Evaluation of Antifungal Potential of Macro-Algae for Controlling Leaf Spot of Tuberose (*Polianthus tuberosa* L.) Incited by *Alternaria polianthi*

ABSTRACT

Aim: To evaluate the antifungal efficacy of various seaweed extracts, particularly *Sargassum myricocystum*, against *Alternaria polianthi*, the pathogen responsible for leaf spot disease in Tuberose (*Polianthes tuberosa* L.)

Methodology: In vitro tests utilized with the dual culture plate method with Potato Dextrose Agar (PDA) medium to assess the inhibitory effects of different seaweed extracts on the mycelial growth of *A. polianthi*. Various concentrations of the extracts were tested, and their effectiveness was measured by the reduction in fungal growth. For in vivo testing, a pot culture experiment was conducted to evaluate the efficacy of these extracts on the disease incidence and growth of Tuberose plants.

Results: The results revealed that the methanol extract of *S. myricocystum* at a 5% concentration demonstrated the highest antifungal activity, significantly inhibiting the growth of *A. polianthi* by 71.34% in vitro. The in vivo application of *S. myricocystum* extract also effectively reduced the incidence of leaf spot disease and improved the overall health of the Tuberose plants. This study highlights the potential of *S. myricocystum* as a sustainable and eco-friendly biocontrol agent in managing fungal diseases in ornamental plants.

Conclusion: The present study demonstrates through the antifungal activity of brown seaweed *S. myricocystum*. The methanol extract of *S. myricocystum* at 5% concentration had immense activity against the foliar pathogen *A. polianthi* causing leaf spot in Tuberose.

1. INTRODUCTION

Tuberose (*Polianthes tuberosa L*.) is one of the most important tropical ornamental bulbous flowering plants known for its attractive and fragrant long lasting flower spikes. It is highly important to the global market [1]. It is widely grown in Karnataka, Gujarat, Tamil Nadu, Haryana, Punjab, Andhra Pradesh, Rajasthan, West Bengal, Assam, and Maharashtra in India [2]. It commercially used for garland making, Aesthetic purpose, birthday ceremony, floral arrangement such as; rangoli, bouquets, boutonnieres, Potpourri. Tuberose flowers are widely utilized in the essential oil and perfume industries [3]. Tuberose cultivation covers 18.12 thousand hectares in India, with 109.78 thousand MT of loose flowers and 91.47 thousand MT of cut flowers produced (Anonymous, 2019). In India the leading states in production are Chattisgarh followed by Assam and Madhya Pradesh. Tuberose is susceptible to many

diseases caused by fungi, bacteria and nematodes. Among fungal diseases, stem rot or tuber rot or sclerotial wilt (*Sclerotium rolfsii*), botrytis spot or blight (*Botrytis elliptica*), blossom blight (*Fusarium equiseti*) and leaf spot (*A.polianthi*) were important [4]. Among them, leaf spot incited by *A. polianthi* is an important fungal disease which reduced the economic value and yield of the plant [5].

2. Material and Methods

2.1 In vitro screening of seaweeds against pathogen causing *Alternaria* leaf spot in tuberose

Dual culture plates with PDA medium were used to study the reduction effect of the tested algae and bio inoculants against mycelial growth of the pathogen *A. polianthi* as described by reference [6]. In each plate, two wells (5 mm in diameter) were made 4cm apart. One well was inoculated with a disc (5 mm) of each pathogen (4 days-old culture). The opposite well was inoculated with each of tested algae extract @ 200 µl and bio inoculants. Plates inoculated only with each of the pathogenic fungi served as a control. All inoculated plates were incubated at 25 ± 2° C for 6 - 10 days. All plates were examined and the linear growth of the pathogens was measured. The growing cultures were observed visually and microscopically for evidence of a reduction. The bio efficacy of *S. weightii* was tested against mycelial growth of *A. polianthi* at different concentration by using well diffusion method.

2.2 Collection and isolation of pathogen

Leaves of Tuberose exhibiting typical symptoms of leaf spot, characterized by concentric rings caused by *A. polianthi* were collected for isolation of pathogens. The pathogen was isolated from the diseased tissues of Tuberose using the tissue segment method (Rangaswami, 1958). The infected portions of diseased leaves were cut into small pieces with a sterilized scalpel and surface sterilized with 0.1% mercuric chloride for 1 minute, then washed three times in sterile distilled water before being placed on PDA medium in a petridish. The plates were incubated at room temperature (28 to 32°C) for 7 days and monitored for the growth of the fungus. The hyphal tips of the growing fungus were aseptically transferred to PDA slants for maintenance of the culture. The pathogen was identified based on cultural and morphological characteristics.

