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# Effect of Antifungal Activity of Seaweed Extracts against Soil Borne Pathogens in Pulses

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***Abstract*** **– Different seaweed extracts viz., *Caulerpa racemosa* (green algae), *Sargassum myricocystum* (brown algae) *Gracilaria edulis* (red algae) were tested against the growth of root rot and seedling rot of pulses causing pathogens *Rhizoctonia solani* and *Macrophomina phaseolina* and its antifungal activity at concentrations of 10, 20 and 30% along with control by poison food technique under *in vitro* conditions. The result revealed that, the methanol extract of *Sargassum myricocystum* at 30 per cent concentration showed significant antifungal activity against soil borne pathogens followed by *Caulerpa racemosa* and *Gracilaria edulis*.It was observed that *Sargassum myricocystum*(30%), significantly inhibited growth of two pathogens which recorded the lowest mycelial growth of *R.solani* (14,26 and 40 mm) and *M.phaseolina* (23,38 and 42 mm)at 48, 72 and 96 h after incubation.**

***Keywords*** **– Seaweeds, *Sargassum Myricocystum, Rhizoctonia Solani*, *Macrophomina Phaseolina* and Antifungal Activity.**

## I. INTRODUCTION

Pulses are the basic ingredient in the diets of a vast majority of the Indian population, as they provide a perfect mix of vegetarian protein component of high biological value when supplemented with cereals. Pulses are also an excellent feed and fodder for livestock. India is the largest producer of pulses in the world, with 24% share in the global production. The important pulse crops are chickpea (48%), pigeonpea (15%), mungbean (7%), urdbean (7%), lentil (5%) and field pea (5%). The major pulse-producing states are Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Karnataka and Andhra Pradesh, which together account for about 80% of the total production. Endowed with the unique ability of biological nitrogen fixation, carbon sequestration, soil amelioration, low water requirement and capacity to withstand harsh climate, pulses have remained an integral component of sustainable crop production system since time immemorial, especially in the dry areas. They also offer good scope for crop diversification (grow profitably in relatively low-input management conditions) and intensification (short growing period). During 2009-10, the country produced 14.66 Mt of pulses from 23.00 M ha area, with an average yield of 637 kg/ha. However, despite its immense importance in sustainable agriculture its global production per hectare remained static over last three decades. The yield gap observed between the potential yield and on-farm yield is mainly due to biotic and abiotic stresses and the lack of efficient management practices. Among biotic stresses soil pathogens such as *Rhizoctonia solani* and *Macrophomina phaseolina*is well known diseases of pulses. Soil borne diseases are the most important in pulses causing heavy losses in seed yield. Most of the soil borne pathogens are difficult to control by conventional strategies such as the use of resistant cultivars and synthetic fungicides (Weller *et al.,* 2002). Hooda and Srivastava (1998) have mentioned that natural fungicides are free from environmental toxicity in comparison to synthetic compounds (Saxena *et al.,* 2005). Application of seaweed as soil amendment for the control of soil borne plant diseases has increased in recent years due to their environment friendly role. Marine algae exhibit antiviral, hypocholesterolemic, hypertensive, antibacterial, anticoagulant, antihelmintic, anticancer, antialgal, cytotoxic and antifungal activities (Saleh *et al*., 1993). Seaweed extracts are known to enhance seed germination, improve plant growth, and induce resistance to frost, fungal and insect attack and increase nutrient uptake from soil (Mohan *et al*., 1994; Venkataraman *et al*., 1993). Seaweeds contain elaborated secondary metabolites that play a significant role in the defense of the host against predators and parasites (Sultana *et al.,* 2008). Present investigation was undertaken to evaluate different seaweed extracts for their antifungal activity against *Rhizoctonia solani* and *Macrophomina phaseolina* in pulses.

## II. MATERIALS AND METHODS

### *Isolation and identification of Rhizoctonia solani and Macrophomina phaseolina*

Infected root samples of pulses were collected from farms around the Agricultural college and Research Institute, Madurai, Tamil Nadu, India. The pathogen was isolated on Potato dextrose agar (PDA) medium from diseased specimen showing typical symptoms. The plates were incubated at room temperature (28 $\pm$2 $^{o}$ C) for five days. The purified cultures were maintained on PDA slants and used for further studies.

