

SUPPRESSION OF BACTERIAL WILT DISEASE BY SOME MARINE MACROALGAL EXTRACTS ISOLATED FROM SAFAGA COAST OF RED SEA, EGYPT

SEHAM M. HAMED¹ and NEVEIN A.S. MESSIHA^{2*}

1. Soil Microbiology Department Soils, Water and Environment Research Institute, ARC, Giza, Egypt.
2. Bacterial Plant Disease Department, Plant Pathology Research Institute, Agricultural Research Center, Cairo, Egypt.

Corresponding author:

*nevein_messiha@yahoo.com

(Manuscript received 15 August 2018)

Abstract

Marine macroalgae are an excellent source of biologically active compounds. Among tested 8 different marine macroalgal species, we intensively studied the antibacterial activity of the methanol extract of four selected algal species; *Ulva lactuca*, *Caulerpa racemosa*, *Acanthophora spicifera* and *Sargassum dentifolium* against *Ralstonia solanacearum*, the causal agent of bacterial wilt. *In vitro* assay showed that *U. lactuca* had the highest antibacterial activity while *Sargassum dentifolium* showed the lowest effect. In contrast, *A. spicifera* extract was the most disease suppressive in the *in vivo* study. The suppressive effects of extracts were generally low in sandy soil compared to clay one. The interrelation between growth inhibition of *R. solanacearum*, wilt suppression and phenolic constitutions in algal extracts were investigated. Results showed that gallic and coumaric acids were correlated with inhibition of the pathogen *in vitro* while hydroxybenzoic, chlorogenic, vanillic, salicylic and ferulic acids were *in vivo* correlated with disease suppression under greenhouse conditions. Use of algal extracts, as a strong antibacterial and antioxidant, can be employed as a part of an integrated program for controlling of plant diseases. Further investigations are needed on the feasibility of application.

INTRODUCTION

Potato brown rot (bacterial wilt) is a serious disease with global economic impacts. The pathogen survives in drainage water for almost 4 months under optimum conditions (Stevens *et al.*, 2018). It survives in plant debris, alternative weed hosts such as, *Portulaca oleracea*, *Rumex dentatus*, *Solanum nigrum* and volunteer potato plants (Farg *et al.*, 2004).

The most important aspects applied to control the disease are avoidance of invasion by the pathogen from old to new areas and protection of the constructed pest free areas (PFAs), through avoidance of disease introduction. Many management regimes were employed with variable degrees of success including, different fertilization regimes (Messiha *et al.*, 2007a), antioxidants (Farg *et al.*, 2017), and cyanobacteria

(Mikhail *et al.*, 2016). Use of biocontrol agent (Elhalag *et al.*, 2016) as well as biological soil disinfections (BSD) (Messiha *et al.*, 2007b) and solarization (Schönfeld *et al.*, 2003) are other non-chemical means to control the disease.

The use of algal products from marine macroalgae as an important source of phenolic compounds was investigated (Mišurcován, 2011). Marine macroalgae were proven to contain active biocontrol agents which can be used successfully in controlling many soil borne pathogens as well as improving plant growth (Ibraheem *et al.*, 2017). Seaweed extract (SWE) is known for its antioxidant properties (Matanjun *et al.*, 2008) as well as a promising source of phytohormones as cytokinins, auxins, polyamines and betaines (Zhang *et al.*, 2003). Cytokinins are considered as antioxidants which inhibit the activity of free radical groups that are responsible for chlorophyll degradation, (Yan, 1993).

Seaweed extract (SWE) was proven to suppress *Ralstonia solanacearum*, increase nutrient uptake and improve crop productivity (Farag *et al.*, 2017). Increasing nutrient uptake would reduce the required nitrogen fertilization (Majeed *et al.*, 2017). The antibacterial potential of SWE is correlated by Zhang *et al.*, (2006) to the high content of polyphenol.

The aim of this study was to evaluate some marine macroalgal extract as a new environment friendly approach that would be integrated into program for controlling potato brown rot.

MATERIALS AND METHODS

Algal material collection

Ulva lactuca Linnaeus and *Caulerpa racemosa* (Chlorophyta), *Sargassum virgatum*, *Sargassum dentifolium* Grunow, *Sargassum latifolium*, *Padina gymnospora* (kütz) vichers, *Hydroclathrus clathratus* (Phaeophyta), and *Acanthophora spicifera* Vahl (Rhodophyta) were collected from the coast of the Red sea, Safaga district, Egypt, located at 26° 44' N and 33° 56' E. The algal samples (fresh samples with variable mass and volume) were washed with tap water to remove adhered epiphytes and associated debris as much as possible, then were cleaned using a brush with 5% ethanol to remove the adhering microflora. The algal specimens were air dried under shade at room temperature and were grind thoroughly by electrical blender and sieved through 0.5 mm² mesh sieve plate. The algal powder specimens were then soaked in methanol before use to estimate their antimicrobial activity.

