Moroccan Journal of Agricultural Sciences
Vol 3 No 1 (2022): (March 2022), 1-9
Crop Protection

Moroccan seaweed polysaccharides elicit defense response and induce protection against Botrytis cinerea in tomato plants

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Eight Polysaccharides Enriched Extracts (PEEs) obtained from Moroccan seaweeds were selected to test their effectiveness on the natural defense of tomato plants. Firstly, we examined the effect of the 8 PEEs at 4 concentrations (0.02, 0.05, 0.1 and 0.2 mg. mL^{-1}) with 3 application methods (T1: root irrigation, T2: foliar spray, and T3: combining the 2 methods T1 and T2) on protein content and plant defense enzyme: Phenylalanine Ammonia-lyase (PAL) on tomato plants in absence of a pathogen. In the second part, we analyzed the ability of PEEs to induce protection against *Botrytis cinerea*, causative agent of gray mold, by testing PEEs on detached tomato leaves. Results showed that the PEEs obtained from *Codium tomentosum*, *Ulva rigida,* and *Bifurcaria bifurcata* at 0.1 mg. mL^{-1} and *Gelidium crinale, Schizymenia dubyi* and *Fucus spiralis* at 0.02 mg. mL^{-1} were the best treatments that significantly stimulated protein content and PAL activity in tomato plants with the three application methods. The same extracts at the same concentrations, in addition of PEEs from *G. pistillata* at 0.02 mg. mL^{-1} were the treatments having the greatest inhibitory effect on the diameter lesion of *B. cinerea* in detached leaves when compared to the control. The principal component analysis showed a correlation between PAL content and the reduction of diameter lesion. The comparison of the application method in our study did not show differences. These findings confirmed that these algal polysaccharides treatments could be a promising method to reduce dependency on synthetic fungicides. The presence of uronic acid and sulfated groups in the extracts could explain the elicitation mechanism induced in plant cells.

Keywords: Tomato plants; PEEs; seaweeds; plant defense, PAL, *Botrytis cinerea*

INTRODUCTION

The phytopathogenic fungus Botrytis cinerea Pers. is one of the most extensively studied necrotrophic fungal pathogen, it has a wide host range including more than 200 species of crops (Williamson et al., 2007; Elad et al., 2016). It is the causative agent of gray mould in tomato and many other economically important crops, such as pepper, eggplant, grape, lettuce and raspberry.

It is a devastating disease that causes losses of $ 10 billion to $ 100 billion annually because it reduces crop yields before harvest or results in postharvest waste and spoilage (De Vega et al., 2021). As a result, B. cinerea has been considered the second most serious phytopathogen worldwide (Dean et al., 2012). Under conditions of high humidity coupled with low temperature, the typical disease symptoms can be visualized on tomato fruit and every part of the tomato plant infected with the pathogen through wounds after pruning and harvesting or through direct penetration (Williamson et al., 2007; Elad et al., 2007; Romanazzi et al., 2014). For several reasons, it is difficult to control B. cinerea; it has a wide host range, various modes of attack and asexual and sexual stages to survive under favorable or unfavorable conditions (Fillinger and Elad, 2016). The sexual spores of B. cinerea are sclerotia, which are essential for survival in an unfavorable environment, and the asexual spores are conidia, which are easily dispersed by wind or water (Brandhoff et al., 2017). Over past decades, the control of gray rot caused by B. cinerea has depended mainly on the application of synthetic fungicides (Adnan et al., 2018). However, two main problems are encountered with the application of fungicides. On one hand, Botrytis tends to change constantly during generations and developed certain resistance to several fungicides, including anilinopyrimidines dicarboximides, benzamide, fenhexamid, diethofencarb, procymidone, pyrimethanil, and hydroxyanilide (Myresiotis et al., 2007; Mosbach et al., 2017; Adnan et al., 2019). Additionally, the abuse and excessive use of fungicides have caused environmental pollution and disruption of ecological ecosystems, as well as a danger to human beings, especially their toxicological residues in tomato fruit (Abbey et al., 2019; Rosero-Hernández et al., 2019). In order to overcome the obstacles resulting from the chemical fungicides, there is an urgent need for alternative measures that could safely and effectively control gray mold disease.

Alternatively, sustainable strategies of stimulation of the natural plant defense can also be achieved by the application of natural elicitors which can be derived from natural compounds (Battacharyya et al., 2015). Plant species have developed an innate immune system with two levels of pathogen recognition (Jones et Dangl, 2006). Levels change in terms of location, pattern recognition and development of the defense response. The first level of recognition is established in the plasma membrane or apoplastic space, plants activate their resistance mechanisms against pathogens by recognizing pathogen-associated molecular patterns (PAMPs) through membrane receptors called ‘Pattern Recognition Receptors’ (PRR) (Jones et Takemoto, 2004; Jones et Dangl, 2006; Dodds et Rathjen, 2010; Claverie et al., 2016).This recognition leads to the activation of downstream signaling events such as oxidative explosion, nitric oxide production, callose deposition, which ultimately will trigger a defense response (Boller et al., 2009). The second level of recognition occurs in the cytoplasm through repeated leucine-rich receptors (NB-LRRs) at the nucleotide binding site that detect pathogen effector proteins (Jones et Dangl, 2006; Boller et al., 2009; Dodds et al., 2010).

Elicitors are those compounds recognized as PAMPS and these trigger the induction of the expression of genes involved in defense responses. Marine organisms can produce elicitors, such as polysaccharides and seaweeds that represent a promising resource of bioactive substances (Vera et al., 2011). For many years, beneficial effects of spraying seaweed extracts on crop plants have been observed, and there is an increasing interest in oligo- and polysaccharides in plant immunity. Sulfated polysaccharides have gained attention in crop protection for their roles as priming agents and elicitors that act as signaling molecules (Shukla et al., 2016; Shukla et al., 2019). Ulvan from green seaweed, laminarin isolated from brown algae as well as carrageenan from red seaweed, and their oligomers derivatives, are extensively studied due to their capacity to enhance various plants defenses (El Modafar et al., 2012; Abouraïch et al., 2015; de Freitas et al., 2015; Ben Salah et al., 2018; Aitouguinane et al., 2020; Shulka et al., 2021).

