Journal of
Microbial & Biochemical Technology

Ammar et al., J Microb Biochem Technol 2017, 9:5
DOI: 10.4172/1948-5948.1000366

Research Article Open Access

Control of Fusarium Dry Rot Incited by *Fusarium oxysporum* f. sp. *tuberosi* Using *Sargassum vulgare* Aqueous and Organic Extracts

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Abstract

Aqueous and organic extracts of *Sargassum vulgare*, removed from four Tunisian coastal sites, were evaluated for their antifungal potential against the soilborne fungus *Fusarium oxysporum* f. sp. *tuberosi*, one of the main causal agents of potato Fusarium dry rot in Tunisia. Assessed using the poisoned food technique on Potato Dextrose Agar, the antifungal activity of *S. vulgare* extracts varied depending on extract types (aqueous/organic), alga sampling sites (Tunis, Monastir, Mahdia1 and Mahdia2) and concentrations used (1-100 mg/ml). Mycelial growth inhibition, noted after 4 days of incubation at 25°C, reached 28.99% using *S. vulgare* aqueous extract at 100 mg/mL. Up to 43% growth inhibition was achieved using 50-100 mg/ml of petroleum ether extract of *S. vulgare* removed from Tunis and Mahdia2, and up to 50% with those from Mahdia1. *S. vulgare* aqueous and organic extracts decreased Fusarium dry rot severity, noted after 21 days of incubation at 25°C, in a concentration dependant manner. Applied prior to pathogen challenge, treatment with aqueous extract (at 100 mg/mL) led to 24 and 30% decrease in lesion diameter and rot penetration over the untreated control. Tubers treated with aqueous extracts of alga sampled from Tunis showed the lowest dry rot severity compared to those from the other sampling sites. Chloroformic and methanolic extracts exhibited the highest disease-suppressive effects relative to untreated control and to the other organic extracts. Applied at 100 mg/mL, the methanolic and chloroformic extracts have lowered disease severity by more than 53 and 55%, respectively. This study clearly demonstrated that this brown alga can be valorized as a potential source of antifungal compounds useful in agriculture.

Keywords: Antifungal activity; Disease severity; Dry rot; *Fusarium oxysporum* f. sp. *tuberosi*; Mycelial growth; *Sargassum vulgare*

Introduction

Fusarium dry rot is caused by several species of the soilborne fungus Fusarium which affects potato (*Solanum tuberosum* L.) tubers during their storage [1]. *F. sambucinum*, *F. solani* var. *coeruleum*, *F. oxysporum*, *F. graminearum, F. avenaceum* and *F. culmorum* were reported as the main *Fusarium* species responsible for this disease. *F. sambucinum* is being the most aggressive one but *F. oxysporum* f. sp. *tuberosi* (FOT) has become in the recent years a serious pathogen of this crop in Tunisia [2,3]. The disease results in significant yield losses. In some cases, up to 60% of tubers were affected and reported yield losses ranged between 6 to 25% [4].

FOT causes dry rot, stem-end rot, vascular wilt and damping-off of potato plants [5]. This fungus was frequently isolated from potato tubers showing dry rot symptoms and from wilted plants [3]. It infects plants through roots or wounds [3,6]. After long-term storage, typical external and internal symptoms of dry rot become more evident. Heavily infected tubers become shriveled and mummified [7].

Cultural practices and storage conditions are known to affect disease incidence and severity. Potato tubers stored at high humidity and temperatures ranging between 15 and 20°C will develop rapidly Fusarium dry rot [4]. Various fungicides were widely used as postharvest treatments for disease control [6-9]. However, disease incidence has increased over years and this phenomena seems to be more linked to the appearance of thiabendazole-resistant strains and to the lack of potato cultivars with high levels of resistance to Fusarium dry rot [2,3,9].