Preparation of algal extracts

The extraction was made according to the method described by Hellio *et al.*, (2001). Five hundred grams of dried algal powders were soaked in 3 L methanol 95%,

at room temperature for 72 h. The collected algal extracts were then filtered through Whatman No.1 filter paper. The obtained filtrates were concentrated under reduced pressure in the rotatory evaporator (GG SENCO) to complete dryness. The dried crude extracts were stored at 4 °C for subsequent use.

Isolation and identification of *R. solanacearum*

Within the activities of the national project entitled "Environmental friendly program for controlling potato brown rot" STDF2905, ten *R. solanacearum* isolates were isolated and purified from different habitats (soil, water and infected potato tubers) from different geographical locations. Wardan, (Giza) and Ganoub El-Tahrir (Behera), sandy soils and Talia (Minufiya), Sids (Beni-Suef), (clay soil). The isolated pathogen was cultured and monitored using modified SMSA selective medium agar plates (Anonymous, 1998). Typical colonies (irregular shape, diffuse slimy white with purple centers) were tested using Immunofluorescence Antibody Staining (IFAS) followed by pathogenicity test on transplanted tomato seedling (cv. Pinto) (Janse, 1988). The most severe isolate was selected for further work. Identification of the selected *R. solanacearum* isolate was confirmed by sequencing the 16S-rDNA gene using an 8-capillary Genetic Analyzer (Applied Biosystem) at the Agricultural Research Center as described by (Farag *et al.*, 2017).

Effect of algal extracts on *R. solanacearum* growth *in vitro*

The methanol extract of eight marine macro algae, were evaluated for their inhibitory effect against *R. solanacearum*.

The inoculum density of *R. solanacearum* was adjusted to ($OD_{600} = 0.3$) by spectrophotometer (Jenway 6300, Essex, UK) to reach final concentration of 0.5×10^9 CFU/ml and 0.5×10^8 CFU/ml. One hundred μ l was spread on the surface of SMSA media. Five wells of 0.5 cm diameter were made in each plate to be filled with each algal extract. Five plates were designated for each algal extract. Algal extract was dissolved in methanol at concentration of 10 mg/ml. Each well was filled with 10, 20, 50 or 100 μ l from the extract with the negative controls being filled with the same volume of methanol only. The plates were incubated at 28°C for 3-7 days and inhibition zone was measured.

Effect of algal extracts on bacterial wilt under greenhouse conditions

Soil was collected from two areas with no history of potato brown rot infestation, Ismailia (light clay soil) and Nubaria (sandy soil). Soil samples were bacteriologically checked to ensure freedom of *R. solanacearum*. One hundred twenty pots (200 cm³ each) were filled with 250 g of either sandy soil or clay soil (equal number of pots). Two tomato seedlings were grown in each pot to ensure having at least one

tomato seedling for each pot. Standardized suspension of the pathogen was mixed with the soil to achieve final concentration of the pathogen at 10^7 CFU/ g. soil. Three leaved tomato seedlings were transplanted to the inoculated soil after being treated with the different algal extracts. Each seedling was immersed in water solution of powdered extract (25 mg) dissolved in 2.5 ml water. The four algae selected were, *U. lactuca*, *C. racemosa*, *A. spicifera* and *S. dentifolium*. The experiment was carried under controlled conditions in the quarantine greenhouse at the Potato Brown Rot Project (PBRP) for 3 weeks. The conditions were adjusted to around 25 ° C during the day and 20°C at night with relative humidity (70-85%) and photoperiod of 14 hours. The effect of four algal extract on potato wilt suppression was evaluated on tomato seedlings. The layout of the experiment is shown in table 1.

Table 1. Layout of the greenhouse experiment for evaluating of the effect of marine macroalgal extracts on potato brown rot suppression.

Treatment	Negative control		Positive control		<i>Ulva lactuca</i>		<i>Caulerpa racemosa</i>		<i>Acanthophora spicifera</i>		<i>Sargassum dentifolium</i>	
	Clay	Sandy	clay	sandy	clay	sandy	clay	sandy	clay	sandy	clay	Sandy
No. of pots	10	10	10	10	10	10	10	10	10	10	10	10

For negative and positive control the seedlings were immersed in water (instead of methanol to avoid phytotoxicity). Negative control, the soil was not inoculated with the pathogen.

