



Usage of brown algae *Padina pavonica* and *Turbinaria decurrens* to control bacterial brown rot disease

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Abstract

Brown algae contain the highest biological activities and considered an important source of bioactive natural substances. Two algae (*Padina pavonica* and *Turbinaria decurrens*) extracted by two solvent (water and ethanol) used to study their antibacterial activity on development brown rot disease *in vitro* and under artificial inoculation condition. The two algae (*P. pavonica* and *T. decurrens*) inhibited the growth of *Ralstonia solanacearum in vitro*. The highest inhibition zone recorded with *P. pavonica* water extraction and the least one was *T. decurrens* ethanol extraction. Two algae extraction decreased area under disease progress curve (AUDPC) and disease severity in all treatments compared with the positive control. Quantity of total and free phenols increasing to reach high amount in *P. pavonica* water and ethanol extractions compared with *T. decurrens* extractions. The suppuration of *R. solanacearum* and its disease severity affected by the kind of extraction solvent used in the extraction of algae and the amount of total and free phenols for each alga. Water and ethanol extraction of *P. pavonica* was more effectiveness in controlling *R. solanacearum in vitro* and under artificial inoculation condition. Further research aiming to identify the specific phenolic compounds which are responsible for higher antibacterial activity and availability of apply the algae in controlling plant diseases.

Keywords: Brown algae, *Ralstonia solanacearum*, *Padina pavonica*, *Turbinaria decurrens*, Brown rot disease.



Introduction

Ralstonia solanacearum is the causal agent of bacterial wilt (brown rot) disease in hundreds of economic plants from different families. Bacterial wilt is more important in temperate region according to quarantine status than yield loss (**Hayward, 1991**). This pathogen is the most common pathogen that affects the exportation of potato crop, especially to the European markets and lead to loss of money (**Kabeil *et al.*, 2008**).

Difficulties are associated with controlling *R. solanacearum* due to its abilities to grow endophytically, survive in soil, especially in the deeper layers, travel along water, and its relationship with weeds. So, **Yuliar *et al.*, (2015)** discusses different diverse options have been reported on control methods against diseases caused by *R. solanacearum* and shows some solutions (eg. integrating a biological control agent and organic matter including simple organic compounds, compost, or plant residue).

Marine algae are one of the largest producers of biomass in the marine environment. They produce a wide variety of chemically active metabolites in their surroundings. These active metabolites, also known as biogenic compounds, produced by several species of marine macro and micro algae, have anti-bacterial, anti-algal, anti-macrofouling and anti-fungal properties, and have other likely uses, e.g. in therapeutics (**Bhadury and Wright, 2004**).

Macroalgae considered a big source of potential compounds useful for controlling new diseases or multi-resistant strains of pathogenic microorganisms such as bacteria, fungus and virus (**Pérez *et al.*, 2016**). Marine algal species have some compounds like sterols, Polysaccharides, peptides, proteins, vitamins, chlorophyllides, phenols, heterocyclic



compounds, halogenated ketones, alkanes and cyclic polysulphides (Mtolera and Semesi, 1996., and Abdel-Raouf *et al.*, 2015). In recent year, seaweeds are used in several applications such as anti-microbial (Chiheb *et al.*, 2011). In Egypt, Farag *et al.*, (2017) proved the suppression effect of seaweed extract (SWE) on *R. solanacearum* whereas increase nutrient uptake and improve crop productivity.

Turbinaria decurrens algae extracts shows presence of alkaloids, terpenes, phenols, tannins, saponins, flavonoids, quinones, proteins, sugars, carbohydrates, alkaloids, coumarins, steroids terpenoids and cardiac glycosides, so it used in many branches: antimicrobial, antidiabetic, antioxidant, hepatoprotective, antifungal, antitumor, anticoagulant, and antibacterial (Deepak *et al.*, 2017). Whereas, brown algae *Padina pavonica* is an important one, extensively used as a feedstock for the production of bio-diesel, in heavy-metal bio-sorption, as a pollution bio-indicator, a trace metal bio-monitor, an anti-oxidant, an anti-cancer drug (by inducing apoptosis of cancer cells), an anti-bacterial agent and a bio-insecticide (El Maghraby and Fakhry, 2014).