Post-harvest applications of chitosan and ß-aminobutyric acid, nitric oxide, Topsin-M and score have been investigated as promising alternatives for disease control. During last decades, attention has also been paid to biocontrol using various microbial agents [8,10,11]. *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp., *Pichia* sp., *Aspergillus* spp. were found to be active against *Fusarium* spp [12-15]. Biondi et al. [16] demonstrated the ability of methanol extracts of the cyanobacterium *Nostoc* strain to suppress the mycelial growth of *F. oxysporum*, *Penicillium expansum*, *Phytophthora cinnamomi*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Verticillium albo-atrum*. The modified montmorillonite particles combined with *T. harzianum* used in dual culture (at 3 and 5%), inhibited FOT by more than 90%. Under greenhouse conditions, this combination, applied as soil and tuber treatments, was also effective in suppressing potato Fusarium wilt [13].

Some plant and seaweed extracts were also explored for their ability to suppress Fusarium dry rot. For instance, *Datura metal* and *Inula* spp. leaf and flower extracts were shown to be effective in reducing FOT mycelial growth. Flower aqueous and leaf methanolic extracts of *Nicotiana glauca* have suppressed pathogen mycelial growth by 24.40 and 21.04%, respectively [17-19]. Significant inhibition of *F. roseum* was achieved using seaweed water extracts especially those from

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Received September 14, 2017; **Accepted** September 21, 2017; **Published** September 28, 2017

Citation: Ammar N, Nefzi A, Jabnoun-Khiareddine H, Daami-Remadi M (2017) Control of Fusarium Dry Rot Incited by *Fusarium oxysporum* f. sp. *tuberosi* Using *Sargassum vulgare* Aqueous and Organic Extracts. J Microb Biochem Technol 9:200-208. doi: 10.4172/1948-5948.1000366

Spirulina platensis and *Chara grovesii* [20]. Moreover, methanolic extract of *Aglaophenia* sp., applied at 0.15% (w/v), was shown able to totally suppress *F. oxysporum* f. sp. *vanillae* [21]. Several studies highlighted the beneficial effects of seaweed extracts on plant growth including enhancement of seed germination, root growth, biomass, plant vigor and yield. It was reported that seaweeds can also increase nutrient uptake and plant nutritional quality. They are able to induce early flowering and fruit ripening and increase fruit production [22]. Furthermore, seaweed metabolites can suppress disease through direct action on causal agents. They can induce plant resistance to pest attack and induce tolerance to abiotic stresses such as salinity and frost [22,23].

Tunisian coastlines were characterized by the abundance of algal biomasses. Many Tunisian studies have highlighted the beneficial effects of seaweed extracts in agriculture [23,24]. Among seaweed species explored, *S. vulgare* (Sargassaceae), the most common and abundant seaweed in the Mediterranean coast, was chosen to be evaluated for its antifungal potential against FOT.

Sargassum genus is well known to produce secondary metabolites with high biotechnological potential. Despite their capacity to produce large amounts of natural products with various biological activities, these macroalgae have been little documented for their antifungal activity against phytopathogenic fungi and especially those infecting potato tubers during storage. Therefore, there is an increasing need to develop alternative strategies for controlling potato post-harvest diseases such as the use of algal extracts as pre-storage tuber treatments. In a previous work, we demonstrated the ability of *S. vulgare* extracts to suppress Pythium leak caused by *Pythium aphanidermatum* [24]. The present research was performed in order to evaluate the effectiveness of these same extracts against FOT based on *in vitro* and *in vivo* bioassays.

Materials and Methods

Fungal material

FOT isolate used in the present study was gratefully provided by the laboratory of Phytopathology of the Regional Center of Research on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia. It was originally recovered from potato tubers exhibiting Fusarium dry rot symptoms. Pathogen cultures were initiated from stock cultures maintained at 4°C. They were grown on Potato Dextrose Agar (PDA) amended with streptomycin sulphate (300 mg/L) and incubated at 25°C for 7 days before use. Pathogen virulence was maintained by inoculation of freshly wounded potato tubers, incubation at 25°C for 21 days and re-isolation on PDA medium.

Plant material

Apparently healthy potato tubers, cv. Spunta the most widely grown cultivar in Tunisia, were selected for the *in vivo* trial. They were chosen based on the absence of physical defects and their uniformity in size, appearance and ripeness. They were previously stored in darkness at 6°C for one month before use. They were brought to room ambient conditions few hours before the beginning of trials. They were thoroughly washed to eliminate adhering soil, superficially sterilized by immersing in a 10% v/v sodium hypochlorite solution for 5 min, rinsed with sterile distilled water (SDW) and air dried.