Disease incidence was presented by measuring AUDPC (area under disease progress curve) and count of the pathogen in rhizosphere using SMSA media (Anonymous, 1998) and IFAS (Janse, 1988). AUDPC were calculated according to percentage of wilted leaves progress per each pot (plant) overtime (Winstead and Kelman, 1952 & Messiha, 2006)

$$\text{Disease index (\%)} = [\sum (ni \times vi) \div (V \times N)] \times 100$$

ni=number of plants representing each disease rating; *vi*=disease rating; *V*=the highest disease rating (5); and *N*=total number of plants. Disease rating was calculated as following scale: 1=no symptoms, 2=one-two leaves wilted, 3= most of leaves wilted, 4=all leaves wilted and 5=whole plant died. (Winstead and Kelman 1952)

Determination of phenolic compounds in algal extracts

Determination of phenolic compounds in algal extracts was made at Al-Azhar University (The Chromatographic analysis unit). Extraction of phenolic compounds from algae was made according to Machu *et al.*, (2015). The phenolic compound content was determined using HPLC column (KROMASIL, 150×4.6 mm), with GBC (UV/vis) detector. Pump GBC LC 1110 pump. Analysis was made using win chromatography software (ver.

1.3). The flow rate was 1 ml/min. The detection was made at UV 280 nm. Elution was made with (methanol: water: tetrahydrofuran: acetic acid), (23: 75: 1: 1)

Statistical analysis

One-way ANOVA was employed to test the significant difference between the inhibition zones of different algal extracts. NPar-test (Mann-Whitney test) was employed to test the difference in disease incidence between tomato treated with different algal extracts and non-treated control. Correlation analysis (1-tailed) was employed to correlate phenolic content of different algal extracts from one side, inhibition zone (*in vitro*) and disease incidence (*in vivo*) from the other side. The used software for different analysis was SPSS23.

RESULTS

Isolation and identification of *R. solanacearum*:

The most aggressive *R. solanacearum* isolate, according to its pathogenicity was that strain isolated from Talia, Minufiya governorate. The isolate showed 95% similarity with *R. solanacearum*, *R. pickettii* and *R. syzygii* according 16S-rDNA gene sequencing (Fig 1). Pathogenicity test confirmed that the recovered isolate was *R. solanacearum*.

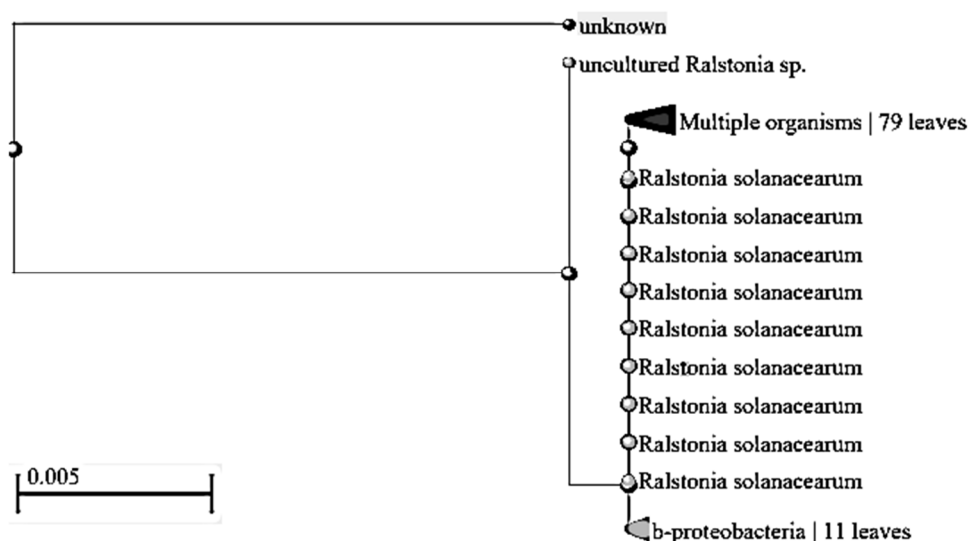


Fig. 1. Neighbor Joining Blast Tree of 16S rRNA database. The unknown refers the *R. solanacearum* isolated from Talia, Minufiya

