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Fungal Growth Inhibition of *Bipolaris sorokiniana* Causal Agent of Wheat Diseases and Activating Defense Systems of Plants Using Algae Extracts

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ABSTRACT

The antifungal properties of extracts of algae were examined to evaluate their ability to protect wheat plants against *Bipolaris sorokiniana*. Our main objectives were to determine their effects on the *in vitro* growth of the pathogen and their aptitude for controlling Helminthosporium leaf blights and activating plant defense responses in the greenhouse. According to the screening carried out on the 23 species of algae, the extracts of the 4 algae (*Fucus spiralis*, *Laminaria Ochroleuca*, *Gracilaria* sp., and *Ellisolandia Elongata*) showed a very high inhibition of fungal growth *in vitro* of *B. sorokiniana*. In the greenhouse, they induced high protection against the fungal strain when applied twice by leaf sprays at 5g/L, 10g/L, and 20g/L, which reduced the alteration index of the leaves by about 75 %, and the stunting index was reduced in plants treated with the ESA. In addition, its protective capacity was associated with the accumulation of H_2O_2, as well as the potentiation of the activity of polyphenol oxidase and peroxidase. After the *in vitro* test of the fractions obtained from the Lancelot fractionation, it was concluded that the molecules responsible for the activity are located in the low molecular weight fraction. Algae extracts (macroalgae) sprayed on plants were reported to reduce the incidence of *Erysiphe polygon* (powdery mildew) on turnips and damping-off of tomato plants, *Botrytis cinerea* (gray rot) on plants of strawberries. These results demonstrated that the aqueous seaweed extracts (ASE) harvested from the coast of El Jadida (Sidi Bouzid) resulted in an improvement of the resistance in wheat plants by both inhibiting the growth of *Bipolaris sorokiniana* and activating plant defense responses.

INTRODUCTION

Wheat is one of the most important staple grains in the world and the main source of calories and vegetable protein in the human diet **(Curtis *et al.*, 2002)**. 735 million tons of wheat were produced worldwide in 2015/2016, which worth around 145 billion US dollars. The Food and Agriculture Organization of the United Nations, in a recent assessment of wheat production, indicates that the current supply of wheat is sufficient for world demand. Nonetheless, future production must increase as the estimated world population in 2050 is anticipated to exceed nine billion people. Thus, annual cereal production is expected to increase by nearly one billion tons. In addition, increased consumption of wheat products in

many countries, and changes in grain quality requirements require additional agricultural production **(Shewry, 2016; Anonymous, 2017)**.

The continued effort to match increases in yield and quality is not without challenges. Climate change, declining availability of suitable agricultural land and a variety of unpredictable abiotic and biotic stresses continue to pose threats to wheat production locally and globally. The search for elite, high-performing cultivars has contributed to a decline in the genetic diversity of wheat, which has led to a perfect storm in the emergence of pathogens to the point that diseases threaten the world's wheat supplies. Pathogenic fungi are a major constraint on wheat production; for instance, leaf blight caused by the fungus *Bipolaris sorokiniana* (Sacc.) Shoemaker which is one of the most important diseases of wheat (*Triticum aestivum* L.) in the world **(Kumar *et al.*, 2002)**. Several parts of the plant can be infected with this fungus, including culms, crowns, coleoptiles, roots and leaves **(Wiese, 1987)**. On the leaves, symptoms are small dark brown lesions that may expand into black oval spots **(Wiese, 1987; Cook & Veseth, 1991)**.

In Morocco, the final production of the three main cereals is estimated at 32 million quintals (17.7 million Qs of soft wheat; 7.9 million Qs of durum wheat and 6.4 million Qs of barley) for the 2019/2020 agricultural campaign, with a decrease of 57% compared to an average year under Plan Maroc Vert (75 million quintals) and a decrease of 39% compared to the season 2018/2019, which was an average year for the production of cereals (52 million quintals) (Ministry of Agriculture, 2020).

A very large amount of cereal is lost due to fungal diseases, among them diseases caused by the species *Bipolaris sorokinina*, which cause losses of 10 to 30% of cereal production **(Ezzahiri, 2001; Oslane *et al.*, 2014)**.

The main measure recommended for the control of diseases caused by *Bipolaris sorokiniana* was the spraying of chemical fungicides. However, the negative effects of these chemical products on the environment and human health have made the development of new alternative control methods a necessity. In this context, alternative management disease using natural compounds is highly fundamental.

Seaweed is an important source of a wide variety of natural products and could be a promising source of new bioactive compounds that can provide protection against these plant diseases. A number of studies have documented *in vitro* antimicrobial effects of algal extracts on plant pathogens **(Kumar *et al.*, 2008; Indira *et al.*, 2013)**.

In addition to the direct antimicrobial effect of algae extracts, evidence indicates that they may suppress the disease or reduce the incidence of the disease on plants *in vivo* **(Mattner, 2013; Mattner, 2014; Wite, 2015)**.

Morocco has a very high diversity of algae, comprising more than 450 species **(Benhissoune *et al.*, 2001; Benhissoune *et al.*, 2002a, b)** because of its long coastline (the Atlantic Ocean and the Mediterranean Sea). Taking this wealth into account, this work is part of the development of algal resources in Morocco. In this context, and trying to reduce chemical pesticides input to control phytopathogens in wheat, the present investigation was carried out to examine the effects of extracts of four algae (*Fucus spiralis*, *Laminaria ochroleuca*, *Gracilaria* sp. and *Ellisolandia elongata*) against Helminthosporium leaf blights (HLB) in wheat. Most published studies have investigated either the direct *in vitro*

antimicrobial role of algae extracts or their activity on plants, but rarely both. Through this work, algae extracts were tested for *in vitro* antimicrobial activities and their ability to protect greenhouse wheat seedlings. Analyses of the activities of two defense enzymes were conducted to determine whether these extracts are capable of stimulating resistance mechanisms in wheat.

MATERIALS AND METHODS

1. Algal materials, preparation of extracts, fungal material, antifungal activity and assessment of inhibition zone

Seaweed was harvested by hand in March/April 2016 on the El Jadida coast (33°33°16'09'N, 8°30' 8°45'W) (Fig. 1). After washing and cleaning, the algae were washed with distilled water, and then dried at room temperature and crushed into a fine powder.