In this context, the present work aim is to investigate the use of polysaccharides extracted from Moroccan seaweeds as elicitors in the control of B. cinerea in tomato. In the first experiment, we investigated the ability of 8 Polysaccharides Enriched Extracts (PEEs) at different concentrations to induce the phenylpropanoid defense pathway, in particular, phenylalanine ammonia-lyase (PAL) activity in the leaves of tomato seedlings in the absence of the pathogen. In the second part, we

tested the effectiveness of the selected 8 PEEs at adequate concentrations on the reduction of the severity of gray rot disease induced by the pathogen B. cinerea using detached leaves of tomato plants.

MATERIALS AND METHODS

Preparation of PEEs

Eight Polysaccharides Enriched Extracts (PEEs) were screened from previous studies for their common beneficial effect on plant growth (Mzibra et al., 2020). These polysaccharides were extracted from 2 green seaweeds (Codium tomentosum and Ulva rigida), 2 brown seaweeds (Bifurcaria bifurcata and Fucus spiralis) and 4 red seaweeds (Chondracanthus acicularis, Gelidium crinale, Gigartina pistillata and Schizymenia dubyi). All these species of seaweed were collected from Moroccan seashore during August 2016 (Table 1) and Polysaccharide's extraction had been carried out using the conventional hot water method under neutral conditions, then precipitated with ethanol, as previously described (Mzibra et al., 2018).

Table 1: Collection site locations of the 8 studied seaweeds

Table 1: Collection site locations of the 8 studied seaweeds

Seaweed	Location	GPS Point	Date of Collection
Chlorophyta			
Codium tomentosum	Guy Ville—El Harhoura	33°56′11′′N, 6°56′45′′W	3 August 2016
Ulva rigida	Ouled Hmimoun—Mohammedia	33°40′34′′ N, 7°26′55 ′′W	3 August 2016
Phaeophyta			
Bifurcaria bifurcata	Sidi Bouzid	33°13′2952′′ N, 8°33′19 ′′W	17 August 2016
Fucus spiralis	Sidi Daoui	33°15′35,11′′ N,8°30′8,75 ′′W	17 August 2016
Rhodophyta			
Chondracanthus acicularis	El Jadida beach	33°14′564′′ N, 8°29′41 ′′W	17 August 2016
Gelidium crinale	El Jadida beach	33°14′564′′ N, 8°29′41 ′′W	17 August 2016
Gigartina pistillata	Skhirat beach	33°52'10''N, 7°3'32''W	03August 2016
Schizymenia dubyi	Ain Sebaa—Casablanca	33°37′14′′ N, 7°32′9 ′′W	3 August 2016

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Characterization of extracted Polysaccharides by Fourier transform infrared spectroscopy

The ATR-FTIR spectra of the polysaccharide was measured in the range of 400 - 4000 cm−1 using a Perkin Elmer FT-IR Spectrometer Spectrum Two Universal ATR. Each spectrum was obtained by adding 16 consecutive scans with a resolution of 4 cm−1. The FTIR spectra were applied to highlight the presence of some characteristic vibration bands related to the organic functional groups of the polysaccharide material.

Plant treatment for detection of enzyme activity and phenylpropanoid compounds

Plant culture and treatment

Tomato plants were cultivated for 4 weeks under controlled conditions (26 °C, photoperiod of 16:8 h at an irradiance of 240 µmol photons m−2 s−1), and treated one time with 100 ml of distilled water (control), or sterile PEEs solutions at 4 concentrations (0.02; 0.05; 0.1 and 0.2 mg mL-1). Three application methods were used for each treatment of PEEs, T1: foliar spray, T2: root

irrigation, and T3: Foliar spray + root irrigation. In the spray application, plants were sprayed in the upper and lower faces of all leaves. 24 hours after the treatment, leaves were collected from the lower, middle and upper parts of each plant, pooled and frozen in liquid nitrogen for enzymatic analyzes. Five replications of plants were tested for each treatment of PEEs.

Preparation of protein extracts

Protein extraction was done according to the method described by El Modafar et al., (2001). Tomato leaves (100 mg) were milled in 1 ml extraction buffer: 100 mM Tris-HCl pH 8.5 containing 14 mM β-mercaptoethanol (w/v). The homogenate was then centrifuged at 13.000 g for 30 min and the supernatant constitutes the enzymatic extract. Protein concentration was determined using bovine serum albumin (BSA) as a standard, according to Bradford (1976). Samples were incubated at room temperature for 30 min, then the absorbance was measured at 595 nm.

Determination of Phenylalanine Ammonia-lyase (PAL) activity

PAL activity was determined according to the technique described by El Modafar et al., (2001), with some modifications. The reaction mixture consisted of 100 µl of enzymatic extract, 1 ml of 100 mM Tris-HCl buffer, pH 8.5, and 200 µl of 100 mM L-phenylalanine. After incubation at 40 °C for 60 minutes, the reaction was stopped by addition of 250 µl of 5N HCl. The formed cinnamic acid was extracted twice by 2 ml of diethyl ether. After evaporation of the ether, the residue was suspended in 500 µl of methanol and the absorbance was determined at 290 nm. In the same way, a standard curve was made with cinnamic acid under the same experimental conditions.

Plant treatment and disease evaluation

The causal pathogen of Botrytis cinerea

The virulence of the isolated strain of B. cinerea was studied according to the method of Wang et al. (1986), by inoculation of 2 months aged leaves detached from tomato plants. The leaves were placed in Petri dishes containing tissue filter papers moistened with sterile distilled water to maintain a humid atmosphere. A 5 mm mycelial explant from an actively growing colony of B. cinerea on PDA was placed centrally on the upper surface of each leaf and incubated in a humid chamber at 23 °C until sporulation.

