The Potential Use of Aqueous Extract of Ulva lactuca seaweed for the Control of the Post-Harvest Citrus Green Mold, in vivo and in vitro conditions

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Abstract:

Seaweed liquid extracts (SLE) were assessed as natural antifungal products for the control of the post-harvest citrus green mold Penicillium digitatum. The SLE of a green alga, *Ulva lactuca*, at different concentration (25, 50, 100 and 200 g L^{-1}) were evaluated for their inhibitory potential of the *in vivo* development of *P. digitatum* on fresh oranges and of the *in vitro* conidia germination and tube elongation of the mold on potato dextrose broth (PDB). The study of their effects on fungus viability on malt extract agar (MEA) was also evaluated. The results revealed that the assessed SLE at 50 and 100 g L^{-1} allowed a potential inhibition of the fungus viability and development. The U. lactuca liquid extract at 50 g L^{-1} allowed a complete *in vivo* protection of the oranges from the mold for the total duration of the study and a 90 % inhibition of spore germination. Moreover, the chemical characterization of the extract showed valuable content in polysaccharides and mainly in ulvan, known for its antifungal effects. These findings could be highly promising to develop an eco-friendly, safe and effective product for citrus fruit post-harvesting protection.

Keywords: algae, *Penicillium digitatum*, postharvest protection, seaweed liquid extracts, *Ulva lactuca*.

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Introduction

Citrus fruit are frequently subjected, during their storage process, to numerous postharvest infections. These infections are generally caused by molds infecting the host mainly by sustained injuries triggered during different stages of harvesting process (Karim et al. 2017).

Among the most commonly known citrus postharvest molds, figure *Penicillium digitatum*. This green citrus mold is mainly accountable for the major financial losses observed during refrigeration, transportation and marketing of citrus fruits especially within the Mediterranean countries (D'Aquino et al. 2013). In order to control this postharvest occurring mold, several chemical fungicides have traditionally been used such as sodium o-phenylphenate, imazalil and thiabendazole (Moretto et al. 2014). However, the intensive use of chemical fungicides stimulated the expansion of resistant isolates of *Penicillium* spp. leading therefore to a decline in their observed efficiency (Kinay et al. 2007). Moreover, the sanitary threats linked to the persistence of these chemicals as residues in the fruit has raised concerns for finding alternative safe and effective strategies, among which biological pest control has gained much consideration (Spadaro and Droby 2016). This latter typically refers to active compounds including among others, proteins, polysaccharides from plants and microorganisms, polysaccharides from algae as well as oligochitosan from animals. These compounds are known to be highly specific and active toward their target while being safe to the environment (Zhao et al. 2017).

Marine algae are characterized by a wide diversity of natural products that can be explored in agricultural fields. In fact, due to their high polysaccharides' content, algae are known to induce a considerable resistance against microbial pathogens by activating the plants defense mechanisms. For this, several antiviral, deworming, antibacterial and antifungal activities were identified in marine algae (Akremi et al. 2017), (Fernando et al. 2016), (Val et al. 2001). For instance, biological, antioxidant and antifungal properties were associated with sulphated polysaccharides isolated from green algae (Adrien et al. 2017), (Guidara et al. 2020). Moreover, different antifungal molecules were isolated from several green algae (Abouraïcha et al. 2015), (Dussault et al. 2016). For instance, significant antifungal activity was observed in the green alga *Ulva lactuca* followed by the brown alga *Sargassum wightii* and the red alga *Kappaphycus alvarezii (Aruna et al. 2010).*

In a previous study, Chbani et al, 2013, showed that green seaweeds are highly efficient toward citrus fruits protection against the postharvest green mold *P. digitatum* (Chbani et al. 2013a).

Accordingly, the aim of this study is to evaluate the antifungal effect of the green seaweed, *U. lactuca*. Different concentrations of the green alga were evaluated *in vivo* and *in vitro* on spore germination as well as on fungus viability on post-harvesting protection of citrus fruits against the green mold *P. digitatum*.

Materials and Methods

Algal material

Fresh *Ulva lactuca* was manually collected from a coastal zone of the Mediterranean, El Mina (34 ° 26 'N - 35 ° 50' E) in Tripoli, Lebanon, on May 1th, 2016.

The alga was cleaned with sea water to remove unwanted and then carried in simply moistened plastic bags to the laboratory where they were washed thoroughly under tap water.

Citrus fruits

Valencia Oranges (*Citrus sinensis* (L.) Osbeck) were harvested from a local orange orchard in Tripoli (Lebanon).

The fruits were selected for their size uniformity and their healthy appearance, and then their surface was directly sterilized with a bleach solution (2%) for 2 minutes. Afterwards, sterilized oranges were washed with tap water and then air- dried at room temperature prior to their usage.

