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RESEARCH PAPER

Antifungal activity of brown, red and green alga seaweed extracts against *Macrophomina phaseolina* (Tassi) Goid., in pigeonpea var. CO (Rg) 7

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Abstract : *In vitro* studies was conducted to evaluate the effect of seaweed extracts of *Caulerpa racemosa* (green alga), *Sargassum myricocystum* (brown alga) and *Gracilaria edulis* (red alga) against the mycelial growth of *Macrophomina phaseolina* at different concentrations of 10, 15, 20, 25 and 30 per cent along with control by poison food technique. The result revealed that extract of *S. myricocystum* showed significant antifungal activity against pathogen followed by *G. edulis* and *C. racemosa*. *S. myricocystum* (30%) extract recorded the lowest mycelial growth (45.2, 50.6, 58.4 and 61.5 mm) at 24, 48, 72 and 96 hrs after incubation. Among the antagonists tested against *Macrophomina phaseolina*, the fungal antagonists *Trichoderma viride* was found to be most effective in reducing the mycelial growth than the bacterial antagonist *Pseudomonas fluorescens.* Both the antagonistic of fungi and bacteria has compatability with seaweed extracts in all the concentrations.

Key Words : Seaweeds, Soil borne pathogen, Red gram, *Macrophomina phaseolina*

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the major grain legume crops of the tropics and subtropics and accounts for about 5 per cent of the world legume production. The peas are rich in source of protein. Soil borne diseases are the most important in pulses causing heavy losses in seed yield. *Macrophomina phaseolina* (Tassi) Goid., a soil inhabiting fungus is an important root pathogen and causes dry root rot/stem canker, stalk rot or charcoal rot of over 400 plant species including pigeonpea (Mahrshi, 1986). *Macrophomina phaseolina* has been recently reported as an emerging phytopathogen (Kaur *et al*., 2012). The disease development is favoured by high temperature (30-35^{0}C) followed by moisture stress (Sandhu *et al*., 1999) and a good source of inoculum (Lodha, 1998). This is a serious problem in late sown or summer crops and in perennial or ratooned pigeonpea. The pathogen posses greater problem in cultivation and causes considerable loss (Bajpal *et al*., 1999). There is growing concern that environmental pollution caused by imbalanced use and misuse of chemical fertilizers and pesticides is directly or indirectly related to human health problems. Consequently, farmers

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in developed countries began to shift from chemical based conventional farming methods towards organic, alternative or low input sustainable agriculture (Bhatia, 2002). The seaweed concentrates are applied to crops as root dips, soil drenches or foliar sprays. Seaweed concentrates are effective biostimulant in many crops including vegetables, trees, flowering plants and grain crops (Stirk *et al.,* 2004). Compounds are extracted from different macroalgae families like green, brown and red algae (Vallinayagam *et al.,* 2009) confirmed earlier for their antifungal activity of seaweeds (Khanzada *et al.*, 2007 and Bhosale *et al.,* 2002). Extracts of the brown algae *Ascophyllum nodosum* applied as a soil drench and foliar sprays have been shown to improve growth rates, reduce pests, consequently increasing crop yields, as well as overall quality of the product (Blunden *et al.,* 1997). Present investigation was undertaken to evaluate different seaweed extracts for their antifungal activity against *Macrophomina phaseolina* in red gram and compatability with antagonist bacteria and fungi.

MATERIAL AND METHODS

Isolation of pathogen :

The pathogen was isolated from the diseased tissues of red gram by tissue segment method (Rangaswami, 1958). The infected portions of diseased plants were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in three changes of sterile water. The surface sterilized tissues were plated on PDA in sterile Petriplates and incubated at room temperature (28 ± 2°C) for 14 days. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The fungus was further purified by single spore isolation and maintained on PDA. The pathogen was identified based on colony character, conidial production and spore morphology.

Collection and preparation of extracts :

The marine alga *Caulerpa racemosa* (green alga), *Sargassum myricocystum* (brown alga) and *Gracilaria edulis* (red alga) collected from Mandapam coast, Tamil Nadu, were washed with seawater initially to remove macroscopic epiphytes and sand particles and then with fresh water to remove adhering salt. The materials were shade dried for 2 weeks followed by oven drying at 40°C for 24 h and powdered. A total quantity of 150 ml of alcohol was added to 20 g powder and kept for overnight with intermittent stirring and extracted through rotary evaporator at 40°C and 45 rpm. The liquid fertilizer was collected and stored in air tight container. The different concentrations were prepared by taking 10, 15, 20, 25 and 30 ml from the stock preparation and mixing with distilled water to get 10, 15, 20, 25 and 30 per cent concentrations.