2.3 Collection of seaweeds

Live and healthy specimens of seaweeds *viz, S.cristaefolium*, *Kappaphycus alvarezii*, *Gracilaria edulis, Caulorpa racemosa* and *Ulva lactuca* were collected from Mandapam coast, Tamil Nadu, India and washed in tap water. To remove any macroscopic epiphytes and extraneous matter and rinsed in distilled water to remove excess salt on the surface. The specimens were spread on blotting paper and shade-dried for 10 days at room temperature. The shade-dried seaweed samples were powdered using mixer grinder and sieved through 0.8 mm sieve plate. The powdered samples were kept in polythene bags, sealed properly and stored at room temperature until further studies (Plate 1).

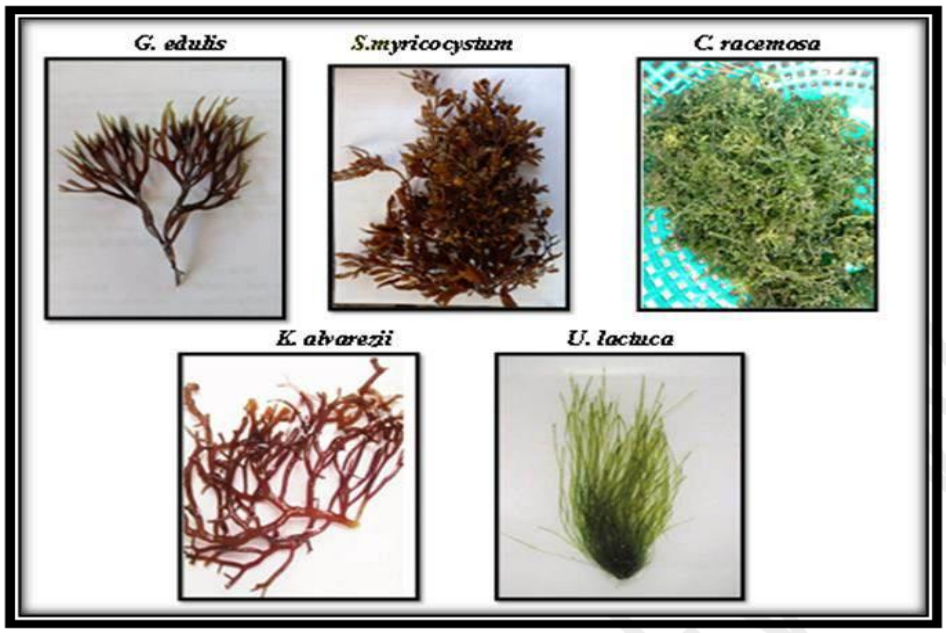

Plate 1. Collection of seaweeds

2.4 Preparation of methanol extract of *S. cristaefolium*

To prepare the seaweed extract, 20 g of partially blended seaweed powder were placed into a Soxhlet apparatus. The seaweed powder was enclosed in a cellulose thimble paper and subjected to reflux for a duration of 12 hours using methanol solvent measuring 150 ml. Following the reflux process, the extracted solvent was filtered through Whatman No.1 filter paper to eliminate any impurities. The resulting solution was then concentrated through evaporation using a rotary evaporator operating at a temperature of 40°C and a speed of 45 rpm, continuing until the solvent was completely evaporated, following the procedure as described. The final extract was diluted with the respective solvents and stored at -4°C for future use [7].

