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# Antimicrobial activities of some brown macroalgae against some soil borne plant pathogens and in vivo management of *Solanum melongena* root diseases

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**ABSTRACT**

Marine macroalgae are rich source of structurally novel and biologically active metabolites that could be used in biological control of some plant diseases. In this study, we evaluated the antimicrobial activities of crude methanolic extracts of three brown macroalgal spices; *Sargassum latifolium*. *Hydroclathrus clathratus* and *Padina gymnospora*, collected from Red Sea, Hurghada coastline, Egypt, against four soil borne plant pathogenic microbes, bacteria; *Ralstonia solanacearum* and *Pectobacterium carotovora*, fungi; *Fusarium solani*, *Rhizoctonia solani*. Minimal inhibition concentration (MIC) was 1.25 mg/disc for three algal species except, *H. clathratus* had no effect against *F. solani* and *R. solani*. The methanolic extract of *P. gymnospora* recorded the highest inhibition zones (18.3, 16.3 mm) against *R. solanacearum* and *P. carotovora* at 5 mg/disc and (24, 21.7 mm) against *F. solani* and *R. solani* at 7 mg/disc, respectively. Phyto-chemical analyses of fatty acids, saccharides and phenolic compounds revealed that, *P. gymnospora* and *S. latifolium* were characterized by the highest proportion of total saturated fatty acids with (58.33 %, 42.78). In contrast, *H. clathratus* showed the highest content of unsaturated fatty acids by 58.21 %. Palmitic acid (C16:0) was the most abundant saturated fatty acid in *S. latifolium, H. clathratus* and *P. gymnospora* extracts by (29.5, 21.4 and 36.4 %), respectively. Interestingly, arachidonic acid (all cis 5,8,11,14-C20:4) was only detected in *P. gymnospora* methanol extract by 2 %. Stachyose represented the highest saccharide fraction in *S. latifolium* and *P. gymnospora* by (67.3 and 56.9 %), respectively; while, Glucouronic recorded the highest saccharide fraction in *H. clathratus* by (42 %). *P. gymnospora* had the highest content of chlorogenic, P-OH-benzoic and e-vanillic with (25.0, 40 and 567.2 ppm) respectively, compared to *S. latifolium* and *H. clathratus*. In addition, in vivo application of the powder of *P. gymnospora*, *S. latifolium* and H. *clathratus*, as soil amendment, in *Solanum melongena* L. (eggplant) pot experiment significantly decreased the percentage of root rotting in *F. solani* infected-soil by 83, 56 and 27 %, respectively. Moreover, *P. gymnospora* enhanced growth performance of eggplant in term of shoot length and plant fresh weight in the infected soil. Moreover, soil-free pathogen treated with *P. gymnospora* and *S. latifolium* powder showed significant increase in root length compared to control as well as increased fruit fresh weight by *P. gymnospora* powder. The considerable antimicrobial/biostimulant activities of *P. gymnospora* are due to its phyto-chemicals composition. This makes it feasible for biological control of some plant diseases and biosimulant agent for plant growth.



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## INTRODUCTION

Pathogenic bacteria and fungi are the most organisms responsible for a considerable loss of plant yield (Sexton and Howlett, 2006). Brown rot disease on potato is the most serious disease affecting potato exportation, caused by numerous bacterial pathogens such as *Ralstonia solanacearum* (brown rot or bacterial wilt) and *Pectobacterium carotovora* subsp. *R. solanacearum* is the most common pathogen that affect the exportation of potato crop, specially to the European markets (Kabeil *et al*., 2008). Similarly, great loss of many crops yield was attributed to many fungal diseases eg. *Fusarium solani, Rhizoctonium solani* (Sharma *et al*., 2004; Chehri *et al*., 2010). Orientations for biological control became recently an effective strategy for fighting plant pathogens using natural product extracts as alternative to chemical fungicide and bactericide due to their safety and negligible environmental impact (Brimmer and Boland, 2003; Galal *et al*., 2011). Attempts to use some medicinal plants as fungicides have been reported by Zafer *et al*., 2002 and Khalil *et al*., (2005) or agriculture waste (rice straw, maize and cotton) against *R. solani* (Osman *et al*., 2011). Marine macroalgae are considered as an excellent source of bioactive compounds with a broad range of biological activities including antibacterial (Haroun *et al*., 1995; Singh, 2010), antifungal (De Felício *et al*., 2010; Al-Saif *et al*., 2014) and antiviral (Bouhlal *et al*., 2010; Ibraheem *et al*., 2012). Many bioactive compounds have been identified in several marine algal species by their antimicrobial activities. Some of these compounds are sterols, terpenoids, polysaccharides, peptides, proteins, vitamins, acrylic acid, terpenes, chlorophyllides, phenols, heterocyclic compounds, halogenated ketones, alkanes and cyclic polysulphides (Mtolera and Semesi, 1996; Taskin *et al*., 2007; Bouhlal *et al*., 2011; Priyadharshini *et al*., 2011; Elsayed *et al*., 2012; Abdel-Raouf *et al*., 2015). Brown macroalgae typically contain high levels of polyphenols which well known for antimicrobial activities (Khaled *et al*., 2012; Peres *et al*., 2012). In addition, other biologically active compounds viz sulphated polysaccharides, polyunsaturated fatty acids (PUFA), sterols, carotenoids and α-tocopherol showed high antimicrobial activities (Wang *et al*., 2011). Methanol extract of *Codium fragile* exhibited strong activity against *Alternaria alternate, Fusarium oxysporium*, *Alternaria brassicicola*, *Ulocladium botrytis* and *Botryotricum piluliferum* (Galal *et al*., 2011).