Seaweed liquid extracts (SLEs)

SLEs were prepared according to Jimenez et al., in 2011 (Jiménez et al. 2011). 200 g of fresh alga was grounded then boiled with 1 liter of ultrapure water while stirring for one hour. The extract was then filtered twice using a double layer sterile muslin cloth and then cooled to room temperature for the preparation of correspondent diluted series. Sterile distilled water was used for the dilutions and series of 200 g L^{-1}, 100 g L^{-1}, 50 g L^{-1} and 25 g L^{-1} were prepared.

Preparation of pathogen inoculum

Fungal isolates of *Penicillium digitatum* were isolated from infected oranges, and then cultivated on Malt Extract Agar (MEA, Sigma–Aldrich) in order to obtain single conidia colonies. For inoculum growth, cultures were kept at 22°C for 7–10 days. Obtained spores were then collected using a sterile spatula, diluted in sterile distilled water, and then mixed

using a vortex for 1 minute. Spore concentration was fixed to 10^8 c/mL using a densitometer (Biosan DEN-1B, McFarland densitometer). The obtained stock solution (10^8 c/mL) was then diluted to two solutions S1= 10^6 c/mL used for the study of SLE effects on spore germination in liquid medium and germ tube elongation, and S2= 2x 10^3 c/mL used for the study of the SLE effects on fungus viability on solid medium (Nicosia et al. 2016).

In vivo antifungal activity of SLE against P. digitatum

The antifungal potential of *U. lactuca* on oranges infected by *P. digitatum* was assessed using three oranges for each of the prepared dilution. For this, each orange was treated with 4 mL of the tested SLE and then placed on an absorbent paper previously soaked with sterile distilled water. Distilled water was used as negative control and Nystatin as positive control (Chbani et al. 2013b).

Two hours after their treatment, oranges were infected with 10 µL of spore suspension of *P. digitatum* prepared using Tween 20 (10^5 spores/mL suspension). Afterwards, treated oranges were covered with a transparent film and stored at room temperature where they were monitored for 8 weeks during which the total number of infected oranges has been recorded.

In vitro antifungal activity of SLE against P. digitatum

The study of the *in vitro* antifungal activity of SLE was conducted according to Li Destri Nicosia et al., in 2016 (Nicosia et al. 2016).

Effects of SLEs on spore germination

In order to study the SLE effects on spore germination and germ tube elongation, 12.5 µL of the solution S1 (prepared at 10^6 c/mL) were added to an eppendorf tubes followed by 25 µL of tested extracts and 12.5 µL of potato dextrose broth (PDB, Sigma–Aldrich). Sterile ultrapure water and Nystatin were respectively used as negative and positive controls. The tubes were then mixed and incubated for 20 hours at 22 °C followed by a vortex in order to homogenize them. Afterwards, 2 µL of the prepared spore suspension were transferred to microscope slides where they were mixed and homogenized with 2 µL of lacto-phenol blue then observed at a magnification of 40 for spore germination and tube elongation. For each slide, three observations were haphazardly done.

Effects of SLEs on fungus viability

In order to evaluate the SLE effects on pathogen viability, 0.5 mL of solution S2 ($2x10^3$ c/mL) were added, along with 0.5 mL of SLE to eppendorf tubes. Sterile ultrapure water and nystatin were respectively used as negative and positive controls. Tubes were smoothly agitated then incubated for 20 hours at 22 °C. Afterwards, tubes were mixed and a volume of 100 µL of each mixture was spread on MEA culture medium containing ampicillin. Finally, cultures were incubated for 4 days at 25 °C and the colonies forming units (CFU) numbers were recorded.

Extract characterization

Total Polysaccharide content analysis

Polysaccharides were analyzed by ion exchange chromatography using a CarboPac PA1 column (Dionex). Potassium hydroxide was used as eluent at 1mL min^{-1}.

The elution was performed using the following gradient: 2mM for 39 minutes, followed by an increase to 10 mM for 2 minutes and then to 100mM for 8min where it remained stable for 3 minutes. Temperature was set to 25 °C and the injected volume to 25 µL.

For the identification of polysaccharides, external standards were used while for the quantification of polysaccharides Fucose, as internal standard, was used.

Ulvan content analysis

Dried *U. lactuca* was used, following the procedure of Bilan et al., in 2006 (Bilan et al. 2006), for ulvan content determination. Accordingly, 20 grams of dry *U. lactuca* were treated with a mixture of methanol (MeOH)/ chloroform ($CHCl_3$)/ water (H_2O) (4:2:1 v/v/v) at room temperature in order to remove colored matter followed by filtration and vacuum drying in order to obtain defatted algal biomass. Afterwards, the method of Mao et al., in 2006 was applied on the resulting product for ulvan extraction (Mao et al. 2006). For this, 400 mL of water were added to the resulting alga and the solution was kept in a hot-water bath for 2 hours where it was subjected to a continuous stirring at 80–90 °C. Subsequently, the aqueous extract was centrifuged, and the supernatant was subjected to filtration followed by concentration on rotary evaporator. After this, the aqueous extract was centrifuged, filtered and precipitated with 3 vol. of absolute ethanol. Resulted precipitate was washed using ethanol, dried at 40 °C, re-dissolved in distilled water, and finally dried in order to obtain the purified ulvan.

