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ANTIFUNGAL ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF SOME SEAWEEDS AGAINST COMMON SOIL-BORNE PLANT PATHOGENIC FUNGI

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Abstract

Total 32 species of different seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta were collected from the coast of Karachi, Pakistan to investigate their antifungal activity. Most of the seaweeds inhibited growth of *Fusarium oxypsorum*, *Macrophomina phaseolina* and *Rhizoctonia solani*. The highest antifungal activities were observed in *Sargasssum tenerrimum* in both aqueous and methanolic extracts as compared to other seaweeds.

Key words: Antifungal activity, Soil-borne pathogens, Seaweeds, Aqueous and Methanolic extract.

Introduction

Ocean has been recognized as a store house of fine chemicals. Marine algae have been closely associated with human life and being exhaustively used in numerous ways as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids (Levring *et al.*, 1969; Chapman, 1970). Macro algae can be classified as green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta) depending on their pigment, nutrient and chemical composition. They serve as an important source of bioactive natural substances (Mabeau & Fleurence, 1993; Marinho-Soriano *et al*., 2006). Seaweeds are considered as source of biological activities. Compounds have been detected in green, brown and red algae with cytostatic, antiviral, antiheliminthic, antifungal and antibacterial activities (Newman *et al.,* 2003). They have secondary metabolites with antiviral, antibacterial and antifungal activities (Caccamese *et al.,* 1980; Del-Val *et al.,* 2001; Perry *et al.,* 1991).

Karachi Coast (100 km) is located on the Arabian Sea. It includes beaches and numerous islands. The coastal waters around Manora, Sandspit, Hawkesbay, Buleji and Paradise Point are inhabited by a variety of marine benthic algae (Shameel & Tanaka, 1992). A variety of marine benthic algae belonging to Chlorophyta, Phaeophyta, and Rhodophyta are associated with different coastal areas of Karachi and have several biological properties such as antibacterial, antifungal, phytotoxic and insecticidal activities (Rizvi & Shameel, 2005).

Soil-borne pathogenic fungi are responsible for considerable plant yield losses than other microorganisms (Sexton & Howlett, 2006) because these fungi can survive in soil for several years by producing sclerotial and other resting bodies. The pathogens cause collar rots, leaf blights, root rots and stem rots in many economically important host crops (Garrett, 1956). In Pakistan, several soil-borne fungi including *Fusarium* spp., produce wilt and root rot disease in almost all the infect plants while *R. solani* and *M. phaseolina* make reduction in growth of plants (Saleem *et al*., 1996; Mushtaq and Hashmi, 1997; Aboshosha *et al*., 2007; Hussain *et al.,* 2013; Usman *et al.,* 2014; Hussain *et al.,* 2015). In this background, the present study was initiated to explore the bioactive potential of major seaweeds infested along the coast of Pakistan.

The main objectives of the present studies were, 1) to identify and examine the texa belonging to Chlorophyta, Phaeophyta and Rhodophyta from marine coast of Karachi, 2) to conduct the antifungal test of all identified specimen by using aqueous and methanolic extract against three common soil-borne pathogenic fungi viz., *F. oxysporum*, *M. phaseolina* and *R. solani*, and 3) to observe the inhibition percentage of seaweeds against these pathogenic fungi.

Materials and Methods

Isolation of soil-borne fungi: *Fusarium oxysporum*, *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated from the roots of egg plants obtained from Department of Botany, Federal Urdu University of Arts, Science & Technology, Karachi. The infected roots were surface sterilized with 1% sodium hypochlorite (NaOCl) for 2 minutes and inoculated on Potato Dextrose Agar (PDA) medium containing Petri plates. The plates were incubated at 28 ±2 °C for five days.

Collection and extract preparation of seaweeds: Thirty two seaweeds were collected from the Manora, Hawksbay and Buliji coast of Karachi, Pakistan during 2012-2014 in different seasons (Table 1). Seaweed species exposed on sand, rocks and floating along the waves, were collected in sterilized plastic bags containing water to prevent evaporation, put into ice box and transferred to laboratory immediately. Fresh seaweeds were rinsed with tap water and polished to remove extraneous (any associated epiphytes, salt, sand, microorganisms) and other suspended materials. Samples were washed thoroughly with seawater as well as surface sterilized with 1% NaOCI. The samples were shade dried for ten days than oven dried at 40±2°C.These dried samples were ground in an electric mixture into powder, stored in polythene bag at room temperature until use.