Antifungal activity :

Poisoned food technique (Schmitz, 1930) was employed to screen the antifungal efficacy of seaweed extracts. Potato dextrose agar medium amended with seaweed extracts (10, 15, 20, 25 and 30%) was autoclaved and poured into sterile Petriplates. Fungal disc of 9 mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of *Macrophomina phaseolina* and the disc were transferred aseptically on PDA plates poisoned with seaweed extracts. A plate only with PDA and fungal disc was considered as control and the diameter of growth of fungus in this plate was used as a control for the calculation of per cent inhibition of test fungus.

Radial growth :

Measurement of the radial growth in centimeters (cm) was done and the radial growth was determined by using the formula Kr according to Reeslev and Kjoller (1995).

$$\text{Radial growth } (K_r) = \frac{[R_1 - R_0]}{[t_1 - t_0]}$$

where, R_0 and R_1 are the colony radial growth at time t_0 and t_1, respectively, determined after 24, 48, 72 and 96 hrs from inoculums.

Inhibition percentage :

The inhibition percentage was calculated measuring the radial growth of the fungus grown on control and amended plates after 24, 48, 72 and 96 hrs after incubation, using the following formula (Harlapur *et al.,* 2007).

$$I\% = 100 \div \frac{[C - T]}{C}$$

where, I per cent = inhibition percentage of pathogen growth, C = average radial growth in control plates and T = average radial growth in plates amended with seaweed extract.

Efficacy of bacteria antagonist under against *Macrophomina phaseolina in vitro* :

Culture of *Pseudomonas fluorescens* obtained from the Department of Plant Pathology, Agricultural College and Research Institute, Madurai and tested for their antagonistic effect on *Macrophomina phaseolina* by dual culture plate technique (Dennis and Webster, 1971). *P. fluorescens* was multiplied on King's B medium (King *et al.*, 1954). A total of nine mm culture disc of the pathogen was placed on the PDA medium in sterilized Petri dish at one side 1.5 cm away from the edge of the plate and incubated at room temperature (28±2°C). Simultaneously test bacteria were streaked on the medium at the opposite side of the plate, 1.5 cm away from the edge of the plate. Potato dextrose agar medium inoculated with the pathogen alone served as the control. The inoculated plates were incubated at room temperature (28±2°C) with three replications. When the control plate showed full growth of the pathogen, the radial growth of the mycelium was measured. The results were expressed as per cent growth inhibition over control.

Efficacy of the fungal antagonist against *Macrophomina phaseolina in vitro* :

Culture of *T. viride* obtained from the Department of Plant Pathology, Agricultural College and Research Institute, Madurai and tested for their antagonistic effect on *Macrophomina phaseolina* by dual culture plate technique (Dennis and Webster, 1971). A total of nine mm mycelial disc of *Macrophomina phaseolina* and *Trichoderma* sp. were placed opposite to each other near the periphery of the Petri plate and incubated at room temperature (28±2°C). After incubation, mycelial growth of the pathogen and inhibition zone was measured as well as in control plates.

Compatibility between antagonistic bacteria and seaweed extracts :

King's B medium was amended with the *Caulerpa racemosa* (green alga), *Sargassum myricocystum* (brown alga) and *Gracilaria edulis* (red alga) with 10, 15, 20, 25 and 30 per cent concentration. Then the antagonistic bacterial isolates were inoculated in the poisoned media. The plates were incubated under room temperature for 48 h and the growth of bacteria was recorded visually and scored either as highly compatible or moderately compatible or not compatible.

Compatibility between antagonistic fungi and seaweed extracts :

PDA medium was amended with the *Caulerpa racemosa* (green alga), *Sargassum myricocystum* (brown alga) and *Gracilaria edulis* (red alga) with 10, 15, 20, 25 and 30 per cent concentrations. Then the antagonistic fungi isolates were inoculated in the poisoned media. The plates were incubated under room temperature and the growth of fungi was expressed in cm.

Data analysis :

The data from various experiments were analysed statistically adopting the procedure described by Panse and Sukhatme (1985). Wherever necessary, the percentage values were transformed to arc sine values before carrying out the statistical analysis.