Total Phenolic content analysis

For the determination of the total phenolic content of the SLE, the method Folin Ciocalteu was applied (Lamuela- Raventós 2018). For this, a 96-well microplate was used and 20 µL of the extract, 10 µL of Folin Ciocalteu reagent and 170 µL of sodium carbonate were added to each of its wells. Afterwards, the plate was incubated at 45 °C for 45 minutes followed by a measurement of the absorbance at 760 nm using a spectrophotometer (Spectrostar, BMG Labtech).

Standard solution of gallic acid was used for plotting a calibration curve that was used for sample analysis. Results were expressed as grams of Gallic Acid Equivalent per 100 g of sample on a dry basis (GAE/100 g d.b.).

Soluble Protein content

Proteins were quantified using the Lowry method (Lowry et al. 1951) using a standard curve of bovine serum albumin (BSA) and a Lowry kit (Lowry reagent, bovine albumin standard and 2-N Folin-Ciocalteu reagents) purchased from Thermo Fisher Scientific. BSA was used for plotting a calibration curve ranging from 0 to 500 µg. mL^{-1}. For this, 0.2 mL of each standard and sample encompassing the crude protein extract was withdrawn and then 1 mL of modified Lowry reagent was added. Samples were then homogenized using a vortex and then incubated for 10 minutes. Afterwards, 100 µL of Folin-Ciocalteu reagent (1 N) was added to the sample that was homogenized on a vortex then incubated for an extra 30 minutes. The absorbance of the blue-colored solution obtained was finally measured at 750 nm using a UV-1800 Shimadzu spectrophotometer.

Statistical analysis

All analyses were done in triplicate. Data were statistically analyzed using Student's t test and analysis of variance (ANOVA); means were compared to negative control at $P < 0.05$.

Results

The percentage of the inhibition of *P. digitatum* recorded on the oranges over 8 weeks is shown in figure 1. Results showed that a total inhibition of the mold was observed with *U. lactuca*

prepared at 50 g. L^{-1} as well as by the positive control Nystatin. For the extract prepared at 100 g. L^{-1} a total inhibition of *P. digitatum* was observed till the last week of the study where this percentage declined to 33.33 %. For *U. lactuca* prepared at 200 g. L^{-1}, a total inhibition of the green mold was observed during the first three weeks then a decline to 66.66 % was recorded for the remaining 5 weeks of the study. Finally, for the SLE prepared at 25 g. L^{-1}, *P. digitatum* was completely inhibited for the first two weeks where the inhibition percentage started to decline to 66.66 % during the third week of the study in order to reach 33.33 % during the fourth week where it remained constant till the end of the study. Regarding water, used at negative control, the total inhibition of the mold was only observed during the first week then started to decline in order to completely disappear at the 6th week of the study.

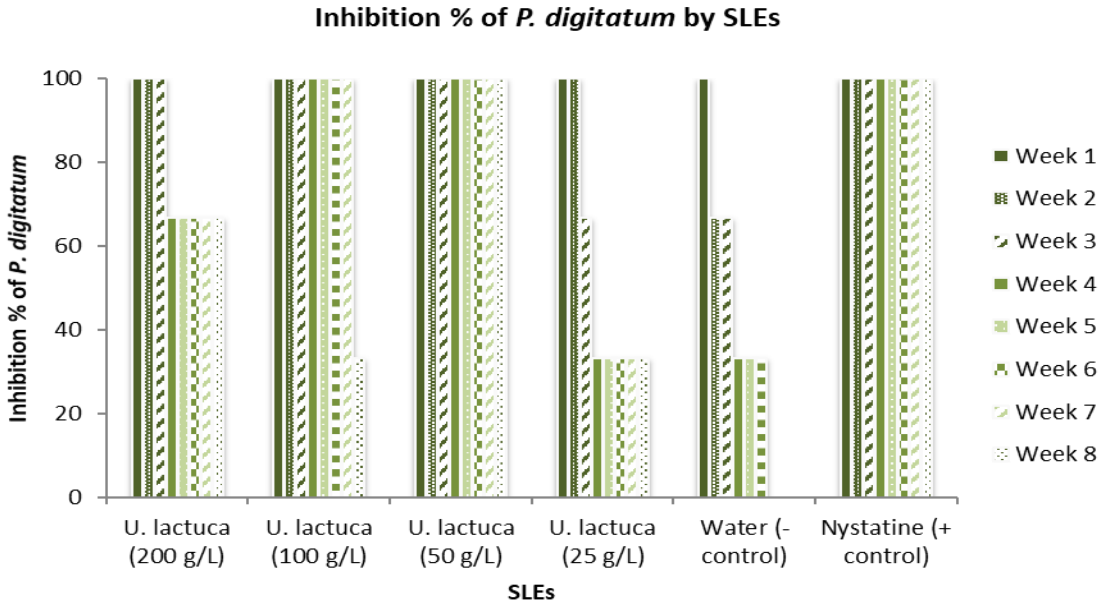


Fig. 1: Effect of *U. lactuca* SLEs at different concentrations on *in vivo* growth of *Penicillium digitatum* for 8 weeks

