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Antifungal Properties of Selected Seaweed and Seagrass Extracts against *Macrophomina phaseolina* Infecting Pigeon Pea

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ABSTRACT

A study was conducted to evaluate *in vitro* efficacy of seaweed (*Sargassum myriocystum* and *Sargassum wightii*) and seagrass (*Cymodocea serrulata* and *Syringodium isoetifolium*) extract against the mycelial growth of *Macrophomina phaseolina* at different concentrations of 5, 10, 15 and 20% along with control by poison food technique. The result revealed that, the extract of *S. wightii* (20%) exhibited the highest suppression of mycelial growth (10, 25 and 38 mm) at 24, 48 and 72 h after incubation. Among the antagonists tested against *Macrophomina phaseolina*, the fungal *Trichoderma viride* was found to be the most effective in reducing mycelial growth than the bacterial antagonist *Pseudomonas fluorescens*. Both the antagonistic fungi and bacteria have compatibility with seaweed and seagrass extracts in the concentrations.

Key words: *Macrophomina phaseolina,* Red gram, Seagrass, Seaweeds, Soil-borne pathogen.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the major grain legume crops of the tropics and subtropics and accounts for about 5% of the world's legume production. The pigeonpeas are rich in protein. Soil-borne diseases are important in pulses causing heavy losses in seed yield. *Macrophomina phaseolina* (Tassi) Goid., a soil-inhibiting 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°C) followed by moisture stress (Amrit *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 poses a 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 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 biostimulants in many crops including vegetables, trees, flowering plants and grain crops (Stirk *et al*., 2004). Compounds extracted from different macroalgae (seaweed and seagrass) families like green, brown and red algae (Vallinayagam *et al*., 2009) were confirmed earlier for their antifungal activity (Khanzada *et al*., 2007; 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 and reduce pests, consequently increasing crop yields, as well as the overall quality of the product (Blunden *et al*., 1997). The present investigation was undertaken to evaluate different seaweed and seagrass extracts for their antifungal activity against *Macrophomina phaseolina* in red gram and compatibility with antagonistic bacteria and fungi.

The pathogen, *Macrophomina phaseolina,* was isolated from the diseased tissues of red gram by tissue segment method (Rangaswami, 1958). Seaweeds and sea grasses were collected from the Mandapam coast, Tamil Nadu. After washing of seaweeds and seagrasses with water they 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 overnight with

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intermittent stirring and extracted through a rotary evaporator at 40°C and 45 rpm. The extract was collected and stored in an airtight container. The different concentrations of 5, 10, 15 and 20% were prepared. A Poisoned food technique (Schmitz, 1930) was employed to screen the antifungal efficacy of seaweed extracts. Radial growth was calculated (Reeslev and Kjoller, 1995) and inhibition percentage was calculated (Harlapur *et al*., 2007). Finally, compatibility between antagonistic bacteria, fungi and seaweed extracts were tested. The data from various experiments were analyzed 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 indicated that a 20% concentration of all seaweeds and sea grasses showed better performance in general. Significant differences were observed in the seaweed extract of *S. wightii* (20%) compared to seagrass and *S. myriocystum*. Mycelia growth of *Macrophomina phaseolina* was lowest in *S. wightii* (20%) (10, 25 and 38 mm after 24, 48 and 72 h) mm followed by *S. myriocystum* (20%) with 20, 28 and 45 mm whereas control recorded the highest mycelial growth of 30, 71 and 90.0 mm after 24, 48 and 72 h respectively (Table 1). The inhibition over control was also highest (63%) for *S. wightii* (20%) followed by in 20% for *S. myriocystum* (56%). The bacterial (*P. fluorescens*) and fungal antagonist (*T.viride*) were found to be compatible with seaweed and sea grass extracts. The presence of growth was not affected by the extracts (Table 2; Fig 1). The compatibility test shows that the seaweed and seagrass extracts can be applied to the plants in combination with biocontrol agents. Bacterial strains of *P. flourescens*, *P. putida* and *P. aeruginosa* have been reported as effective bio-control agents of various soil fungi (Validov *et al*., 2005). The use of antagonistic organisms against *Macrophomina* root rot has been well-documented in several crops (Raguchander *et al*., 1998). Cotton seeds soaked in seaweed solution (1:500 *Sargassum wightii* for 12 h) provided considerable resistance to seedlings against *Xanthomonas campestris* (Raghavendra *et al*., 2007). In carrot application of SLF (seaweed liquid fertilizer) enhanced the 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 defense-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 cucumbers (Jayaraman*et al*., 2011). Brown algae have shown 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). Brown seaweeds contain high amounts of flavonoid 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 favor