RESULTS AND DISCUSSION

Among the concentrations, 30 per cent showed better performance compared to 20 and 10 per cent. Significant differences were observed in the seaweed extract of *Sargassum myricocystum* (30%) inhibited the mycelial growth of *Macrophomina phaseolina* which recorded lowest mycelial growth of 45.2, 50.6, 58.4 and 61.5 mm followed by *Gracillaria edulis* (30%) with 55.0, 60.3, 62.7 and 65.0 mm whereas in control the highest mycelial growth of 78.1, 83.3, 87.5 and 90.0 mm after 24, 48, 72 and 96 hrs, respectively (Table 1). Cotton seeds soaked in seaweed solution (1:500 *Sargassum wightii* for 12 h) provided seedlings with considerable resistance against *Xanthomonas campestris* (Raghavendra *et al.*, 2009). Seaweed extract of *Sargassum myricocystum* inhibited radial growth in the range of 23 to 42 per cent after 24 hrs, 21 to 39 per cent after 48 hrs, 24 to 33 per cent after 72 hrs and 23 to 32 per cent after 96 hrs compared to control (Table 2 and Fig. 1). Jayaraj *et al.* (2008) found that the seaweed in

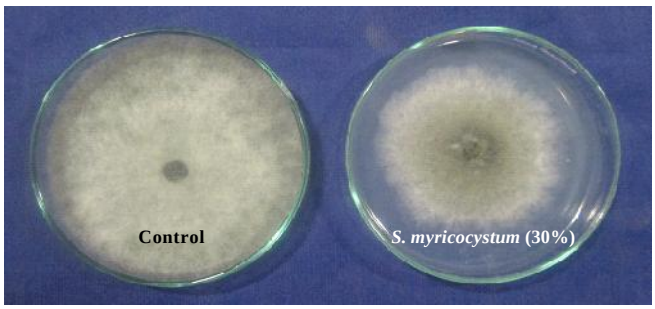

Fig. 1 : Efficacy of seaweed extracts against the mycelial growth of *Macrophomina phaseolina in vitro* condition

Table 1 : Effects of seaweed extracts on mycelial growth (mm) of *Macrophomina phaseolina in vitro*

Treatments	Mycelial growth (mm)				
	Hours				
	24	48	72	96	Mean
Sargassum myricocystum (10%)	60.2	65.4	66.8	69.7	65.5
Sargassum myricocystum (15%)	56.8	62.5	65.9	68.3	63.4
Sargassum myricocystum (20%)	52.4	60.1	62.6	66.9	60.5
Sargassum myricocystum (25%)	53.7	58.4	60.5	63.3	59.0
Sargassum myricocystum (30%)	45.2	50.6	58.4	61.5	53.9
Gracilaria edulis (10%)	69.3	71.2	72.7	74.7	72.0
Gracilaria edulis (15%)	66.5	68.9	70.1	72.8	69.6
Gracilaria edulis (20%)	62.1	64.7	69.1	71.5	66.9
Gracilaria edulis (25%)	59.5	62.5	63.6	68.2	63.5
Gracilaria edulis (30%)	55.0	60.3	62.7	65.0	60.8
Caulerpa racemosa (10%)	74.4	76.2	78.9	82.6	78.0
Caulerpa racemosa (15%)	70.9	73.3	75.8	78.0	74.5
Caulerpa racemosa (20%)	68.9	71.5	73.6	76.5	72.6
Caulerpa racemosa (25%)	66.5	69.0	71.5	74.0	70.3
Caulerpa racemosa (30%)	61.7	63.8	67.3	70.1	65.7
Control	78.1	83.3	87.5	90.0	84.7
Mean	62.6	66.4	69.2	72.1	67.5
	C		T		C × T
S.E. ±	0.70846		0.35423		1.41692
C.D. (P=0.05)	1.397**		0.698**		2.794**

** indicate significance of value at P=0.05

Table 2 : Effects of seaweed extracts on inhibition over control of *Macrophomina phaseolina in vitro*