This study aimed to evaluate the efficacy of two brown algae against *R. solanacearum* in vitro and their ability to reduce the development of brown rot disease under greenhouse conditions.

Materials and Methods

Source of algae:

Two algae (*Turbinaria decurrens* and *Padina pavonica*) and their extractions were obtained kindly from National Institute of Marine Science and Fisheries, Hurghada, Red Sea Governorate, and Environmental Biotechnology Department, College of Biotechnology, Misr University for Science and Technology.



Source of *R. solanacearum* isolate:

Virulent isolate of *R. solanacearum* was obtained from the collection of Bacterial Diseases Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt. Virulent isolate was previously isolated from infected potato tubers and was identified using Immunofluorescent Antibody Staining (IFAS) and PCR technique using specific primer (Shoala and Abd-El-Aziz, 2019).

Effect of two algae on *R. solanacearum* in vitro:

Algae (*P. pavonica* and *T. decurrens*) extracted with cold water and ethanol screened for their ability to inhibition *R. solanacearum* growth by two methods (Stonier, 1960) as the following.

(1) Papers discs:

Plates containing triphenyle tetrazolium chloride (TTC) medium seeded with *R. solanacearum* isolate using appropriate amount of broth culture (3ml of 10^8 colony forming units (cfu) /ml suspension for flask containing 250 ml medium) as inoculum. Discs of sterilized filter paper (whatman3) were immersed in each concentration of algae extractions (1 and 2ml /250ml water). Three plates were designated for each treatment (have discs immersed in algae extractions). Three plates have discs immersed in ethanol only and water as a negative control. All plates incubated for 48hrs at 28°C and inhibition zone was measured.

(2) Dilution Plates:

Plates containing King's B (KB) medium have algae (1 and 2ml /250ml medium). Other plates have KB medium with ethanol and other containing KB medium only (negative control). A loopful of *R. solanacearum* (24 hrs- old) streaked on surface of each plate. Three plates



were designated for each treatment. All plates incubated for 48hrs at 28°C, then observed the growth of bacteria on plates surface.

Effect of two algae on disease severity under artificial inoculation condition:

A suspension of bacterial isolate was prepared by inoculation in nutrient agar (NG) broth and incubated at 28°C for 48 hrs and adjusted to 10^8 cfu / ml. Pots (20cm diameter) filled with sterilized soil and irrigate by water before 24hrs from infestation with *R. solanacearum*. Tomato seedlings (cultivar Beto 86 and 28 day-old) were soaked for one hour in algae suspension (1 and 2ml from each alga / 250ml water) before transplanting in infested soil. Tomato seedlings soaked in water only before transplanted (as negative control) and other seedlings transplanted directly in infested pots (as positive control). Ten replicates were used for each treatment. Disease incidence was represented by measuring AUDPC which were calculated according to percentage of wilted leaves progress per plant overtime (**Winstead and Kelman, 1952.** and **Messiha, 2006**). The disease severity (DS) was recorded after 21 days from sowing in infested soil, where DS was calculated as the following:

$$\text{Disease Severity (\%)} = [\sum (n_i \times v_i) \div (V \times N)] \times 100$$

n_i =number of plants representing each disease rating; v_i =disease rating; V =the highest disease rating (5); and N =total number of plants. Disease rating was calculated as following scale: 0= no symptoms, 1= one-two leaves wilted, 2= three or four leaves wilted, 3= most leaves wilted, 4=all leaves wilted and 5=whole plant died. (**Winstead and Kelman 1952**).

Statistical analysis:

Completely randomized design was used in all experiments. The collected data were analyzed using one-way ANOVA as outlined by (**Gomez and Gomez, 1984**). For performing the mentioned statistical



analysis, SPSS version 13.0 (SPSS Inc., Chicago, IL, USA) statistical packages were used. Duncan's multiple range test (**Duncan, 1955**) was used to compare the means at probability (*P*) level 0.05.