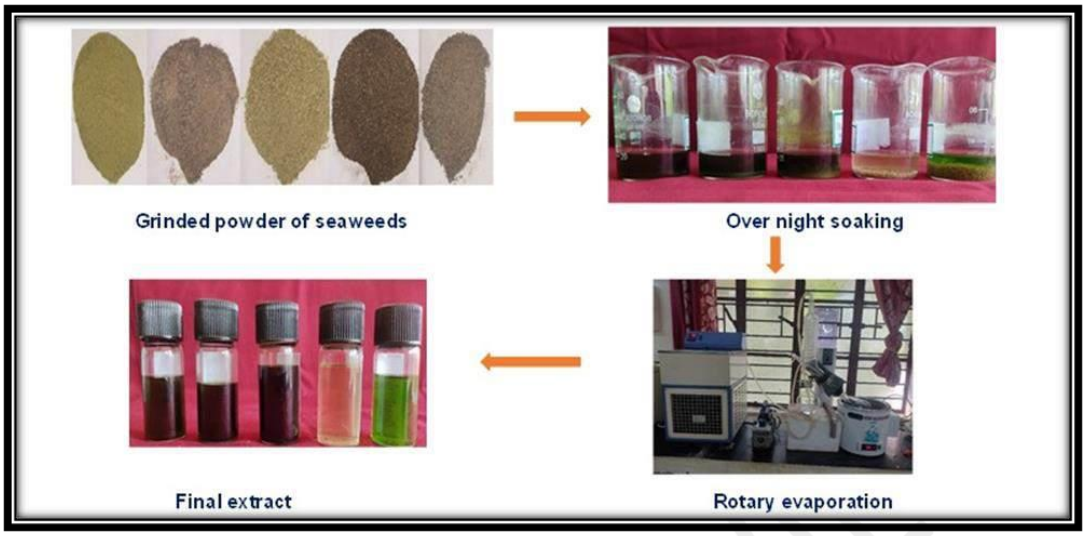

Plate 2. Seaweeds extraction by Rotary evaporation method

***2.5 In vitro* screening of seaweeds against pathogen causing *Alternaria* leaf spot in tuberose**

Dual culture plates with PDA medium was used to study the reduction effect of the tested algae and bio inoculants against mycelial growth of the pathogen *A.polianthi* as described by Dennis and Webster (1971) [6]. In each plate, two wells (5 mm in diameter) were made 4cm apart. One well was inoculated with a disc (5 mm) of each pathogen (4 days-old culture). The opposite well was inoculated with each of tested algae extract at 200 µl and bio inoculants. Plates inoculated only with each of the pathogenic fungi served as a control. All inoculated plates were incubated at 25 ± 2° C for 6 -10 days. All plates were examined and the linear growth of the pathogens was measured. The growing cultures were observed visually and microscopically for evidence of a reduction. The bio-efficacy of *S. myricocystum* was tested against mycelial growth of *A. polianthi* at different concentration by using well diffusion method. The percentage inhibition (PI) of mycelial growth was calculated using the formula suggested by reference [8].

2.6 Evaluating the efficacy of seaweed extract against onion twister blight under pot culture conditions

The experiment was conducted at the Department of Plant Pathology, Agricultural College and Research Institute, Madurai. To assess the effectiveness of the seaweed extract against *Alternaria* leaf spot in potted tuberose plants. To test the impact of bio stimulants, namely, *S. myricocystum* and *K. alvarezii* spray was performed at 45 and 60 days after planting. According to a predefined schedule, each treatment was repeated three times using a CRD. The treatment details *viz*., T_1 - Foliar spray of *K. alvarezii* (1%) 45 and 60 DAP; T2-oliar spray of *K. alvarezii* (3%) 45 and 60 DAP ; T_3 - Foliar spray of *K. alvarezii* (5%) 45 and 60 DAP T_4 - Foliar spray of *S.myricocystum* (1%) 45 and 60 DAP ; T_5 - Foliar spray of *S. myricocystum* (3%) 45 and 60 DAP ; T_6 - Foliar spray of *S. myricocystum* (5%) 45 and 60 DAP T_7 - Foliar spray of

Propiconazole 0.1%(25EC) 45 and 60 DAP(chemical check); T_8 - Foliar spray of *Bacillus subtilis*(0.1%) 45 and 60 DAP (bio control check); T_9 - control.

2.7 Statistical Analysis

To evaluate the mean differences among the treatments, an analysis of variance (ANOVA) was conducted and Duncan's Multiple Range Test at a significance level of 5% was employed [9]

3. RESULTS AND DISCUSSION

3.1 Screening of different solvents of seaweed extract at 10 % against the *A.polianthi* (*Invitro)*

The primary objective of this study was to assess the effectiveness of seaweed extracts specifically bio stimulants in combating the pathogen causing the twister blight of onion. In this study, the different solvents *viz.*, methanol, ethyl acetate, hexane and acetone were used to collect the extract from five distinct seaweeds *viz., S. cristaefolium*, *K. alvarezii*, *G. edulis,C. racemosa* and *U. lactuca*. Among the seaweed extracts examined, *S. cristaefolium* stood out by displaying an unparalleled and remarkable suppression of mycelial growth of *A.polianthi*. Among the different solvents extracts, the methanol extract of *S. cristafolium* significantly inhibited the mycelial growth (2.43 cm) which recorded the maximum growth inhibition of 73.00 per cent reduction over control, followed by methanol extract of *K. alvarezii* which exhibited the maximum inhibition of mycelial growth of 59.22 per cent reduction over control (Figure. 1; Table. 1). The Ethyl acetate extract of *U. lactuca* showed the least inhibition, recording 11.11 % reduction over control and still exhibited some degree of antifungal activity. The experimental results revealed that among various solvents of seaweed extracts, methanol extract of 10 % *S. cristaefolium* showed the least mycelial growth (2.43 cm) of the pathogen under *in vitro* conditions. In contrast, *K. alvarezii* recorded the second-highest reduction in mycelial growth with 3.67 cm and a reduction rate of 59.22 per cent. Among the five seaweed extracts, *S. cristaefolium* exhibited unparalleled and remarkable suppression of mycelial growth of *C.gloeosporioides* followed by *K. alvarezii*, as recorded by reference [10]. Ambika and Sujatha (2015) [11] reported that *S. myriocystum* extract effectively inhibited the mycelial growth of *Alternaria porri* in onion and *C. gloeosporioides* in sugarcane. According to reference [12] found that *K. alvarezii* was resistant to chilli anthracnose caused by *C. gloeosporioides*.