Treatments	Inhibition over control (%)				
	Hours				
	24	48	72	96	Mean
Sargassum myricocystum (10%)	22.92 (28.60)	21.49 (27.61)	23.66 (29.10)	22.56 (28.35)	22.66 (28.42)
Sargassum myricocystum (15%)	27.27 (31.48)	24.97 (29.98)	24.69 (29.79)	24.11 (29.40)	25.26 (30.17)
Sargassum myricocystum (20%)	32.91 (35.00)	27.85 (31.85)	28.46 (32.24)	25.67 (30.44)	28.72 (32.40)
Sargassum myricocystum (25%)	31.24 (33.98)	29.89 (33.14)	30.86 (33.74)	29.67 (33.00)	30.42 (33.47)
Sargassum myricocystum (30%)	42.13 (40.47)	39.26 (38.79)	33.26 (35.22)	31.67 (34.24)	36.58 (37.21)
Gracilaria edulis (10%)	11.27 (19.61)	14.53 (22.40)	16.91 (24.28)	17.00 (24.35)	14.93 (22.73)
Gracilaria edulis (15%)	14.85 (22.66)	17.29 (24.57)	19.89 (26.48)	19.11 (25.92)	17.79 (24.94)
Gracilaria edulis (20%)	20.49 (26.91)	22.33 (28.20)	21.03 (27.29)	20.56 (26.96)	21.10 (27.34)
Gracilaria edulis (25%)	23.82 (29.21)	24.97 (29.98)	27.31 (31.50)	24.22 (29.48)	25.08 (30.05)
Gracilaria edulis (30%)	29.58 (32.94)	27.61 (31.69)	28.34 (32.16)	27.78 (31.80)	28.33 (32.15)
Caulerpa racemosa (10%)	4.74 (12.57)	8.52 (16.97)	9.83 (18.27)	8.22 (16.66)	7.83 (16.25)
Caulerpa racemosa (15%)	9.22 (17.67)	12.00 (20.26)	13.37 (21.44)	13.33 (21.41)	11.98 (20.25)
Caulerpa racemosa (20%)	11.78 (20.07)	14.17 (22.11)	15.89 (23.49)	15 (22.78)	14.21 (22.14)
Caulerpa racemosa (25%)	14.85 (22.66)	17.17 (24.48)	18.29 (25.32)	17.78 (24.94)	17.02 (24.36)
Caulerpa racemosa (30%)	21.00 (27.27)	23.41 (28.93)	23.09 (28.72)	22.11 (28.04)	22.40 (28.24)
Mean	21.20 (27.41)	21.70 (27.76)	22.33 (28.20)	21.25 (27.45)	21.62 (27.70)
	C		T		C × T
S.E. ±	0.238		0.123		0.477
C.D. (P=0.05)	0.471**		0.243**		0.942**

** indicate significance of value at P=0.05

carrot plants reduced leaf blights caused by *Alternaria* and *Botrytis* as effectively as the fungicide chlorothalonil. In carrot application of SLF enhanced activities of chitinase, B-1-3 flucanase, polyphenol oxidase and lipoxynase which are factors regulating plant disease. 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 SLF application (Jayaraman *et al.*, 2011). The commercial extract from the brown seaweed *Ascophyllum nodosum* was found to reduce fungal diseases in cucumber (Jayaraman *et al.*, 2011). Brown algae showed effectiveness in controlling plant diseases. The laminarin polysaccharide isolated from *Laminaria digitata* is able to elicit host defense responses in plants (Klarzynski *et al.*, 2000). Extracts of *Spatoglossum asperum* and *Spatoglossum* sp. inhibited the radial growth of the fungi such as *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani in vitro* when used at 6 mg^{-1} disc. The extract of the seaweed *Ascophyllum nodosum* stimulates the activity of peroxidases and phytoalexin synthesis in some plants of commercial value, increasing their resistance. The *Ulva fasciata* extract is able to effectively reduce the number of colonies in powdery mildew. The brown seaweeds shows high antifungal activity as compared to red and green algae. The brown seaweeds contain high amount of flavanoid and phenolic compounds could be the reason for antifungal activity (Cowan *et al.*, 1999). Seaweed could also affect cell metabolism through the induction of the synthesis of antioxidant molecules which could favour plant growth and plant resistance to stress (Zhang and Schmidt, 2000). The water extract of *Padina tetrastromatica* and ethanolic extract of *Padina tetrastromatica* and *Sargassum tennerrimum* showed

Table 3 : Effect of antagonists on the mycelial growth of *Macrophomina phaseolina in vitro* (Dualculture technique)

Sr. No.	Treatments	*Mycelial growth (mm)	Per cent reduction over control
1.	*Trichoderma viride*	30.1	66.5 (54.63)
2.	*Pseudomonas fluorescens*	41.2	54.2 (47.41)
3.	Control	90.0	–
	S.E. ±	1.24	
	C.D. (P=0.05)	2.81**	

* Mean of three replications ** indicate significance of value at P=0.05

Table 4 : Compatibility of seaweed extracts with antagonistic fungi and antagonistic bacteria

Seaweeds	*Trichoderma viride*	*Pseudomonas fluorescens*
	Mycelial growth(mm)	Compatibility level
Sargassum myricocystum (10%)	66.2	+++
Sargassum myricocystum (15%)	72.4	+++
Sargassum myricocystum (20%)	76.7	+++
Sargassum myricocystum (25%)	82.4	+++
Sargassum myricocystum (30%)	86.9	+++
Gracilaria edulis (10%)	65.0	+++
Gracilaria edulis (15%)	69.6	+++
Gracilaria edulis (20%)	75.4	+++
Gracilaria edulis (25%)	77.9	+++
Gracilaria edulis (30%)	81.5	+++
Caulerpa racemosa (10%)	61.3	++
Caulerpa racemosa (15%)	65.7	++
Caulerpa racemosa (20%)	71.8	++
Caulerpa racemosa (25%)	76.3	++
Caulerpa racemosa (30%)	80.2	++
Control	90.0	++
Mean	74.9	–
S.E. ±	1.56	–
C.D. (P=0.05)	3.15**	–