Preparation of extracted antifungal bioassay: The dried samples (16g) were extracted in 100 ml of each solvent i.e. methanol and water at 28°C. After 24 hours, extract was filtered through Whatman filter paper no 1. The methanolic and aqueous extracts of seaweeds were tested against soil-borne fungi by poison food technique

against *F. oxysporum*, *M. phaseolina* and *R. solani* under lab conditions. Seaweeds extracts at 2.2 ml of each methanol and water stock solution (16% concentration) were added in PDA and poured in sterilized Petri plates for significant results. For antifungal activity, 5 mm disc of actively growing culture of a test fungus placed at the center of Petri plates. Each treatment was replicated three times and Petri plates were incubated at 28±2ºC. After five days of inoculation, radial growth of mycelium were measured and compared with the results in control. The following formula was used to calculate the percent inhibition for each fungus.

$$\text{Percent inhibition} = \frac{Y - Z}{Y} \times 100$$

where Y = Mycelial growth of pathogen alone (control),
Z = Mycelial growth of pathogen along with antagonist

Results and Discussion

Thirty two seaweeds were collected from different localities of Karachi coast (Table 1).

Aqueous extracts of 30 of the 32 seaweeds collected during the present studies showed significant inhibition in the growth of *F. oxysporum.* Whereas *Colpomenia sinousa* and *Iyengaria stellata* were no affected. Maximum (86%) inhibition was found in *P. tetrastromatic* followed by 82% in *C. iyengarii*. When same seaweeds were used against *M. phaseolina*, the maximum inhibition in growth (86%) was observed in *S. tenerrimum* as compared to other seaweeds. However, aqueous extracts of seaweeds against *R. solani* were found effective. Maximum inhibition in *R. solani* growth (74%) was observed in *S. tenerrimum* as compared to other seaweeds extracts (Fig. 1).

In methanolic extracts, *F. oxysporum* was highly inhibited by almost all species of seaweeds except *C. sinousa* and *I. stellata* in which no activity was found. Maximum inhibitions (98%) were observed in *S. tenerrimum* followed by 89% in *S. bindarri*. *S. tenerrimum* also significantly inhibited *M. phaseolina* growth (88%) as compared to other seaweeds. However, methanolic extracts of different seaweeds were found less effective against *R. solani* as compared to *F. oxysporum* and *M. phaseolina*. The maximum inhibition was found (77%) in *S. tenerrimum* seaweed as compared to other seaweeds (Fig. 2).

Table 1. List of thirty two seaweeds used for antifungal activity.

Serial code	Seaweeds name	Common name
A	*Calliblepharis floresii*	Flat Twig alga, Y-Twig Alga
B	*Caulerpa paltata*	Papyrus Print Alga
C	*C. racemosa*	Green Grape Alga
D	*C. scalepiliformis*	-
E	*C. taxifolia*	-
F	*Centroceras* sp.	Flat Twig alga, Y-Twig Alga
G	*Ceramium* sp.	Pottery seaweed, Hairy pottery seaweed, Staghorn felt, Flat Twig alga, Y-Twig Alga
H	*Chaetomorpha antinnin*	Hog's bristle, Green excelsior
I	*Codium iyengarii*	Sea staghorn, Sponge seaweed, Spongy coushion, Dead man's fingers, Encrusting *codium*, Green Grape Alga
J	*Colpomenia sinousa*	Oyster thief, Pocket thief, Round brown bag
K	*Cystoseira indica*	Bladder weed, Rainbow bladder weed, Woody chain bladder, Northern bladder chain, Bladder chain
L	*Dictyota dicotoma*	Forded Sea Tumbleweeds
M	*Geladium pulchrum*	brown sea parsely, Red lace, Flat Twig alga, Y-Twig Alga
N	*Gracilaria costicata*	Ceylon moss, Chinese moss, Sea string, Sewing thread, Red spaghetti, Flat Twig alga, Y-Twig Alga
O	*Halimeda tuna*	Large Leaf Watercress Alga
P	*Halymenia porphyaeformis*	Flat Twig alga, Y-Twig Alga
Q	*Hypnea muciformis*	Green tips, Flat Twig alga, Y-Twig Alga
R	*Iyengaria stellata*	-
S	*Jania adhaereus*	Flat Twig alga, Y-Twig Alga
T	*Jolyna laminariodes*	-
U	*Laurencia pinatifida*	Pepper dulse (in Scotland), Sea laurel
V	*Melanothamnus afaqhusinii*	-
W	*Padina tetrastromatica*	Peacock's tail
X	*Porphyra perforators*	Karengo (in New Zealand), Laverbread (in Wales), Laver, Red fringe, Red jabot laver, Red laver, Slack (in Scotland), Sloke (in Ireland, Nori, Kelp-fringing nori
Y	*Sargasssum tenerrimum*	Gulf weed, Wireweed
Z	*S. bindarri*	Sea-lentil, Gulf Weed, *Sargassum* Weed, White-vein *Sargassum*, Sargasso Weed
A1	*S. swightii*	Gulf weed, Wireweed
B1	*Sciani husimini*	Burgundy Crust Alga
C1	*Steochospermum polypolides*	-
D1	*Udotia* sp.	Spindleweed, Fuzzy Tip Alga
E1	*Ulva rigida*	Green dried laver, green laver, Sea-Iattuce, Spindleweed, Fuzzy Tip Alga
F1	*Valaniopsis* sp.	-