### *Collection and Preparation of extracts*

The marine *Caulerpa racemosa* (green algae),*Sargassum myricocystum* (brown algae) *Gracilaria edulis* (red algae) collected from Mandapam coast, Tamil Nadu, India were washed with sea water initially to remove macroscopic epiphytes and sand particles finally with fresh water to remove adhering salt then shade dried for 4 - 5 days followed by oven drying at 40$^{o}$C for 24 h and powdered from which 100g powder was taken and 100 ml of alcohol was added then kept it for overnight with intermittent stirring and extracted through rotary evaporator with 40$^{o}$C and 45 rpm and seaweed extracts collected and stored in air tight container. The different concentrations were prepared by taking 10, 20, and 30 ml

of the stock preparation and dissolved in distilled water to give 10, 20, and 30 % concentrations.

*Antifungal Activity of seaweeds extracts against mycelial growth of Rhizoctonia solani and Macrophomina phaseolina*

Poisoned food technique was employed to screen the antifungal efficacy of seaweed extracts. Potato dextrose agar media amended with seaweed extracts (10%, 20% and 30%) were autoclaved and poured into sterile petriplates. Fungal disc of 5mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of *Rhizoctonia solani* and *Macrophomina phaseolina* and the discs were transferred aseptically on PDA plates poisoned with seaweed extracts. The medium without incorporating the extract served as control. The inoculated plates were incubated at 25 °C and colony diameter was measured and recorded 48, 72 and 96 h after incubation. Three plates per replication were maintained for each treatment. The experiment was repeated two times and average colony diameter was noted. The per cent inhibition of mycelial growth was calculated.

*Statistical analysis*

The statistical analysis of the experiment data was carried out by adopting the standard method as described by Gomez and Gomez (1984).

## III. Results and Discussion

*In vitro* effect of seaweed extracts on the mycelial growth of tested fungal pathogens is presented in table 1and 2. Among the extracts tested, *Sargassum myricocystum* at 30% concentration significantly inhibited the growth of test pathogens and recorded the minimal mean mycelial growth of *Rhizoctonia solani* and *Macrophomina phaseolina*(26.6 and 34 mm) with accounted of 61.44 and 58.53 per cent reduction over control followed by *Caulerpa racemosa* (30%) showed its efficacy and recorded considerable reduction in mycelial growth (52 and 56.33mm) which accounted 24.63 and 31.70 per cent respectively. *Gracilaria edulis* (30%) was least effective by recording the lowest reduction of 18.84 and 13.41 per cent over control.

The effect of seaweed extracts on mycelial growth of *Rhizoctonia solani* and *Macrophomina phaseolina* under *invitro* revealed that, *Sargassum myricocystum* (30%)was able to reduce the mycelial growthof above two pathogens at the end of the experiment at 96 h after incubation (Plate 1 and 2). The percentage reduction of mycelial growth over control at 30% concentration was 61.44 and 58.53 per cent respectively. Ara *et al.* (1996) reported that *Sargassum spp.,* and biocontrol agents significantly reduced the infection of root infecting fungi on sunflower. Comparing the rate of mycelial growth in medium amended with *Sargassum myricocystum* aqueous extract compared with that of the control. It could be concluded that, the pathogen grew freely on the control medium, establishing itself and using up the food, while on the "poisoned food" of PDA containing the seaweed extract, growth was significantly reduced. The inhibitory effects of the extracts might be due to the antifungal properties present in the seaweed. The soil amendment with seaweeds *Stokeyia indica, Padina pavonia* (brown), *Solieria robusta* (red), at 1% w/w reduced *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* infection on okra roots. *Codium iyengarii* (green) at 0.5 % w/w was effective against *F. Solani. (*Sultana*et al.,* 2005).Seaweed extracts were found beneficial in reducing disease through induction of defence enzymes and also the presence of the carbohydrate laminarin (a 1, 3β - glucan, structurally similar to fungal cell – wall components) may result in both the stimulation of fungal antagonists and elicit a plant defense response. In carrot, the application of SLF enhanced activities of chitinase, B-1-3 flucanase, polyphenol oxidase and lipoxynase which are factors regulating plant disease. Carrot plants sprayed with seaweed showed less disease due to *Alternaria* and *Botrytis* compared to Salicylic acid and the control (Jayaraj *et al.,*2008). Pepper plants treated with an extract of the marine algae *Ascophyllum* had enhanced foliar resistance to *Phytophthora capsici* (Lizzi *et al.,* 1998). Similar results were found in cucumber which showed enhanced activities of various defence-related enzymes including chitinase, B-1, 3-glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and lipoxygenase due to seaweed liquid fertilizer application (Jayaraman *et al.,* 2011). In this study, results on the seaweed extracts showed that, *Sargassum myricocystum*at 30% concentration inhibited mycelial growth of the test pathogens. This finding coincide with that of Viqar sultana *et al.*(2002) who reported that, suppression of root rotting fungi and root knot nematode of chili by a red algae *Solieria robusta* (Sultana *et al*., 2008). Seaweed extracts have been reported to increase plant resistant to pests and diseases, improve plant growth, yield and quality (Pardee *et al*., 2004).