The main goals of the present study include screening and evaluating the potency of methanolic extract of three brown macroalgal species for in vitro antibacterial and antifungal activities against four soil borne pathogenic bacteria and fungi. Identifying the phyto-chemical composition of the algal extracts, to lay out the most active compounds among three marine species. Investigating of the in vivo management of eggplant root rotting disease using algal powders/extracts.

## MATERIALS AND METHODS

### *Algae sampling:*

Algal samples were collected from Hurghada along the Red Sea coast, Egypt south (25°04′N 34°54′E), during April 2015. The collected samples were stored in plastic bags and transported to the laboratory in ice box. The samples were initially washed thoroughly with sea water to remove sand and any adhering substances and then washed thoroughly with fresh water to remove salts. The algal species were identified based on the schemes reported in the literature (Bold, 1978; Aleem, 1993 and Coppejans *et al*., 2009) and saved in the lab of Phycology, Botany and Microbiology Department, Faculty of Science, Beni-Suef University.

### *Preparation of algal extract:*

The macroalgal specimens were air dried under shade for 72 hours. The algal biomass then grinded by the electrical mixer and then fed to a Soxhlet extractor fitted with a 500 ml round bottomed flask and a condenser. The extraction was done on a water bath for 12 h with 300 ml of 99% methanol and 30 g from each powdered samples separately. The crude extracts were filtered in Whatman filter paper No.1 and the obtained filtrates were concentrated under reduced pressure in the rotatory evaporator (GG SENCO) for complete dryness. The dried crude extracts were stored at 4 °C for testing their antimicrobial activities and phyto-chemical characterization.

### *Target microorganisms and culture conditions:*

The pathogenic bacteria, *R. solanacearum* and *P. carotovora* were kindly given by Dr. Nevein Messiha, Bacterial Diseases Research Department, Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. The bacterial strains were grown in Kings B medium, with the following constituents; 20 g peptone, 1.5 g $K_2HPO_4$, 1.5 g $MgSO_4$, 15 ml glycerol and 20 g agar in 1L distilled water, pH was adjusted at 7.2-7.4. The two fungal strains; *F. solani* and *R. solani* were obtained from Ornamental, Medicinal and Aromatic Plants Diseases Department, PPRI, ARC. The fungal species were grown in PDA medium at 30 °C for 72 h.

***Determination of antimicrobial activities:***

The antibacterial activities of the algal extracts were achieved using agar-disc diffusion technique as described by Nilsson. (1978). Minimum inhibitory concentration (MIC) was conducted by testing three different concentrations of the crude methanolic extract i.e. control, 1.25, 3.0 and 5.0 mg/disc while, the antifungal activities were assessed using control, 1.25, 3.0 ,5.0 and 7.0 mg/disc. Pure methanol and Topsin-M (chemical fungicide) were used as negative and positive controls, respectively. All measurements were conducted in triplicates.

***Phyto-chemical analyses:***

***Fatty acids analysis:***

Fatty acids composition was determined using a gas-liquid chromatography (Hewlett Packard Model 6890) according to A.O.A.C. (2000). Analysis of fatty acid methyl esters was preceded on capillary column INNO wax (polyethylene glycol) with 30.0 m×530 µm×1.0 µm dimensions. Nitrogen was used as carrier gas for gas phase analysis, with flow rate 15 ml/min, average velocity 89 cm/sec and pressure 8.2 psi. The flame ionization detector temperature was maintained at 280 °C with hydrogen and air flow rates of (30 mi/min and 300 ml/min), respectively. Column temperature was adjusted at 240 °C, initial temperature was set at 100 °C for 10 min, after which point it was increased up to 240 °C at a rate of 10 °C/min then was hold at 240 °C. Injection temperature was maintained at 280 °C with split ratio and split flow (8:1 and 120 ml/min), respectively.

***Phenolic compounds and polysaccharides analyses:***

The phenolic compounds and polysaccharides content in the methanolic extracts of marine algal samples were determined according to method described by Goupy *et al*. (1999) and Zielinski *et al*. (2014) respectively. The detections were conducted using high performance liquid chromatography (HPLC Agilent 1200 series,USA) equipped with Quaternary pump, Auto sampler, column compartment was set at 35 °C, multi wavelength detector set at 330 nm, 280 nm.

**In vivo *management of eggplant root rotting disease:***

***Eggplant nursery preparation:***

Eggplant (*Solanum melongena* L.), obtained from Sids Research Station, were soaked in water for 48h then covered with a sheet of cloth, stored for 48 h for nursery preparation. The nursery was fertilized with the uniform recommended dose of NPK fertilizers. At 30 days aged seedling, 4 healthy seedlings of equal size were uprooted and transplanted in eggplant pot experiment.

***Egg plant pot experiments:***

***Evaluation of the influence of algal powder amendments on management of eggplant root disease:***

In pot experiment, the soil was naturally infested with 70 ml of $1\times10^6$ cfu (cfu = colony forming units) of *F. solani* as determined by soil dilution (Nash and Snyder, 1962). 3 kg of infested soil was transferred to plastic pots (20 cm diam). Soil was amended with dry algal powder in ratio (1.0 gm:1.0 Kg soil) wt/wt. Pots were watered to allow decomposition of the organic substrate. The experiment comprised of 9 different treatments accordingly; [Topsin-M (chemical fungicide), *F. solani* infected- soil, *S. latifolium*, *S. latifolium* with *F.solani*, *H. clathratus*, *H. clathratus* with *F. solani*, *P. gymnospora*, *P. gymnospora* with *F. solani* and control (soil free from amendment or infection)]. In more details, algal powder of *S. latifolium*, *P. gymnospora* and *H. clathratus* were tested separately in soil treated with/without *F. solani*. Fungicide (Topsin-M) was mixed with infected soil and used as a positive control. Soil without algal amendments or fungicide was used as negative control.