+++: Highly compatible, ++: Moderately compatible, +: Slightly compatible, – : No compatible ** indicate significance of value at P=0.05

activity against *Fusarium solani* and *F. oxysporum* by well diffusion and disc diffusion method, respectively (Asnad and Abbass, 2014). 70 per cent of antifungal activity was recorded in brown seaweed, 30 per cent in red seaweed and only 15 per cent in green seaweeds.

The effect of bacterial antagonists and fungal antagonist were tested against the growth of *Macrophomina phaseolina* by following dual culture technique *in vitro*. Among these antagonists the fungal antagonist *viz., Trichoderma viride* was found to be most effective by recording 66.5 per cent reduction over control. *Pseudomonas fluorescens* recorded the mycelial growth reduction of 54.2 per cent over control (Table3). Use of antagonistic organisms against Macrophomina root rot has been well documented in several crops (Mukhopadhyay, 1987 and Raguchander *et al*., 1998).

The effect of different concentrations of seaweed extracts was tested for their compatibility with fungal antagonist of *T. viride* and bacterial antagonist of *P. fluorescens* under *in vitro* condition. The results revealed that the growth of *T. viride* was found to be not sensitive to the extracts of seaweeds *Sargassum myricocystum, Caulerpa racemosa* and *Gracilaria edulis.* Among the concentrations 30 per cent in all species recorded highest growth compared to other concentrations. Mycelial growth of *T. viride is* 87, 82 and 80 mm for *Sargassum myricocystum* (30%), *Caulerpa racemosa* (30%) and *Gracilaria edulis* (30%), respectively (Table 4 and Fig. 2). Sundravadana (2002) reported that the seed and soil application of *T. viride* significantly controlled the blackgram root rot caused by *M. phaseolina*. Sreedevi *et al.* (2011) reported that *T .viride* inhibited fungal growth upto 69 per cent.

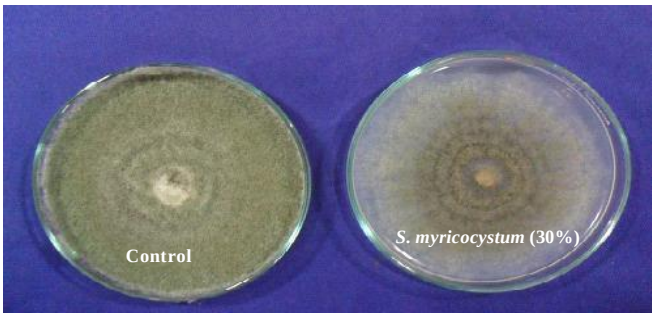

Fig. 2 : Compatibility between antagonistic fungi and seaweed extracts

The bacterial antagonist of *P. fluorescens* found to be compatible with seaweed extracts *Sargassum myricocystum*, *Caulerpa racemosa* and *Gracilaria edulis* in all concentrations evidenced by the presence of growth. The colonies of *P. fluorescens* were not affected by the extracts of above seaweeds with high degree of compatibility (Table 4 and Fig. 3). Bacterial starins of *P. flourescens*, *P. putida* and *P. aeruginosa* have been reported as effective bio-control agents of various soil fungi (Vaidov *et al.*, 2005).

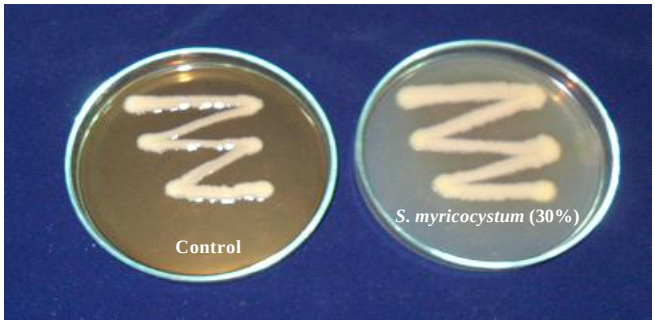

Fig. 3 : Compatibility between antagonistic bacteria and seaweed extracts

Conclusion :

Sargassum myricocystum seaweed extract at 30 per cent concentration could be effectively controlled and inhibited the mycelial growth of Macrophomina *phaseolina* in red gram under *in vitro* studies.

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