Effect of PEEs treatment on gray mold on detached tomato leaves

Evaluation of the effect of PEFs on the severity of B. cinerea was studied on detached leaves from tomato plants aged 2 months according to the technique of Aziz et al. (2003). Each leaf was soaked in 10 mL of PEEs solutions at different concentrations (0.02; 0.05; 0.1 and 0.2 mg. mL-1) or sterile distilled water for control leaves, and put in Petri dishes containing wet filter papers at the rate of 3 leaves per dish. After 24 hours, B. cinerea infection was carried out on each leaf by depositing 5 µL of conidia suspension prepared beforehand, then the leaves were incubated at 23 °C. Spore suspension was prepared by adding 10 ml of sterile distilled water containing 0.05% (w / v) Tween 80 on a 10-days old B. cinerea culture and the suspension was then collected in a sterile tube and adjusted to a concentration of 5 x 105 conidia mL-1 using a Malassez cell. Quantification of disease development was measured as the average diameter (two perpendicular diameters) of lesions formed after 7 days of infection.

Statistical analysis

For protein and PAL content, comparison of multiple means was determined by analysis of variance ANOVA using the LSD test of IBM SPSS statistics 22 (SPSS InC., Chicago, IL, USA). Comparison of diameter lesions on detached leaves and the principal component analysis (PCA) of the correlation table obtained between variables including PEEs, Protein content and PAL activity were carried out

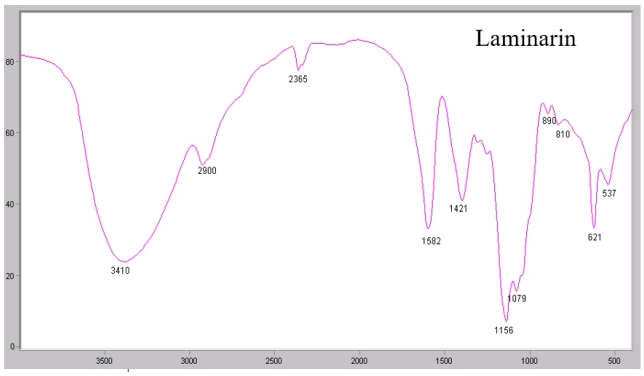
using R software version 3.5, package: MixOmics, FactoMineR, Factoextra, and FactoMineR.

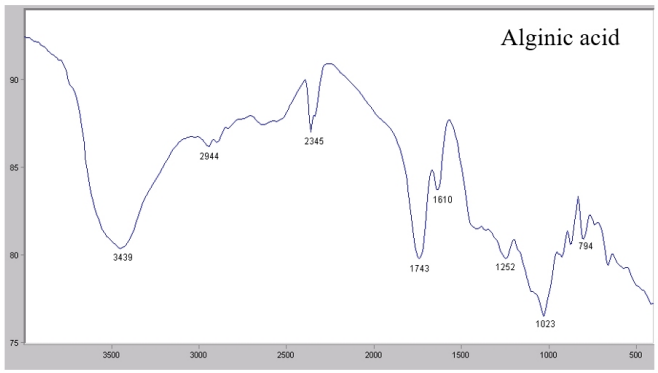
RESULTS

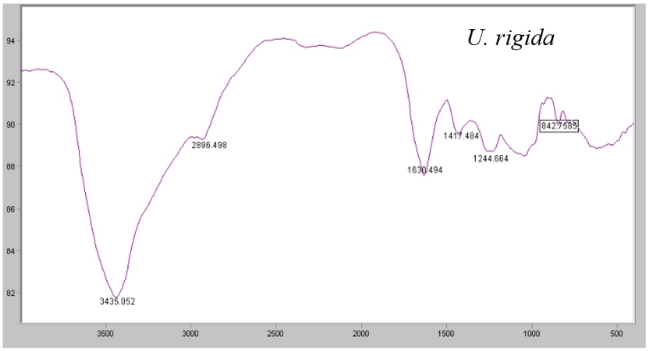
FTIR Analysis

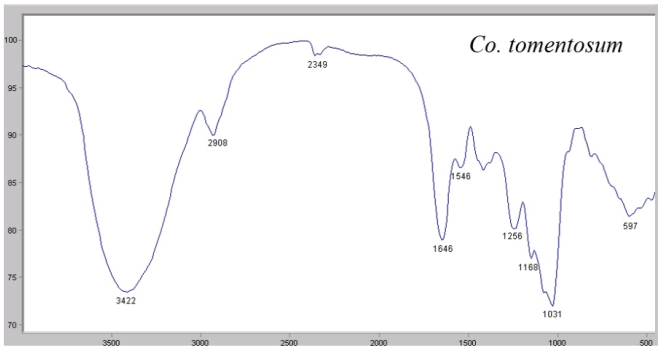
FTIR spectroscopy is one of the important analytical techniques widely used to study molecular structures and conformations of macromolecules to identify vibrations between different atoms in molecules. The spectrum obtained between 400 and 4000 cm-1 could be used to analyze the structural characteristics of polysaccharides, including glucosidic bonds and functional groups (Chen et al., 2021).

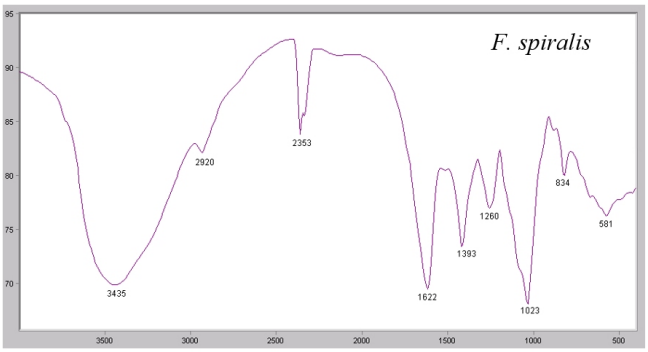
The FTIR spectra of polysaccharide standards (alginic acid and laminarin) and the eight seaweed polysaccharides in the range 350 - 4000 cm-1 are presented in figure 1. Each peak was assigned a functional group. Common to all polysaccharide standards and seaweed samples, two bands appeared in the 4000-2000 cm-1 region of the FTIR spectra: a broad band centred between 3500-3200 cm-1 assigned to hydrogen bonded O-H stretching vibrations and a weak signal at 2900-2800 cm-1 due to C-H stretching vibrations (Fernando et al., 2017). In addition, the medium to strong IR absorption bands at 1700-1600 cm-1 due to the carboxylate O-C-O bond stretching vibrations (Fernando et al., 2017) which is within the spectral range for uronic acid (Elnahas et al., 2017), this band was also common for all polysaccharides.