Inhibitory potential of algal extracts on *R. solanacearum* in vitro

In the present study, *in vitro* assay was conducted using methanol extracts of marine macroalgae against *R. solanacearum*, at two different concentrations of the pathogen 0.5×10^8 CFU/ml and 0.5×10^9 CFU/ml for 24 h of incubation on nutrient agar medium. Among the eight different marine macroalgal species, *U. lactuca*, *C. racemosa*, *A. spicifera* and *S. dentifolium* showed the highest antimicrobial activities. No inhibition zone was recorded for less than 0.5 mg (50 μ l) for all tested algal extracts. The effect was most clear at lower pathogen concentrations. Our results showed that, at low pathogen concentration (0.5×10^8 , 24h), *U. lactuca*, *C. racemosa* and *A. spicifera* extracts showed comparatively greater inhibition activity by (10 \pm 0.6 mm), (9 \pm 0.5 mm) and (8 \pm 1.0 mm), however *S. dentifolium* showed the least inhibition zone by (4 \pm 0.7 mm) ($P < 0.001$). Meanwhile, at high pathogen concentrations (0.5×10^9 , 24h), *U. lactuca* showed the highest significant inhibition zone by (5 \pm 0 mm) compared to (4 \pm 0.4 mm) by *C. racemosa*, $P = 0.024$, (3 \pm 0.6 mm) by *A. spicifera*, $P = 0.004$ and (2 \pm 0.3 mm) of *S. dentifolium*, $P < 0.001$ (table 2).

Table 2. Inhibition zone associated with different algal extracts

Algal extract	Inhibition zone of 0.5 mg algal extract in mm	
	0.5×10^8 CFU/ml **	0.5×10^9 CFU/ml
<i>Ulva lactuca</i> ^a	10 \pm 0.6 ²	5 \pm 0 ^{*3}
<i>Caulerpa racemosa</i> ^b	9 \pm 0.5 ²	4 \pm 0.4 ⁴
<i>Acanthophora spicifera</i> ^c	8 \pm 1.0 ²	3 \pm 0.6 ⁵
<i>Sargassum dentifolium</i> ^d	4 \pm 0.7 ¹	2 \pm 0.3 ⁶

*average of 5 replicates (mean \pm SE)

**100 μ l from the *R. solanacearum* inoculum density spread on the surface of the media

1 & 2 $P < 0.001$

3 & 4 $P = 0.024$

3 & 5 $P = 0.004$

3 & 6 $P < 0.001$

4 & 6 $P = 0.01$

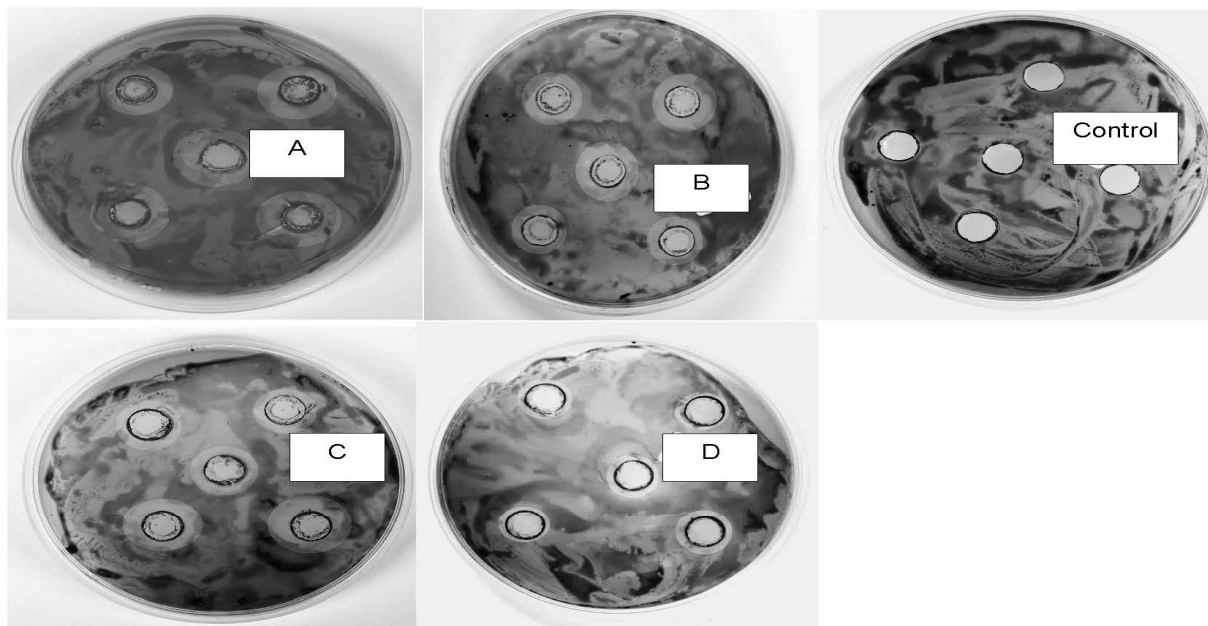


Fig. (2) Antimicrobial activity of the methanolic extract of: (A) *Ulva lactuca linnaeus* C. Agardh Legolis, (B) *Acanthophora spicifera*, (C) *Caulerpa racemose*, (D) *Sargassum dentifolium* Grunow and Control (methanol only) against soil borne pathogenic bacteria *R. solanacearum* after 24 h of incubation.