Algal material: Sampling and preparation for extraction

S. vulgare samples were collected on February 2014 from four different ecological sites along the Tunisian coast (Table 1). They were removed at 1 m-depth beneath the sea surface. Algal samples were collected by hand, gently washed with sea water and putted in plastic bags before being transferred to the laboratory.

Locality	Geographic location	GPS coordinate
Tunis	North of Tunisia	N 36°51'53.041" E10°21'14.4"
Mahdia1	Center-East of Tunisia	N 35°30'15.942" E 11°4'42.035"
Mahdia2		N 35°30'13.278" E 11°4'34.371"
Monastir		N 35°46'47.754" E 10°47'9.312"

Table 1: Sampling sites of the brown alga *Sargassum vulgare* used in the current study.

Once brought to laboratory, samples were thoroughly rinsed several times with tap water, to remove marine epiphytes and sand particles, and shade-dried for three weeks at 25 ± 2°C. Dry algal materials were grounded into fine powder and stored at 4°C until further use.

Aqueous and organic extraction

Algae were subjected to aqueous extraction according to Oryan et al. [25]. Powder samples of 200 g each were soaked in 2 L of SDW and incubated for 24 h under ambient conditions (25 ± 2°C). Extracts were filtered twice through Whatman N°1 sterile filter paper and further sterilized by filtration through micro-filter (0.22 µm pore size). Collected aqueous extracts were stored at 4°C until future use.

For organic extraction, a 250 g sample of grounded alga was subjected to a series of maceration in methanol (500 mL) for 2 to 3 days under ambient room conditions according to Saidana et al. [26]. After filtration, the solvent was evaporated using a rotary evaporator under reduced pressure (at 60°C). The dried algal residue was further subjected to successive extractions using three solvents (of 350 mL each) with increasing polarities (namely petroleum ether, chloroform and ethyl acetate). All obtained extracts were filtered and evaporated using a rotary evaporator under reduced pressure and different temperatures depending on solvents used for extraction (i.e., at 35°C for petroleum ether, 60°C for chloroform and at 75°C for ethyl acetate). All dry residues were quantified and separately dissolved into 1 mL of Dimethyl sulfoxide (DMSO) and stored at 4°C until further use.

Test of the antifungal potential of *S. vulgare* extracts against *F. oxysporum* f. sp. *tuberosi*

S. vulgare aqueous and organic extracts were evaluated for their antifungal potential against FOT using the poisoned food technique on PDA medium amended with streptomycin sulphate (300 mg/L). Appropriate amounts (1-100 mg/mL) of each *S. vulgare* extract (obtained from the four different sampling sites) were added to molten PDA medium. Extract-amended medium was aseptically poured into Petri plates (9 cm in diameter).

SDW (for aqueous extracts test) and DMSO (for organic extracts test) were used as negative controls. After medium solidification, three agars plug (6 mm in diameter), cut from 7 day old cultures and were equidistantly placed in each plate. There were three replicate plates for each individual treatment and the whole experiment was repeated at least twice.

The mean diameter of FOT colony was noted after 4 days of incubation at 25°C. The percentage of mycelial growth inhibition was calculated as:

$$I=(C-T/C) \times 100$$

Where I: Growth inhibition (in %), C: Colony diameter of pathogen in control plates and T: Pathogen colony diameter in extract-amended plates.

Test of the disease-suppression ability of *S. vulgare* extracts

Disinfected potato tubers were wounded (at 6 mm depth) at two sites along the tuber longitudinal axis using a sterile cork borer (6 mm in diameter). Algal treatments were applied 2 h before pathogen challenge by injecting 100 µL of each *S. vulgare* extract (aqueous or organic extracts applied at the different concentrations tested) in the performed wounds. Tuber inoculation was made by deposing in each wound a 6 mm agar plug colonized by FOT removed from a 7 day old culture. Negative control tubers were inoculated with pathogen and treated with equal volume (100 µL) of SDW (for aqueous extracts test) and DMSO (for organic extracts test). Positive control tubers were inoculated with FOT and treated with SDW. Five potato tubers (with two inoculation sites each) were used for each individual treatment. After pathogen challenge and treatments, all potato tubers were incubated for 21 days at 25°C.

