Comparative studies on brown, red and green alga seaweed extracts for their antifungal activity against *Fusarium oxysporum* f.sp. *udum* in Pigeon pea var. CO (Rg)7 (*Cajanus cajan* (L.) Mills.)

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ABSTRACT

In vitro studies was conducted to evaluate the effect of seaweed liquid extracts of *Caulerpa racemosa* (green alga), *Sargassum myricocystum* (brown alga) and *Gracilaria edulis* (red alga) at different concentrations of 10, 15, 20, 25 and 30% along with control against the mycelial growth of *Fusarium oxysporum* f. sp. *udum* by poison food technique. 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 (28.1, 33.9, 34.7, 39.4 and 44.3 mm) at 24, 48, 72, 96 and 108 hrs after incubation. Among the antagonists tested against *Fusarium oxysporum* f.sp. *udum*, 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.

MS History: .25.09.2014 (Received)-08.11.2014 (Revised)-18.11.2014 (Accepted)

Citation: S. Ambika and K. Sujatha. 2014. Comparative studies on brown, red and green alga seaweed extracts for their antifungal activity against *Fusarium oxysporum* f.sp. *udum* in Pigeon pea var. CO (Rg)7 (*Cajanus cajan* (L.) Mills.). *Journal of Biopesticides*, 7(2):167-176.

Key words: Seaweeds, soil borne pathogen, red gram, *Fusarium oxysporum* f.sp. *udum*.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Mills.) is one of the major legume crops grown in the tropics and sub-tropics, and accounts for about 5% of world legume production. The largest producer is India, where the dried pea is the favoured choice for the preparation of *dhal*. Due to cultivation of pigeonpea in diversified climatic situations in various geographical areas it is attacked by more than 100 pathogens (Nene *et al*., 1989 and Raju *et al.,* 2008**)**. Soil borne diseases are the most important in pulses causing heavy losses in seed yield. *Fusarium* wilt caused by *Fusarium udum* is the most important soil borne disease of pigeonpea capable of causing 30-100% loss in grain yield (Reddy *et al.,* 1990) and the incidence occurs at flowering and crop maturity stages (Kannaiyan and Nene, 1981). The disease was first reported from Bihar state in India (Butler, 1906). Pigeonpea wilt is widely prevalent throughout the world and more important in India (Kannaiyan and Nene 1981) and the annual crop losses due to wilt alone have been estimated about 36 million US dollars in India (Kannaiyan *et al.,* 1984). Most of the soils borne pathogens are difficult to control by conventional strategies such as the use of resistant cultivars and synthetic fungicides (Weller *et al.,* 2002). To avoid the implication of yield losses due to plant diseases, variety of control measures presently are in use. The chemical compounds are most commonly used for the controlling of plant diseases. No doubt the use of chemicals has been found very effective in controlling plant fungal diseases but some major problems threaten to limit the continued use of fungicides.

The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are ecofriendly and effective (Mohana *et al.,* 2011). In recent years, there have been many reports of macro

algae derived compounds that have a broad range of biological activities, such as antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic and antimitotic activities (Demirel *et al.,* 2009).The majority of these compounds are terpenes and polyphenols (Blunt *et al.,* 2006). Marine bioactive substances extracted from seaweeds have been used for several decades to enhance plant growth and productivity (Rathore *et al.,* 2009). Brown seaweed extract is a well known plant growth stimulator, which improves general plant health and enhances plant resistance to nematodes, pests and fungal diseases (Jayaraj *et al.,* 2008). Present investigation was undertaken to evaluate different seaweed extracts (red, brown and green) for their antifungal activity against *Fusarium oxysporum* f.sp. *udum and their* compatibility with *Trichoderma viride* and *Pseudomonas fluorescens*.

MATERIALS 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 Petri plates and incubated at room temperature ($28 \pm 2^{\circ}C$) 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 *C. racemosa*, *S.myricocystum*and *G. edulis* were collected from Mandapam coast (Lat. 09^{o} 17.417'N; Long. 079^{o} 08.558'E), 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 $40^{o}C$ for 24 h and powdered. A 150 ml of alcohol was added to 20 g powder and kept for overnight with intermittent stirring and extracted through rotary evaporator at $40^{o}C$ and 45 rpm (Bhosle *et al.,* 1975 with slight modification). 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 of the stock preparation and mixing with distilled water to get 10, 15, 20, 25 and 30 % concentrations.