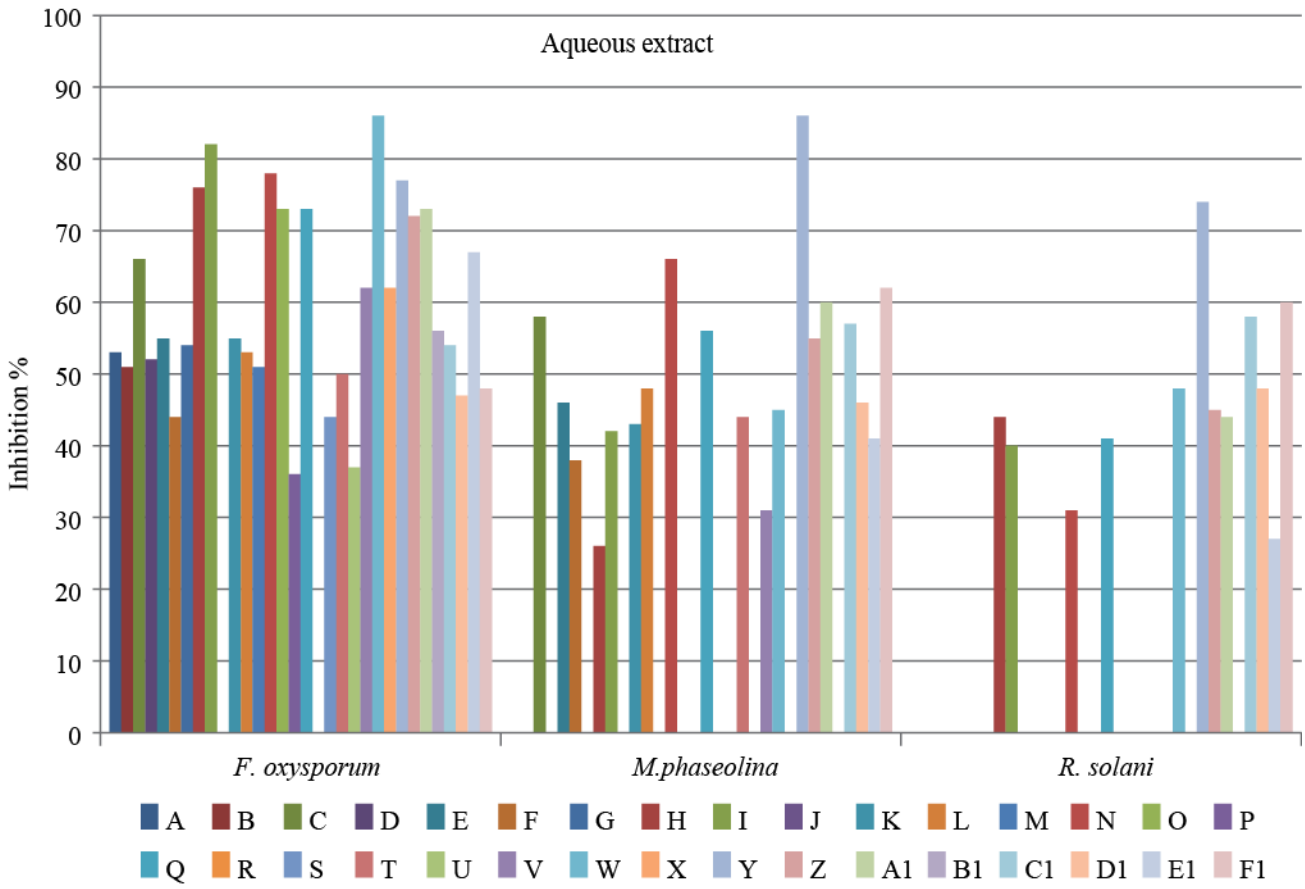

Fig. 1. Inhibition percentage of different fungi with aqueous extract of all selected seaweeds **A** to **F1** as described in Table. 1.