At the end of the incubation period, Fusarium dry rot severity was determined based on the external and internal extent of the induced decay. Symptomatic lesions were evaluated externally by measuring two perpendicular diameters of rot lesion and the mean diameter was considered for each inoculation site. Then, tubers were cut along the longitudinal axis and across the inoculation site to measure rot width (W, mm) and depth (D, mm). Penetration (P, mm) of rotted tissues within tubers was estimated using the following formula where:

$P=[(W/2) + (D-6)] / 2.$

Statistical Analyses

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0. They were subjected to one-way analysis of variance (ANOVA) according to a completely randomized factorial design.

For the *in vitro* trial of aqueous extracts, alga sampling sites and the tested concentrations were the both fixed factors whereas for the test of organic extracts, three fixed factors were considered (namely alga sampling sites, types of organic extracts, and the tested concentrations). Each individual treatment was replicated thrice and the whole experiment was repeated at least twice.

For the test of the effect of aqueous extracts on Fusarium dry rot severity (based on rot lesion diameter and penetration), the alga sampling sites and the tested concentrations were the both fixed factors whereas for that of organic extracts, three factors were fixed (namely alga sampling sites, types of organic extracts, and tested concentrations). Each individual treatment was replicated ten times (5 tubers × 2 inoculation sites). Each of the *in vivo* bioassay was repeated twice and result from one representative experiment was reported in the current study. Means were separated using LSD or Duncan's Multiple Range tests at $P \leq 0.05$.

Results

Antifungal potential of *S. vulgare* aqueous extracts against FOT

ANOVA analysis performed for the colony diameter of FOT, recorded after 4 days of incubation at 25°C, revealed a significant (at $P \leq 0.01$) effect depending on alga sampling sites and tested concentrations. No significant interaction was noted between both fixed factors.

Data given in Figure 1 show that *S. vulgare* aqueous extract inhibited pathogen growth in a concentration-dependent manner. In fact, this extract was found to be more active when used at 100 mg/mL where pathogen mycelial growth was inhibited by 28.99% relative to control, compared to 20.33-24.59% recorded with the other tested concentrations.

Figure 2 shows that for all concentrations combined, aqueous extract of *S. vulgare* sampled from Mahdia1 were the most effective in decreasing growth of target pathogen, followed by those originating from Monastir, Mahdia2 and Tunis sites.

Antifungal potential of *S. vulgare* organic extracts against FOT

Analysis of variance revealed that FOT colony diameter grown on PDA amended with *S. vulgare* organic extracts varied significantly (at $P \leq 0.01$) depending on alga sampling sites, type of organic extracts, and tested concentrations. A significant interaction was detected between these three fixed factors. Overall, tested organic extracts whatever their types, alga geographical origin, and tested concentrations had significantly suppressed FOT radial growth by 14.84-51.04% as compared to the control (Table 2).

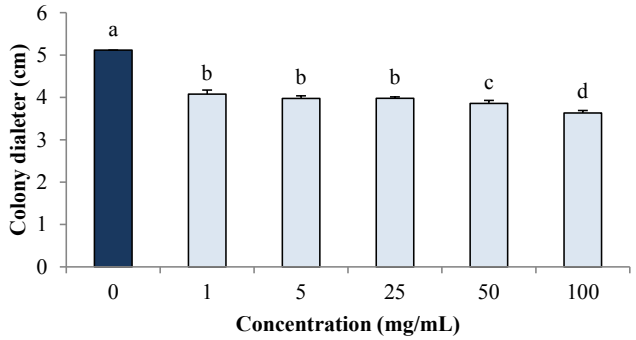

Figure 1: Effect of different concentrations of aqueous extracts from *Sargassum vulgare* (all sampling sites combined) on *Fusarium oxysporum* f. sp. *tuberosi* mycelial growth noted after 4 days of incubation at 25°C.