Activity of phenolic compounds from algae:

A- Phenolic Extraction:

Algae were extracted with water or ethanol. The dried residue was dissolved in isopropanol 50% and kept in freezer till analysis. The extracts were used, later for analysis of phenols.

B- Determination of total and free Phenolic:

To determine the content of total and free phenolics the Folin–Ciocalteu method was used (**Simons and Ross, 1971**). To an aliquot of the extract, diluted Folin–Ciocalteu reagent was added. Then, to each sample, sodium carbonate (20%) and water were added. Total and free phenolic content was measured spectrophotometrically at 520 nm, using chatichole as standard.

Results

Effect of two algae on the growth of *R. solanacearum* in vitro:

Data in Table (1) and figure (1) showed that the two extractions of two algae can inhibit *R. solanacearum* growth on TTC medium. Whereas, the highest inhibition zone recorded with water extraction of *P. pavonica* (24and 21mm) followed with *T. decurrens* (21and 19mm) at concentration 2 and 1ml from each alga / 250ml water. Inhibition zone was 15and 12mm with *P. pavonica* and the least one is *T. decurrens* 13and 11mm with ethanol extraction at concentration 2 and 1ml from each alga / 250ml water respectively. Although, inhibition zone was zero when used ethanol or water only.

Table (1): Diameter of inhibition zone for different concentration of water and ethanol extraction of two algae on the growth of *R. solanacearum* *in vitro*.

Algae	Concentration (ml/ 250ml medium)	Diameter of inhibition zone (mm)	
		Water*	Ethanol*
<i>P. pavonica</i>	1	21	12
	2	24	15
<i>T. decurrens</i>	1	19	11
	2	21	13
control	-	0	0

* Extraction type

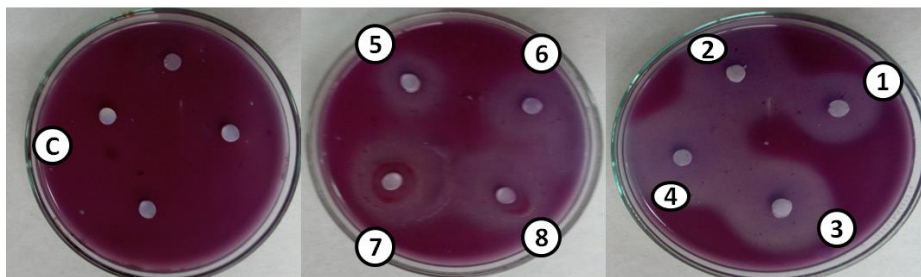


Fig (1): Inhibition zone (mm) of *R. solanacearum* by using water and ethanol extraction of *T. decurrens* and *P. pavonica* algae *in vitro*.

1: 1ml *T. decurrens* water extraction/250ml water- 2: 2ml *T. decurrens* water extraction/250ml water- 3: 1ml *P. pavonica* water extraction/250ml water- 4: 2ml *P. pavonica* water extraction/250ml water- 5: 1ml *T. decurrens* ethanol extraction/250ml water- 6: 2ml *T. decurrens* ethanol extraction/250ml water- 7: 1ml *P. pavonica* ethanol extraction/250ml water- 8: 2ml *P. pavonica* ethanol extraction/250ml water- C: Control with water and ethanol only.

On the other hand, Figure (2 and 3) showed that no growth of *R. solanacearum* observed on surface of streaked plates with the two algae in two extractions at concentration 2ml of algae /250ml medium. However, in 1ml of algae /250ml growth of *R. solanacearum* on surface of plate is very weak compared with control.