Effect of algal extracts on bacterial wilt under greenhouse conditions

The onset of symptoms was detected after 3 weeks and within 14 days wilted plants died, when the experiment was terminated. Healthy and dead plants were checked for the presence of the pathogen in plant tissues. In general, the disease incidence as expressed by wilt severity and AUDPC was more severe in sandy soil as compared to clay soil at ($P=0.007$ and 0.001) for disease severity and AUDPC respectively (Table 3 and Fig 3).

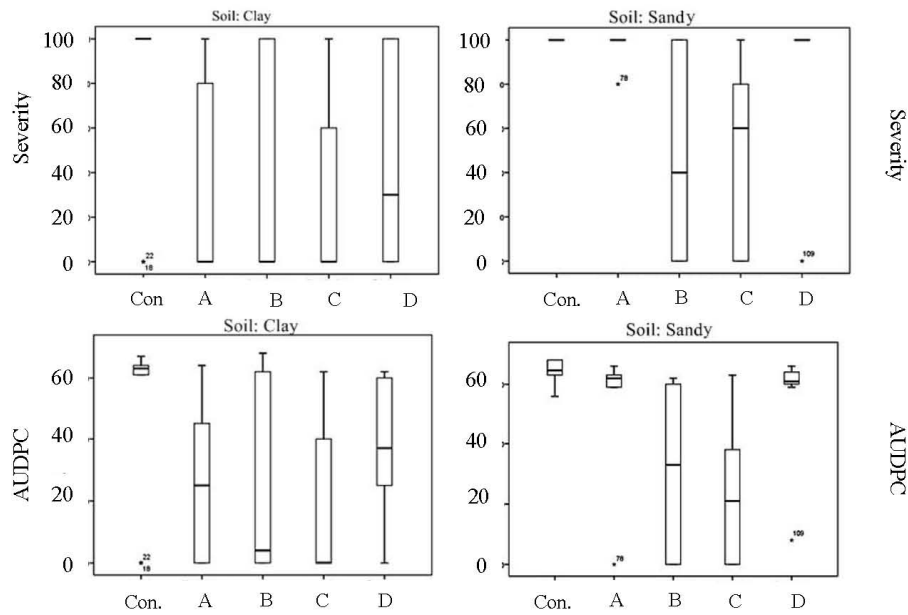


Fig. (3a) Effect of different algal extracts on disease severity and area under disease progress curve (AUDPC) under greenhouse conditions in two different soil types (clay and sandy)

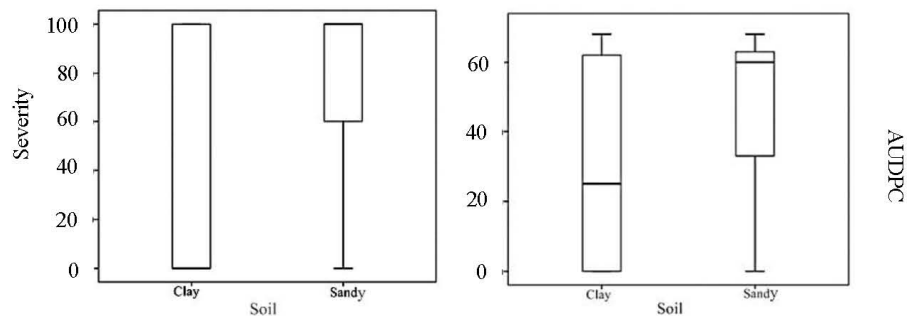


Fig. (3b) Effect of different soil types (clay versus sand) on disease incidence under greenhouse conditions.

(A) *Ulva lactuca linnaeus* C. Agardh Legolis, (B) *Acanthophora spicifera*, (C) *Caulerpa racemose*, (D) *Sargassum dentifolium* Grunow and (Con.) Control.