Antifungal activity

Poisoned food technique (Schmitz, 1930) was employed to screen the antifungal efficacy of seaweed extracts. Potato dextrose agar media amended with seaweed extracts (10, 15, 20, 25 and 30%) were autoclaved and poured into sterile Petriplates. Fungal disc of 9mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of *Fusarium oxysporum* f. sp. *udum* 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 (standard) and the diameter of growth of fungus in this plate was used as a control for the calculation of percent 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).

Radial growth $(K_r) = (R_1 - R_0) / (t_1 - t_0)$

Where, R_0 and R_1 are the colony radial growth at time t_0 and t_1 respectively, determined after 24, 48, 72, 96 and 108 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, 96 and 108 hrs after incubation, using the following formula (Harlapur *et al.,* 2007).

$I\% = 100 \times (C - T) / C$

Where, I% = 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

Culture of *Pseudomonas fluorescens* obtained from the Department of Plant Pathology, AC&RI, Madurai and tested for their antagonistic effect on *F.o.* f.sp. *udum* by dual culture plate technique (Dennis and Webster, 1971).*Psuedomonas fluorescens* was multiplied on King's B medium (King's *et al.*, 1954). A total of 9 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^{o}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^{o}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

Culture of *T. viride* obtained from the Department of Plant Pathology, AC&RI, Madurai and tested for their antagonistic effect on *F.o.* f.sp. *udum* by dual culture plate technique (Dennis and Webster, 1971). A total of nine mm mycelial disc of *F.o.* f.sp. *udum* and *Trichoderma* sp. were placed opposite to each other near the periphery of the petri plate and incubated at room temperature (28±2^{0}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 *C. racemosa , S. myricocystum* and *G. edulis* with 10, 15, 20, 25, 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, 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 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 AND DISCUSSIONS

Among the concentrations, 30% showed better performance. Significant difference were observed in the seaweed extarct of *S. myricocystum* (30%) inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *udum* which recorded lowest mycelial growth of 28.1, 33.9, 34.7, 39.4 and 44.3 mm followed by *G. edulis* (30%) with 33.2, 38.1, 40.5, 47.6 and 53.1 mm where as in control the highest mycelial growth of 48.5, 62.4, 81.9, 85.2 and 90 mm after 24, 48, 72, 96 and 108 hrs was recorded (Table- 1). Cotton seeds soaked in seaweed solution (1:500 *S.wightii* for 12 h) provided seedlings with considerable resistance against *Xanthomonas campestris* (Raghavendra *et al.,* 2007).Seaweed extract of *S. myricocystum* showed inhibited radial growth in the range of 19 to 42% after 24 hrs, 20 to 46% after 48 hrs, 34 to 58% after 72 hrs, 27 to 54% after 96 hrs and 27 to 51 after 108 hrs compared to control (Table 2). Jayaraj *et al.* (2008) found that the seaweed in carrot plants reduced leaf blights caused by

Table 1. Effects of seaweed extracts on mycelial growth (mm) of *Fusarium oxysporum* f. sp. *in vitro.*

	Mycelial growth (mm)					
	Hours					
	24	48	72	96	108	Mean
Sargassum myricocystum (10%)	39.4	49.7	54.2	62.1	65.7	54.22
Sargassum myricocystum (15%)	38.2	45.7	49.4	57.5	61.6	50.48
Sargassum myricocystum (20%)	35.3	36.8	43.3	46.8	52.8	43.00
Sargassum myricocystum (25%)	30.1	34.7	38.3	42.4	45.8	38.26
Sargassum myricocystum (30%)	28.1	33.9	34.7	39.4	44.3	36.08
Gracilaria edulis (10%)	43.7	53.4	58.8	69.3	73.8	59.80
Gracilaria edulis (15%)	40.5	47.1	56.5	60.7	67.2	54.40
Gracilaria edulis (20%)	37.3	42.8	47.7	55.1	62.0	48.98
Gracilaria edulis (25%)	36.4	39.8	44.9	53.6	59.4	46.82
Gracilaria edulis (30%)	33.2	38.1	40.5	47.6	53.1	42.50
Caulerpa racemosa (10%)	43.0	58.1	62.7	72.6	79.2	63.12
Caulerpa racemosa (15%)	41.5	50.4	55.7	63.8	71.2	56.52
Caulerpa racemosa (20%)	40.2	44.7	51.3	58.7	68.3	52.64
Caulerpa racemosa (25%)	39.0	41.0	45.8	54.0	61.6	48.28
Caulerpa racemosa (30%)	37.7	39.7	44.7	50.7	58.3	46.22
Control	48.5	62.4	81.9	85.2	90.0	73.60
Mean	38.26	44.89	50.65	57.47	63.39	50.93
	C	T	C X T			
SEd	0.273	0.489	1.095			
CD (0.05)	0.539**	0.964**	2.157**			