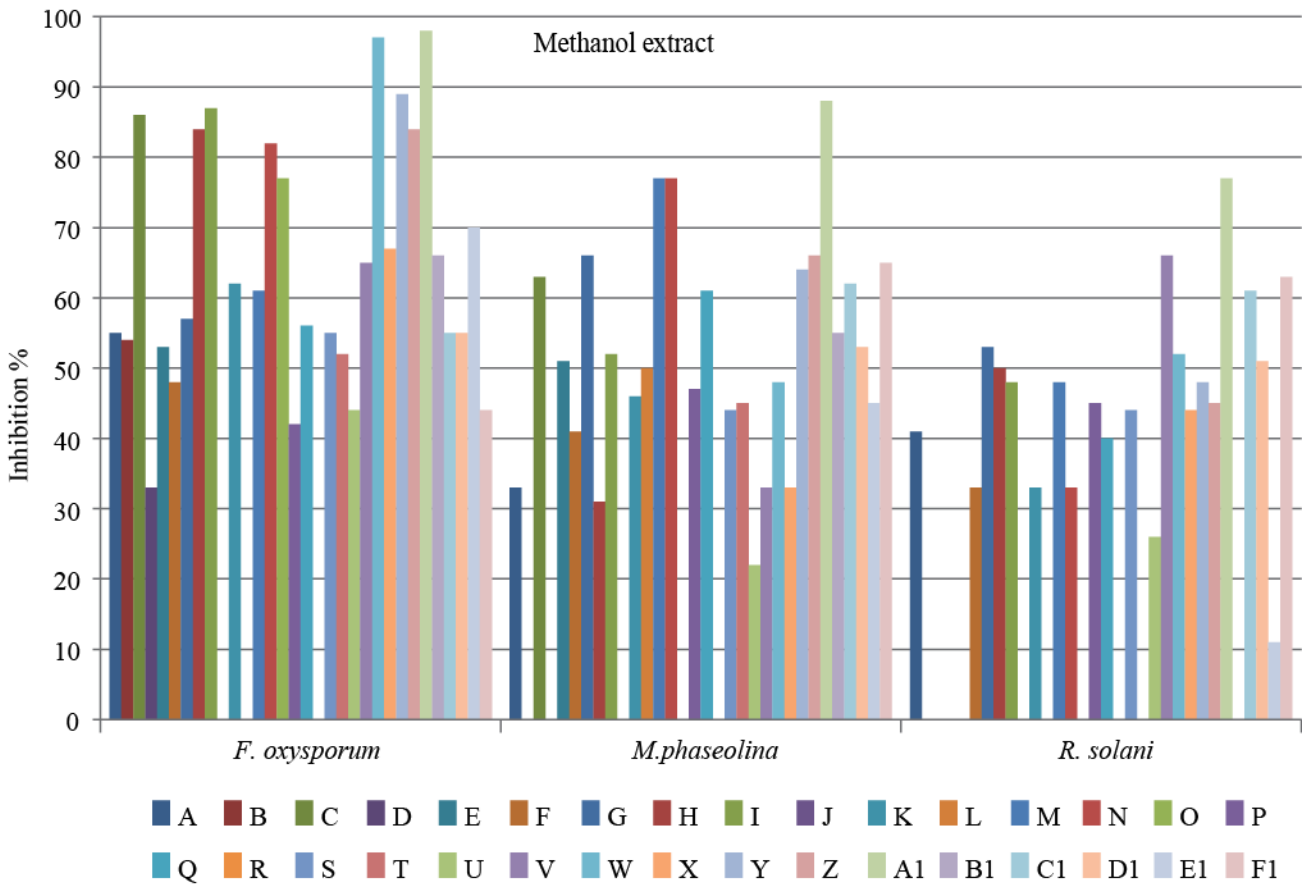

Fig. 2. Inhibition percentage of different fungi with methanonlic extract of all selected seaweeds **A** to **F1** as described in Table. 1.