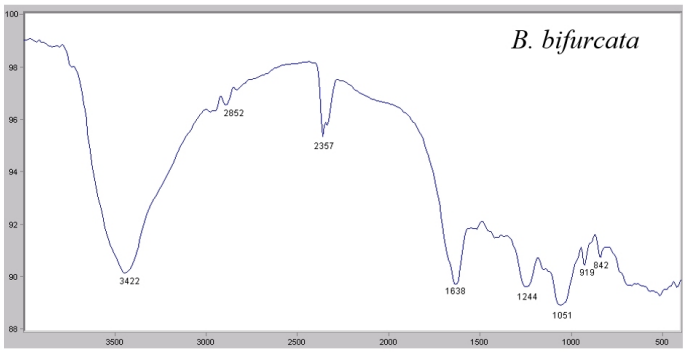

Laminarin
3410
2900
2365
1582
1421
1079
1156
890
810
621
537

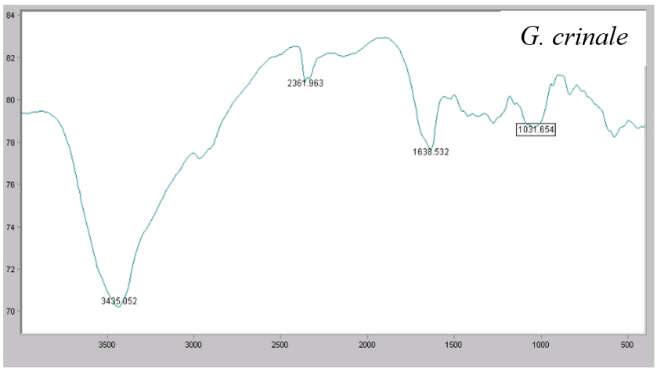

Alginic acid
3439
2944
2345
1743
1610
1252
1023
794

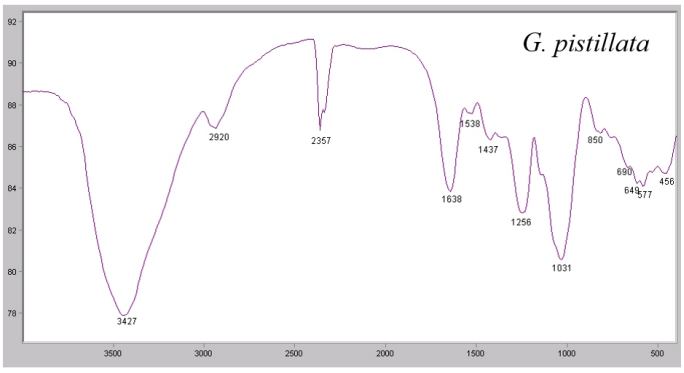

U. rigida
3435.052
2896.498
1630.494
1411.484
1244.664
842.585

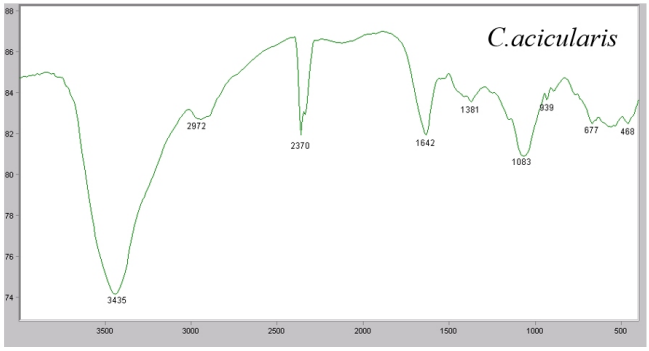

Co. tomentosum
3422
2908
2349
1646
1546
1256
1168
1031
597


F. spiralis
3435
2920
2353
1622
1393
1260
1023
834
581


B. bifurcata
3422
2852
2357
1638
1244
1051
919
842


G. crinale
3435.052
2361.963
1638.532
1031.654


G. pistillata
3427
2920
2357
1638
1538
1437
1256
1031
850
690
649
577
456


C.acicularis
3435
2972
2370
1642
1381
1083
939
677
468

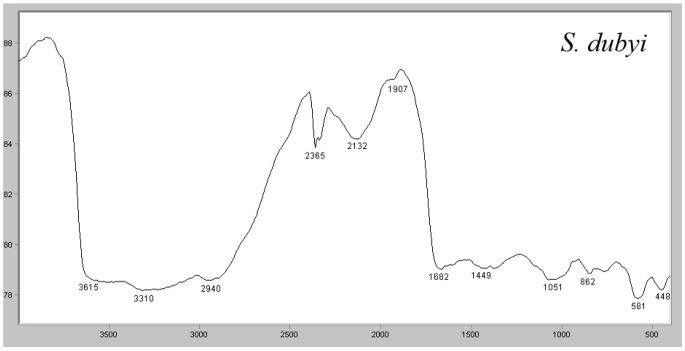

S. dubyi
3615
3310
2940
2365
2132
1907
1682
1449
1051
862
581
448

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Figure 1: FTIR spectrum of extracts of the seaweed species obtained from green seaweed Codium tomentosum, Ulva rigida, red seaweeds: Bifurcaria bifurcata and Fucus spiralis and brown seaweeds: Chondracanthus acicularis, Gelidium crinale, Gigartina pistillata, Schizymenia dubyi

The broadened peak between 1280-1120 cm-1 indicates the existence of a significant amount of sulfate in the polysaccharides (S=O stretching) (Gómez-Ordóñez et al., 2011; Fernando et al., 2017; Chen et al., 2021), this intense absorption in this spectral region was commonly observed in extracts obtained from U. rigida, Co. tomentosum, F. spiralis, S. dubyi, B. bifurcata and alginic acid as a standard. The IR band between 890-800 cm-1, shows the C-O-S bonding vibration and further confirms the presence of a sulfate group (Gómez-Ordóñez et al., 2011; Fernando et al., 2017; Cardoso et al., 2019), it was present in PEEs of U. rigida, F. spiralis, G. pistillata, S. dubyi, B. bifurcata and Laminarin as a standard.

