Plate 2 *In vitro* Evaluation of *Ulva lactuca* (Green macro algal extract)against*Sclerotium rolfsii*(SrALR)



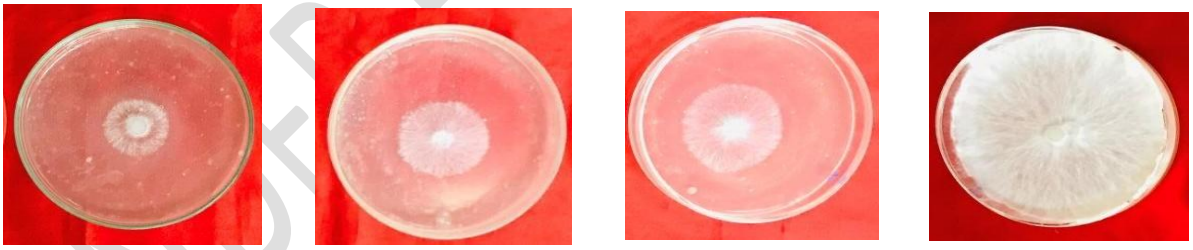
10%Conc. **5%Conc.** **2.5%Conc.** **Control**

Table4*In vitro* EvaluationofRed macro algalextract against*Sclerotiumrolfsii*(SrALR)

S.No	Treatment	Mycelialgrowth(cm)(5DAI)					
		2.5%	Percentinhibitionovercontrol	5%	Percentinhibitionovercontrol	10%	Percentinhibitionovercontrol
1	*Acanthophoraspicifera*	4.87[b](12.74)	45.89	4.42[d](12.14)	50.89	3.91[d](11.41)	56.56
2	*Gracilariasalicornia*	4.02[a](11.57)	55.34	3.37[a](10.58)	62.56	2.08[a](8.30)	76.89
3	*Janiarubens*	5.27[c](13.26)	41.45	4.87[e](12.74)	45.87	4.33[e](12.01)	51.89
4	*Kappaphycusalvarezii*	4.56[b](12.33)	49.33	3.98[c](11.51)	55.78	3.24[c](10.36)	64.00
5	*Hypneamusciformis*	4.19[a](11.81)	53.44	3.65[b](11.02)	59.45	2.78[b](9.60)	69.12
6	Control		9.00				

Meanofthreereplications
Valuesinthecolumnfollowedby samesuperscript lettersdonotdiffersignificantlyat5%levelbyDuncan'smultiplerangetest(DMRT)

Plate 3 *In vitro* Evaluation of *Gracilaria salicornia* (Red macro algalextract)against*Sclerotium rolfsii*(SrALR)



10%Conc. **5%Conc.** **2.5%Conc.** **Control**

3.2 In vitro evaluation of different solvent extracts of macro algae against *Sclerotium rolfsii* (SrALR)

Different macro algal extracts were prepared by using two different solvents viz., Methanol and Chloroform, to investigate for their efficacy compared with aqueous macro algal extracts under poison food technique (Table 5). The results revealed that methanol macro algal extracts were significantly inhibited the test pathogen of *S. rolfsii*. Among the six macro algal extracts, methanol extracts of *Sargassum wightii* recorded maximum mycelial inhibition (88.22 %) and on par with aqueous extracts recorded 66.44 % of mycelial inhibition @ 10% concentration compared to other macro algal extracts.

Table5*In vitro*evaluationofdifferent solventextractsof macroalgaeagainst *Sclerotium rolfsii*(SrALR)

S.No	Treatment	Mycelialgrowth(cm)(5DAI)								
		Aqueousextract			Methanolextract			Chloroformextract		
		2.5%	5%	10%	2.5%	5%	10%	2.5%	5%	10%
1	*Sargassumwightii*	57.89	82.78	85.78	70.22	85.11	88.22	50.11	58.67	74.22
2	*Padina gymnospora*	48.22	61.11	66.44	50.78	64.44	70.33	43.11	50.00	58.56
3	*Enteromorphaintestinalis*	40.11	49.67	54.22	42.33	52.33	57.67	33.78	40.89	44.56
4	*Ulvalactuca*	46.44	70.78	74.67	66.44	73.89	78.56	48.67	56.00	65.67
5	*Gracilariasalicornia*	43.78	58.78	61.44	46.89	60.44	65.56	39.67	44.22	49.78
6	*Hypneamusciformis*	35.22	44.22	46.00	37.44	44.67	48.67	29.56	34.78	40.67
7	Control	9.00								

Meanofthreereplications
Valuesinthecolumnfollowedbysamesuperscriptlettersdonotdiffersignificantlyat5%levelbyDuncan'smultiplerangetest(DMRT)

4. Discussion

In this present study, ourfindingsareaccordancewithearlierreportsbyKarthiketal.[6],they studied efficacy of seaweed liquid fertilizer (SLF) *Turbinaria ornata* and*Ulvareticulata*againstthesoilbornepathogen*S.rolfsii*andreportedthatzoneofmycelialinhibitionwasnoticedafter72hoursofincubation.Sarkar et al. [14]and Latifehet al. [10] stated that macro algal fertilizer or seaweed liquid fertilizer from algal group viz., *Sargassum polyphyllum, Gelidiopsis* sp., *Padina tetrastomatica*, and *Gracilaria corticata*, which exhibited antagonistic effect against various plant pathogens such as *Rhizoctonia solani, Macrophomina phaseolina, Sclerotium rolfsii* and *Alternaria solani*.Sujatha et al. [16] stated that the antifungal activity of macro algal extract against soil borne pathogens in pulses. The results revealed that methanol extract of *S. myricocystum* have a strongest antifungal ability against different soil borne pathogens like *Rhizoctonia solani* and *Macrophomina phaseolina*. Rupapara et al. [13] reported that methanol extracts of *Sargassum johnstonii* showed a significant antimicrobial activity against plant pathogens due to the presence of various bioactive antimicrobial compounds viz., alkaloid, protein carbohydrates, and phenolics.Macro algae contain the different bioactive molecules in secondary metabolites such as terphenes, phenols, alkaloids, fatty acids and polysaccharides that have ability tosignificantly reduce the mycelial growth of *S. rolfsii* under in vitro conditions [5].

5. Conclusion

These studies show that the methanol solvent extraction of macro algae from *Sargassum wightii* has most effective inhibitory action against examined pathogen of *S. rolfsii*. Future researches in *S. wightii*are needed to determine their bioactive compounds and specifically investigate their anti-microbial activity against plant pathogens.

7. Reference

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