The algal species under study were identified as: **Phaeophyceae:** *Cystoseira humilis, Bifurcaria bifurcata, Fucus spiralis, Sargassum muticum, Sargassum vulgaris, Laminaria ochroleuca*; **Rhodophyceae:** *Osmundea pinnatifida, Gelidium* sp.1, *Plocamium cartilagineum, Hypnea musciformis, Gelidium pulchellum, Ellisolandia elongata, Gracilaria* sp., *Corallina officinalis, Gelidium* sp.2, *Gracilaria cervicornis, Bornetia secundiflora, Halopitys incurvus, Gymnogongrus norvegicus* and **Chlorophyceae:** *Enteromorpha ramulosa, Ulva rigida, Ulva* sp. and *Codium elongatum.*

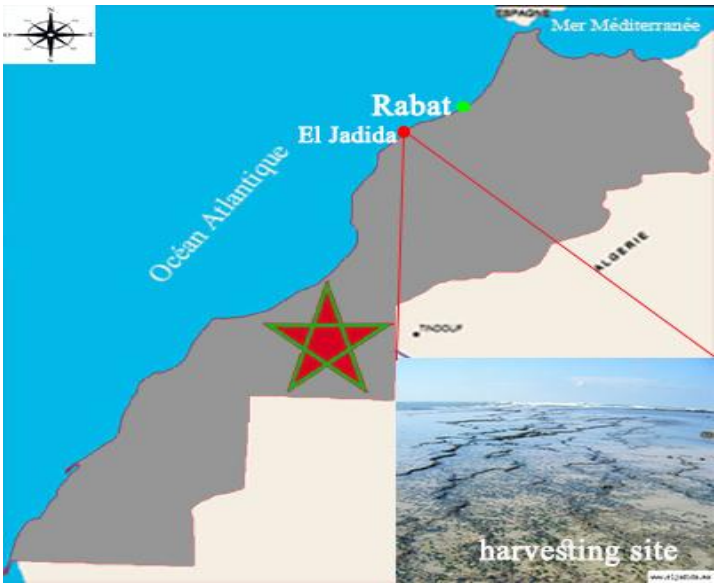


Fig. 1. Location of the collection site Sidi

The dried seaweed powders were extracted into different solvents, namely methanol, butanol, methanol/dichloromethane (50/50), dichloromethane and hexane, as described in the study of **Caccamese and Azzolina (1979)**. After the evaporation of the solvents in a rotary

evaporator under reduced pressure at 45°C, the raw extracts obtained were stored at 4°C until further use.

The strain used on this study (*Bipolaris sorokiniana*) was obtained from the laboratory of Phytopathology of the National Institute of Agronomic Research (INRA), Settat-Morocco.

The agar disc diffusion test was used to evaluate the antifungal activity of algal extracts. Three fungi colonies were removed with a metal loop from the original culture Petri dishes and were placed in a test tube containing 5ml of nutrient broth. A suspension of 10^6 spores per ml was obtained after an overnight culture (evaluated by the absorbance value of 0.5 at 620nm), the suspension was diluted 100 times, and the fungal density was adjusted to 0.2×10^4 spores per mL with sterile distilled water to inoculate Petri dishes containing culture media Mueller-Hinton. The organic extracts were tested using cellulose discs (diameter 6 mm), for which a quantity of 500µg of the algal extract (solubilized in 20 µL of suitable solvent) was deposited on the disc. After the evaporation of the solvent, these pellets were then applied directly in Petri dishes previously inoculated with the fungal strain *B. sorokiniana.* The fungi were incubated for 36 hours at 24°C. After that, the diameters of inhibitory zones were then measured. Discs impregnated with a standard antifungal (Amphotericine B (50µg/mL)) were used as reference, and discs impregnated with each solvent were used as control. All tests were performed in triplicate.

2. Plant material, effect of algae extracts on wheat seeds germination, elicitation, inoculation of wheat plants and determination of disease incidence and disease severity

Wheat (*Triticum durum*) seeds from the variety "KARIM" were disinfected with 5% commercial bleach for 4 minutes before rinsing thoroughly with sterile distilled water. Seedlings were cultivated in a greenhouse under a 12h photoperiod at 26°C and 70% relative humidity in black plastic poly pots (12.5 cm diameter and 17 cm length) containing a sterile combination of peat and sand (3:1).

In the greenhouse, wheat was used for Helminthosporium leaf blight biocontrol experiments. Wheat seeds were cleaned and separated into 100-seed batches. The control group consisted of seeds that were placed in Petri dishes with a paper filter wet in 4mL of water. The remaining batches were treated for 24 hours with 4mL of 5, 10, or 20g/L aqueous seaweed extract. Following that, all batches were watered every day for 7 days with 5mL of water. The percentage of germination was measured on the eighth day.

The plants were sprayed 28 days after planting with distilled water (DW) or seaweed extracts (5, 10 and 20 g of powder L^{-1}) until runoff. Plants were sprayed with DW or algae extracts again four days after the initial treatment (5, 10 and 20 g of powder L^{-1}). Three days later they were inoculated by the strain *Bipolaris sorokiniana* (10^7conidia mL^{-1}) or with DW (Mock inoculation). The treated plants were sprayed until runoff from the aerial part with the spore suspension. For control plants, samples were sprayed with sterile distilled water. All of the plants were placed in a three-block randomized block design, with one experimental unit

consisting of eight plants. The experiment was carried out three times, and the results of a typical experiments were recorded.

The quantitative and qualitative assessment of the disease was determined from 45 plants based on external and internal symptoms. Several parameters were taken into account to assess the degree of expression of the disease. The leaf alteration index (FAI) was measured at regular intervals for three weeks. It reflected the leaf damage on each plant. A grade (N) was assigned to each cotyledon and leaf according to the following scale: (0) absence of leaf symptoms; (1) yellowing or partial necrosis of the cotyledon; (2) cotyledon scar; (3) leaf yellowing; (4) wilting or necrosis of leaves; and (5) attributed to leaf scar **(Daayf *et al*., 1995)**. The FAI was then calculated for each inoculated plant using the formula FAI=((N)/(4 + 5n)), where n is the number of leaves on the plant; 5 is the maximum score for each leaf, and 4 is the maximum score for the cotyledons. The stunting index (SI) was calculated by measuring epicotyl elongation 3 weeks after inoculation using the following formula: $SI=((Epl_c-Epl_x)/Epl_c) \times 100$, where Epl_c and Epl_x represent the average epicotyl lengths for control plants and inoculated plants, respectively.

3. Biochemical assays/determination quantity of H_2O_2 and enzymatic activities

Algae extracts (5g/L, 10g/L, and 20 g/L) or DW were sprayed on 28-day-old wheat plants till runoff. Six days later, a second treatment with algal extracts or DW was added. Three days after, plants were challenged by leaf-inoculation with the strain *Bipolaris sorokiniana* (10^7 conidia mL^{-1}) or with DW (Mock inoculation). Plants that had been treated and inoculated were organized in a randomized three-block configuration, with each experimental unit containing three plants (three plants for enzymatic activities and the other three plants for H_2O_2 determination). Each sample is composed of a group of apical leaves taken from three distinct plants at 0, 3, 6, 9, 12, 15 and 18 days after the first treatment. The samples were frozen and kept at 80°C until needed.

The leaves collected at 0, 3, 6, 9, 12, 15 and 18 days after the first elicitation were used for hydrogen peroxide determination as described by **Alexieva *et al*. (2019)**. The leaves (200 mg) were ground in 3ml of 0.1 percent trichloroacetic acid and centrifuged at 5000g for 15 minutes. The supernatant was then mixed with 0.5mL of 10mM potassium phosphate buffer (pH 7) and 1mL of 1M potassium iodide. Via comparing the supernatant's absorbance at 390 nm to a standard calibration curve, the H_2O_2 content was determined.