PEEs effect on protein content and PAL activity

The effect of Polysaccharides Enriched Extracts (PEEs) on plant proteins content was presented in figure 2. The significant increase was shown in the 3 modes of application of PEEs obtained from U. rigida applied at concentrations 0.05 and 0.1 mg. mL-1 (Figure 2-A), B. bifurcata (0.1 and 0.2 mg. mL-1), F. spiralis (0.02 mg. mL-1) (Figure 2-B) and G. crinale (0.02; 0.05 and 0.1 mg. mL-1) (Figure 2-C). However, PEEs obtained from Co. tomentosum and S. dubyi affected proteins content by all concentrations used in this assay (0.02; 0.05; 0.1 and 0.2 mg. mL-1). Furthermore, the differentiation in application mode, particularly on some parts of tomato plant, improved significantly protein contents, as for the root irrigation of PEEs obtained from U. rigida at 0.02 mg. mL-1 (Figure 2-A), F. spiralis (0.05 and 0.1 mg. mL-1) (Figure 2-B), G. pistillata and G. crinale at 0.2 mg. mL-1 (Figure 2-C). Also, foliar application (T2) of PEEs obtained from U. rigida at 0.02 mg. mL-1 (Figure 2-A), G. crinale at 0.2 mg. mL-1 and C. acicularis at 0.05 mg. mL-1 (Figure 2-C) induced a significant increase in protein content of tomato plants.

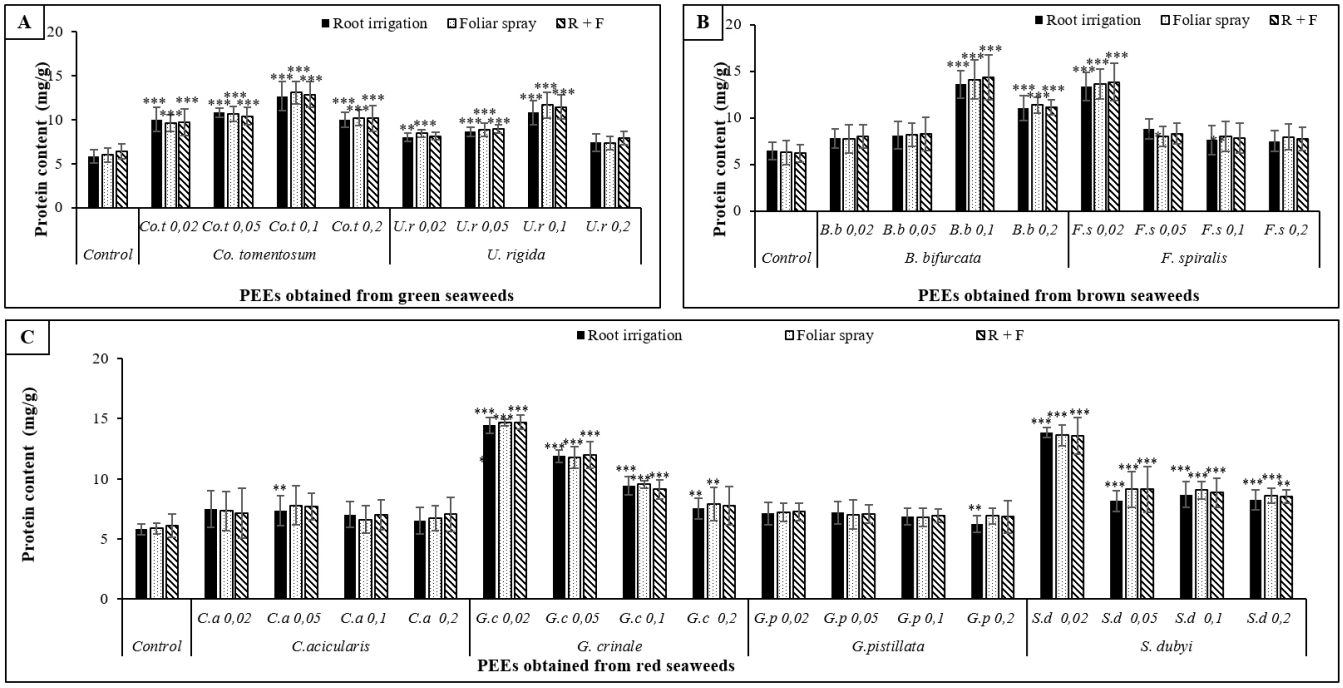

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Figure 2: Protein content in control tomato plants and in plants treated with 8 Polysaccharides Enriched Extracts (PEEs) obtained from green seaweed (A): Codium tomentosum, Ulva rigida,

brown seaweeds (B): Bifurcaria bifurcata and Fucus spiralis and red seaweeds (C): Chondracanthus acicularis, Gelidium crinale, Gigartina pistillata, Schizymenia dubyi, at 4 concentrations (0.02, 0.05, 0.1 and 0.2 mg mL-1). Protein content is expressed as micrograms of protein per per 1 g of leaves fresh weight. Data are expressed as mean ± SE of three replicates. Bars indicate the standard errors. Significant difference compared to the control treatment by ANOVA analysis ($p \leq 0.05$) ***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$

Regarding the effect of PEEs biostimulants on plant PAL activity, the results illustrated in figure 3 showed a significant increase in PAL activity in plants treated with the three application methods of PEEs obtained from Co. tomentosum at 0,2 and 0,1 mg. mL-1, U. rigida at 0,1 mg. mL-1, B. bifurcata at 0.05; 0.1 and 0.2 mg. mL-1, F. spiralis at 0.02 and 0.05 mg. mL-1, G. crinale at 0.02 and 0.05 mg. mL-1 and S. dubyi at all concentrations. In addition, this significant increase was also shown by the root irrigation by PEEs obtained from B. bifurcata, F. spiralis and C. acicularis at 0.1, 0.02 and 0.05 mg. mL-1, respectively. However, PAL activity was significantly stimulated with the foliar application of PEEs obtained from

Co. tomentosum and U. rigida at 0.05 mg. mL-1, B. bifurcata at 0.02 mg. mL-1 and F. spiralis at 0.1 mg. mL-1.