***Evaluation of the influence of sole algal extract or combined with algal powder amendments on management of eggplant root rotting disease***:

In this experiment, treatments were conducted using algal extracts alone or combined with algal powder amendments. Seedling roots of 3 weeks old were soaked in crude methanol extracts of respective marine algal species for 10 minutes. The seedlings were then transplanted immediately into soil amended with/without 9 different treatments as detailed earlier. This experiment was conducted concomitant with the previous experiment. The pots were kept randomized on a greenhouse bench. Plants were watered 2-3 days intervals depending upon of plant requirements. All treatments were conducted in three biological replicates and arranged in a complete randomized design according to (Gomez and Gomez, 1984).

***Plant analyses:***

***Chlorophyll assay:***

The chlorophyll *a*, *b* contents were determined in plant cells in seedling of fifth week age growth according to the method described by Metzner *et al*. (1965). A clean plant tissue from the middle leaf was weighted, cut into small pieces and mixed thoroughly with 10 ml of methanol and petroleum ether (10:3 v/v).

Chlorophyll was extracted by heating the test tubes in water bath for 15 min at 50 °C. The pure chlorophyll extract was obtained by centrifugation the mixture for 10 min at 3000 rpm. The optical density was measured at absorbance 663, 644 nm using mixture of methanol and petroleum ether as blank. The chlorophyll concentration (µg/ml) of each plant extract was calculated using the following equations:

(1) Chl *a* = $(10.3\times E_{663})-(0.918\times E_{644})$

(2) Chl *b* = $(19.7\times E_{644})-(3.87\times E_{663})$

Where, 10.3, 0.918, 19.7 and 3.87 are the conversion constants.

***Disease symptoms and growth parameters analyses:***

The effects of algal amendments or Topsin-M against root rotting fungi as well as plant growth were evaluated by uprooting of 12 weeks old plant and the developed symptoms were recorded. Incidence of fungal infection (% of root rooting) was evaluated according to the method described by Sultana *et al*. (2011). 1 cm long root pieces from tap roots (five pieces from each plant) were surface disinfested with 1 % Ca $(OCl)_2$ solution and plated onto potato dextrose agar amended with penicillin (100,000 units/L) and streptomycin (0.2 g/L), after incubation for 5 days at 28°C, colonies of *F. solani* were counted. All measurements were conducted in three biological replicates.

***Statistical analyses:***

Statistical analysis was performed using the ANOVA in the SPSS 20 software (IBM Corporation, Armonk, New York, USA) and means were compared using the least significant difference (LSD) according to Gomez and Gomez (1984).

***Results:***

***Identification of the marine macroalgal species:***

Taxonomy of the three collected marine algal species to schemes reported in the literature. Searches revealed that marine species were belonging to phaeophyta (brown algae) accordingly; (A) *Padina gymnospora* (Kützing) Sonder, (B) *Hydroclathrus clathratus* (C. Agardh) M.Howe and (C) *Sargassum latifolium* (Turner) C.Agardh.

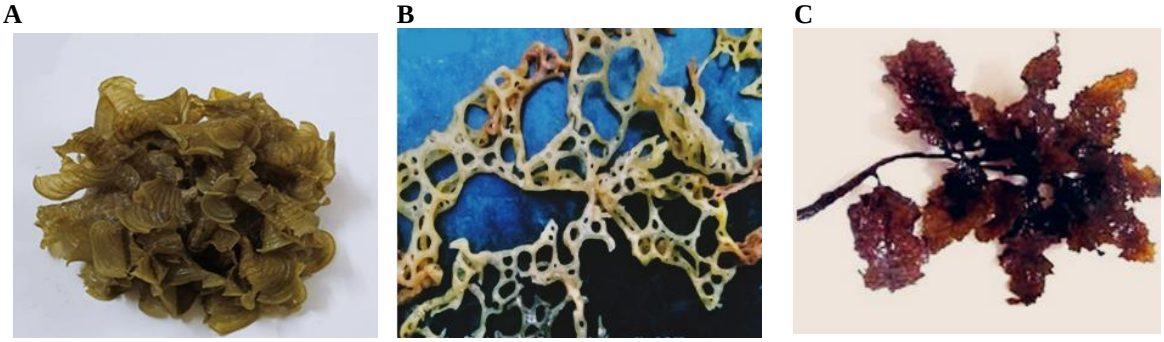


**Fig. 1:** Thallus morphology of the brown macroalgal species collected from Red Sea, Hurghada coastline. (A) *Padina gymnospora* (Kützing) Sonder, (B) *Hydroclathrus clathratus* (C. Agardh) M.Howe and (C) *Sargassum latifolium* (Turner) C.Agardh.

***Antimicrobial activity:***

The antimicrobial activities of *S. latifolium*, *H. clathratus* and *P. gymnospora* methanolic extracts against soil pathogenic bacteria are in (Table 1). Data showed that, the two bacterial soil borne pathogen were susceptible to the all algal extracts of brown macroalgae. The recorded inhibition zones increased proportionally with increasing the concentration of the crude extracts. *P. gymnospora* recorded the highest inhibition zones by (18 and 16 mm) at 5mg/dick against *R. solanacearum* and *P.carotovora*, respectively, followed by *S. latifolium* (12.7 and 16 mm) and *H. clathratus* (15 and 11 mm) at the same concentration. The antifungal activities of *S. latifolium*, *H. clathratus* and *P. gymnospora* are shown in (Table 2). *P. gymnospora* also recorded the highest inhibition zones by (24.7 and 21.7 mm) at 7 mg/disc against *F. solani* and *R. solani*, respectively followed by *S. latifolium* by ( 20 and 18 mm) at the same concentration. On contrary, *H. clathratus* showed negative result against both *F. solani*, *R. solani.*