According to **Moerschbacher *et al*. (1986)**, peroxidase (POX) activity was spectrophotometrically measured at 470nm and expressed as micromoles per minute per milligram of protein. The activity of polyphenol oxidase (PPO) was measured using the technique of **Masia *et al*. (1998)** and expressed a ΔDO per minute per milligram of protein.

RESULTS

1. *In vitro* antifungal activity of extracts of 23 algae

The results of the antifungal test of each extract (Methanol, Butanol, Dichloromethane/Methanol (50/50), Dichloromethane, and Hexane) against *Bipolaris sorokiniana* are summarized in Fig. (2).

With respect to the thirteen red algae tested, the majority of the species showed a positive activity against *Bipolaris sorokiniana* (Fig. 2a), an important activity was observed in the methanolic extract of *Gracilaria* sp. with a diameter of inhibition of 33,5 mm, followed by the dichloromethane extract of *Ellisolandia elongata* (26 mm), the hexane extract of *Gracilaria* sp. (23.5 mm), the methanol extract of *Gelidium* sp.2 and the dichloromethane / methanolic extract of *Gracilaria* sp. (23 mm). Significant activity against *Bipolaris sorokiniana* was obtained in the hexane extract of *Ellisolandia elongata*, the methanolic extract of *Gracilaria cervicornis* and *Halopitys incurvus*, the dichloromethane / methanolic extract of *Gelidium* sp.1 and the butanolic extract of *Bornitias ecundiflora* (Fig. 2a).

The same results were observed for the brown algae; the majority of species showed a positive activity against *Bipolaris sorokiniana*. An important activity was observed in the methanol extract of *Fucus spiralis* (29 mm), followed by dichloromethane/methanol extract of *Laminaria ochroleuca* (23 mm) and Butanol extract of *Fucus spiralis* (22,5 mm). Significant inhibition with diameter of inhibition higher than 20mm was observed in the dichloromethane and dichloromethane/methanol extract of *Fucus spiralis* whish is successively 20 and 20,5mm. compared to the antifungal control (Figure 2b).

Of the four green algae tested, the best activity against *Bipolaris sorokiniana* was recorded in the methanolic extract of *Enteromorpha ramulosa* and dichloromethane/methanol extract of *Ulva rigida* (20,5 mm), followed by the dichloromethane/methanol extract of *Enteromorpha ramulosa*, with a diameter of inhibition equals to 20mm (Fig. 2c).

Results make it possible to conclude that the majority of the brown algae represent a moderate activity (approximately 15mm), while only 2 species (*Fucus spiralis* and *Laminaria ochroloca*) represent an activity greater than or equal to 20mm in diameter; on the other hand, the majority of red algae (*Gelidium* sp., *Gelidium* sp2., *Gracilaria* sp., *Gracilaria cervicornis*, *Ellisolandia elongata* and *Halopitys incurvus*) has an antifungal activity greater than or equal to 20mm in at least one solvent used.

Fig (2) shows that the best solvents of extraction are the methanol and the mixture dichloromethane/methanol. This is why these two solvents were selected to carry out the extractions for the continuation of the work.

Effect of seaweed extracts on wheat seeds germination

The influence of aqueous seaweed extracts on germination parameters such epicotyl length, root length and germination rates were investigated (Table1). Germination rates were not significantly different between the control and ASE from the four algae when used at 5g/L, 10g/L or 20g/L. However, all treatments resulted in a 2.5-fold increase in root length. Following that, all treatments significantly improved the germination index, regardless of the aqueous seaweed extract and concentration utilized. These little changes; however, were not substantial. This result showed that seaweed extracts are not toxic to plants.

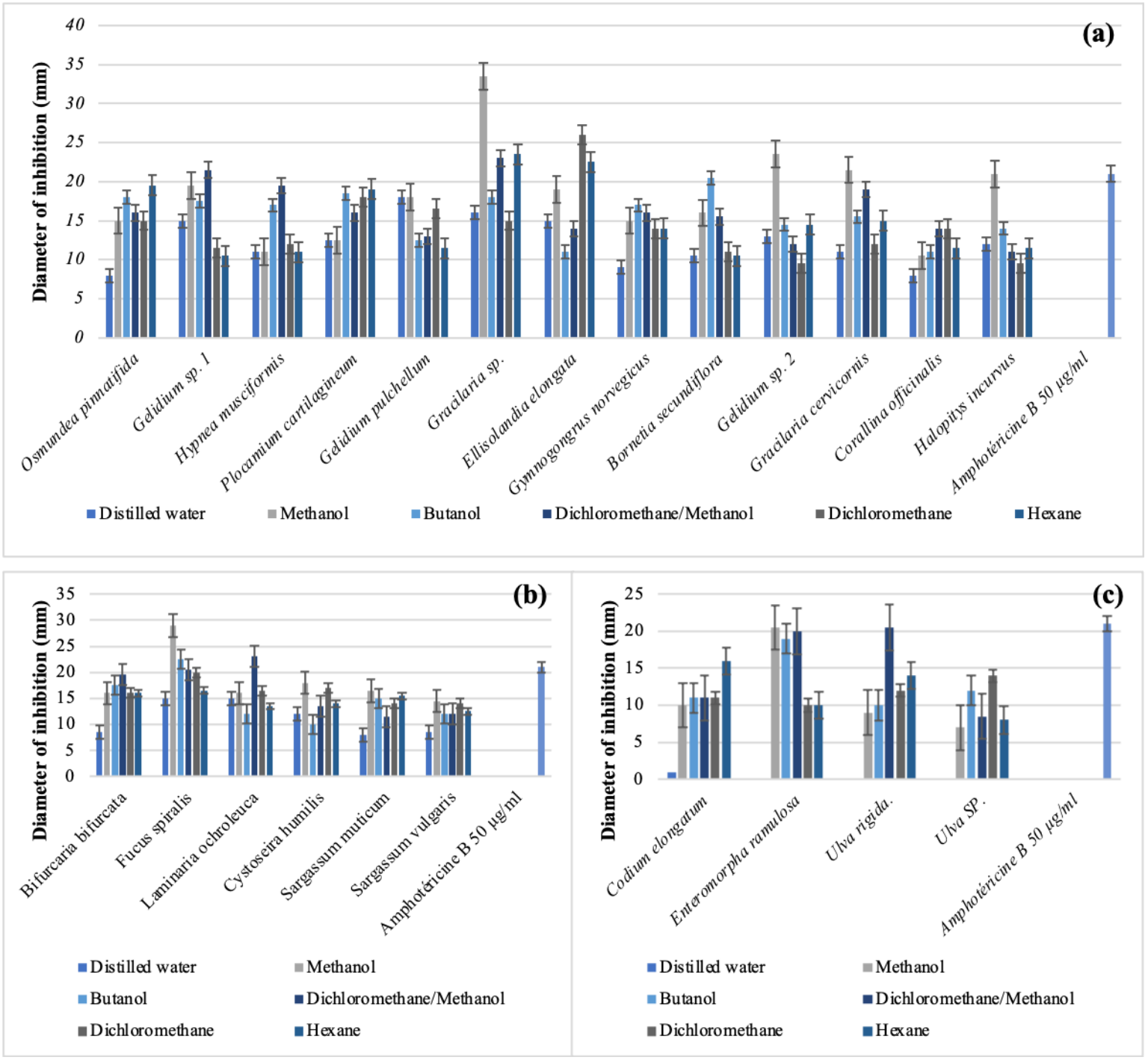


Fig. 2. Antifungal activity of (a) red seaweeds, (b) brown seaweeds and (c) green seaweeds extracts against *Bipolaris sorokiniana*

Table 1. Effect of seaweed extracts on germination parameters of wheat (*Triticum durum*) seeds soaked with distilled water (DW) or aqueous seaweed extracts (ASE) from *Fucus spiralis, Laminaria ochroleuca* and *Gracilaria* sp., or from *Ellisolandia elongata* at 5, 10 or 20 gram of powder per liter of water.

