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

ABSTRACT

Tuberose (*Polianthes tuberosa L.)* is one of the major commercial flower crops cultivated in Tamil Nadu. Although it has been cultivated for a long time, the significant increase in productivity is yet to be achieved. It is well recognized that the disease constitutes a major constraint in increasing the flower production. The incidence of leaf spot disease caused by the fungal pathogen *Alternaria polianthi* is a severe problem that leads to reduced growth and flower yield. The occurrence of seaweeds *viz., Sargassum myricocystum* (Brown algae), *Kappaphycus alvarezii, Gracilaria edulis* (Red algae), *Caulorpa racemosa* (Green algae) and *Ulva lactuca* (Green algae) from Munakkadu, Thonithurai, and Vedhalai at Mandapam, Rameshwaram taluk where as in Chennai *Chaetomorpha antennia* were collected*. In vitro* evaluation of seaweeds revealed that methanol extract of *Sargassum myricocystum* (5%) found to be effectively inhibited the mycelial growth of *Alternaria polianthi* which showed 70.33 per cent inhibition over control. A pot culture experiment was conducted to evaluate the efficacy of seaweeds on incidence of leaf spot was revealed that foliar spray of *Sargassum myricocystum* @ 3% two spray at 45 and 60 days after planting was significantly reduced the leaf spot which recorded disease incidence of 21.65 PDI.

Keywords: Leaf spot, Seaweeds, Tuberose, Sargassum myricocystum

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 is commercially used for garland making, aesthetic purpose, birthday ceremonies, floral arrangements such as; rangoli, bouquets, boutonnieres and 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 Chhattisgarh 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 that reduced the plant's economic value and yield [5].

2. Material and Methods

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

In this study, dual culture plates with PDA medium were used to determine the algae and bioinoculants tested that affected the pathogen *A. polianthi* mycelial growth, as explained in [6]. Each plate had two wells that were 5 mm across and 4 cm apart. Each pathogen (4-day-old culture) was injected into one well using a disc (5 mm) size. Each of the tested algae extracts (200 µl) and bioinoculants were put into the well on the other side. Control plates were those that were only infected with one of the infectious fungi. All infected plates were kept at 25 ± 2° C for 6 - 10 days. All plates were evaluated and the linear growth of the pathogens was recorded. The growing cultures were watched visually and microscopically for signs of a reduction. The bio effectiveness of S. myricocystum was studied against mycelial growth of A. polianthi at different levels by using well diffusion method [8].

2.2 Collection and isolation of pathogen

Tuberose leaves with concentric rings, a typical symptom of leaf spot produced by *A. polianthi*, were collected in order to isolate the pathogens. Using the tissue segment procedure, the pathogen was isolated from the Tuberose diseased tissues (Rangaswami, 1958). After being cut into tiny pieces using a sterilized scalpel and surface sterilized for one minute with 0.1% mercuric chloride, the diseased leaves were cleaned three times in sterile distilled water and then put on PDA medium in a Petri dish. For seven days, the plates were incubated at room temperature (28 to 32°C), and the fungus's development was observed. To maintain the culture, the hyphal tips of the developing fungus were aseptically transferred to PDA slants. Based on physiological and cultural characteristics, the pathogen was identified.

2.3 Collection of seaweeds

Seaweeds such as *S. myricocystum, Kappaphycus alvarezii, Gracilaria edulis, Caulerpa racemosa*, and *Ulva lactuca* have been collected and cleaned in tap water from the Mandapam coastline in Tamil Nadu, India. The specimens were healthy and alive. Wash it in pure water to get rid of any extra salt on the surface and to get rid of any large epiphytes. These specimens were placed on blotting paper and allowed to dry at room temperature for 10 days in the shade. The samples of shade-dried seaweed were ground up in a mixing grinder and then put through a 0.8 mm sieve plate. For further study, the powdered materials were preserved at room temperature in polythene bags that had been carefully sealed. (Plate 1).