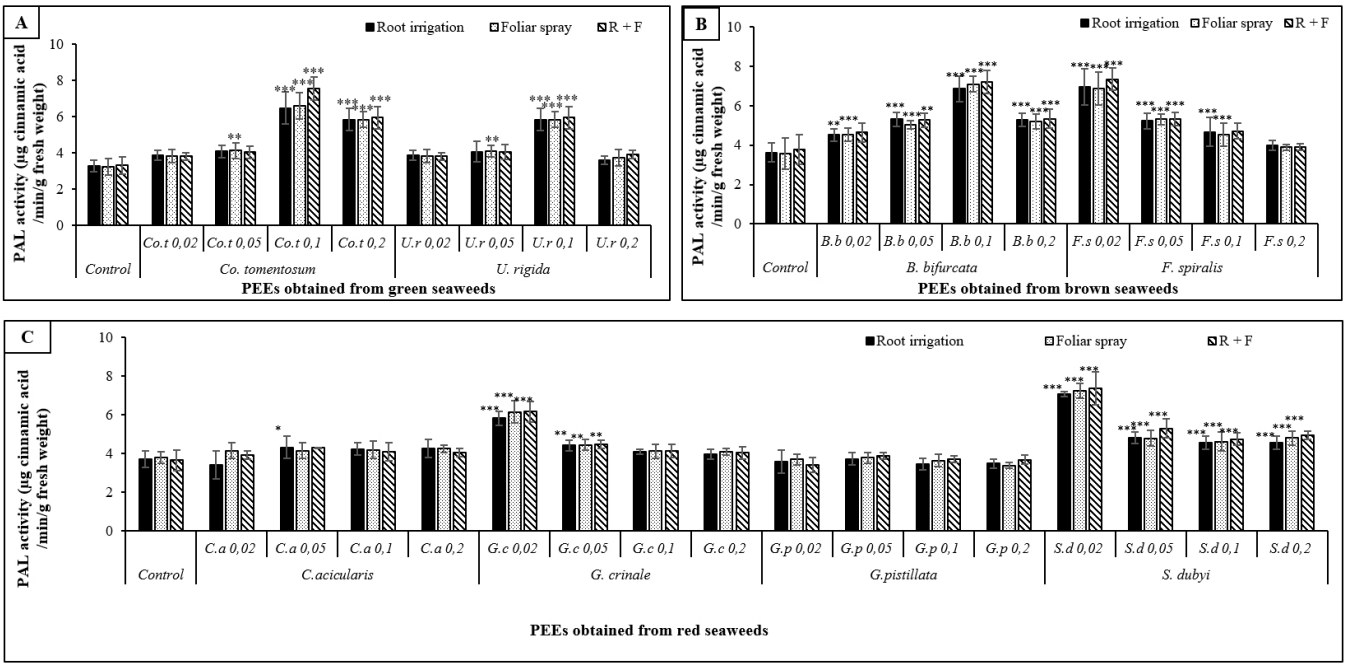

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Figure 3: PAL activity in control tomato plants and in plants treated with 8 Polysaccharides Enriched Extracts (PEEs) obtained from (A): Codium tomentosum, Ulva rigida, brown seaweeds (B): Bifurcaria bifurcata and Fucus spiralis and red seaweeds (C): Chondracanthus acicularis, Gelidium crinale, Gigartina pistillata, Schizymenia dubyi, at 4 concentrations (0.02, 0.05, 0.1 and 0.2 mg. mL-1). PAL activity is expressed as micrograms of cinnamic acid per minutes per 1 g of leaves fresh weight. Data are expressed as mean ± SE of three replicates. Bars indicate the standard errors. Significant difference compared to the control treatment by ANOVA analysis ($p \leq 0.05$) ***$p \leq 0.001$; ** $p \leq 0.01$; *$p \leq 0.05$

To conclude, the PEEs obtained from Co. tomentosum and U. rigida, and B. bifurcata at 0.1 mg. mL-1 and PEEs obtained from G. crinale, S. dubyi and F. spiralis at 0.02 mg. mL-1 were the best treatments inducing significant stimulation of protein content and significant elicitation of PAL activity in tomato plants with the tree application methods.

PEEs effect on gray mold on detached tomato leaves

As shown in figure 4 (A, B and C), the pathogenic effect caused by B. cinerea on detached tomato leaves were found in all treatments using different concentrations of PEEs (0.02; 0.05; 0.1 and 0.2 mg. mL-1). However, there is significant differences in the diameter lesions between leaves treated by PEEs and control leaves ($p<0.05$). Compared to control leaves, the inhibition effect on the gray mold was especially remarkable with 71, 6 and 77,0 %, using 0.1 mg. mL-1 of PEEs obtained from Co. tomentosum, U. rigida and B. bifurcata, respectively. Additionally, the smallest concentration of PEEs (0.02 mg. mL-1) obtained from G. crinale, G. pistillata, S. dubyi and F. spiralis had also decreased the lesion diameter by 75; 71; 71 and 76 %, respectively.