Algae	Concentration	Root length (cm)	Epicotyl length (cm)	Germination rates (%)
Fucus spiralis	5 g/L	14.017±1.02	12.888±0.60	97.78±2.25
	10 g/L	15.088±0.92	13.558±0.50	97.78±2.15
	20 g/L	13.688±1.1	13.558±0.50	93.33±5.6
Laminaria ochroleuca	5 g/L	11.533±0.84	13.938±0.60	93.33±4.55
	10 g/L	11.083±0.92	13.896±0.40	93.33±3.66
	20 g/L	9.679±0.76	12.854±0.30	100.00±0.00
Gracilaria sp.	5 g/L	14.221±0.96	13.104±0.40	100.00±0.00
	10 g/L	14.354±1.12	15.013±0.50	100.00±0.00
	20 g/L	13.958±0.87	14.096±0.45	91.11±5.67
Ellisolandia elongata	5 g/L	13.979±1.05	12.754±0.65	95.56±4.23
	10 g/L	13.313±0.98	13.271±0.50	100.00±0.00
	20 g/L	10.717±1.13	11.896±0.45	93.33±3.65
Control (DW)		10.645±0.50	12,25±0,25	95.56±3.35

The percentage of germination was recorded from 100 seeds. Data are means and standard deviations of three replicates.

2. Protective effects of ASE on wheat disease caused by Bipolaris sorokiniana.

In the greenhouse, ASE were tested for their potential to protect wheat seedlings against *B. sorokiniana*. "Spray treatment" is the technique employed. The disease was assessed based on leaf alteration index and the stunting index.

3 days before bipolaris inoculation, plants were sprayed twice with AES at 5, 10, and 20g/L 5 to test the suppressive effect of ASE (Fig. 3).

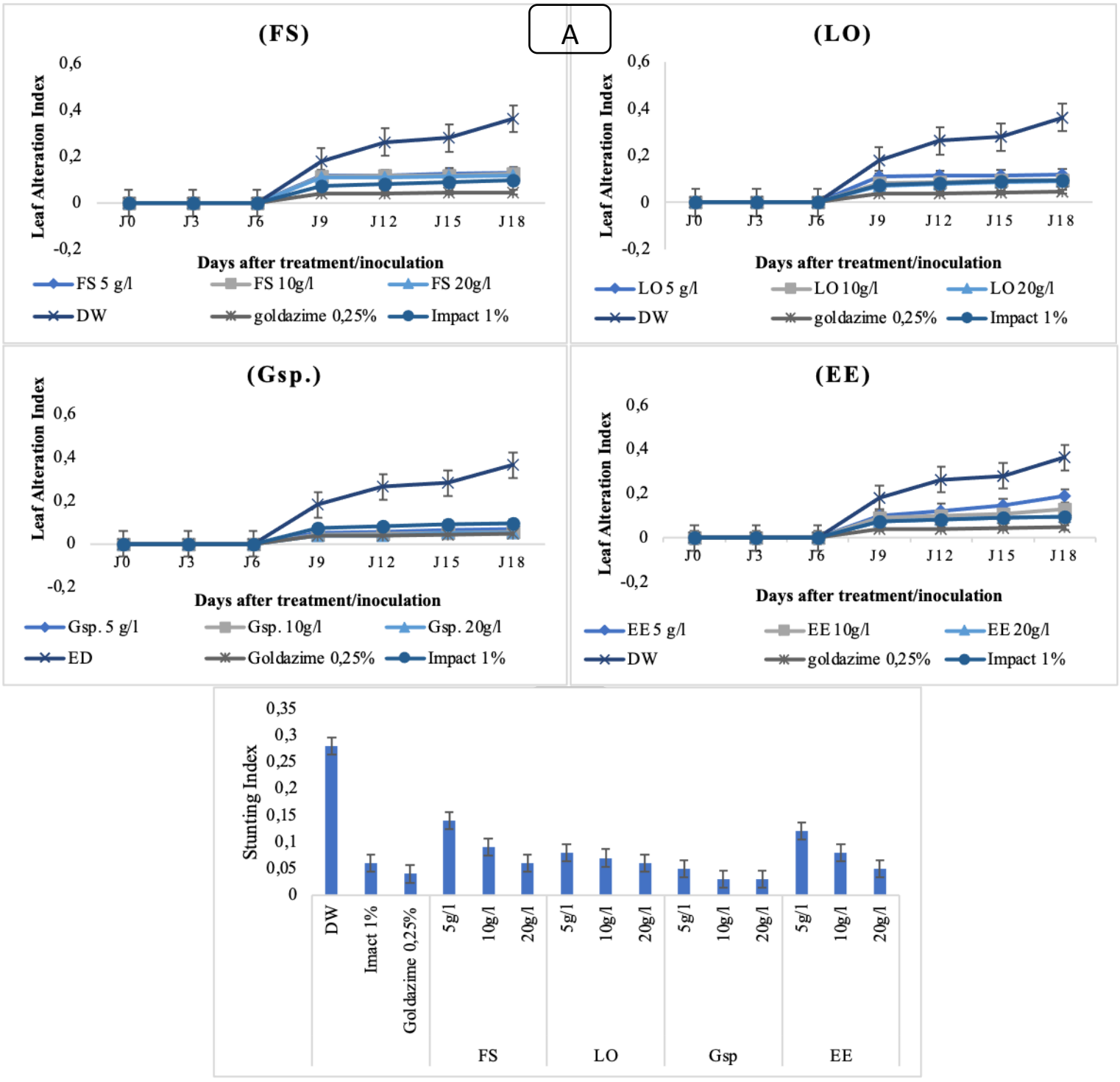


Fig. 3. The effect of treatment with ASE at 5g/L, 10g/L, or 20 g/L or distilled water on the incidence and severity of sickness caused by *Bipolaris sorokiniana* (control). Seedlings were treated twice on day 0 and day 6. On the seventh day, plants were inoculated with *Bipolaris sorokiniana* strain at 10^7 spore mL^{-1}. (A) Foliar alteration index and (B) stunting index were determined for 24 plants after treatment with extracts of (FS) *Fucus spiralis*, (LO) *Laminaria ochroleuca*, (Gsp) *Gracelaria* sp. and (EE) *Ellisolandia elongata*.
Values are means ± standard deviations.