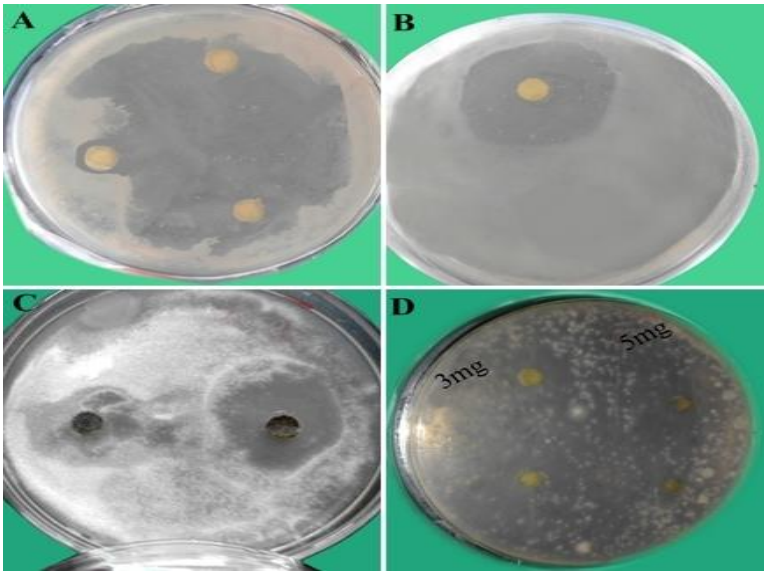


**Fig. 2:** Antimicrobial activities of the methanolic extracts of *Padina gymnospora* against soil borne pathogenic bacteria; (A) *Pectobacterium carotovora*, (B) *Ralstonia solanacearum* and fungi; (C) *Fusarium solani* and (D) *Rhizoctonium solani* after of 24 h of incubation.

**Table 1:** The antibacterial activities of different methanolic extract concentrations of *Sargassum latifolium*, *Hydroclathrus clathratus* and *Padina gymnospora* against some soil borne pathogenic bacteria using disc diffusion method. Data points are the mean average of three replicates ± S.D.

| | *Sargassum latifolium*. Inhibition zone (mm) | | | *Hydroclathrus clathratus* Inhibition zone (mm) | | | *Padina gymnospora* Inhibition zone (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial pathogens | 1.25 mg/disc | 3.0 mg/disc | 5.0 mg/disc | 1.25 mg/disc | 3 mg/disc | 5 mg/disc | 1.25 mg/disc | 3 mg/disc | 5 mg/disc | L.S.D |
| *Ralstonia solanacearum* | 10.0±1.0 | 12.3±2.3 | 12.7±2.1 | 10.7±1.5 | 14.0±1 | 15.0±4.4 | 13.0±2 | 16.7±2.1 | 18.3±2.5 | 1.88 |
| *Pectobacterium carotovora* | 10.3±1.5 | 15.7±2.5 | 16.3±1.6 | 9.7±2.1 | 10.0±2 | 11.3±2.5 | 9.7±0.6 | 16.0±3.6 | 16.3±2.5 | 1.81 |

**Table 2:** The antifungal activities of different methanolic extract concentrations of *Sargassum latifolium*., *Hydroclathrus clathratus* and *Padina gymnospora* against some soil borne pathogenic fungi. Data are mean of three biological replicates ± S.D.

| | *Sargassum latifolium*. Inhibition zone (mm) | | | | *Hydroclathrus clathratus* Inhibition zone (mm) | | | | *Padina gymnospora* Inhibition zone (mm) | | | | Topsin M Positive Control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fungal pathogen | 1.25 mg/disc | 3.0 mg/disc | 5.0 mg/disc | 7.0 mg/disc | 1.25 mg/disc | 3.0 mg/disc | 5.0 mg/disc | 7.0 mg/disc | 1.25 mg/disc | 3.0 mg/disc | 5.0 mg/disc | 7.0 mg/disc | 24.3±4.0 | L.S.D |
| *F. solani* | 10.3±1.5 | 16.7±1.5 | 17.0±2.0 | 20.3±1.5 | 0.0 | 0.0 | 0.0 | 0.0 | 10.3±0.6 | 16.7±3.1 | 17.3±2.1 | 24.7±2.5 | | 1.56 |
| *R. solani* | 11.0±1.0 | 12.7±2.1 | 16.3±1.5 | 18.0±2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 11.0±1.0 | 18.7±1.5 | 20.0±1.0 | 21.7±1.5 | | 1.39 |

***Phyto-chemical analyses:***

Fatty acids profile analysis in Table (3) revealed that *P. gymnospora* had the highest total saturated fatty acids composition with 58.33% followed by *S. latifolium* with 42.78. In contrast, *H. clathratus* showed the highest content of unsaturated fatty acid composition by 58.21 followed by *S. latifolium* by 57.22. Palmitic acid (C16:0) was the most abundant saturated fatty acid in *S. latifolium, H. clathratus, P. gymnospora* by ( 29.5, 21.4 and 36.4 %), respectively. Oleic acid (C18:0) recorded the highest content of mono saturated fatty acid by (30.4, 21.4 and 30.3 %) in *S. latifolium, H. clathratus, P. gymnospora*, respectively. Interestingly, arachidonic acid (all cis 5, 8, 11, 14- C20:4) was only detected in *P. gymnospora* extract with 2 %.