U. lactuca showed significant decrease 65% ($P=0.013$) and 51% ($P=0.053$) in wilt severity and AUDPC respectively, in clay soil and was less effective in sandy soil showing only 13% ($P=0.038$) decrease in AUDPC. *C. racemosa* showed a trend of 50% decrease in wilt severity ($P=0.075$) in clay soil and was more effective in sandy soil with 52% ($P=0.005$) and 51% ($P=0.001$) decrease in wilt severity and AUDPC respectively. *A. spicifera* was the most effective algal extract with 70% ($P=0.006$) and ($P=0.005$) decrease in wilt severity and AUDPC in clay soil and 56% and 59% ($P<0.001$) decrease in sandy soil. *S. dentifolium* showed the least disease suppression with only 42.5% trend of decrease in wilt severity ($P=0.096$) and significant 29% decrease in wilt severity ($P=0.022$) in clay soil and was less effective in sandy soil with only 12% decrease in AUDPC ($P=0.063$).

Determination of phenolic compounds in algal extracts

Data in table (3) revealed that, *A. spicifera* showed the highest phenolic compound content (176.067 $\mu\text{g/gm}$), while *S. dentifolium* showed the lowest content (124.129 $\mu\text{g/gm}$). Interestingly, *A. spicifera*, had the highest components of hydroxybenzoic chlorogenic, vanillic, salicylic, ferulic and cinammic followed by *C. racemosa*. On the other hand, *U. lactuca* contained the highest content of gallic, resorcinol and coumaric acid.

Hydroxybenzoic acid showed significant negative correlation with disease incidence as expressed by AUDPC in clay soil, sandy soil as well as wilt severity in sandy soil (-0.9, $P=0.03$), and (-0.89, $P=0.05$) and (-0.81, $P=0.1$) respectively. Similarly, chlorogenic acid showed significant negative correlation with disease incidence only in sandy soil, being (-0.97, $P=0.02$) for wilt severity and (-0.97, $P=0.02$) for AUDPC. Vanillic acid showed negative correlation with disease incidence (-0.97, $P=0.02$) only in sandy soil. Salicylic acid showed negative correlation with wilt severity (-0.84, $P=0.08$) and AUDPC (-0.98, $P=0.01$) for clay soil and was less significant for wilt severity in sandy soil (-0.79, $P=0.1$). Ferulic acid showed significant negative correlation with disease incidence only in sandy soil, being (-0.94, $P=0.03$) for wilt severity and (-0.91, $P=0.04$) for AUDPC.

Interestingly, gallic acid showed significant positive correlation with inhibition zone (antibacterial potential *in vitro*) with (+0.79, $P=0.1$) at low inoculum density of the pathogen (0.5×10^8 CFU) and (+0.84, $P=0.08$) at high inoculum density (0.5×10^9 CFU) (Table 3). Similarly, coumaric showed significant positive correlation with antibacterial potential *in vitro* by (+0.9, $P=0.07$) at both low and high inoculums density of the pathogen. Resorcinol acid showed negligible ratio for different tested algal extracts (Table 3).

Hydroxybenzoic showed significant positive correlation with salicylic acid and Cinammic (+0.98, $P=0.01$) and (+0.9, $P=0.05$) respectively. Cinammic acid was

represented by a considerably little amount in the different algal extracts and its correlation with disease suppression may related to a combined effect of hydroxybenzoic, salicylic and cinammic acid (Table 3). Chlorogenic acid showed positive correlation with vanillic and freulic acids being (+0.996, $P=0.002$), (+0.82, $P=0.09$) respectively.

Table 3. Phenolic acid constitution of different algal extracts and effect on the pathogen and disease severity