In vitro antifungal activity of SLE against P. digitatum

Effects of SLEs on spore germination

The alga extracts were tested for their ability to inhibit the germination of *P. digitatum* spores. Spores were considered germinated when elongation tubes were formed. The inhibition percentage was estimated by the comparison of tested extracts with water used as negative control.

The tested extracts showed a significant inhibition of *P. digitatum* germination in comparison with water. In fact, the spore germination was inhibited to 90 % with *U. lactuca* liquid extract at 50 g L^{-1}, while the inhibition percentage was 85, 70 and 10% respectively with the *U. lactuca* liquid extracts at 100, 200 and 25 g L^{-1}.

Figures 2 and 3 show the effects of *U. lactuca* crude extracts at different concentrations on spore germination.

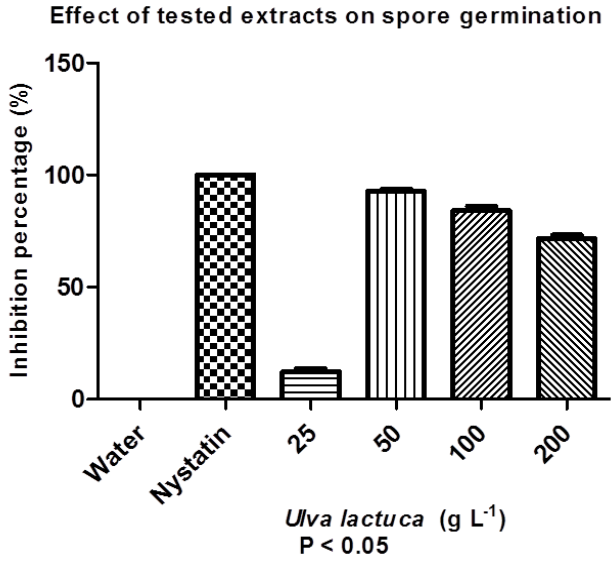
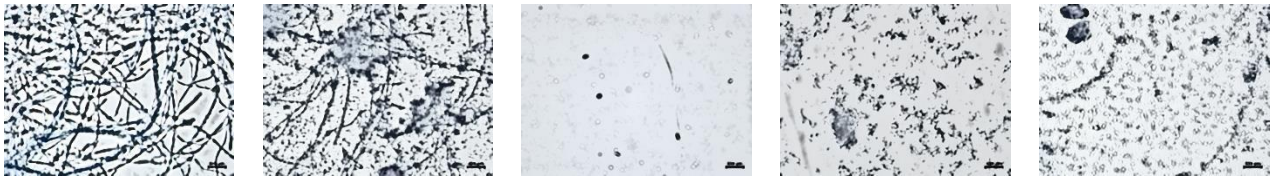


Fig. 2: Inhibition percentages of the germination of *P. digitatum* incubated for 20 h at 22 °C in PDB containing *Ulva lactuca* extracts at different concentrations, water as negative control and Nystatine as positive control. Results were statistically significant according to ANOVA test (P<0.05)



A B C D E

Fig. 3: Germination of *P. digitatum* incubated for 20 h at 22 °C in PDB containing *Ulva lactuca* extracts at different concentrations (B=25 g L^{-1}, C= 50 g L^{-1}, D= 100 g L^{-1}, E= 200 g L^{-1}), and water as negative control (A). Results were statistically significant according to ANOVA tests (P< 0.05)

3.2. 2. Effects of SLEs on fungus viability

After being incubated for 4 days at 25 °C, the number of colonies forming units (CFU) was recorded for all tested extracts.

Figure 4 represented the inhibition percentage of CFU calculated based on the number of colonies observed with water used as a negative control. The results showed that the SLE at 50 g. L^{-1} allowed a high inhibition of the mold CFU (90%), while this inhibition seems to be less effective with the lowest concentration tested (16%).