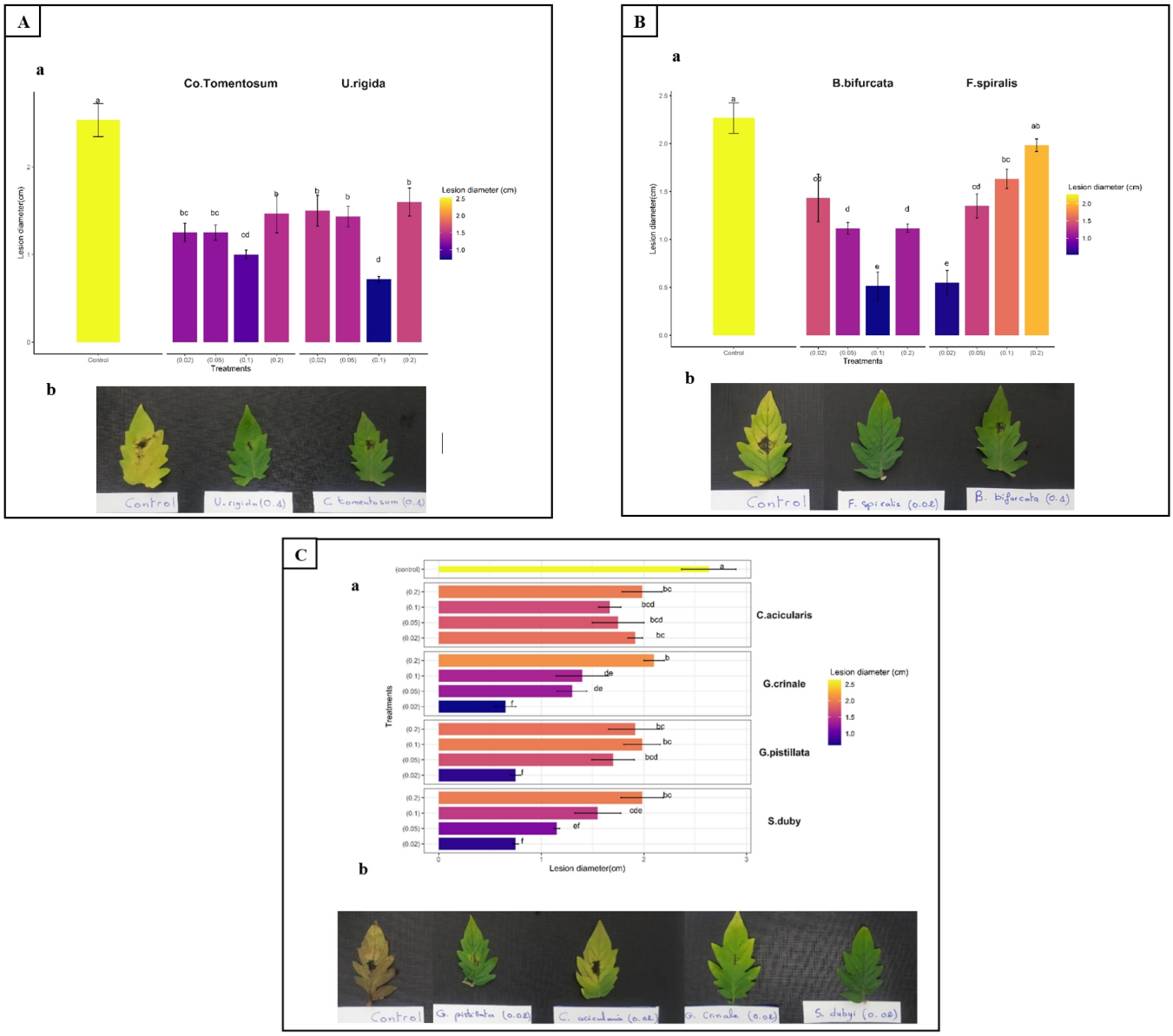

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Figure 4: The effect of Polysaccharides Enriched Extracts (PEEs) obtained from green seaweed (A) Codium tomentosum, Ulva rigida, red seaweeds (B): Bifurcaria bifurcata and Fucus spiralis and brown seaweeds (C): Chondracanthus acicularis, Gelidium crinale, Gigartina pistillata, Schizymenia dubyi on detached tomato leaves after 7 days infection by B. cinerea. a: The average lesion diameter (two perpendicular diameters) after 7 days of B. cinerea inoculation of detached tomato

leaves treated by PEEs at 4 concentrations (0.02, 0.05, 0.1 and 0.2 mg. mL-1). Data were obtained from three biological replicates. b: Lesion development of B. cinerea infection on tomato leaves treated by PEEs showing the significant inhibitory effect compared to control leaves

DISCUSSION

Research to reduce the application of chemical pesticides in agriculture through the discovery of new natural compounds is urgently needed in order to respond to the increasing demand for

pesticide-free food and to meet the needs of sustainable agriculture (Lykogianni et al., 2021). The use of algal polysaccharides in agriculture has been widely reported as elicitors of natural plant defense (Agarwal et al., 2021). In this study, we have studied the effect of eight polysaccharides extracts obtained from Moroccan seaweeds at four concentrations with three different applications methods on protein content and PAL activity in tomato plants. In the second assay, the eight PEEs were tested for inhibitory activity in tomato detached leaves against the aggressive plant pathogen Botrytis cinerea, which causes gray mold rot in fresh horticultural crops, resulting in heavy economical losses worldwide.

Our results showed that the PEEs obtained from Co. tomentosum, U. rigida, and B. bifurcata at 0.1 mg. mL-1 and G. crinale, S. dubyi and F. spiralis at 0.02 mg. mL-1 were the best treatments by significantly stimulating protein content and PAL activity in tomato plants with the three application methods (Figure 2 and Figure 3). The same extracts at the same concentrations, in addition of PEEs from G. pistillata at 0.02 mg. mL-1, were the treatments having the greatest inhibitory effect on the lesion diameter of B. cinerea in detached leaves when compared to the control (Figure 4). Our results corroborated those reported by Zhang et al., (2015), who reported that seaweeds algino-oligosaccharides could induce the enzyme activity of PAL in rice plants as defense against Magnaporthe grisea. Similarly, the treatment of olive and tomato plants by Ulvan extracted from Ulva lactuca significantly induced PAL activities against Verticillium wilt and lycopersicicum, respectively (Ben Salah et al., 2018). Likewise, polysaccharides extracted from microalga have the same effect. Rachidi et al, (2021) reported that The PAL activity have been improved in tomato plants leaves treated by polysaccharides extracted from several microalgae such as P. triocnutum, Desmodesmus sp., P. triocnutum and Porphyridium sp.

The analysis of the PEEs from the studied seaweeds was previously published and revealed the presence of high sugar content and sulfated groups in the extracts (Mzibra et al., 2018). Infra-red analysis revealed the footprint of some known polysaccharides extracted from the green, brown and red seaweed species with the presence of sulfated groups. FTIR analysis confirmed the presence of uronic acids in the composition of the eight polysaccharides. Molecules containing uronic acid and sulfate groups are known to trigger plant defense responses against pathogens (De Borba et al., 2021; Limaet al., 2021), this finding reinforces the proposition that the recognition of the seaweed PEEs by tomato plant may be associated with uronic acid residues and sulfate groups.