Saccharides type and content varied from algal species to another. The saccharides analysis is shown in Table (4), in more details, stachyose represented the highest saccharide proportion in *S. latifolium* and *P. gymnospora* by (67.3 %, 56.9 %), respectively; while, glucouronic recorded the highest saccharide fraction in *H. clathratus* by (42 %) compared to the other constituents. The highest content of xylose, sucrose, mannose and arabinose were recorded in *P. gymnospora* extract with (0.07, 0.17, 0.3 and 0.07 %), respectively. In contrast, *S. latifolium* recorded the highest content of glucose, galactose, fructose, mannitol, sorbitol with (1.3, 1.4, 0.45, 1.73, 0.04), respectively compared to the other algal extract.

Phenolic compounds analysis in Table (5) revealed that, the tested algal extracts showed variable phenolic compound content with species dependent manner. *S. latifolium* showed the highest content of pyrogallol,

gallic, 4-amino-benzoic, catechein, epicateachein, caffeinc, caffeic, vanillic and ellagic with 252.0, 15.7, 4.85, 159, 30.8, 59.2, 7.0, 275.8, 63.3 ppm, respectively. While *H. clathratus* recorded the highest content of protocatchuic, catechol, p-coumaric, ferulic, iso-ferulic, alpha-coumaric, benzoic, 3,4,5, methoxy cinnamic, coumarin, salycilic, cinnamic with 25.5, 7.8, 24.9, 29.3 , 19.3, 7.4, 225.9, 22.7, 16.9, 212.5, and 8.2 ppm, respectively. In contrast, *P. gymnospora* recorded the highest content of chlorogenic, P-OH-benzoic and e-vanillic with 25.0, 40 and 567.2 ppm, respectively.

**Table 3:** Fatty acids profile (percentage of total fatty acids) of the methanolic extract of *Sargassum latifolium*, *Hydroclathrus clathratus* and *Padina gymnospora*. Data points are the mean average of three replicates ± S.D.

| Fatty acids % | *S. latifolium* | *H. clathratus* | *P. gymnospora* |
|---|---|---|---|
| Lauric acid (C12:0) | 1.55±0.1 | 3.01±0.3 | 3.38±0.37 |
| Myristic acid (C14:0) | 8.06±0.5 | 5.26±0.48 | 14.04±0.9 |
| Palmitic acid (C16:0) | 29.46±3.0 | 21.36±2.9 | 36.35±4.5 |
| Palmitoleic (C16:1) | 4.92±0.2 | 4.14±0.2 | 3.28±0.1 |
| Stearic acid (C18:0) | 3.71±0.1 | 12.16±1.0 | 4.56±0.5 |
| Oleic acid (C18:1) | 30.36±3.0 | 21.39±2.1 | 30.30±2.5 |
| Linoleic acid (C18:2) (9,12-Octadecadienoic acid) | 8.39±1.6 | 15.39±2.0 | 4.19±0.6 |
| Linolenic acid (C18:3) (9,12,15-Octadecatrienoic acid) | 13.55±1.8 | 17.29±2.0 | 1.90±0.02 |
| Arachidonic acid (all cis 5,8,11,14- C20:4) | ---- | ---- | 2.00±0.3 |
| Total Saturated Fatty Acids | 42.78±3.8 | 41.79±4.0 | 58.33±5.0 |
| Total Unsaturated Fatty Acids | 57.22±5.0 | 58.21±3.5 | 41.67±3.0 |

Data in Table (6) indicated significant decrease in all growth characteristics in *F. solani* infected-soil where, Chl *a* and *b* contents decreased by 66 % and 62.5 % compared to control (soil-free pathogen) after 30 days of eggplant transplanting. Similarly shoot, root length and plant fresh weight were significantly decreased by 16, 50 and 47.2 %, respectively. On contrary, percentage of root rotting significantly increased by 10.8-fold. Meanwhile, treating the infected soil with the powder of *P. gymnospora* had the similar effect of the chemical fungicide (Topsin-M) on Chl *a* and *b* contents. Insignificant differences were observed in both shoot length, plant fresh weight and root rooting percentage of eggplant cultivated in *F. solani* infected-soil, treated with *P. gymnospora* when compared to control soil. Moreover, *P. gymnospora* amended-soil recorded the lowest percentage of root rotting by 11.7 % compared to 30% and 50% achieved by *S. latifolium* and *H. clathratus* treated-soil, respectively. Interestingly, the soil-free pathogen treated with *P. gymnospora* and *S. latifolium* powders significantly increased root length by 15 % and 6 %, respectively compared to control (Fig. 3). In addition, *P. gymnospora* increased the fruit fresh weight by 3.6 %. Furthermore, treating the soil-free pathogen with *P. gymnospora*, *H. clathratus* and *S. latifolium* powders decreased the percentage of root rotting by 0 %, 2.7 % and 3.7 %, respectively, compared to 6.3 % of the control soil. On contrary pre-soaking the seedling for 10 min ,before transplanting, in the methanol extracts of *S. latifolium*, *H. clathratus* and *P. gymnospora* as well as combining pre-soaking in algal extracts with soil-amended with algal powders completely inhibited the plant growth (Data are not shown).

**Table 4:** Saccharides profile (percentage of monosaccharides, disaccharides and polysaccharides) of the methanolic extract of *Sargassum latifolium*, *Hydroclathrus clathratus* and *Padina gymnospora*. Data points are the mean average of three replicates ± S.D.