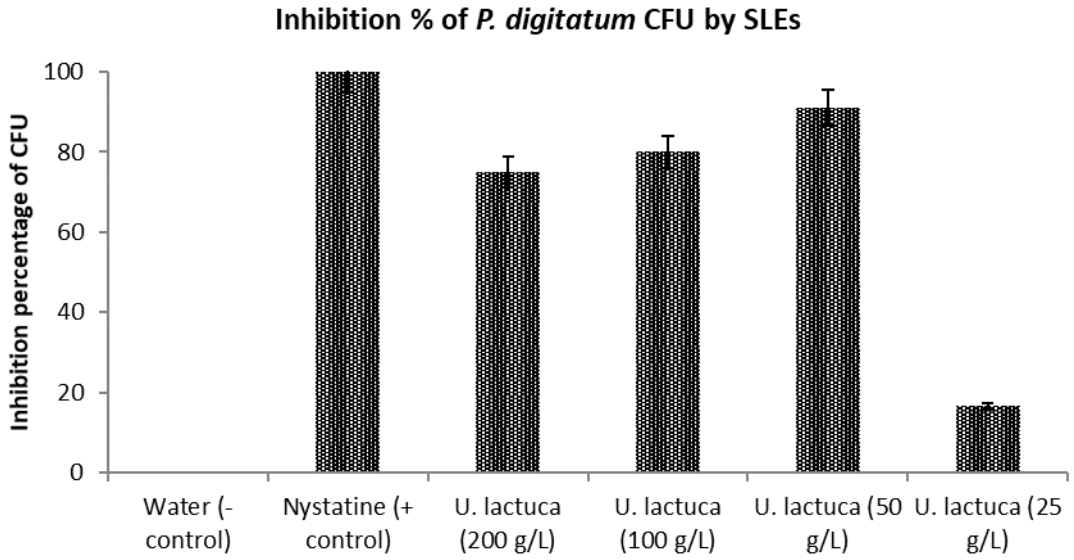


Fig. 4: Inhibition percentage of CFU obtained on MEA after 4 days of incubation. Results were statistically significant according to ANOVA tests ($P< 0.05$)

Extract characterization

The analysis of total polysaccharides in the SLE of the tested *U. lactuca* showed a total concentration of 76.89 mg. L^{-1}. The analysis of these polysaccharides showed that they were mainly formed from Fructose (66.64 mg L^{-1}), Glucose (5.9 mg L^{-1}) and Rhamnose (4.35 mg L^{-1}). Regarding the ulvan analysis, contents were determined from algal biomass and results showed that the ulvan content in the tested *U. lactuca* was 18.5 (g/100g DM). For polyphenols and soluble proteins, results showed that their total amount was found to be 6 and 5.4 g/100 g respectively.

Discussion

In order to control postharvest infections of citrus fruits caused by *P. digitatum*, a direct treatment of the fruits after their harvest may be effective for their protection while being harvested and packaged disabling therefore the fungal pathogen to pass through the wounds and infect the host (Nicosia et al. 2016), (Tayel et al. 2009), (Zheng et al. 2015).

In this study, the antifungal activity of the green seaweed, *U. lactuca*, against *P. digitatum* was assessed. The SLE effects on spore germination and on fungus viability were tested. The results showed a strong antifungal activity against *P. digitatum* germination. In fact, the results provided by the two tests were coherent; the SLE at 50 and 100 g L^{-1} seemed to be of a high interest in the process of post-harvested protection of citrus fruits from *P. digitatum*. This effect was shown by the ability of these extracts to inhibit the appearance of green mold of fresh citrus fruits for up to 8 weeks without any appearance of the mold neither any damage observed at the tissues of the fruits. These results were in correlation with those observed on PDB for the effects of the SLEs on the spore germination of the fungus after 20 hours of incubation. Moreover, the use of these extracts on MEA seems to inhibit the development of the fungus. However, even if the SLEs at 25 g L^{-1} seems to be less efficient, this extract showed a weak ability to inhibit the germination of *P. digitatum* spores after 4 days of incubation (up to 16%). In fact, the infective life cycle of *P. digitatum* initiates with the germination of conidia on the surface of the fruits (Vilanova et al. 2016). Hence, when the growth cycle of the pathogen is limited to multiplication and not developed to germination, the infectious life cycle will be arrested. Moreover, it is also noted that visible mycelium appears soon after germination is over and accordingly the conidia fungal germination should be considered as a key step during the control of antifungal infection (Kalai et al. 2017).

The results obtained are in correlation with several studies showing the potential effects of biological extracts in the *P. digitatum* inhibition process. For instance, the *Eugenia caryophytae* plant crude extract allowed up to 100% reduction of *P. digitatum* hyphal growth providing thus a total protection of citrus fruit (Sukorini et al. 2013). Furthermore, the use of the essential oils of the citrus plant *Citrus reticulata*, for the bio control of citrus green mold, *P. digitatum*, showed a potential role in fruit protection by limiting the mycelial growth of the fungus (Tao et al. 2014). Likewise, some bioactive compounds, such as carotenoids, alkaloids, flavonoids, fatty acids, saponins, amino acids and carbohydrates, widely found in seaweeds could be highly effective in the inhibitory action against fungal and microbial pathogens (Bhagavathy et al. 2011). Moreover, the use of seaweeds as natural fungicides can be due to