Phenolic acids ($\mu\text{g/gm}$)	Algal strains				Retention Time (min)
	<i>Ulva lactuca</i>	<i>Caulerpa racemosa</i>	<i>Acanthophora spicifera</i>	<i>Sargassum dentifolium</i>	
Hydroxybenzoic ^a	8.14	9.27	10.18	6.14	1.3
Gallic ^b	41.42	30.55	33.71	27.22	2.1
Resorcinol ^c	0.041	0.028	0.037	0.019	2.8
Chlorogenic ^d	25.33	35.98	38.89	30.31	3.3
Vanillic ^e	6.66	10.66	12.53	7.97	4.1
Coumaric ^f	29.52	20.04	23.17	15.11	5
Salicylic ^g	19.12	19.55	22.15	13.82	5.9
Ferulic ^h	15.55	25.26	29.12	20.93	7.5
Cinammic ⁱ	3.19	4.18	6.28	2.61	8
Total	148.971	155.518	176.067	124.129	
					Untreated control (PC)
<i>In vitro</i> inhibition of <i>R. solanacearum</i> (0.5×10^8 CFU/ml)*¹	10 \pm 0.6	9 \pm 0.5	8 \pm 1.0	4 \pm 0.7	0 \pm 0
<i>In vitro</i> inhibition of <i>R. solanacearum</i> (0.5×10^9 CFU/ml)*²	5 \pm 0	4 \pm 0.4	3 \pm 0.6	2 \pm 0.3	0 \pm 0
Wilt severity in clay soil³	28 \pm 14	40 \pm 16	24 \pm 13	46 \pm 16	80 \pm 13
% decrease compared to PC	65	50	70	42.5	
AUDPC in clay soil⁴	25 \pm 8	26 \pm 11	15 \pm 8	36 \pm 8	51 \pm 9
% decrease compared to PC	51	49	71	29	
Wilt severity in sandy soil⁵	98 \pm 2	48 \pm 16	44 \pm 13	90 \pm 10	100 \pm 0
% decrease compared to PC	2	52	56	10	
AUDPC in sandy soil⁶	56 \pm 6	32 \pm 9	24 \pm 8	57 \pm 6	46 \pm 6
% decrease compared to PC	13	51	59	12	

*0.5 mg of methanolic algal extract

Significant correlations:

^{a,4}(-0.9, $P=0.03$), ^{a,5}(-0.81, $P=0.1$), ^{a,6}(-0.89, $P=0.05$), ^{b,1}(+0.79, $P=0.1$), ^{b,2}(+0.84, $P=0.08$),
^{c,1}(+0.84, $P=0.08$), ^{c,3}(-0.94, $P=0.03$), ^{c,4}(-0.78, $P=0.1$), ^{d,5}(-0.97, $P=0.02$), ^{d,6}(-0.97, $P=0.02$)
^{e,5}(-0.97, $P=0.02$), ^{e,6}(-0.97, $P=0.02$), ^{f,1}(+0.85, $P=0.07$), ^{f,2}(+0.86, $P=0.07$), ^{f,3}(-0.82, $P=0.09$),
^{i,4}(-0.91, $P=0.05$), ^{i,5}(-0.84, $P=0.08$), ^{i,6}(-0.925, $P=0.08$), ^{h,5}(-0.94, $P=0.03$), ^{h,6}(-0.91, $P=0.04$),
^{g,3}(-0.84, $P=0.08$), ^{g,4}(-0.98, $P=0.01$), ^{g,5}(-0.79, $P=0.1$),
^{a,9}(+0.98, $P=0.01$), ^{a,i}(+0.9, $P=0.05$), ^{b,c}(+0.92, $P=0.04$), ^{b,f}(+0.99, $P=0.005$),
^{c,9}(+0.79, $P=0.1$), ^{c,f}(+0.99, $P=0.01$), ^{d,e}(+0.99, $P=0.07$), ^{d,h}(+0.996, $P=0.002$), ^{d,i}(+0.82, $P=0.09$),
^{e,h}(+0.98, $P=0.008$), ^{e,i}(+0.9, $P=0.048$).

DISCUSSION

Marine macroalgae are considered to be a rich source of bioactive compounds showing great antimicrobial activities. In the present study, maximum inhibition zones were recorded for *U. lactuca* followed by *A. spicifera* and *C. racemosa* while *S. dentifolium* showed the least inhibition zone. The antibacterial activity of *S. latifolium* against *R. solanacearum* and *P. carotovora* was previously addressed by Ibraheem *et al.*, (2017), where they also reported that, the most effective antibacterial activity resided in the methanolic extract. In the present work, some phenolic compounds are directly correlated with antibacterial activity *in vitro* such as gallic and coumaric acids while others correlated with disease suppression such as hydroxybenzoic, chlorogenic, vanillic, salicylic and ferulic acids. Alves *et al.*, (2013) showed that 2,4-dihydroxybenzoic, vanillic and p-coumaric acids had antibacterial activity (MIC = 1 mg/ml) against *E. coli*. Phenolic compounds are known for their antioxidant and bactericidal potential and marine macroalgal products are known for being rich in phenolic compounds (Machu *et al.*, 2015, Hamed *et al.*, 2018). The antibacterial properties of the gallic acid, extracted from pomegranate and acacia fruit, against *R. solanacearum* was proven by Farag *et al.*, (2015). In this study, cinnamic acid showed negative correlation with disease incidence. Cinnamic acid is known to participate in the formation of lignin which may explain this correlation (Guzman, 2014). The disease suppressive effect of phenolic acids may be attributed to enhancing the formation of lignin (Hahlborck and Sheel, 1989) and inhibit some microorganisms which are resistant to conventional antibiotic (Alves *et al.*, 2013).