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 have shown effectiveness in controlling

Fig 1.Spores of *Fusarium oxysporum f.sp. udum*

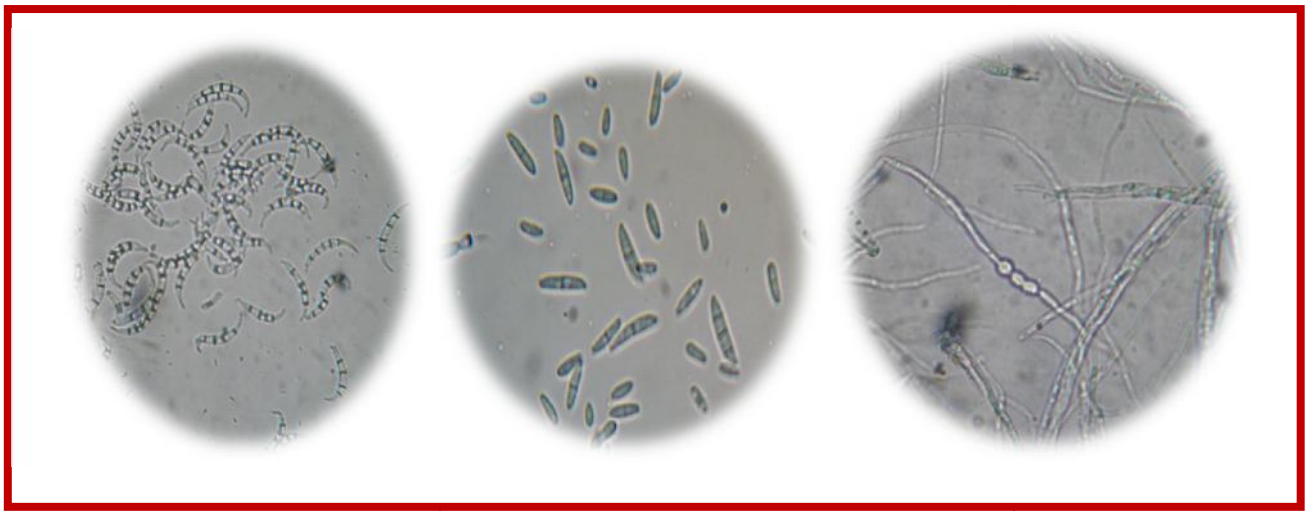


Table 2. Effects of seaweed extracts on inhibition over control of *Fusarium oxysporum* f. sp. *udum in vitro*.

Seaweed	Inhibition over control (%)					
	Hours					
	24	48	72	96 hr	108	Mean
Sargassum myricocystum (10%)	18.76	20.35	33.82	27.11	27.00	25.40
Sargassum myricocystum (15%)	21.24	26.76	39.68	32.51	31.56	30.35
Sargassum myricocystum (20%)	27.22	41.03	47.13	45.07	41.33	40.35
Sargassum myricocystum (25%)	37.94	44.39	53.24	50.23	49.11	46.98
Sargassum myricocystum 30%)	42.06	45.67	57.63	53.76	50.78	49.98
Gracilaria edulis (10%)	9.90	14.42	28.21	18.66	18.00	17.83
Gracilaria edulis (15%)	16.49	24.52	31.01	28.76	25.33	25.22
Gracilaria edulis (20%)	23.09	31.41	41.76	35.33	31.11	32.54
Gracilaria edulis (25%)	24.95	36.22	45.18	37.09	34.00	35.48
Gracilaria edulis (30%)	31.55	38.94	50.55	44.13	41.00	41.23
Caulerpa racemosa (10%)	11.34	6.89	23.44	14.79	12.00	13.69
Caulerpa racemosa (15%)	14.43	19.23	31.99	25.12	20.89	22.33
Caulerpa racemosa (20%)	17.11	28.37	37.36	31.10	24.11	27.61
Caulerpa racemosa (25%)	19.59	34.29	44.08	36.62	31.56	33.22
Caulerpa racemosa (30%)	22.27	36.38	45.42	40.49	35.22	35.95
Mean	22.53	29.92	40.70	34.72	31.53	31.88
	C	T	C X T			
SEd	0.313	0.181	0.701			
CD (0.05)	0.618**	0.357**	1.383**			