their bioactive compounds, for instance, El Baky et al., 2008 showed antimicrobial activity of the extract Dichloromethane/methanol from *U. lactuca* isolated from the Egyptian coast (Abd El-Baky et al. 2008). As well, significant antifungal activity was observed in the green alga *U. lactuca* followed by the alga Brown *S. wightii* and red alga *K. alvarezii* (Aruna et al. 2010). Furthermore, many substances have been recognized as antimicrobial agents based on marine algae such as aliphatic compounds, terpenes and acrylic acid (Lavanya and Veerappan 2011). Besides, it was shown that the cyclic eudesmol, a sesquiterpene isolated from the green alga *Chondria oppositiclada*, exerts a high antimicrobial effect against *Staphylococcus aureus* and *Candida albicans* (El Gamal 2010), (Fenical and Sims 1974).

Among the functional compounds identified from marine algae, natural pigments have retained special attention. In fact, these pigments have shown different beneficial biological activities as antioxidants, anti-cancer, anti-inflammatory, anti-obesity, anti-angiogenic as well as neuroprotective activities (Pangestuti and Kim 2011). In addition, the high polysaccharide content of seaweeds acts as inducers of plant defense responses leading to their resistance against microbial pathogens. For example, oligosaccharides of the green algae, *U. lactuca*, particularly ulvan and oligulvans, induced, in tomato plants, natural systemic defenses and acquired systemic resistance dependent on salicylic acid by a reduction in the development of wilting by *F. oxysporum* reducing the mortality of treated tomato plants (El Modafar et al. 2012). In this work, the characterization of the tested *U. lactuca* showed a high content in polysaccharide (76.89 mg L^{-1}) and in ulvan particularly (18.5 g/100g DM). Our results are in accordance to many previous works, showing that polysaccharides and ulvans are accountable for the antifungal effects and the defense mechanisms exerted by marine algae (Alves et al. 2013), (Mirzadeh et al. 2020), (Thanh et al. 2016). The observed antifungal activity exerted by the tested SLE could be attributed to the presence of polysaccharides and ulvan as it was shown that these latter produced significant inhibitory rates on mycelial growth of the fungus attacking olive trees (Ben Salah et al. 2018) and were used an alternative in the sustainable postharvest management of papaya due to the potential antifungal effect they showed (Chiquito-Contreras et al. 2019). These findings were also proved by Salim et al., in 2020 showing that ulvans fractions, extracted from *U. lactuca* exhibited the highest inhibition zone diameters, among other algal extracts, while being tested against the fungal strain *P. digitatum* (Salim et al. 2020).

Otherwise, the green alga *U. lactuca* Linnaeus was taken and evaluated for its antimicrobial activity and fractions obtained showed greater antifungal activity than the extract against

Aspergillus niger and *C. albicans* (Alang et al. 2009). In fact, recently, various antifungal molecules have been isolated from the green algae *Caulerpa racemosa*, *U. lactuca*, *Penicillus capitatus* and the brown alga *Lobophora variegata* (El-Hossary et al. 2017), (Shobier et al. 2016).

Conclusion

In conclusion, this study showed that SLE of *Ulva lactuca* could be of a high potential in the process of post-harvesting control of the green citrus fruit's mold *Penicillium digitatum*. Moreover, among the different concentrations tested, 50 and 100 g L^{-1} showed a good inhibitory activity of *P. digitatum* germination and multiplication. Thus, in order to protect citrus fruit from fungicides, SLEs act as an inhibitor of the fungus germination, limit it to the multiplication stage and inhibit the initiation of the infection process. In addition, the characterization of the alga showed that polysaccharides and ulvan could be mainly considered as the main actors of the observed antifungal effect. All these findings may be promising for the use of marine algae as alternatives for harmful chemical pesticides.

Acknowledgements

The authors acknowledge "AZM & SAADE" association and the Lebanese University for funding the project

References

Abd El-Baky, H.H., El Baz, F.K., El-Baroty, G., 2008. Evaluation of marine alga Ulva lactuca L. as a source of natural preservative ingredient. Am Eurasian J Agric Environ Sci 3, 434-444.

Abouraïcha, E., El Alaoui-Talibi, Z., El Boutachfaiti, R., Petit, E., Courtois, B., Courtois, J., El Modafar, C., 2015. Induction of natural defense and protection against Penicillium expansum and Botrytis cinerea in apple fruit in response to bioelicitors isolated from green algae. Scientia Horticulturae 181, 121-128.

Adrien, A., Dufour, D., Baudouin, S., Maugard, T., Bridiau, N., 2017. Evaluation of the anticoagulant potential of polysaccharide-rich fractions extracted from macroalgae. Natural product research 31, 2126-2136.