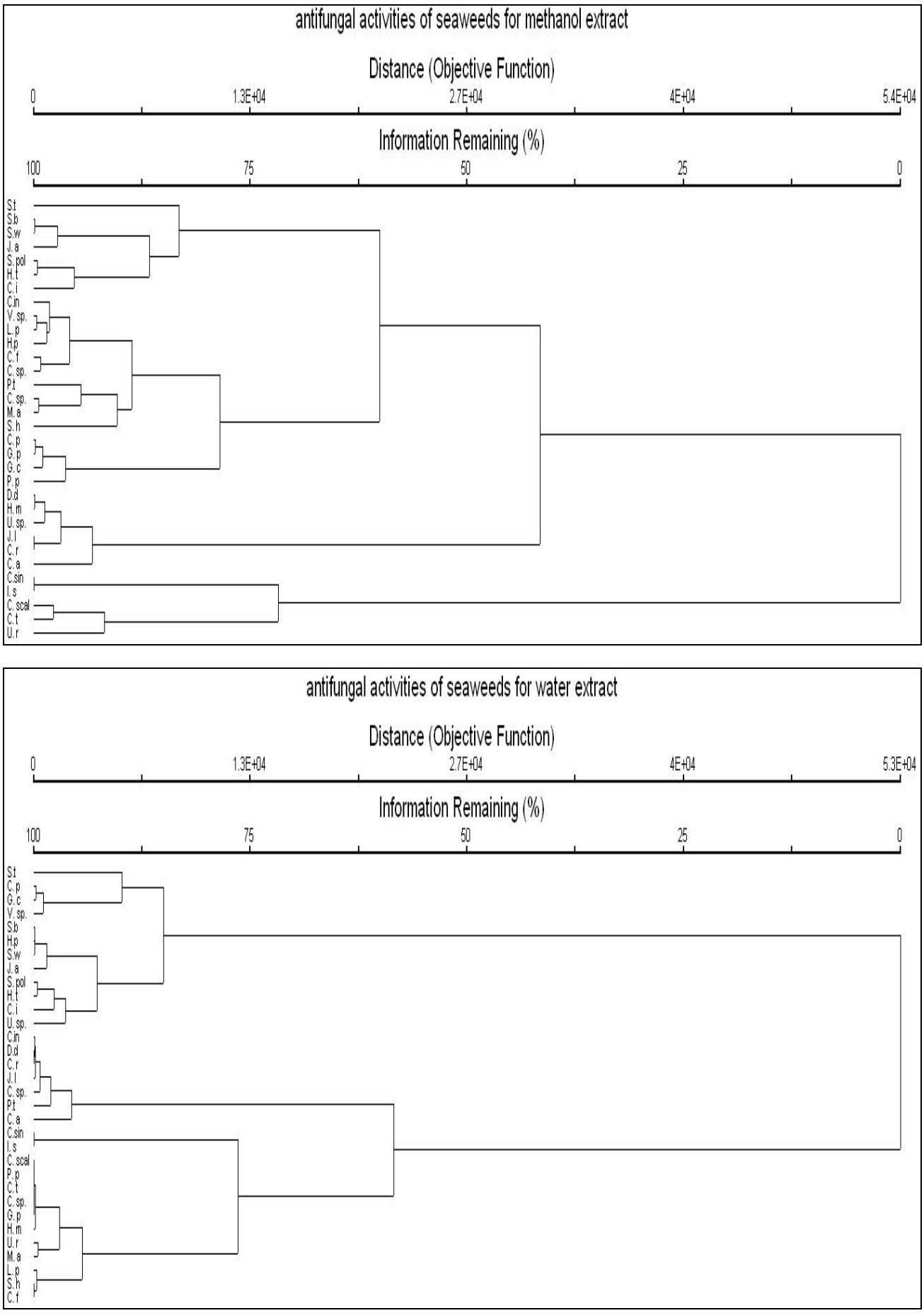

Fig. 3. Dendrogram obtained from Ward's agglomerative cluster analysis of aqueous and methanol extract of 32 stands of different seaweeds.