The disease incidence was higher in sandy soil as compared to clay soil. The lower effect of different algal extracts in sandy soil may be attributed to the excess reactive oxygen species (ROS) produced in plants which was probably less than the amount of available antioxidants. Most algal extracts were more effective in clay soil as compared to sandy soil with the exception of *C. racemosa*, which was more effective in sandy soil. The disease suppressive effect of SWEs was correlated partially to enhancing nutrient uptake by plant (Farag *et al.*, 2017). Reactive oxygen species (ROS) is one of the most significant signals of pathogenesis (Torres *et al.*, 2006). The excess ROS is controlled by a complex defense mechanism of antioxidants by the living organism, when the amount of ROS exceeds the available antioxidant, the organism become diseased (Machu *et al.*, 2015). The total determined phenolic compounds was high in *A. spicifera* and low for *S. dentifolium* which may explain the highest potential of disease suppression associated with *A. spicifera* as compared to *S. dentifolium*. Positive correlation between hydroxybenzoic class of the phenolic compounds (such as gallic acid and 4-hydroxybenzoic) and antioxidant capacity of water soluble compounds was proven by Machu *et al.*, (2015). Hydroxybenzoic acid is an essential component of

building lignin (Machu *et al.*, 2015) which may explain its role in plant defense against plant pathogens. Both hydroxybenzoic and gallic phenolic compounds showed the highest proportion in *U. lactuca* and *A. spicifera* and were the lowest in *S. dentifolium* which may in part explain the varied effect of the different algal extracts on disease suppression. Vanillin is produced from ferulic acid (Plaggenborg *et al.*, 2006) which could suggest the relation between ferulic and vanilic acid in the analyzed phenolic compound content of different extracts.

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تنشيط مرض الذبول البكتيري باستخدام بعض مستخلصات الطحالب البحرية الكبيره المعزولة من ساحل سفاجا، البحر الاحمر، مصر

سهام موسى محمد حامد و نيفين مسيحه

1. قسم الميكروبيولوجيا في التربة ، معهد بحوث التربة والمياه والبيئة ، مركز البحوث الزراعية ، الجيزة ، مصر .
2. قسم أمراض النبات البكتيرية ، معهد بحوث أمراض النبات ، مركز البحوث الزراعية ، القاهرة ، مصر .

تعد الطحالب البحرية مصدر ممتاز للمركبات النشطة بيولوجيا. من بين 8 أنواع مختلفة من الطحالب البحرية التي تم اختبارها، قمنا بعمل دراسة مكثفة لاختبار النشاط المضاد للبكتيريا باستخدام مستخلص الميثانول لأربعة سلالات طحلبية مختارة. حيث تم اختبار كفاءة الاولفا لاكتيوكا، كوليربا راسيموزا، اكانسوفورا سبيسيفيرا و سارجاسوم دينتيفوليوم ضد بكتيريا رالستونيا سولاناسيريوم (المسبب الرئيسي لمرض الذبول البكتيري). اظهر الفحص المختبري ان الاولفا لاكتيوكا هي الاعلى في النشاط المضاد للبكتيريا بينما سارجاسوم دينتيفوليوم كانت اقلهم تأثيرا. في المقابل ، كان مستخلص اكانسوفورا سبيسيفيرا أكثر تنشيطا للمرض في الدراسة الحيوية. بشكل عام كانت التأثيرات المثبطة للمستخلصات منخفضة في التربة الرملية مقارنة بالتربة الطينية. تم بحث العلاقة بين تنشيط نمو رالستونيا سولاناسيريوم ، تنشيط مرض الذبول و محتوى المركبات الفينولية في مستخلصات الطحالب. وأظهرت النتائج أن حمضى الجالييك والكيوماريك كانت مرتبطة مع تنشيط مرض الذبول البكتيري في المختبر بينما ارتبط كلا من حمض هيدروكسى بنزويك، حمض كلوروجينك ، حمض قانيليك ، حمض ساليسيلك حمض الفيلوروك مع تنشيط المرض في الظروف الحيوية خلال تجربة الصوبة . وعليه، يمكن استخدام مستخلصات الطحالب البحرية، كمضاد قوي للبكتيريا و للأكسدة في النبات، كجزء من البرنامج المتكامل للتحكم في الأمراض النباتية. هناك حاجة لمزيد من البحث حول امكانية و جدوى التطبيق.