Akremi, N., Cappoen, D., Anthonissen, R., Verschaeve, L., Bouraoui, A., 2017. Phytochemical and in vitro antimicrobial and genotoxic activity in the brown algae Dictyopteris membranacea. South African Journal of Botany 108, 308-314.

Alang, G., Kaur, R., Singh, A., Budlakoti, P., Singla, P., 2009. Antimicrobial activity of Ulva lactuca extracts and its fractions. Pharmacology (online) 3, 107-117.

Alves, A., Sousa, R.A., Reis, R.L., 2013. In vitro cytotoxicity assessment of ulvan, a polysaccharide extracted from green algae. Phytotherapy Research 27, 1143-1148.

Aruna, P., Mansuya, P., Sridhar, S., Kumar, J.S., Babu, S., 2010. Pharmacognostical and antifungal activity of selected seaweeds from Gulf of Mannar region. Recent Research in Science and Technology.

Ben Salah, I., Aghrouss, S., Douira, A., Aissam, S., El Alaoui-Talibi, Z., Filali-Maltouf, A., El Modafar, C., 2018. Seaweed polysaccharides as bio-elicitors of natural defenses in olive trees against verticillium wilt of olive. Journal of Plant Interactions 13, 248-255.

Bhagavathy, S., Sumathi, P., Bell, I.J.S., 2011. Green algae Chlorococcum humicola-a new source of bioactive compounds with antimicrobial activity. Asian Pacific Journal of Tropical Biomedicine 1, S1-S7.

Bilan, M.I., Grachev, A.A., Shashkov, A.S., Nifantiev, N.E., Usov, A.I., 2006. Structure of a fucoidan from the brown seaweed Fucus serratus L. Carbohydrate research 341, 238-245.

Chbani, A., Mansour, R., Mawlawi, H., Gmira, N., 2013a. In vitro and in vivo evaluation of anti-phythopathogenic activity and anti-adhesive properties of three Macro Algae against Penicillium digitatum. Science Lib Journal 5, 1-23.

Chbani, A., Mawlawi, H., Zaouk, L., 2013b. Evaluation of brown seaweed (Padina pavonica) as biostimulant of plant growth and development. African Journal of Agricultural Research 8, 1155-1165.

Chiquito-Contreras, R.G., Murillo-Amador, B., Carmona-Hernandez, S., Chiquito-Contreras, C.J., Hernandez-Montiel, L.G., 2019. Effect of Marine Bacteria and Ulvan on the Activity of Antioxidant Defense Enzymes and the Bio-Protection of Papaya Fruit against Colletotrichum gloeosporioides. Antioxidants 8, 580.

D'Aquino, S., Fadda, A., Barberis, A., Palma, A., Angioni, A., Schirra, M., 2013. Combined effects of potassium sorbate, hot water and thiabendazole against green mould of citrus fruit and residue levels. Food Chemistry 141, 858-864.

Dussault, D., Vu, K.D., Vansach, T., Horgen, F.D., Lacroix, M., 2016. Antimicrobial effects of marine algal extracts and cyanobacterial pure compounds against five foodborne pathogens. Food Chemistry 199, 114-118.

El-Hossary, E.M., Cheng, C., Hamed, M.M., Hamed, A.N.E.-S., Ohlsen, K., Hentschel, U., Abdelmohsen, U.R., 2017. Antifungal potential of marine natural products. European journal of medicinal chemistry 126, 631-651.

El Gamal, A.A., 2010. Biological importance of marine algae. Saudi pharmaceutical journal 18, 1-25.

El Modafar, C., Elgadda, M., El Boutachfaiti, R., Abouraicha, E., Zehhar, N., Petit, E., El Alaoui-Talibi, Z., Courtois, B., Courtois, J., 2012. Induction of natural defence accompanied by salicylic acid-dependant systemic acquired resistance in tomato seedlings in response to bioelicitors isolated from green algae. Scientia Horticulturae 138, 55-63.

Fenical, W., Sims, J.J., 1974. Cycloeudesmol, an antibiotic cyclopropane containing sesquiterpene from the marine alga, Chondria oppositiclada Dawson. Tetrahedron Letters 15, 1137-1140.

Fernando, I.S., Nah, J.-W., Jeon, Y.-J., 2016. Potential anti-inflammatory natural products from marine algae. Environmental Toxicology and Pharmacology 48, 22-30.

Guidara, M., Yaich, H., Amor, I.B., Fakhfakh, J., Gargouri, J., Lassoued, S., Blecker, C., Richel, A., Attia, H., Garna, H., 2020. Effect of extraction procedures on the chemical structure, antitumor and anticoagulant properties of ulvan from Ulva lactuca of Tunisia coast. Carbohydrate Polymers 253, 117283.

Jiménez, E., Dorta, F., Medina, C., Ramírez, A., Ramírez, I., Peña-Cortés, H., 2011. Anti-phytopathogenic activities of macro-algae extracts. Marine Drugs 9, 739-756.

