Short Communication

Short note on the effects of ethanolic extracts of selected South African seaweeds on the growth of commercially important plant pathogens, *Rhizoctonia solani* Kühn and *Verticillium* sp.

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This paper describes the biological activity of ethanolic extracts from some commonly abundant seaweeds from KwaZulu-Natal, South Africa. The pour-plate method was used to determine the effects of selected seaweed extracts on the growth of two phytopathogens; *Verticillium* sp. and *Rhizoctonia solani*. Extracts from *Caulerpa filiformis* and *Ulva rigida* (Chlorophyta); *Zonaria tournefortii* (Phaeophyta); and *Hypnea spicifera, Gelidium abottiorum* and *Osmundaria serrata* (Rhodophyta) inhibited fungal growth by more than 50%. The extracts from the rhodophytes, *Spyridia cupressina* and *Beckerella pinnatifida* showed the weakest antifungal activity of the seaweeds tested. The pour plate method demonstrated both fungal growth promotion and inhibition, due to the seaweed extracts. At low concentrations (1:16 and 1:32 dilutions) the *H. spicifera* extract promoted the growth of *R. solani*.

Keywords: Biological activity, phytopathogens, seaweed extracts, fungal growth promotion and inhibition, South African seaweeds.

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Introduction

Secondary metabolites of some seaweeds have been reported to show biological activity (inhibition) when tested against various viruses (Neushul 1990), bacteria and fungi (Pesandro 1989). However, the growth promotion of test organisms has not been tested in previous studies. Although the extracts of numerous seaweeds have been studied, very few southern African species have been tested (Vlachos *et al.* 1996).

This paper examines the effects of extracts from selected South African seaweeds on the growth of two plant pathogens, *Verticillium* sp. and *Rhizoctonia solani* Kühn. Agricultural productivity is limited in part by phytopathogens and their control is a potential application of antibiotics from macroalgae (Ireland *et al.* 1993).

Methods

Commonly occurring and abundant seaweeds were collected in June 1995 from two sites in KwaZulu-Natal (sub-tropical, East Coast region of South Africa). The following seaweeds were collected from Reunion Rocks (Durban): Chlorophyta: *Caulerpa filiformis* (Suhr) Hering and *Ulva rigida* C. Ag., Phaeophyta: *Sargassum incisifolium* (Turner) J. Ag., Rhodophyta: *Gelidium abbottiorum* R.E. Norris and *Hypnea spicifera* (Suhr) Harv. The following were collected from Palm Beach (on the southern KwaZulu-Natal coast): Rhodophyta: *Spyridia cupressina* (Harvey) Kutzing, *Osmundaria serrata* (Suhr) R.E. Norris and *Beckerella pinnatifida* J. Ag., Phaeophyta: *Anthophycus longifolia* (Turner) Kützing and *Zonaria tournefortii* (Lamour.). Seaweeds from Reunion Rocks were transported to the laboratory in an insulated bag under cool conditions and stored at 5°C for one week before extracts were made. The material from Palm Beach was air dried for a week before extraction.

The extraction method used was adapted from Chesters and Stott (1956) and Papadopoulos (1989). Samples were rinsed with sterile, distilled water to remove surface salt. Rinsed seaweed (100 g) was pulverised with 300 ml ethanol (96%). The seaweed-ethanol mixture was heated in a water bath at 70°C for four hours. After filtration through Whatman No. 1 filter paper, the ethanol was evaporated off at 70°C, and the extract diluted with sterile distilled water so that it represented a final concentration of 20 g (fresh mass) alga per ml. The extract was then stored at 5°C for up to a month before being used.

The pour plate method (Tariq 1991) was used to test the growth response of the selected fungi to the algal extracts. One ml of extract or sterile distilled water (control) was mixed with 9 ml Potato Dextrose Agar 3.9% (w/v) (Biolab). The two phytopathogens used in this study, *Rhizoctonia solani* and *Verticillium* sp., were obtained from the South African National Fungus Collection, Pretoria. Plugs (5 mm) from stock cultures were inoculated centrally onto 9 cm PDA plates. The plates were incubated at 25°C for three days and the colony radii were measured on each day to determine growth rates (mm d^{-1}). The sizes of the fungal colonies were obtained by measuring the distances from the central plug to the edge of the colony across two lines drawn at right angles to each other. The means of the two measurements were used to calculate the growth rates. Five replicates were used for each treatment and for the controls (no extract).

The extracts from each site producing the highest growth inhibition were selected to test the effect of concentration on fungal growth (see results). Extracts from *Osmundaria serrata* and *Hypnea spicifera* were used in serial dilutions (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32) made with sterile distilled water and tested against the test fungi in the same way as the undiluted extracts.