Such polysaccharides can be recognized by plant cells as PAMPs that activate their resistance mechanisms against pathogens. In this sense, Aitouguinane (2020) indicated that fucoidans isolated from Moroccan Bifurcaria bifurcata and Fucus spiralis induced natural defense in the roots of date palm by the significant increase in PAL activity, phenols and lignin in roots. Interestingly, this elicitor effect seems to be linked to the sulfated groups in the extracted polysaccharide.

Resistance reactions induced in tomato leaves as a result of PEEs applications could be explained by the induction of secondary metabolism PAL, which is considered as an important enzyme related to defense reactions in plants under stress and the key element in the lignification process that converts the phenylalanine to trans-cinnamic acid, the first step in the general phenylpropanoid way (Reichert et al., 2009). This enzyme is frequently studied in plant defense research known to correlate with the occurrence of induced resistance. Indeed, PAL activity is accompanied by the

biosynthesis of active metabolites such as Phytoalexins, lingnins, favonoids, phenolics compounds and salicylic acid leading to plant defense against microbial phytopathogens (Hsieh et al., 2010; Battacharyya et al., 2015).

The PCA represented in the figure 5 showed the correlation between PAL content and the reduction of lesion diameter. The only exception contradicting this explanation was shown with the G. pistillata extract, which significantly inhibited the lesion diameter of B. cinerea without stimulating the PAL activity. In our previous studies, the PEE of the red seaweed G. pistillata was the only extract which inhibited tomato seed germination (Mzibra et al., 2020). Accordingly, this may be explained by the fact that this extract contains some bioactive compounds which decrease the lesions of Botrytis without affecting PAL activity and needs further investigation.

Algae polysaccharides and oligo-saccharides are applied mainly by foliar spray, root soaking or drench and leaf infiltration (Shukla et al., 2021). The comparison of the application method in our study did not show differences as presented in figure 4. The best treatment at the identified concentration increased the PAL activity and reduced the lesion diameter of the fungal pathogen regardless the application mode.

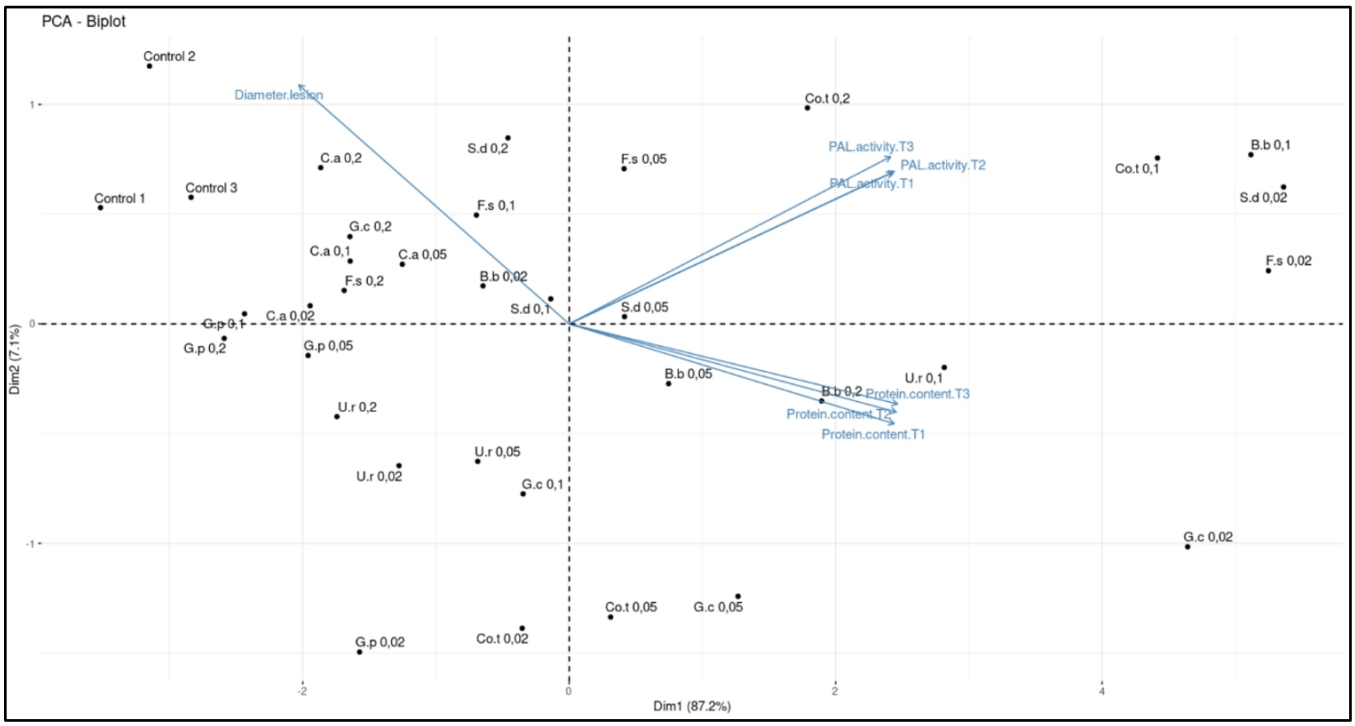

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Figure 5: Principal component analysis between the following variables: Protein content at the three application methods (T1, T2 and T3), PAL activity at the three application methods (T1, T2 and T3) and Diameter lesion of B. cinerea

CONCLUSION

The results from this showed that the application of PEEs obtained from Co. tomentosum, U. rigida, and B. bifurcata at 0.1 mg. mL-1 and G. crinale, S. dubyi and F. spiralis at 0.02 mg. mL-1 may be able to modulate antioxidant enzymes and activate the phenolic metabolism pathway leading to an effective control of gray mold in tomato plants. This study demonstrates that those algae polysaccharides can be an abundant, renewable and safety source of elicitors, which suggests that these treatments could partially replace the use of synthetic fungicides, until further studies to test

these natural algal polysaccharides on large-scale conditions are successfully conduced. The presence of uronic acid and sulfated groups in the extracts could explain the elicitation mechanism induced in plant cells.

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