plant diseases. The laminarin polysaccharide isolated from *Laminaria digitata* is able to elicit host defense responses in plants (Klarzynski *et al.*, 2000). 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). Using organic solvents which are able to extract a large quantity of lipophilic compounds (glycolipid, phenolic-terpenoidsds, unsaturated-fatty acids and hydroxylated unsaturated-fatty acids), the higher antifungal activity found in ethanol extracts, compared to water extracts (Hanaa *et al.*, 2008). The water extract of *Padina tetrastromatica* and ethanolic extract of *Padina tetrastromatica* and *Sargassum tennerrimum* showed activity against *Fusarium solani* and *F. oxysporum* by well diffusion and disc diffusion method, respectively (Asnad and Tanver, 2014).

Table 3. Effect of antagonists on the mycelial growth of *Fusarium oxysporum* f.sp.*udum in vitro.* (Dual culture technique)

Fungi	*Mycelial growth (mm)	Per cent reduction over control
Pseudomonas fluorescens	68.5	*23.8* (29.20)
Trichoderma viride	*32.5*	63.8 (53.01)
Control	*90.0*	-
SEd		1.41
CD (0.05)		3.19**

Fig 2.Efficacy of seaweed extracts against the mycelial growth of *Fusarium oxysporum* f. sp. *udum in vitro* condition.

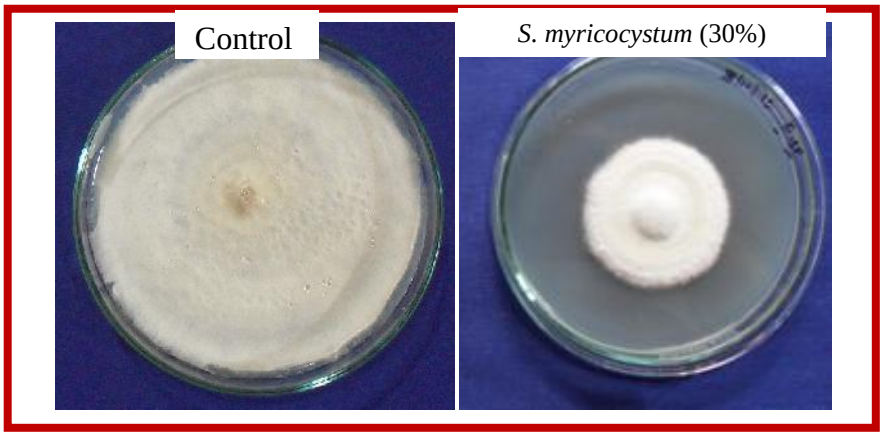


Fig 3.Effect of antagonists on the mycelial growth of *Fusarium oxysporum* f. sp. *udum in vitro* (Dual culture technique)