The changes in fungal growth due to the algal extracts were calculated using the following formula:

$$GrowthIndex = 100 - \left(\frac{E}{C} \times 100\right)$$

where E is the growth rate of the fungus on the agar-extract medium (experiment) and C is the growth rate of the fungus on agar (control). In this way the controls had growth indices of zero. Positive values indicate growth inhibition and negative values growth promotion

One-way Analysis of Variance and Tukey-Kramer tests (SYSTAT Version 5.03) were used to compare the original fungal growth rates on seaweed extract and control plates. For the dilution experiments the $\log_{10}$ values of the growth rates were used in regression analysis of the means (Microsoft Excel).

Results

Both fungi showed significant growth inhibition ($p \leq 0.001$) on all seaweed extracts when compared with growth on control plates, except when grown on the extract of *S. cupressina*. *R. solani* grown on *B. pinnatifida* extracts showed no significant inhibition compared with the control (Table 1). Extracts of *Z. tournefortii* and *O. serrata* produced the highest fungal growth inhibition of the seaweeds collected from Palm Beach (site 2), while *H. spicifera* produced the highest fungal growth inhibition of the seaweed collected from Reunion Rocks (site 1).

With an increase in seaweed extract dilution of *H. spicifera* and *O. serrata*, there was an increase in fungal growth (Figures 1 and 2). The high r^2 values obtained from the regression analyses

Table 1 Percentage fungal growth inhibition in response to seaweed extracts. Values are the means of five replicates (± SE)

	Percentage growth inhibition	
Algae	*Verticillium* sp.	*Rhizoctonia solani*
Site 1 - Reunion Rocks	($F_{5,24} = 23.7$, $p \leq 0.001$)	($F_{5,24} = 67.6$, $p \leq 0.001$)
Gelidium abbottiorum^{Rh}	21.5% ± 1.3^{a}	56.0% ± 4.8^{b}
Sargassum heterophyllum^{Ph}	29.2% ± 3.0^{a}	37.2% ± 3.6^{a}
Caulerpa filiformis^{Ch}	29.5% ± 3.2^{a}	57.8% ± 0.9^{b}
Ulva rigida^{Ch}	32.3% ± 2.9^{a}	59.1% ± 2.5^{b}
Hypnea spicifera^{Rh}	34.7% ± 1.2^{a}	69.4% ± 1.4^{c}
Site 2 - Palm Beach	($F_{5,24} = 246.9$, $p \leq 0.001$)	($F_{5,24} = 226.5$, $p \leq 0.001$)
Spyridia cupressina^{Rh}	4.8% ± 0.9^{Aa}	3.8% ± 2.0^{Aa}
Beckerella pinnatifida^{Rh}	16.5% ± 3.8^{b}	0.3% ± 1.9^{Aa}
Anthophycus longifolia^{Ph}	16.6% ± 1.7^{b}	18.6% ± 1.7^{b}
Zonaria tournefortii^{Ph}	64.9% ± 0.7^{c}	74.9% ± 1.3^{c}
Osmundaria serrata^{Rh}	77.8% ± 2.0^{d}	71.0% ± 2.6^{c}

Ch = Chlorophyta, Ph = Phaeophyta, Rh = Rhodophyta
A = growth rates not significantly different from the control. The same small letters show no significant difference between growth rates in each column from each side.

show that as the concentrations of the seaweed extracts were decreased exponentially, there was a corresponding exponential increase in the growth rates of the test fungi. At low dilutions of *H. spicifera* extract there was an increase in the growth rate of *R. solani* compared with the control (Figure 1).

Discussion

The data provides good preliminary evidence for the antifungal properties of some South African seaweed extracts (Table 1). The antibacterial activities of some South African seaweed have also recently been demonstrated (Vlachos *et al.* 1996). These authors also tested the efficacy of different extraction methods. They established that boiling milled seaweeds in 80% ethanol yielded the highest activity indices, while those made with ground seaweed extracted with diethyl ether yielded the lowest. Extraction methods thus need to be optimised in order to make valid comparisons with other studies. Another aspect which needs investigation is the seasonality of antibiotic activity of seaweed extracts (Vidyavathi & Sridhar 1991).

At very low concentrations, extracts of *H. spicifera* caused stimulation in the growth of *R. solani* (Figure 1). Growth promotion could be detected since the pour-plate method allowed the measurement of inhibition and promotion of fungal growth. Other methods (where the extract is applied in wells or on disks) give little quantitative data on growth promotion because the test fungi overgrow the wells or disks.

Compounds that promote fungal growth, especially at low concentrations, might be useful in fungal cultivation (e.g. mushrooms; Stamets & Chilton 1983) and industrial fermentation (e.g. citric acid production using *Aspergillus niger;* Alexopoulos & Mims 1979). Promotion of fungal growth at low extract concentrations may be economically viable because of the small quantities of seaweed extract needed. The extract obtained from *H. spicifera* shows promise in this regard and warrants further study (Figure 1). The authors have not found any reports where diluted seaweed extracts promoted the growth of a test organism. This may be because most studies on the biological activity of seaweed extracts used wells or disks, thus any growth promotion would not readily be noticed. In addition, other workers have concentrated on obtaining antibiotics from macroalgae and, thus, did not test for growth promotion. The observed promotion in fungal growth may have been due to either small quantities of a very active compound, or large quantities of a weakly active compound. It has been suggested that seaweed extracts be evaporated in future to determine the amount of solid material in them. It would also be necessary to verify the growth promoting properties of seaweed extracts on a number of different growth media. This is because the growth medium which was used (PDA) would probably have affected the sensitivity of the fungi to the seaweed extracts because of the supply of hormones and vitamins by the medium. If the medium is adequate in these then the seaweed extracts would not produce a response, but may do so if the medium is deficient in these.