From the 1st day of inoculation, the index of foliar alteration climbed steadily in control plants, reaching 0.46 after 18 days (Fig. 3A). Symptoms appeared three to four days after inoculation in plants treated with different doses of ASE, and the leaf alteration index was three to four times lower than that recorded in the control during 18 days following inoculation, resulting in a percentage of protection of roughly 75 percent.

At the end of the experiment, the stunting index was measured (Fig. 3B). Plants treated with DW witnessed a 28 percent decrease in growth induced by *B. sorokiniana.* This was decreased greatly in wheat plants treated with various doses of ASE, ranging from 3% with concentrations of 10g/L and 20g/L of *Gracilaria* sp. extract to 14% with a concentration of 5g/L of *Fucus spiralis* extract.

3. Activation of the oxidative burst by ASE

The kinetics of H_2O_2 generation were studied in order to see if the aqueous seaweed extract (ASE) may cause an oxidative burst (Fig. 4).

The effect of aqueous seaweed extracts on H_2O_2 accumulation is shown in Fig. (4). In inoculated and treated plants with the reference pesticides, 3 days after the first spray application of Impact at 1% or Goldazime at 0.25%, a transitory increase was detected. After a second treatment, another significant increase was observed on the 6th, 9th and 12th days. *Bipolaris sorokiniana* enhanced the H_2O_2 content slightly in the control treated with distilled water on day 9. However, inoculated plants pre-treated with 5 mg/mL, 10 mg/mL, or 20 mg/mL of aqueous seaweed extracts from *Fucus spiralis*, *Laminaria ochroleuca*, *Gracilaria* sp., or *Ellisolandia elongata* had significantly greater content. Likewise, *Bipolaris sorokiniana* inoculation enhanced H_2O_2 accumulation in plants pre-treated with 20mg/ mL aqueous seaweed extracts on study days 3, 6 and 9. In inoculated plants, considerable increase in H_2O_2 content was observed at the end of the experiment after treating with 20mg/ of mL of aqueous extracts from *Laminaria ochroleuca* or *Ellisolandia elongate*, compared to the control. In inoculated plants, a slight increase of H_2O_2 content was recorded on days 3, 6 and 9 after treatment with 5mg/ mL of different aqueous seaweed extracts and on day 12 after treatment with aqueous extracts of *Laminaria ochroleuca* (Fig. 4).

4. Potentiation of POX and PPO activities by ASE

Wheat plants were inoculated with 10^7 conidia.mL^{-1} of *Bipolaris sorokiniana* after being sprayed with the algal extracts or DW. The results are the means and standard deviations of three replicates.

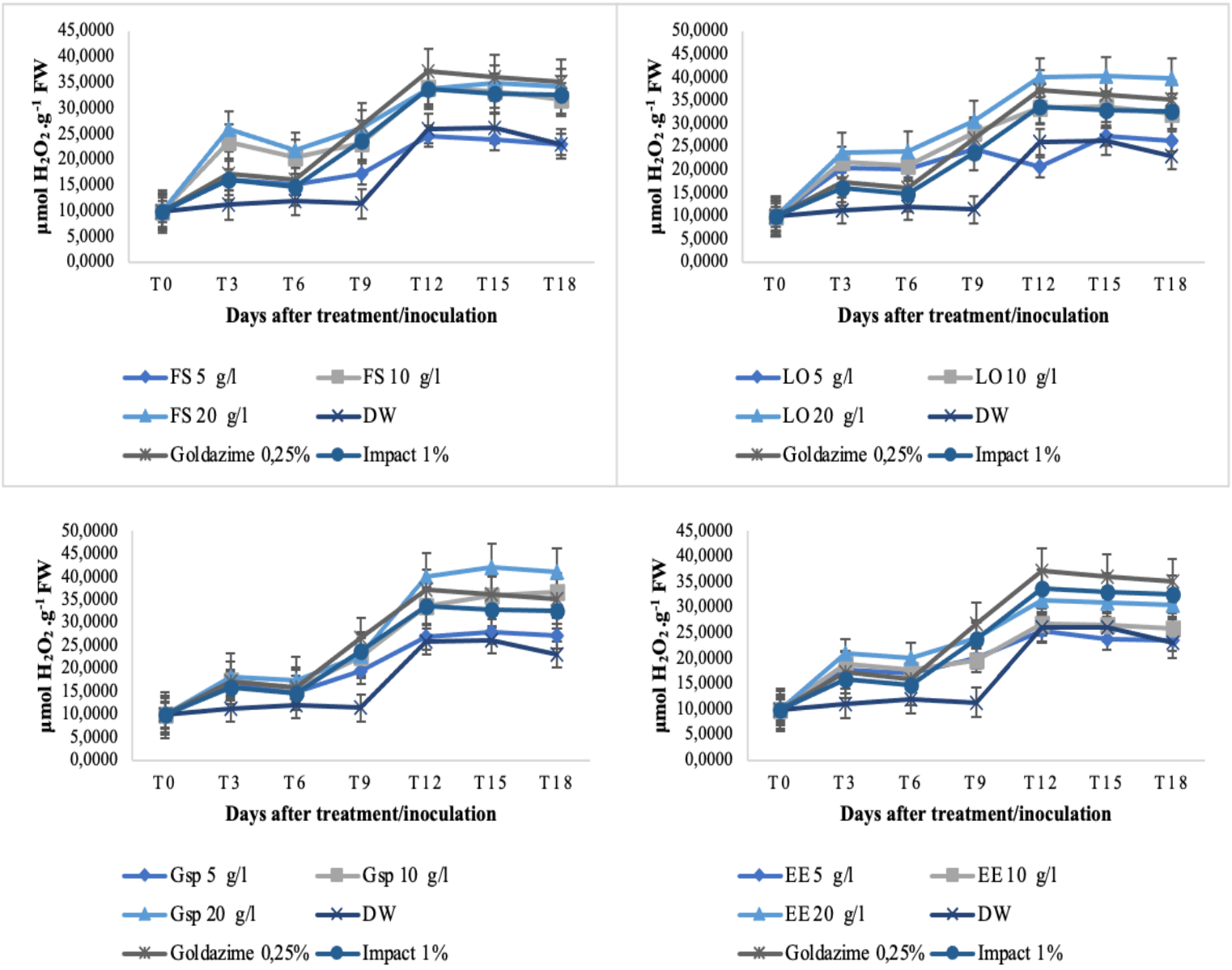


Fig. 4. Time course examination of H_2O_2 buildup in *Triticum aestivum* seedlings following spray treatment (Preventive application) with aqueous seaweed extracts from (a) *Fucus spiralis* (FS), (b) *Laminaria ochroleuca* (LO), (c) *Gracilaria* sp., (Gsp), or (d) *Ellisolandia elongata* (EE) at 5mg/ mL, 10mg/ mL or 20mg/ mL

The plants are inoculated by spraying the aerial part with a spore suspension of the strain *Bipolaris sorokiniana* previously adjusted to 10^8 conidia.mL−1. Control plants were infected with distilled water. Data are means ± standard deviations of three replicates.