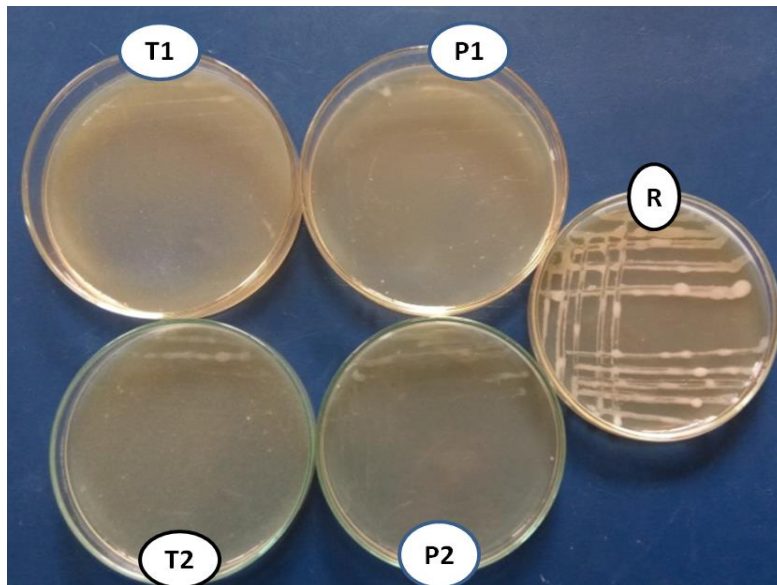


Fig. (2): Effect of water extraction for *T. decurrens* and *P. pavonica* algae on the growth of *R. solanacearum* in vitro.

T1: 2ml of *T. decurrens* algae /250ml media –**P1:** 2ml of *P. pavonica* algae /250ml media

T2: 1ml *T. decurrens* algae /250ml media – **P2:** 1ml of *P. pavonica* algae /250ml media

R: *Ralstonia solanacearum* only

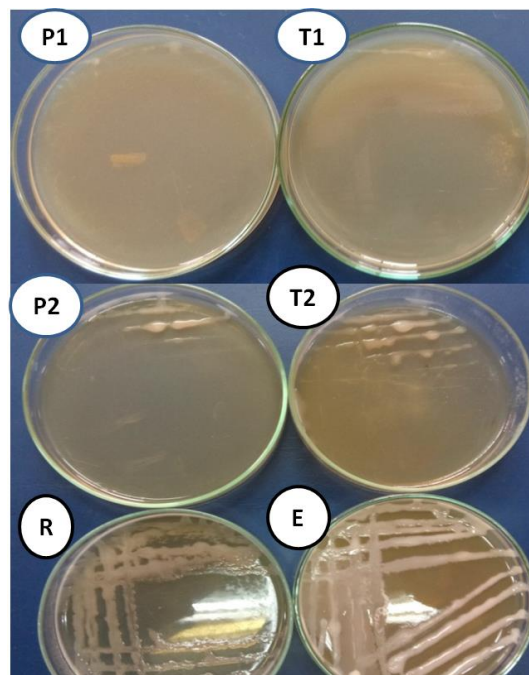


Fig. (3): Effect of ethanol extraction for *T. decurrens* and *P. pavonica* algae on the growth of *R. solanacearum* in vitro.

T1: 2ml of *T. decurrens* algae /250ml media –**P1:** 2ml of *P. pavonica* algae /250ml media

T2: 1ml *T. decurrens* algae /250ml media – **P2:** 1ml of *P. pavonica* algae /250ml media

R: *R. solanacearum* only - **E:** Ethanol only



Effect of two algae on disease severity under artificial inoculation condition:

From Table (2) can concluded that AUDPC and disease severity were decreased in all algae treatments (ranged from 1 to 9.7) compared with *R. solanacearum* treatment (positive control, 24.5). The least AUDPC recorded when used *P. pavonica* and *T. decurrens* at concentration 2ml/250ml in the water and ethanol extractions (1, 1.3, 1.2 and 1.5) respectively followed by the other concentration (2, 5.6, 7.8 and 9.7) respectively. In all treatments AUDPC recoded significant differences with positive control. But, non- significant differences showed between all algae extractions uses at concentration 1ml/250ml (ranged 2 to 9.7) except *P. pavonica* (water extraction, 2). Also, all treatments at 2ml/250ml (ranged 1 to 1.5) and *P. pavonica* water extraction didn't show any significant differences among them.

Table (2): Effect of *T. decurrens* and *P. pavonica* algae as water and ethanol extracted on brown rot disease development in pots experiment.