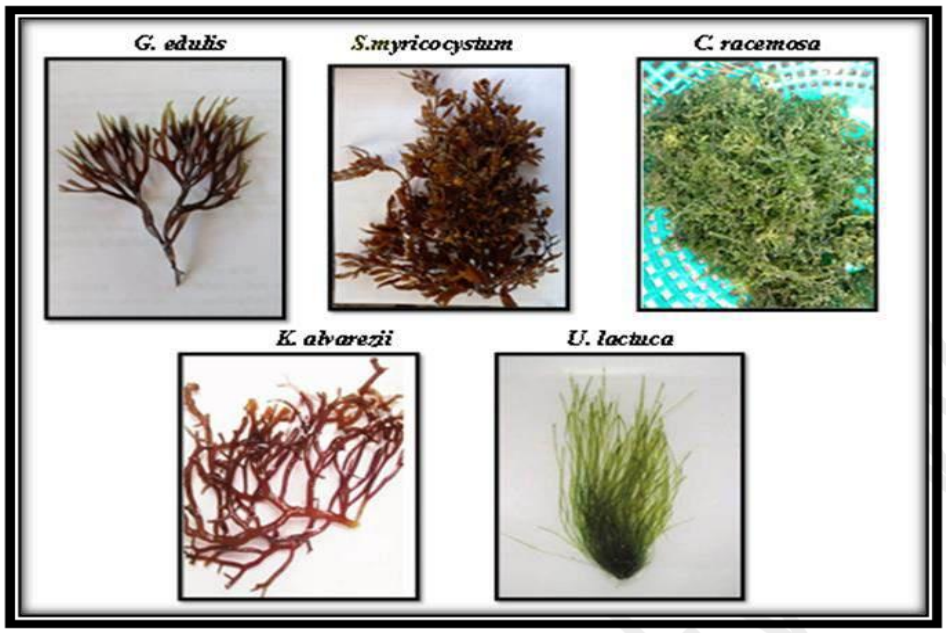

Fig 1. Collection of seaweeds

2.4 **Preparation of methanol extract of *S. myricocystum***

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 mixed with the respective solvents and kept at -4°C for future use [7].

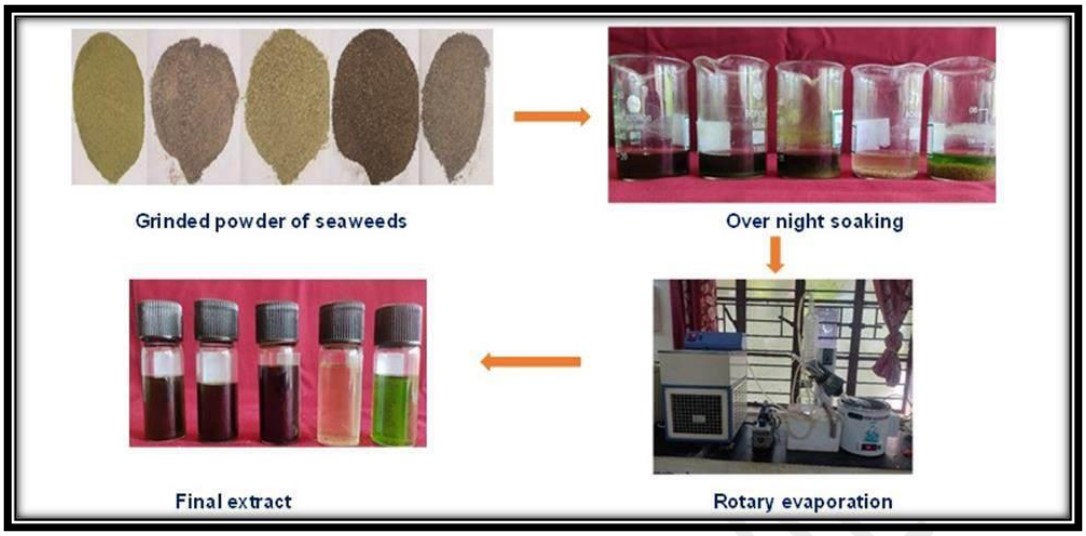

Fig 2. Seaweeds extraction by Rotary evaporation method

2.5 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 biostimulants, 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- Foliar 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.

2.6 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 main aim of this study was to evaluate the effectiveness of seaweed extracts, especially 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 different seaweeds viz., *S. myricocystum, K. alvarezii, G. edulis, C.racemosa* and *U. lactuca*. Among the seaweed extracts tested, S. myricocystum stood out by exhibiting a distinctive and remarkable reduction of mycelial growth of *A. polianthi*. Among the various solvent extracts, the methanol extract of *S. myricocystum* significantly restricted the mycelial growth (2.43 cm) which recorded a 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 % reduce over control and still showed some degree of antifungal activity. The experimental results indicated that among different solvents of seaweed extracts, methanol extract of 10 % *S. myricocystum* showed the least mycelial growth (2.43 cm) of the pathogen under in vitro conditions. In contrast, *K. alvarezii* reported 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. myricocystum* showed a distinctive and remarkable suppression of mycelial growth of *C.gloeosporioides* followed by *K. alvarezii*, as reported by reference [10]. Ambika and Sujatha (2015) [11] found that S. myriocystum extract successfully 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 myricocystum	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

Fig 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 biocontrol 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 outcomes were in line with the foliar spray and root drench, which significantly reduced disease in greenhouse cucumber plants at concentrations of 0.5 and 1% of Ascophyllum nodosum extract, respectively [13]. Furthermore, it was shown that 10% brown algae extract from *S. myriocystum* inhibited the mycelial growth of *C. gloeosporioides* under *in vitro* [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

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 causes 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 a safe bioinoculant for sustainable agriculture technology.

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