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| Saccharide profile (%) | Test results of Saccharides (%) | | |
|---|---|---|---|
| | *Sargassum latifolium* | *Hydroclathrus clathratus* | *Padina gymnospora* |
| Stachyose | 67.31±3.0 | 29.572±1.0 | 56.876±2.3 |
| Glucouronic | --- | 41.981±2.0 | 4.753±0.7 |
| Galacturonic | --- | --- | --- |
| Maltose | --- | 0.108±0.03 | --- |
| Sucrose | 0.141±0.004 | --- | 0.165±0.02 |
| Glucose | 1.300±0.1 | 0.182±0.01 | 0.184±0.04 |
| Xylose | --- | --- | 0.074±0.002 |
| Galactose | 1.386±0.2 | 0.437±0.03 | 0.217±0.005 |
| Mannose | 0.176±0.06 | 0.095±0.00 | 0.299±0.01 |
| Arabinose | --- | --- | 0.071±0.002 |
| Fructose | 0.447±0.1 | 0.156±0.04 | 0.108±0.02 |
| Mannitol | 1.725±0.3 | 0.272±0.1 | 0.566±0.18 |
| Sorbitol | 0.041±0.001 | 0.0356±0.002 | 0.031±0.001 |

**Table 5:** Phenolic compounds profile of the methanolic extract of *Sargassum latifolium*, *Hydroclathrus clathratus* and *Padina gymnospora*. Data points are the mean average of three replicates ± S.D.

| Content of Phenolic compounds (ppm) | *Sargassum latifolium* | *Hydroclathrus clathratus* | *Padina gymnospora* |
|---|---|---|---|
| Pyrogallol | 252.04±8.2 | 87.03±4.6 | 48.05±3.9 |
| Gallic | 15.70±2.6 | 3.68±1.1 | 7.18±1.8 |
| 4-Amino-benzoic | 4.85±1.78 | 4.12±1.0 | 3.70±0.9 |
| Protocatchuic | 20.37±4.6 | 25.49±4.0 | 20.92±3.2 |
| Catechein | 158.95±6.3 | 102.85±5.0 | 65.87±2.0 |
| Catechol | 7.06±1.04 | 7.79±2.0 | 3.71±0.87 |
| Chlorogenic | 9.69±2.0 | 23.64±4.3 | 25.02±3.8 |
| Epicateachein | 30.78±5.1 | 22.26±3.7 | 18.67±2.5 |
| P-OH-benzoic | 37.32±2.3 | 39.03±3.0 | 39.95±3.6 |
| Caffeinc | 59.24±4.0 | 37.20±3.7 | 26.65±2.8 |
| Caffeic | 7.01±1.5 | 2.83±0.9 | 2.21±0.6 |
| Vanillic | 275.75±9.6 | 78.67±6.8 | 105.04±7.5 |
| P-Coumaric | 8.07±2.4 | 24.90±2.6 | 21.81±1.9 |
| Ferulic | 19.16±2.7 | 29.26±2.04 | 16.76±2.0 |
| Iso-ferulic | 4.72±1.2 | 19.28±3.6 | 4.76±1.3 |
| e-Vanillic | 141.99±4.7 | 510.29±10.3 | 567.19±12.0 |
| Ellagic | 63.26±3.1 | 30.53±2.6 | 50.75±4.2 |
| Alpha-coumaric | 3.96±0.9 | 7.36±1.3 | -- |
| Benzoic | 77.16±2.0 | 225.92±5.5 | 160.38±6.1 |
| 3,4,5,methoxy cinnamic | 6.24±1.5 | 22.68±2.3 | 7.73±1.8 |
| Coumarin | 6.43±0.7 | 16.92±2.0 | 7.59±1.4 |
| Salycilic | 57.67±2.6 | 212.50±9.7 | 139.91±7.6 |
| Cinnamic | 2.34±0.6 | 8.16±1.1 | 4.45±0.35 |

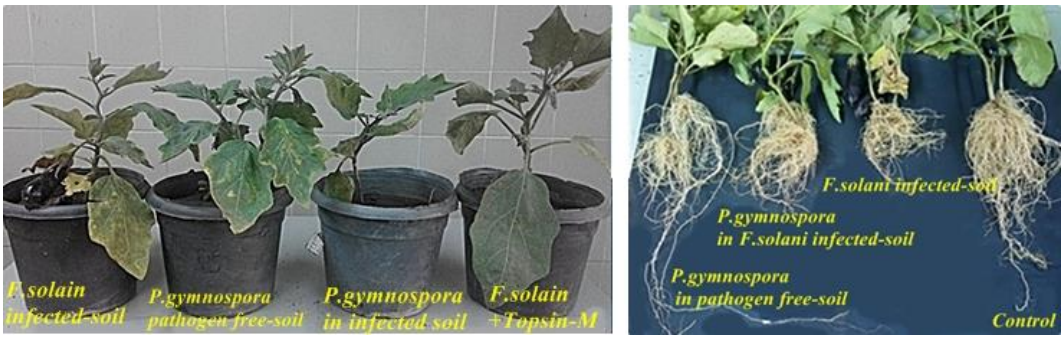


**Fig. 3:** Influence of *Padina gymnospora* treatment or chemical fungicide (Topsin-M) on eggplant growth performance; shoot and root length cultivated in control soil (pathogen-free) or *Fusarium solani* infected-soil. The developed symptoms were recorded by uprooting of 12 weeks old plant.