Extraction	Algae	Concentration (ml/250ml water)	AUDPC*	Disease Severity(%)
Ethanol	<i>P. pavonica</i>	1	7.8 b	48 ab
		2	1.2 c	12 cd
	<i>T. decurrens</i>	1	9.7 b	54 ab
		2	1.5 c	14 cd
Water	<i>P. pavonica</i>	1	2 c	16 bcd
		2	1 c	8 d
	<i>T. decurrens</i>	1	5.6 b	28 abc
		2	1.3 c	10 d
Control +			24.5 a	98 a

* Area Under Disease Progress Curve

On the other hand, data in the same Table indicated the decreased of disease severity in all treatments (ranged 8% to 48%) compared with positive control (98%). The treatment with water extraction of *P. pavonica* followed by *T. decurrens* recorded the least disease severity 8



and 10% when uses at concentration 2ml/250ml respectively. Then, followed by treatment with ethanol extraction of *P. pavonica* and *T. decurrens* 12 and 14% at the same concentration.

At concentration 1ml/ 250ml of algae the disease severity were 16, 28, 48 and 54% when used *P. pavonica* and *T. decurrens* extracted by water and ethanol respectively. No significant differences showed in all treatment when using two extractions of two algae at 1ml concentration, also no significant differences recorded among all treatment at the same concentration and positive control. However, significant differences showed among all treatments at concentration 2ml and positive control, and also among these treatments and the treatments at concentration 1ml except treatment with *P. pavonica* water extraction.

Total and free phenolic quantity:

Data in Table (3) revealed that algae were extracted by water have high amount of total and free phenols compared with algae were extracted by ethanol. The highest one algae is *P. pavonica* (water extraction) in total and free phenols 3.070 and 2.193mg/ml respectively, followed by *T. decurrens* (water extraction) and *P. pavonica* (ethanol extraction) 2.516, 1.596, 1.891 and 1.316mg/ml respectively. While, the least one algae *T. decurrens* (ethanol extraction) were 1.431 and 1.287mg/ml.

Table (3): The quantity of total and free phenolic compounds in two extractions algae.

Extractions	Algae	PHENOLIC COMPOUNDS (mg/ml)	
		TOTAL	FREE
Ethanol	<i>P. pavonica</i>	1.891	1.316
	<i>T. decurrens</i>	1.431	1.287
Water	<i>P. pavonica</i>	3.070	2.193
	<i>T. decurrens</i>	2.516	1.596



Discussion

Ralstonia solanacearum causing bacterial wilt disease in hundreds of economic plants from different families. This pathogen is affects the exportation of potato crop, to the European markets and that lead to lose a lot of money (**Kabeil et al., 2008**). Methods of controlling *R. solanacearum* due to its abilities to grow endophytically, survive in soil, especially in the deeper layers, travel along water, and its relationship with weeds. So, **Yuliar et al., (2015)** discusses different methods to control the diseases caused by *R. solanacearum* like uses of antioxidants (**Farag et al., 2017**), and cyanobacteria (**Mikhail et al., 2016**) to shows some solutions. In recent year, seaweeds are used in several applications such as anti-microbial (**Chiheb et al., 2011**) and produce a large number of antibacterial and antifungal materials, also they almost never threaten the environment (**Kulik, 1995**) . In Egypt, **Farag et al., (2017)** proved the suppression effect of seaweed extract (SWE) on *R. solanacearum* whereas increase nutrient uptake and improve crop productivity.

Brown algae have some of the valuable medicinal components such as: Anti-biotics and anti-oxidant, and most of the compounds of brown seaweeds show anti-bacterial activities (**Mtolera and Semesi, 1996** and **Blunt, 2006**).