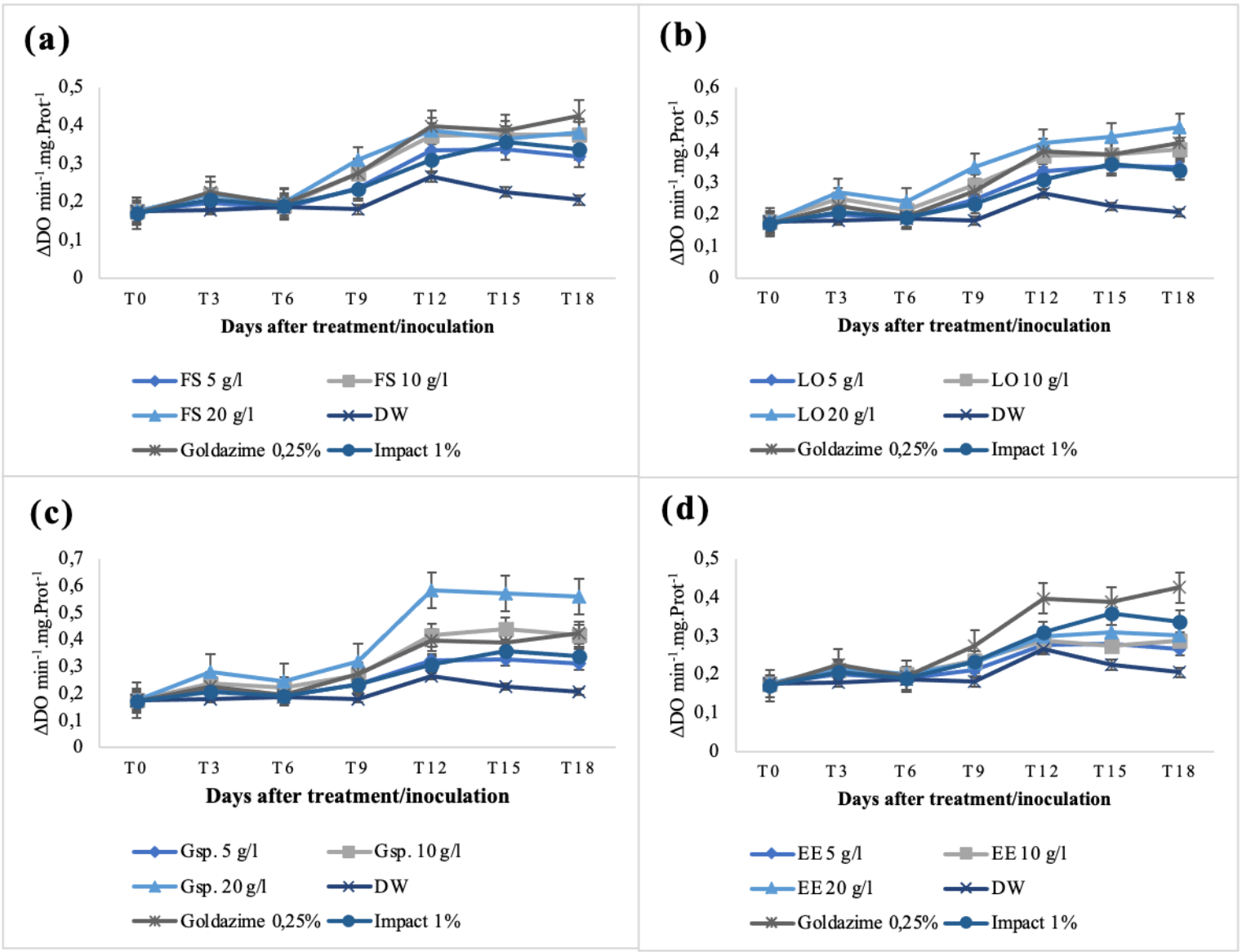
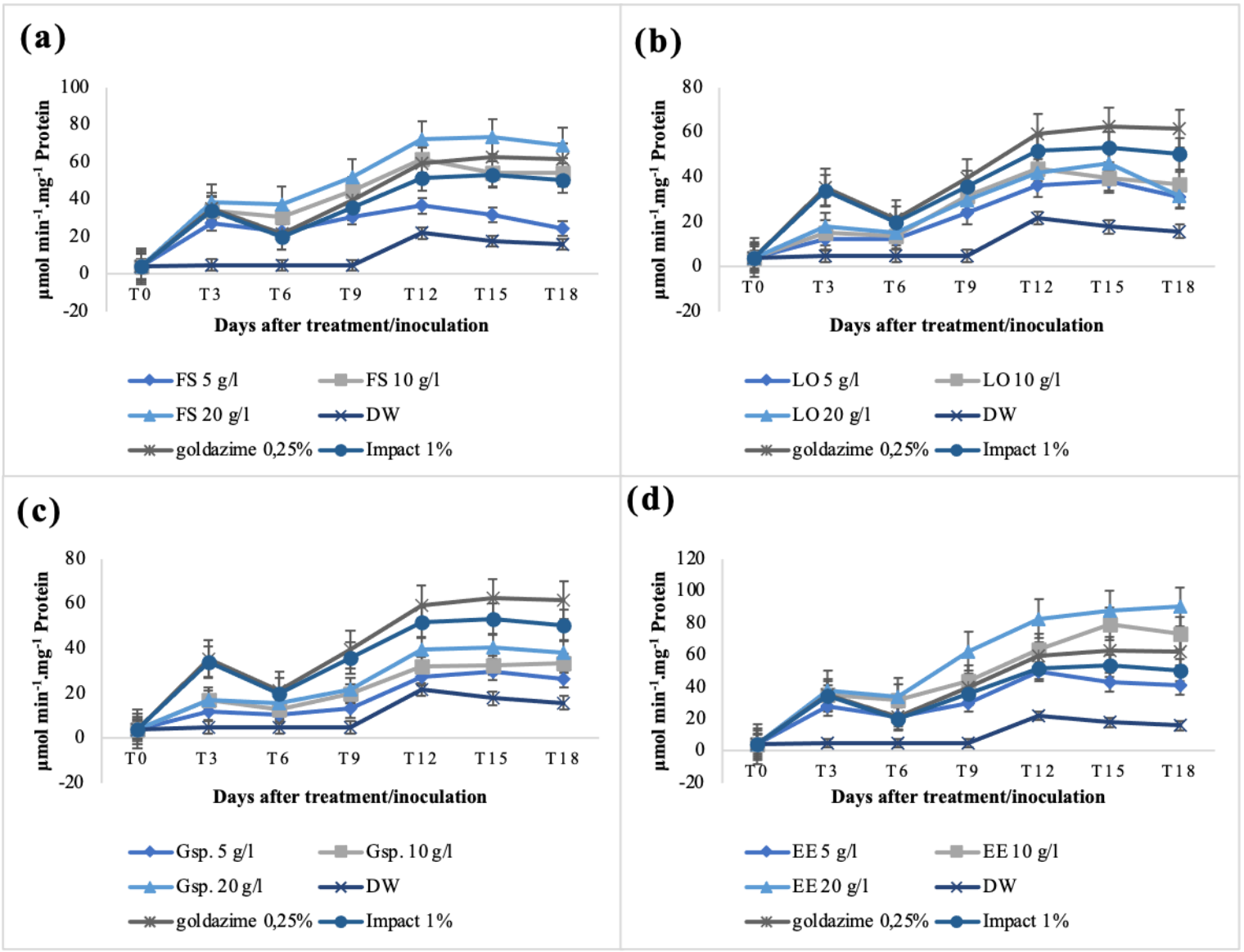