Agglomerative cluster analysis of stands (Ward's method) for aqueous and methanolic extract: The dendrogram resulting from cluster analysis for aqueous extract of different selected seaweeds using Ward's method is shown in Fig. 3. The dendrogram of aqueous extract discloses two main groups at a squared Euclidean distance. Group I comprising of 12 stands, is characterized by the predominance of *S. tenerrimum* (86%) and *C. iyengarii* (82%). Group II includes maximum 20 stands. It is dominated by *P. tetrastromatica* (86%). The dendrogram resulting from cluster analysis for methanolic extract of seaweeds data discloses two main groups at a squared Euclidean distance. Group I comprising of 27 stands, is characterized by the predominance of *S. tenerrimum* (98%), *S. bindarri* (89%) and *C. Iyengarii* (87%). Group II that includes only five stands was dominated by *U. rigida* (70%); *C. taxifolia* (53%) had low abundance in this group (Fig. 3).

The ANOVA for effect of aqueous and methanolic extracts of different seaweeds on selected soil-borne fungi showed highly significant differences in treatments ($F=3.41$, $p<0.001$) in inhibiting the growth of *F. oxysporum*, *M. phaseolina* and *R. solani* ($F=19.09$, $p<0.001$). However in methanolic extract, all most all treatments showed highly significant differences ($F=5.88$, $p<0.001$) and helped to suppressed the growth of the test fungi.

Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens chemical defense mechanisms against herbivore; for example, grazer-induced mechanical damage triggers the production of chemicals that acts as feeding detergents or toxins in seaweeds (Watson & Cruz-Rivera, 2003). The sea offers a reservoir of useful seaweeds with biodynamic activities. Several studies of marine plant extracts have exhibited a variety of antimicrobial activities (Shameel & Tanaka, 1992; Alam *et al.,* 1994; Shameel *et al.,* 1996; 2000; Aliya & Shameel, 1999; Hameed *et al.,* 2000; 2001; Rizvi & Shameel, 2003).

Pesando & Caram (1984) and Reichelt & Borowitzka (1984) have reported that extracts from brown algae show higher degrees of anti microbial activity. Also, pholorotannins, phenolic compounds and cliterpenediol (crinitol) are reported to be produced by brown algae *Sargassum critaefolium*, *S. tortile*, *Ecklonia kurome*, *E. bicyclis* and *Cystoseira crinite* and also exhibit antifungal activity (Vairappan *et al.,* 2004). The majority of the compounds isolated from marine algae are responsible for the antimicrobial activity. These compounds are (König *et al.,* 1999a; Etahiri *et al.,* 2001; Wang *et al*., 2007; Smyrniotopoulos *et al.,* 2008), phenolic and lipidic by nature in seaweeds (Paul *et al*., 1987; Al-Fadhli *et al.,* 2006).

Ali *et al.* (2000) reported that extracts from *Codium iyengarii* displayed good activity, and in other observations this extract showed significant antifungal activity against a variety of pathogens. The ethanol extract from *S. tenerrimum* showed 100% inhibition of the fronds at a concentration of 500 mg/ml. It appears that different species of the same genus act variably. Probably they accumulate different natural products, which may be responsible for phytotoxic activity (Ali *et al.,* 2000; Rizvi & Shameel, 2003). The present study closely related and confirms the results of Ali *et al.* (2000) and Rizvi & Shameel (2003).

Conclusion

In present study, three common soil-borne fungi *viz.*, *F. oxysporum, M. phaseolina* and *R. solani* were tested against the extracts of thirty two seaweeds. Maximum inhibitory activities were recorded against *F. oxysporum* in almost all extracts of selected seaweeds. On the other hand, similar extracts of these species of seaweeds did not show any inhibitory activity against *M. phaseolina* and *R. solani*. It is concluded that methanolic extract is highly effective rather than aqueous extract so methanol extract can be applied against the soil-borne fungi and it has entirely bioactive compounds for antifungal potential.

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