This study demonstrates the potential of South African seaweed extracts to provide antimicrobial compounds that inhibit the growth of fungal plant pathogens. Algal extracts exhibiting good biological activity need to be purified and characterised in order to determine which compounds caused the observed promotion or inhibition of fungal growth. The purified secondary products may then be subject to further testing of the plant-pathogen-environment triangle (Isaac 1992), where the seaweed

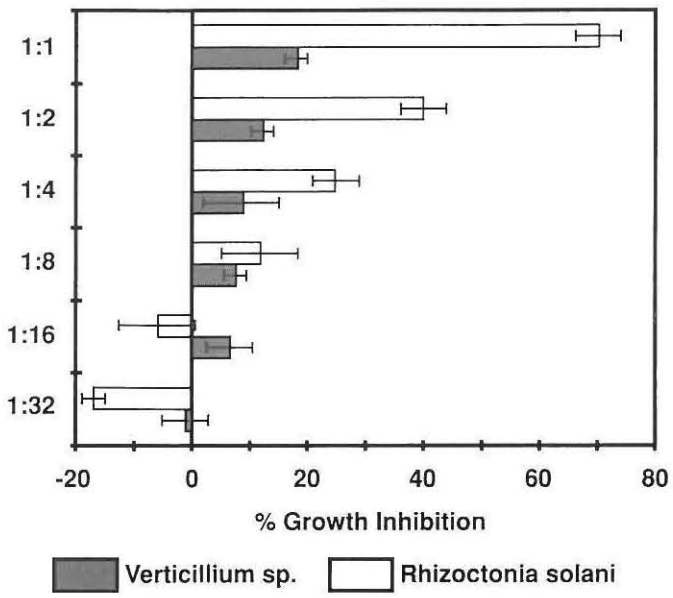

Figure 1 Effects of increasing dilution of *Hypnea spicifera* extract on *Verticillium* sp. and *Rhizoctonia solani*. The growth index values are the means of five replicates (±SE). Regression of means were performed on the growth rate data for each fungus: for *R. solani* $r^2 = 0.972$, $F_{14} = 141.448$, $p = 0.0003$ (y = 0.017x -1.114) and for *Verticillium* sp. $r^2 = 0.927$, $F_{14} = 66.275$, $p = 0.0012$ (y= 0.076x -1.517).

products would be an environmental variable. The synthesis of organic compounds analogous to those made by seaweeds can then proceed and be used commercially without exploiting seaweed resources.

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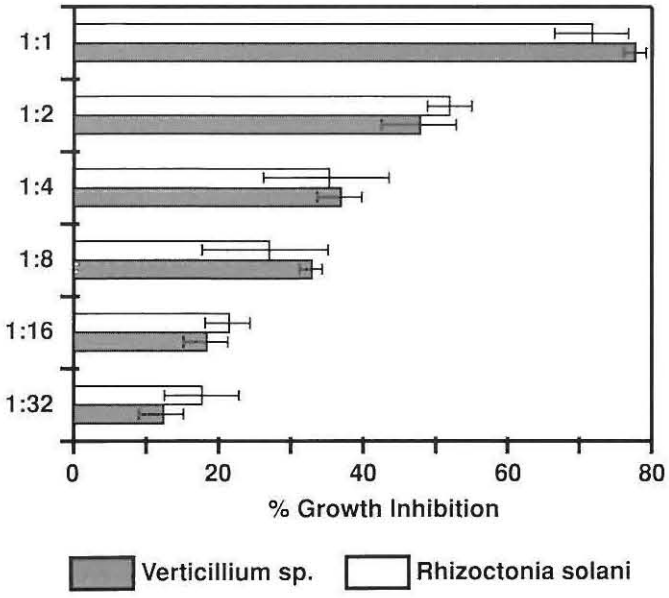

Figure 2 Effects of increasing dilution of *Osmundaria serrata* extract on *Verticillium* sp. and *Rhizoctonia solani*. The growth index values are the means of five replicates (±SE). Regression of means were performed on the growth rate data for each fungus: for *R. solani* $r^2 = 0.919$, $F_{14} = 45.646$, $p = 0.0025$ (y = 0.026x -1.728) and for *Verticillium* sp. $r^2 = 0.911$, $F_{14} = 38.506$, $p = 0.0034$ (y = 0.025x -1.755).