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Table 1: Effects of seaweed extracts on mycelial growth (mm) of *Rhizoctonia solani*

| Treatments | Mycelial growth (mm) | | | | Percent inhibition over control |
|---|---|---|---|---|---|
| | Hours | | | | |
| | 48 | 72 | 96 | Mean | |
| *Gracilaria edulis* (10%) | 41 | 70 | 85 | 65 | 5.7 |
| *Gracilaria edulis* (20%) | 33 | 66 | 82 | 60 | 13.04 |
| *Gracilaria edulis* (30%) | 28 | 62 | 79 | 56 | 18.84 |
| *Caulerpa racemosa*(10%) | 35 | 61 | 80 | 59 | 14.49 |
| *Caulerpa racemosa*(20%) | 31 | 58 | 75 | 55 | 20.02 |
| *Caulerpa racemosa*(30%) | 29 | 55 | 72 | 52 | 24.63 |
| *Sargassum myricocystum*(10%) | 30 | 58 | 71 | 53 | 23.18 |
| *Sargassum myricocystum* (20%) | 29 | 55 | 68 | 51 | 26.08 |
| *Sargassum myricocystum*(30%) | 14 | 26 | 40 | 26.6 | 61.44 |
| Control | 45 | 73 | 90 | 69 | - |
| CD(0.05) | 2.34 | 6.72 | 5.51 | 4.23 | 16.24 |

Table2. Effects of seaweed extracts on mycelial growth (mm) of *M. phaseolina*

| Treatments | Mycelial growth (mm) | | | | % inhibition over control |
|---|---|---|---|---|---|
| | Hours | | | | |
| | 48 | 72 | 96 | Mean | |
| *Gracilaria edulis* (10%) | 67 | 80 | 87 | 78 | 4.8 |
| *Gracilaria edulis* (20%) | 64 | 76 | 84 | 74 | 9.7 |
| *Gracilaria edulis* (30%) | 61 | 72 | 81 | 71 | 13.41 |
| *Caulerpa racemosa* (10%) | 64 | 75 | 80 | 73 | 10.97 |
| *Caulerpa racemosa* (20%) | 60 | 70 | 77 | 69 | 15.85 |
| *Caulerpa racemosa* (30%) | 36 | 59 | 74 | 56 | 31.70 |
| *Sargassum myricocystum* (10%) | 51 | 65 | 74 | 63 | 23.17 |
| *Sargassum myricocystum* (20%) | 41 | 58 | 67 | 55 | 32.92 |
| *Sargassum myricocystum*(30%) | 23 | 38 | 42 | 34 | 58.53 |
| Control | 73 | 85 | 90 | 82 | - |
| CD(0.05) | 3.69 | 5.05 | 4.50 | 4.78 | 1.63 |

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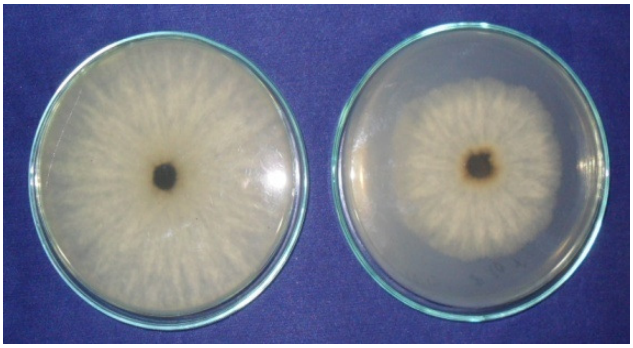
**Control** *Sargassum myricocystum* (30%)

Plate 1: Effects of sea weed extracts on mycelial growth (mm) of *R.solani* at 96 h after incubation



**Control** *Sargassum myricocystum* (30%)

Plate 2: Effects of seaweed extracts on mycelial growth (mm) of *M. phaseolina* at 96 h after incubation