Fig. 5. polyphenol oxidase (PPO) activity in wheat plants sprayed with aqueous seaweed extract from (a) *Fucus spiralis* (FS), (b) *Laminaria ochroleuca* (LO), (c) *Gracilaria* sp. (Gsp), or (d) *Ellisolandia elongata* (EE) at 5mg/ mL, 10mg/ mL, or 20 mg/mL

A second plant defensive response marker was polyphenol oxidase activity. It was tested with wheat plants sprayed twice with aqueous seaweed extracts from *Fucus spiralis*, *Laminaria ochroleuca*, *Gracilaria* sp., *or Ellisolandia elongata* at 5 mg/mL, 10 mg/mL, or 20 mg/mL at 7 and 3 days before inoculation with *Bipolaris sorokiniana* (Figure 5). When compared to the inoculated control and pre-treated with benchmark pesticide at 4 days post-inoculation, *Bipolaris sorokiniana* caused a slight but significant increase in PPO activity in plants pre-treated with distilled water. These activities were highly enhanced in plants pre-treated with 5 mg/mL, 10 mg/mL or 20 mg/mL aqueous seaweed extracts on days 3 and 7 before inoculation. Elevated PPO activities around two times higher than in the control-treated with distilled water were recorded from mock-

inoculated plants pretreated with 20mg/ mL aqueous seaweed extracts of *Laminaria ochroleuca* and *Gracilaria* sp. on days 3 and 5 after treatment, respectively. The pathogen slightly activated PPO activity in the control on day 4. However, in plants pre-treated with aqueous seaweed extracts, increases were stronger. The maximum of activity



was reached within 5 days with 20 mg/mL of *Laminaria ochroleuca* extract (Fig. 5).

Fig. 6. Evolution of peroxidase activity in *Triticum aestivum* seedlings following spraying with aqueous seaweed extracts from (a) *Fucus spiralis* (FS), (b) *Laminaria ochroleuca* (LO), (c) *Gracilaria* sp., (Gsp), or (d) *Ellisolandia elongata* (EE) at 5 mg/mL, 10 mg/mL, or 20 mg/mL

Four-week-old seedlings were pre-treated with aqueous seaweed extracts or DW (control) on days 7 and 3 before inoculation. A spore solution of the *Bipolaris sorokiniana* strain, adjusted to 10^8 conidia m^{L1}, was used to inoculate the plants. Data are means and standard deviations of three replicates.

We also looked at the kinetics of peroxidase activation after inoculation with *Bipolaris sorokiniana* to see if aqueous seaweed extracts may stimulate plant defenses. Plants sprayed twice with 5, 10 or 20mg/ mL aqueous seaweed extracts on days 7 and 3 before *Bipolaris sorokiniana* inoculation were examined for time course investigation of peroxidase activity (Fig. 6). On day 4 following the initial spray treatment with distilled water, a modest increase in peroxidase activity was detected in inoculated and non-treated *Triticum aestivum* plants.

A significant increase in peroxidase activity was seen in pre-treated and inoculated plants, 1 day after the initial spray treatment with 5, 10 or 20mg/ mL of *Fucus spiralis* or *Ellisolandia elongata* extract (Fig. 6a, d). On the third day, a second spray treatment with 10 and 20mg/ ml of *Fucus spiralis* or *Ellisolandia elongata* extract increased significantly peroxidase activity. In plants pre-treated with *Fucus spiralis* extract, this activity diminished after the sixth day. In inoculated plants sprayed with *Laminaria ochroleuca* extract, a significant rise of peroxidase activity was observed in different concentrations on days 1 and 3 after treatment but less important in comparison with the controls (Fig. 6b). While, with *Gracilaria* sp. extracts, a substantial peroxidase activity was obtained on the fourth day following the first treatment but less important than the controls (Fig. 6c). Similarly to what was seen with other extracts, peroxidase activity was significantly enhanced in inoculated and pre-treated plants with control pesticides on day 1 after the first treatment. These results suggest that aqueous seaweed extracts stimulated plants for enhanced peroxidase activity.

1. **Localization of the molecules responsible for the antifungal activity**

The results show that the compounds active against the *Bipolaris sorokiniana* strain are localized in the fractions of the low molecular weight molecules as well as a polysaccharide fraction of the algae *Ellisolandia elongata.*

Table 2. Activity of fractions from fractionation of lancelot of four algae (*Fucus spiralis*, *Laminaria ochroleuca*, *Gracilaria* sp. and *Ellisolandia elongata*) on the development of the strain *Bipolaris sorokinianain in vitro*

	Diameter of inhibition (mm)			
Algae	Lipids	MLMW*	Polysaccharides	Proteins
Fucus spiralis	14±2,0	18±2,0	7±0,5	7±1,0
Laminaria ochroleuca	10±1,0	16±1,5	7±1,0	7±0,0
Gracilaria sp.	8±0,0	15±1,0	7±0,0	7±0,0
Ellisolandia elongata	10±1,5	22±2,0	15±1,5	11±1,0

*MLMW: Molecule of Low molecular Weight

DISCUSSION

The present findings agree with those of **Pesando and Garam (1994)** who found that, among the 12 species tested, those pertaining to the phaeophyceae family were the most active against *Bipolaris sorokiniana*, compared to Rhodophyceae and Chlorophyceae; the same result was reported in the studies of **Kumar *et al*. (2008)** and **Lakhdar *et al*. (2015)**.

Marine algae are a source of structurally distinct natural compounds with pharmacological and biological activity **(Schwartsmann *et al*., 2001)**. Seaweeds are high in polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids, which have a variety of biological activity **(Rodriguez-Bernaldo de Quiros *et al*., 2010; Priyadharshini et *al*., 2011)**. They exhibit varied antibacterial activity depending on their solubility and polarity in various solvents.

According to **Shanmughapriya *et al*. (2008)**, the extract from all species of Chlorophyta, Rhodophyta and Phaeophyta taxa were effective against the yeast *Candida albicans*. Previous studies **(Kosanic *et al*., 2015)** indicated that *Ulva lactuca* extract exhibited excellent antibacterial action, suppressing fungus even at low doses.