Table 1: Efficacy of seaweed extracts on mycelial growth (mm) of *Macrophomina phaseolina in vitro* and compatibility of seaweed extracts with antagonistic fungi and bacteria.

Treatments	Concentrations (%)	Mycelial growth (mm)			
		24 h	48 h	72 h	Mean
S. wightii	5	16	29	43	29
S. wightii	10	15	36	53	35
S. wightii	15	13	33	51	32
S. wightii	20	10	25	38	24
S. myriocyctum	5	20	60	70	50
S. myriocyctum	10	16	37	62	38
S. myriocyctum	15	14	35	54	34
S. myriocyctum	20	12	28	45	28
S. isoetifolium	5	20	64	72	52
S. isoetifolium	10	18	55	65	46
S. isoetifolium	15	17	51	60	43
S. isoetifolium	20	14	45	51	37
C. serrulata	5	21	65	73	53
C. serrulata	10	18	53	72	48
C. serrulata	15	18	50	66	45
C. serrulata	20	16	47	60	41
Control		30	71	90	64
		C	**T**		**C × T**
S.E.±		0.317	0.860		1.393
C.D. (P=0.05)		0.629**	1.62**		2.401**

+: Compatible; ** Indicates the significance of value at P=0.05.

Table 2: Efficacy of seaweed extracts on Inhibition over control (%) of *Macrophomina phaseolina in vitro* and compatibility of seaweed extracts with antagonistic fungi and bacteria.

Treatments	Concentrations (%)	Inhibition over control (%)				Comparability	
		24 h	48 h	72 h	Mean	*T. viride*	*P. fluorescens*
S. wightii	5	46.67	59.15	52.22	52.68	+	+
S. wightii	10	50.00	49.30	41.11	46.80	+	+
S. wightii	15	56.67	53.52	43.33	51.17	+	+
S. wightii	20	66.67	64.79	57.78	63.08	+	+
S. myriocyctum	5	33.33	15.49	22.22	23.68	+	+
S. myriocyctum	10	46.67	47.89	31.11	41.89	+	+
S. myriocyctum	15	53.33	50.70	40.00	48.01	+	+
S. myriocyctum	20	60.00	60.56	50.00	56.85	+	+
S. isoetifolium	5	33.33	9.86	20.00	21.06	+	+
S. isoetifolium	10	40.00	22.54	27.78	30.11	+	+
S. isoetifolium	15	43.33	28.17	33.33	34.94	+	+
S. isoetifolium	20	53.33	36.62	43.33	44.43	+	+
C. serrulata	5	30.00	8.45	18.89	19.11	+	+
C. serrulata	10	40.00	25.35	20.00	28.45	+	+
C. serrulata	15	40.00	29.58	26.67	32.08	+	+
C. serrulata	20	46.67	33.80	33.33	37.93	+	+



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

plant growth and plant resistance to stress (Zhang and Schmidt, 2000).

CONCLUSION

Seaweed, *Sargassum wightii,* extract at 20% concentration could effectively control the mycelial growth of *Macrophomina phaseolina* infecting red gram.

Conflict of interest: None.

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