**Table 6:** Growth characteristics of eggplant as affected by marine macroalgal amendments or chemical fungicide under *fusarium solani* infected-soil, pathogen free soil (control) after 90 days of transplanting

| Treatments | Control | Topsin-M chemical fungicide | *Fusarium solani* | *Sargassum latifolium.* | *Sargassum latifolium.* and *Fusarium solani* | *Hydroclathrus clathratus* | *Hydroclathrus clathratus* and *Fusarium solani* | *Padina gymnospora* | *Padina gymnospora* and *Fusarium solani* | L.S.D |
|---|---|---|---|---|---|---|---|---|---|---|
| Chl(*a*)* (mg/ml) | 1.07 ±0.2 | 0.90 ±0.13 | 0.36±0.01 | 0.68±0.12 | 0.31±0.04 | 0.65±0.1 | 0.23±0.08 | 0.77±0.11 | 0.82±0.08 | 0.09 |
| Ch(*b*)* (mg/ml) | 0.40±0.09 | 0.32 ±0.04 | 0.15±0.02 | 0.29±0.02 | 0.11±0.01 | 0.27±0.07 | 0.11±0.01 | 0.28±0.05 | 0.31±0.04 | 0.04 |
| Shoot length (cm) | 31.0±4.58 | 33.0 ±3.0 | 26.0±4.0 | 30.67±0.58 | 28.0±1.0 | 26.0±3.6 | 22.0±4.0 | 30.0±2.65 | 29.0±1.00 | 2.49 |
| Root length (cm) | 33.0±4.58 | 36.3± 1.53 | 16.7± 3.06 | 35.0± 3.0 | 15.3± 1.52 | 31.0± 1.0 | 11.0± 1.0 | 38.0± 9.1 | 27.0± 2.0 | 3.15 |
| Root rotting percentage (%) | 6.3 ± 1.53 | 0.33± 0.6 | 68.3± 12.5 | 3.66± 1.52 | 30.0± 17.3 | 2.7± 2.1 | 50.0± 10.0 | 0.0± 0.0 | 11.7± 2.9 | 6.83 |
| Plant fresh weight gm | 30.1±10.4 | 32.0± 3.0 | 15.9± 7.4 | 24.8± 5.0 | 20.7± 3.2 | 23.7± 4.9 | 13.4± 0.5 | 28.0± 9.0 | 25.3± 9.6 | 5.48 |
| Fresh seedling weight (gm) | 28.0 ± 1.4 | 30.0± 0.0 | 34.0± 1.4 | 28.5± 0.7 | 24.5± 0.7 | 27.5± 3.5 | 30.5± 0.7 | 29.0± 1.4 | 24.2± 1.2 | 0.89 |

(* ) means data were taken after 30 days of plant growth.
Data points are the mean average of three replicates ± S.D.

## *Discussion:*

Marine macroalgae are a potential source of many biocidal and pharmaceutical agents (Tuney *et al*., 2006; Subba *et al*., 2010). In the present study, methanolic extract of *P. gymnospora* recorded the highest antimicrobial activities against the soil plant pathogenic bacteria; *R. solanacearum* and *P. carotovora* and the

soil fungi; *F. solani* and *R. solani*. Similar findings were reported by Arunkumar *et al*. (2005); Subba *et al*. (2010); Galal *et al*., (2011); Pandithurai *et al*. (2015) by using methanol/ethyl acetate extracts of marine macroalgae; *Codium fragile, Padina gymnospora, Spatoglossum asperum ,Sargassum ilicifolium, Sargassum wightii, Padina tetrastromatica* and *Spatoglossum asperum* against *Fusarium oxysporium, Alternarai alternate, Alternaria brassicicola, Ulocladium botrytis, Botryotrichum piluliferum* and *Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Xanthomonas oryzae pv. oryzae* . The present study was contrasted to (Xavier *et al* ., 2012) where, methanolic extract of marine brown algae, *Padina gymnospora* and *Sargassum wightii* showed no activity against the bacterial species of *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi*.

In our study, *P. gymnospora* exhibited significant antimicrobial activity compared to *S. latifolium.* and *H. clathratus* which might be attributed to its unique chemical composition of polyunsaturated fatty acids. Where, arachidonic acid was only detected in *p. gymnospora* methanolic extract but not for *sargassum latifolium* and *H. clathratus.* Arachidonic acid is an elicitor of phytoalexins in plants and may be used for preventing plant disease (Bostock *et al*. 1981).

Additionally, the lipids and fatty acids from marine algae could play an important role in the formation of many other bioactive secondary metabolites since some fatty acids showed antibacterial activities (Barbosa *et al*., 2007; Oh *et al*., 2008). In the present study, palmitic acid was the most abundant saturated fatty acid in the three tested marine algal species. Consistent with our results, the predominant fatty acid, palmitic acid obtained from green algal extracts of *Enteromorpha flexuosa* and *Sargassum pallidum* exhibited antibacterial activity against the plant pathogenic bacterium *Xanthomonas oryzae* pv.oryzae which causes bacterial blight of rice (Arunkumar *et al*., 2001;Gerasimenko *et al*. 2014). Similar finding was reported by Bazes *et al*., (2009) saturated and unsaturated fatty acids with a predominance of myristic, palmitic, oleic and eicosapentaenoic acids were responsible for the antibacterial activities of different brown algal extracts. In addition, antimicrobial activity of *Sargassum muticum* extract against bacteria and diatoms was attributed to palmitic acid which constituted 21.5 % of the total fatty acids. (Bazes *et al*., 2009).

It is worth mentioning that, other phenolic metabolites might play a role in antimicrobial defense where, in this study, *P. gymnospora* recorded the highest content of e-vanillic, chlorogenic, p-OH-benzoic while, *S. latifolium* showed the highest content of pyrogallol, gallic, 4-amino-benzoic, catechein, epicateachein, caffeinc, caffeic, vanillic and ellagic. In agreement, other studies indicated inhibitory activity of crude extract of the millet polyphenols against pathogenic microbes, *Serratia pneumoniae*, *Yersinia enterocolitica*, *Streptococcus pyogenes*, *Proteus mirabilis* and *Serratia marcescens*gallic was attributed to caffeic, ferulic, protocatechuic and p-hydroxy benzoic acids in extract (Chethan, 2008).