Algae extracted by water have the largest inhibition zone at the two concentrations used (1 and 2 ml /250 ml), in two algae (*P. pavonica* and *T. decurrens*) respectively. Followed by Ethanol extractions at the same previously arrangement. The inhibition zone refers to the marine algae especially brown algae antibacterial activity. Whereas, marine algae are the largest producers of biomass in the marine environment, they produce



a wide variety of chemically active metabolites in their surroundings. These active metabolites, also known as biogenic compounds, produced by several species of marine macro and micro algae, have anti-bacterial and anti-fungal properties and many marine algal have bioactive compounds, some of these compounds are sterols, Polysaccharides, peptides, proteins, vitamins, phenols and alkanes (**Bhadury and Wright, 2004., Elsayed et al., 2012., Abdel-Raouf et al., 2015).**

In pots experiment, the extraction by water in two algae was more effective in the two concentrations were used compared with extraction by ethanol, and *P. pavonica* algae was effectiveness compared with *T. decurrens* algae. The amount of phenols contains from any algae depending on the extracted solvent uses, which make variation between the effects of algae in the different extraction (**El-Shazoly and Fawzy 2018., and Naja et al., 2012)**

Quantities of total and free phenols extraction from two algae were different. The highest amount of them was *P. pavonica* extracted by water followed by *T. decurrens* and *P. pavonica* extracted by ethanol and the last one *T. decurrens* respectively. These differences among the phenols amount in each alga certain the differences between *P. pavonica* and *T. decurrens* to inhibit the growth and development of *R. solanacearum*. Whereas, Different extracts of the *Turbinaria* shows the presence of alkaloids, phenols, tannins and flavonoids, It is used as antimicrobial, antioxidant, antifungal, antiulcer, antitumor and antibacterial (**Raj Kumar and Bhavan, 2017).** But, *Turbinaria* species has a low level of phenolic and tannins compounds (**Bittick et al., 2009**), this level depending on the solvent used in the extraction (**Sami. et al., 2019).**



However, *P. pavonica* has been important algae, extensively used as a feedstock for the production of bio-diesel, in heavy-metal biosorption, as a pollution bio-indicator, a trace metal bio-monitor, an anti-oxidant, an anti-cancer drug (by inducing apoptosis of cancer cells), an anti-bacterial agent and a bio-insecticide (**El Maghraby and Fakhry, 2014**). Also, the highest amount of phenolic compound was found in the extract obtained from *P. pavonica* this extract also showed the highest antioxidant activity (**Hamza et al., 2014** and **Naja et al., 2012**).



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استخدام الطحالب البنية بادينا بافونيكاً و توربيناريا ديكيرينس لمقاومة مرض العفن البنى البكتيري

رباب محمد عبد العزيز

قسم بحوث الامراض البكتيرية- معهد بحوث امراض النباتات- مركز البحوث الزراعية- الجيزة- مصر.

الملخص

تعتبر الطحالب البنية من أهم مصادر المقاومة الحيوية لما تحتويه من مواد حيوية نشطة عديدة. وقد استخدمت مستخلصات الطحالبين بادينا بافونيكاً و توربيناريا ديكيرينس في الماء و الكحول الايثيلي لدراسة تأثيرهما المضاد على تقدم مرض العفن البنى في المعمل وتحت ظروف العدوى الصناعية. وقد استطاع هذان الطحلبان تثبيط نمو البكتيريا رالستونيا سولاناسيرم في المعمل. واعطى المستخلص المائي للطحلب بادينا بافونيكاً عند استخدامه أعلى منطقة تثبيط للبكتيريا بينما كان الاقل تثبيطاً للمستخلص الكحولي للطحلب توربيناريا ديكيرينس. وانخفضت الشدة المرضية ومنحنى تقدم المرض في جميع المعاملات النباتية بمستخلصات الطحالبين مقارنة بالمعاملة الموجبة للمرض. ووجد ان كمية الفينولات الكلية والحررة في مستخلصات بادينا بافونيكاً أكبر من كميتها في توربيناريا ديكيرينس. وقد أثرت المواد المستخدمة في الاستخلاص وكمية الفينولات الكلية والحررة على قدرة الطحالب على وقف تقدم الرالستونيا سولاناسيرم وخفض شدتها المرضية. وكان الطحلب بادينا بافونيكاً أكثر كفاءة في مقاومة مرض العفن البنى معملياً وتحت ظروف العدوى الصناعية. لذلك نهدف لزيادة الدراسة لتعريف المركبات الفينولية الخاصة والمسئولة عن زيادة التأثير المضاد للبكتيريا وايضا محاولة تطبيق استخدام الطحالب في مقاومة الأمراض النباتية.