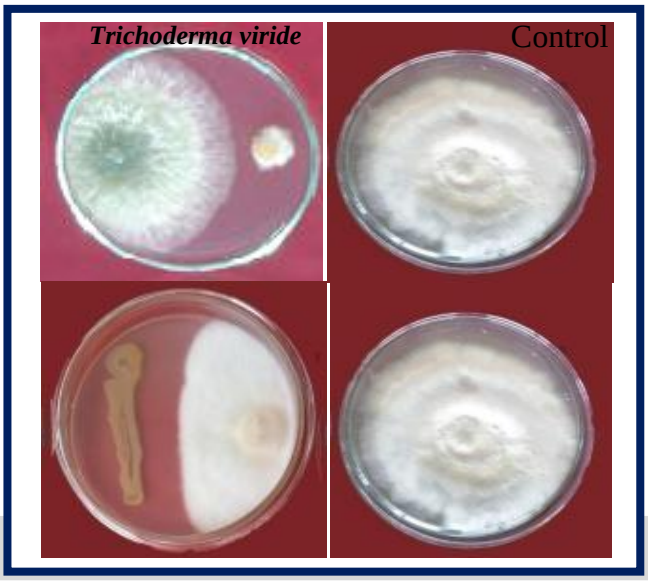
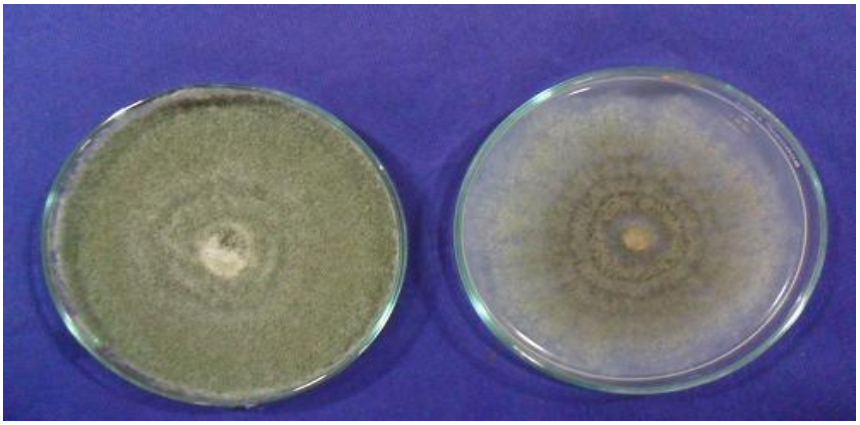
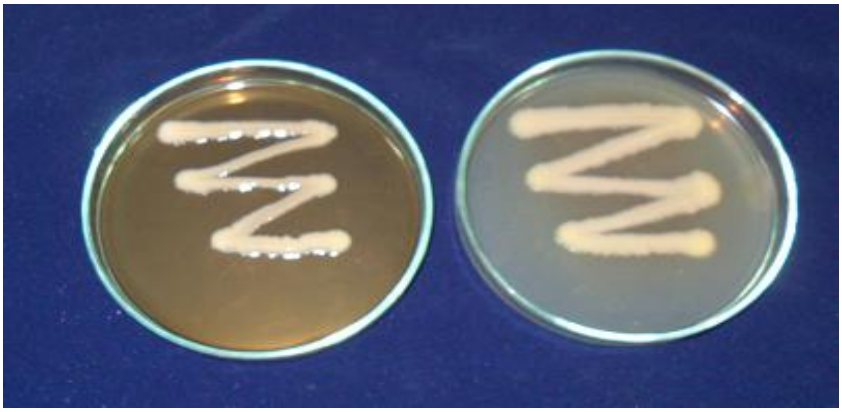


Fig 4. Compatibility between antagonistic fungi and seaweed extracts.



Control *S. myricocystum* (30%)

Fig 5. Compatibility between antagonistic bacteria and seaweed extracts.



S. myricocystum 30%) Control

The effect of bacterial antagonists and fungal antagonist were tested against the growth of *Fusarium oxysporum* f. sp.*udum* by following dual culture technique *in vitro*. Among these antagonists the fungal antagonist *viz., Trichoderma viride* was found to be most effective by recording 63.8 per cent reduction over control. *Pseudomonas fluorescens* recorded the mycelial growth reduction of 23.8 per cent over control (Table 3, Fig. 2). The result was supported by Naik *et al.* (2010) in *Fusarium oxysporum* f. sp. *vanillae.*

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 *S. myricocystum,Caulerpa racemosa* and *Gracilaria edulis.* Among the concentrations 30% 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). Rajendrandran and Ranganathan, (1996) reported that *T. viride, T. harzianum, T. hamatum, T. koningii* and *T. pseudokoningii* were antagonistic to *Fusarium oxysporum* f. sp. *cepae* which causes basal rot in onion. *Trichoderma harzianum* was

Table 4. Compatibility between antagonistic fungi *Trichoderma viride* and antagonistic bacteria *Pseudomonas fluorescens* with seaweed extracts

	Trichoderma viride Mycelial growth (mm)	*Pseudomonas fluorescens* 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	
SEd	1.56**	
CD (0.05)	3.15 **	

+++: Highly compatible, ++: moderately compatible, +: slightly compatible -: No compatible

antagonistic to *F. oxysporum* in plates and pots by reducing the population of *F. oxysporum* (Goodwin-Egein and Arinzae, 2001). Chandel (2011) reported that *T. harzianum* exhibited 68 per cent reduction in the growth of *F.oxysporum* f. *sp. dianthi* followed by *T.viride* and *T.hamatum*, while the least control was observed with *Bacillus subtilis*. The bacterial antagonist of P. *fluorescens* found to be compatible with seaweed extracts *S.myricocystum*, *C.racemes a*and *G. edulis* in all concentrationsevidenced 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). Sundaramoorthy and Balabaskar (2013) showed that *P. fluorescens* has an acceptable performance against *F. oxysporum*. *S. myricocystum* seaweed extract at 30% concentration effectively controlled and inhibited the mycelial growth of *F. oxysporum* f.sp. *udum* in red gram under *in vitro* studies.

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