Table 1. *Invitro* screening of seaweeds against *Alternaria polianthi*

Seaweed extract	Mycelial growth (cm)	Percent inhibition (%)
Sargassum cristaefolium	2.67	70.33
Kappaphycus alvarezii	4.53	49.66
Gracilaria edulis	4.93	45.18
Ulva lactuca	7.12	20.88

Caulorpa racemosa	8.34	7.33
Trichoderma viride	1.56	82.66
Bacillus subtilis	1.13	87.44
Control	9.00	-
CD(*P*=0.05%)	0.89	7.79

Plate 3. Screening of seaweeds and bio control agents against *A. polianthi* (*In vitro*)

Table 2.Antifungal activity of *S.myricocystum* against *A. polianthi* at different concentration

Different concentration (%)	Mycelial growth (cm)	Per cent inhibition over control (%)
3	5.63	37.45
5	5.12	43.11
7	4.56	49.34
10	2.58	71.34
Control	9.00	-
CD(*P*=0.05%)	0.39	6.37
SEd	0.15	3.13

3.2 Evaluation on efficacy of the effective bio agents and seaweeds against *Alternaria* leaf spot of tuberose in pot culture condition

The pot culture experiment was conducted with nine treatments comprising different seaweeds and bio control agent in three replications for the management of foliar diseases of tuberose. The result showed that foliar spray of Propiconazole (25EC) at 45 and 60 days after planting (chemical check) effectively reduced the incidence of leaf spot mildew which recorded 9.54 PDI. Among the seaweeds, the result showed that the minimum disease severity of *Alternaria* leaf spot (21.65 PDI) was recorded in foliar spray of *S. myricocystum* (3%) at 45 and 60 days after planting followed by foliar spray of *S.myricocystum* (1%) at 45 and 60 days after planting which recorded 24.23 PDI (Table 3). These results were consistent with the foliar spray and root drench, respectively at 0.5 and 1 per cent of *Ascophyllum nodosum* extract, effectively reducing disease in greenhouse cucumber plants [13]. Additionally, the report of brown algae extract from *S. myriocystum* at 10 per cent reduced the mycelial growth of *C. gloeosporioides* under *in vitro* condition [11].

Table 3. Effects of seaweed extracts on *Alternaria* leaf spot of sesame under pot culture condition

Tr.No	Treatments	*Alternaria* leaf spot (PDI)
T_1	Foliar spray of *Kappaphycus alvarezii* (1%) 30 days after sowing and 10 days intervals	55.24
T_2	Foliar spray of *Kappaphycus alvarezii* (3%) 30 days after sowing and 10 days intervals	36.17
T_3	Foliar spray of *Kappaphycus alvarezii* (5%) 30 days after sowing and 10 days intervals	30.45
T_4	Foliar spray of *Sargassum myricocystum*(1%) 30 days after sowing and 10 days intervals	43.14
T_5	Foliar spray of *Sargassum myricocystum* (3%) 30 days after sowing and 10 days intervals	24.23
T_6	Foliar spray of *Sargassum myricocystum*(5%) 30 days after sowing and 10 days intervals	21.65
T_7	Foliar spray of Propiconazole0.1% (25EC)30 days after sowing and two spray at 10 days intervals (chemical check)	9.54
T_8	Foliar spray of *Bacillus subtilis* (0.1%) 30 days after sowing and two spray at 10 days intervals (bio control check)	15.27

T_9	Control (untreated)	88.11
	SEd	0.75
	CD(p=0.05)	1.15

p

4. CONCLUSION

The present study demonstrates the antifungal activity of brown seaweed *S. myricocystum* collected from Mandabam, Ramanathapuram district in Tamil Nadu. The methanol extract of *S. myricocystum* at 5% concentration had immense activity against the foliar pathogen *A. polianthi* which cause leaf spot in Tuberose. Moreover, in vivo application of *S. myricocystum* in pot culture experiment significantly decreased disease incidence of leaf spot and enhanced the growth of tuberose. The unique phyto-chemical composition of *S. myricocystum* i.e. fatty acids, saccharides and phenolic compounds could possibly be the main reason of its high antimicrobial and bio stimulant activities. These properties make *S. myricocystum* a potentially brown macroalga for disease control and safe bioinoculant for sustainable agriculture technology.

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