Kalai, S., Anzala, L., Bensoussan, M., Dantigny, P., 2017. Modelling the effect of temperature, pH, water activity, and organic acids on the germination time of Penicillium camemberti and Penicillium roqueforti conidia. International journal of food microbiology 240, 124-130.

Karim, H., Boubaker, H., Askarne, L., Cherifi, K., Lakhtar, H., Msanda, F., Boudyach, E., Aoumar, A.A.B., 2017. Use of Cistus aqueous extracts as botanical fungicides in the control of Citrus sour rot. Microbial pathogenesis 104, 263-267.

Kinay, P., Mansour, M.F., Gabler, F.M., Margosan, D.A., Smilanick, J.L., 2007. Characterization of fungicide-resistant isolates of Penicillium digitatum collected in California. Crop Protection 26, 647-656.

Lamuela- Raventós, R.M., 2018. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. Measurement of Antioxidant Activity & Capacity. Recent Trends and Applications, 107-117.

Lavanya, R., Veerappan, N., 2011. Antibacterial potential of six seaweeds collected from gulf of mannar of southeast coast of India. Advances in Biological Research 5, 38-44.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. Journal of biological chemistry 193, 265-275.

Mao, W., Zang, X., Li, Y., Zhang, H., 2006. Sulfated polysaccharides from marine green algae Ulva conglobata and their anticoagulant activity. Journal of applied phycology 18, 9-14.

Mirzadeh, M., Arianejad, M.R., Khedmat, L., 2020. Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: A review. Carbohydrate Polymers 229, 115421.

Moretto, C., Cervantes, A.L.L., Batista Filho, A., Kupper, K.C., 2014. Integrated control of green mold to reduce chemical treatment in post-harvest citrus fruits. Scientia Horticulturae 165, 433-438.

Nicosia, M.G.L.D., Pangallo, S., Raphael, G., Romeo, F.V., Strano, M.C., Rapisarda, P., Droby, S., Schena, L., 2016. Control of postharvest fungal rots on citrus fruit and sweet cherries using a pomegranate peel extract. Postharvest Biology and Technology 114, 54-61.

Pangestuti, R., Kim, S.-K., 2011. Biological activities and health benefit effects of natural pigments derived from marine algae. Journal of functional foods 3, 255-266.

Salim, D., de Caro, P., Merah, O., Chbani, A., 2020. Control of Post-harvest Citrus Green Mold using Ulva lactuca Extracts as a Source of Active Substances. International Journal of Bio-resource and Stress Management 11, 287-296.

Shobier, A.H., Ghani, S.A.A., Barakat, K.M., 2016. GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. The Egyptian Journal of Aquatic Research 42, 289-299.

Spadaro, D., Droby, S., 2016. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. Trends in Food Science & Technology 47, 39-49.

Sukorini, H., Sangchote, S., Khewkhom, N., 2013. Control of postharvest green mold of citrus fruit with yeasts, medicinal plants, and their combination. Postharvest Biology and Technology 79, 24-31.

Tao, N., Jia, L., Zhou, H., 2014. Anti-fungal activity of Citrus reticulata Blanco essential oil against Penicillium italicum and Penicillium digitatum. Food Chemistry 153, 265-271.

Tayel, A., El-Baz, A., Salem, M., El-Hadary, M., 2009. Potential applications of pomegranate peel extract for the control of citrus green mould. Journal of Plant Diseases and Protection 116, 252-256.

Thanh, T.T.T., Quach, T.M.T., Nguyen, T.N., Luong, D.V., Bui, M.L., Van Tran, T.T., 2016. Structure and cytotoxic activity of ulvan extracted from green seaweed Ulva lactuca. International Journal of Biological Macromolecules 93, 695-702.

Val, A., Platas, G., Basilio, A., Cabello, A., Gorrochategui, J., Suay, I., Vicente, F., Portillo, E., Río, M., Reina, G., 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). International Microbiology 4, 35-40.

Vilanova, L., Teixidó, N., Torres, R., Usall, J., Viñas, I., Sánchez-Torres, P., 2016. Relevance of the transcription factor PdSte12 in Penicillium digitatum conidiation and virulence during citrus fruit infection. International journal of food microbiology 235, 93-102.

Zhao, L., Feng, C., Wu, K., Chen, W., Chen, Y., Hao, X., Wu, Y., 2017. Advances and prospects in biogenic substances against plant virus: A review. Pesticide biochemistry and physiology 135, 15-26.

Zheng, S., Jing, G., Wang, X., Ouyang, Q., Jia, L., Tao, N., 2015. Citral exerts its antifungal activity against Penicillium digitatum by affecting the mitochondrial morphology and function. Food Chemistry 178, 76-81.

Arabian Journal of Medicinal and Aromatic Plants,

ISSN 2458-5920, www.ajmap.info