Pandian *et al*. (2011) disclosed that the methanolic extract of *Acanthaphora spicifera* showed very high antifungal and antibacterial activity, and methanolic extracts showed antifungal and antibacterial activity similar to that of Ciprofloxacin and Amphotericin, respectively. **Ballesteros *et al*. (1982)** recorded antifungal activity in extracts of the *Dictyota*, *Padina* and *Sargassum* algae species, as well as antimicrobial activity in methanolic extracts of 71 species of algae from the Mediterranean area.

Regarding our results on fungi species, the methanol extract of *Gracilaria* sp., *Fucus spiralis* and *Enteromorpha ramulosa*, dichloromethane/methanol extract of *Enteromorpha ramulosa* and *Ulva rigida* showed the best inhibition against *Bipolaris sorokiniana*. Antifungal action is likely owing to the polarity of these solvents and their constituents in varying concentrations, as shown in Fig. (2). **Tuney *et al*. (2006)** cited results that might be linked to the existence of ethanol-soluble bioactive metabolites, which could also have happened with the dichloromethane and methanol extracts of the algae studied in this investigation.

These differences in activity may be due to the sensitivity of the strains **(Perez *et al*., 1990)**, the efficiency of the extraction methods to recover the active metabolites, the seasonal variations (**Vidyavathi & Sridhar, 1991)** and the solvents used (**Tuney *et al*., 2006)**. For this, various solvents were applied in this study, with the aim of selecting the best solvent producing a maximum of bioactive compounds responsible for the antimicrobial activity.

In this study, we demonstrated the efficiency of aqueous seaweed extracts treatments from *Fucus spiralis*, *Laminaria ochroleuca*, *Gracilaria* sp. and *Ellisolandia elongata* in reducing Helminthosporium leaf blights disease. This is the first published study, to our knowledge, on the capacity of natural seaweed extracts directly reducing this disease on wheat plants. This effect seems to be tied from the direct antimicrobial activity since the

aqueous seaweed extracts used for protection experiments strongly inhibited the growth of *Bipolaris sorokiniana*.

Algae are high in bioactive compounds, including terpenes, phenolic compounds, and phenolic lipids, all of which have antibacterial properties **(Prabha *et al.*, 2013; Suleria et *al.*, 2015)**. Seaweed extracts are considered to be biostimulants; they are high in water-soluble such as sulphated fucans and laminarin **(Li *et al.*, 2008**; **Stadnik *et al.*, 2014)**. These polysaccharides act as elicitors of systemic defense in plants **(Klarzynski et *al.*, 2000; Mercier *et al.*, 2001)**.

In the present study, we showed that the two enzymes (polyphenol oxidase and peroxidase) that catalyze the synthesis of phenolic compounds involved in the construction of physical barriers against pathogens, were activated by aqueous extracts of algae **(Hiraga *et al.*, 2001; Mayer, 2002)**.

Interestingly, the enhancement of the activity of enzymes involved in plant defenses by seaweed extracts have been well described by several authors **(Zhang *et al.*, 2003; Aziz *et al.*, 2003 ; Raghavendra *et al.*, 2007 ; Jayaraj *et al.* 2008; Paulert et *al.*, 2010; Hernández-Herrera *et al.*, 2014; Abkhoo & Sabbagh, 2016; Abouraïcha *et al.*, 2016)**. This pretreatment with aqueous extracts of algae switches plants into an alarmed state of defense, allowing them to upgrade their defensive responses against diseases **(Conrath et *al.*, 2015)**.

Activation of PPO is involved in strengthening the cell wall and inhibiting pathogens. Its activation leads to quinones, known for their toxicity towards pathogens **(Mayer, 2006)**. Additionally, PPO activity may be related to the accumulation of reactive oxygen species (ROS) **(Mayer, 2002; Thipyapong *et al.*, 2004)** and the overall values of redox potential **(Webb *et al.*, 2014)**. Its presence could also be beneficial by downregulating photosynthesis **(Trebst & Depka, 1995)**. Therefore, the increased activity of PPO may contribute to the higher protection observed with aqueous extracts of algae.

Similarly, as compared to untreated controls, the aqueous seaweed extracts induced higher levels of accumulation of H_2O_2, which might explain their higher levels of protection against *Bipolaris sorokiniana*. Hydrogen peroxide serves as a signaling molecule in plants under stressful situations. However, for cell homeostasis, its buildup should be closely managed because larger levels are harmful to plants. Notably, numerous strategies via enzymatic and non-enzymatic pathways are implicated in order to avoid its harmful effects **(Foyer & Noctor, 2005)**. The most significant peroxidase in plants for detoxifying H_2O_2 to water is ascorbate peroxidase **(Foyer, 1996)**. In this investigation, we demonstrated that aqueous seaweed extracts significantly increased activity. Following inoculation with *Bipolaris sorokiniana*, these extracts were able to prepare wheat plants for increased POX activity, allowing them to improve their defense responses.

Several investigations have found that derived-elicitors of algal extracts generate reactive oxygen species and antioxidant defenses **(Aziz *et al.*, 2003; Zhang *et al.*, 2003; Jayaraj *et al.*, 2008; Paulert *et al.*, 2010; Abouraïcha *et al.*, 2016)**. However, H_2O_2 is engaged in the oxidation of phenolic compounds catalyzed by POX and PPO activities

involved in plant defense. It also plays a function in plant defense against pathogens through its direct antibacterial impact and in cellular signal transmission **(Lamb & Dixon, 1997)** .

In addition, the aqueous seaweed extracts used were able to prepare wheat plants for a greater POX activity upon inoculation with *Bipolaris sorokiniana*, allowing them to improve their defense responses. Peroxidases have a role in a variety of cellular activities in plants, including stress responses and growth regulation via modulating hormone cell wall metabolism and antioxidant defense **(Jouili *et al*., 2011)**. Remarkably, they are engaged in a wide range of physiological processes throughout the plant's life cycle **(Passardi *et al*., 2005)**. Thus, POX is engaged in suberin and lignin formation, auxin metabolism, phytoalexin synthesis, the metabolism of ROS and RNS and the cross-linking of cell wall components. The bioactive compounds (antifungal and antibacterial) produced by POX-mediated reactions is continuously increasing. POX have been demonstrated to catalyze the production of bioactive compounds **(Ros Barceló & Pomar, 2002)**, which play a function in plant defense by assisting in the synthesis of phytoalexins. Thus, some lignans/neo-lignans, hordatines and viniferins are well-known bioactive (anti-fungal) compounds originating from POX-mediated reactions, and their importance *in vivo* has been widely discussed **(Waffo-Teguo *et al*., 2001; Ros Barceló & Pomar, 2002).**

CONCLUSION

Based on the current results,it was concluded that, the aqueous seaweed extracts (ASE) harvested from the coast of El Jadida (Sidi Bouzid) caused an improvement in the resistance of wheat plants by both inhibiting the growth of *Bipolaris sorokiniana* and activating plant defense responses.

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