The bioactivity guided fractionation of *Acacia arabica* and *Pomegranate granatum* resulted in the isolation of bioactive compounds, 3,5-dihydroxy-4-methoxybenzoic acid, gallic acid and epicatechin which, displayed significant antimicrobial activities against *R. solanacearum* (MIC values 0.5–9 mg/ml). Also, structurally related benzoic acids inhibited growth of *R. solanacearum* (Farag *et al*. 2015). Furthermore, the biological activity of ellagic acid extracted from marine macro algae (seaweeds) toward some pathogenic strains bacteria (*Staphylococcus epidermatis, Bacillus cereus, Klebsiella pneumonia* and *Salmonella* was more effective than gentamycin and streptomycin (Ghudhaib *et al*., 2010).

In addition, the phenolic compounds of the dried crude extracts of seaweed/plant sources were responsible for their antimicrobial activities and could inhibit various foods born pathogen. This might be due to antifungal impact of these compounds against spores germination and could suppress mycelium growth at the early stage (El-Mehalawy, 2003; Cox *et al*., 2010; Plaza *et al*., 2010 and Osman *et al*., 2011) or shifting cell permeability (Wen-Bao *et al*., 2000) and their effect on fungal enzymes activities (Tuney *et al*., 2006). The phenolic compounds in brown algae play a primary role in the structure of cell walls and are generally considered to be a chemical defense against grazers, bacteria, fungi and other epiphytes (Le Lann *et al*. 2008; Plouguerne *et al*. 2006).

Literatures elsewhere, attributed the antimicrobial activities of some marine species to Laminarin (a storage carbohydrate) which can elicit a defense response to tobacco plants (Klarzynski *et al*., 2000; Vera *et al*., 2011) and blackberry cells (Patier *et al*. 1993). In contrast, our study revealed that, stachyose represented the highest saccharide fraction in *S. latifolium* and *P. gymnospora* while, Glucouronic was principle saccharide fraction in *H. clathratus*. High mannitol content might be involved in antibacterial activity of *S. latifolium* against *R. solanacearum* and *P.carotovora*. In accordance, (Stoop *et al*. 1996; Prabhavathi and Rajam, 2007) indicated the important role of mannitol in storage of carbon and energy, regulation of coenzymes, osmoregulation, free-radical scavenging and enhanced resistance to pathogens.

***In vivo biocidal & biostimulant effect of marine macroalgae:***

Treating of *F. solani* infected-soil with *P. gymnospora*, *S. latifolium* and *H. clathratus* powders significantly decreased the percentage of root rotting of eggplant. Moreover, *P. gymnospora* amendment enhanced growth performance of eggplant in term of shoot length and plant fresh weight in the infected soil.

Interestingly, the soil-free pathogen treated with *P. gymnospora* or *S. latifolium* powder showed significant increase in root length/fruit fresh weight compared to control. Furthermore, percentage of root rotting was decreased to 0 %, 2.7 % and 3.7 % by *P. gymnospora*, *H. clathratus* and *S. latifolium*, respectively. Brown algae extracts, as well as algae themselves increased the productivity of a variety of agricultural plants, including potato, grasses, citrus plants, tomato, beet, and legumes. These extracts contained a good deal of mineral substances, amino acids, vitamins, and phytohormones, such as auxin, cytokinines, and gibberellins (Stirk and Van Staden 1997a, 1997b). The application of macroalgae agricultural biostimulants (ABs) on crop plants enhanced rooting, crop height, fruit yields, tolerance to freezing, drought and salt, photosynthetic activity and resistance to fungi, bacteria and viruses (Sharma *et al*., 2014). ABs act on the physiology of the plant through diverse pathways to improve crop, yields, quality and post-harvest (EBIC, 2012). In this context, application of *Spatoglossum variabile*, *Stokeyia indica* and *Melanothamnus afaqhusainii* powders significantly decreased growth of fungi; *F. solani*, *Macrophomina phaseolina* attacking watermelon and eggplant; on one hand it improved the plant growth in soil that naturally infested with root rotting fungi on the other hand (Baloch *et al*., 2013). Moreover, vine and shoot length of watermelon, shoot length and fresh shoot weight of eggplant were increased by seaweed treatment as compared to control or Topsin-M. Seaweeds treated plants also showed earlier fruiting and decreased levels of root rotting fungi compared to control or fungicide treated plants. (Sultana *et al*., 2009; 2011). Seaweeds induced synthesis of antioxidant molecules which could favor plant growth and plant resistance to stress (Zhang and Schmidt, 2000).

In contrast, pre-soaking the seedling of eggplant in methanol extracts before transplanting into soil amended with/without powder amendments had a suppressive effect on seedling growth. We hypothesize that to the constituents of the studied algal extracts which might have high osmotic pressures that hinder water transport in roots cells.

***Conclusion:***

In conclusion, the present study demonstrates through the antimicrobial activities of three brown macroalgae, *S. latifolium*. *H. clathratus* and *P. gymnospora* collected from Red Sea, Hurghada coastline. *P. gymnospora* extract had immense activity against the soil borne pathogenic bacteria *R. solanacearum*, *P. carotovora* and fungi; *F. solani*, *R. solani*. Moreover, in vivo application of *P. gymnospora* powder in eggplant pot experiment significantly decreased the percentage of root rotting in *F. solani* infected soil and enhanced eggplant shoot length and plant fresh weight. The unique phyto-chemical composition of *P. gymnospora* i.e. fatty acids, saccharides and phenolic compounds could possibly the main reason of its high antimicrobial and biostimulant activities. These properties make *P. gymnospora* is a potentially promising brown macroalga for disease control and safe multifaced bioinoculant for sustainable agriculture technology.

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