Concentration 0 mg/mL: (Control) PDA medium unamended with algal extracts but amended with 1 mL of SDW. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

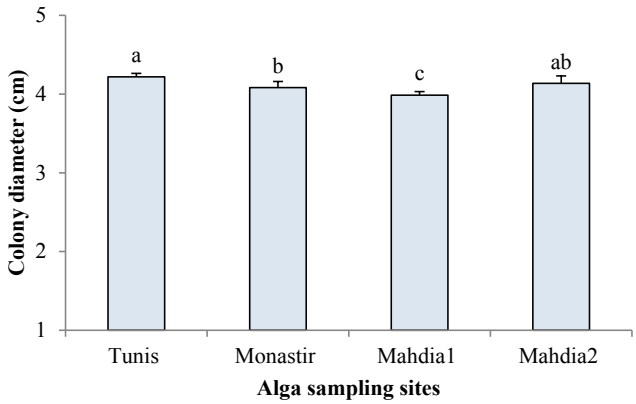

Figure 2: Effect of aqueous extracts from *Sargassum vulgare* collected from different sampling sites (all concentrations combined) on *Fusarium oxysporum* f. sp. *tuberosi* mycelial growth noted after 4 days of incubation at 25°C.

Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

Sites	Tunis				Monastir				Mahdia1				Mahdia2				***Average per concentration
Extracts type	PE	MeOH	$CHCL_3$	EtOAc	PE	MeOH	$CHCL_3$	EtOAc	PE	MeOH	$CHCL_3$	EtOAc	PE	MeOH	$CHCL_3$	EtOAc	
0	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a	5.62 a
1	4.55 b (18.99)	4.78 b (14.84)	4.62 b (17.80)	4.68 b (16.62)	4.47 b (20.47)	4.72 b (16.02)	4.67 b (16.91)	4.62 b (17.80)	4.55 b (18.99)	4.77 b (15.13)	4.67b (16.91)	4.73 b (15.73)	4.58 b (18.40)	4.52 b (19.58)	4.57 b (18.69)	4.78 b (14.84)	4.64 b (17.36)
5	3.55 c (36.80)	4.77 b (15.13)	4.63 b (17.51)	4.63 bc (17.51)	3.60 c (35.91)	4.65 b (17.21)	4.52 b (19.58)	4.68 b (16.62)	3.37 c (40.06)	4.62 bc (17.80)	4.63 b (17.51)	4.72 b (16.02)	4.43 b (21.07)	4.48 b (20.18)	4.50 b (19.88)	4.72 b (16.02)	4.41 c (21.55)
25	3.47 c (38.28)	4.63 bc (17.51)	4.65 b (17.21)	4.55 bc (18.99)	3.15 d (43.92)	4.52 bc (19.58)	4.23 c (24.63)	4.45 bc (20.77)	2.87 d (48.96)	4.43 bc (21.07)	4.58 b (18.40)	4.62 bc (17.80)	3.30 c (41.25)	4.37 b (22.26)	4.47 b (20.47)	4.75 b (15.43)	4.19 d (25.41)
50	3.15 d (43.92)	4.48 bc (20.18)	4.43 b (21.07)	4.48 bc (20.18)	3.12 d (44.51)	4.25 cd (24.33)	4.18 c (25.52)	4.28 cd (23.74)	2.78 d (50.45)	4.45 c (20.77)	4.47 bc (20.47)	4.57 bc (18.69)	3.08 d (45.10)	4.37 b (22.26)	4.52 b (19.58)	4.43 c (21.07)	4.07 e (27.61)
100	3.00 d (46.59)	4.32 c (23.15)	4.28 b (23.74)	4.43 c (21.07)	3.10 d (44.81)	4.13 d (26.41)	4.13 c (26.41)	4.17 d (25.82)	2.75 d (51.04)	4.53 c (19.29)	4.22 c (24.93)	4.40 c (21.66)	3.12 d (44.51)	4.28 b (23.74)	4.45 b (20.77)	4.35 c (22.55)	3.98 f (29.15)
*Average per site and per extract type	3.89 b	4.77 a	4.71 a	4.73 a	3.84 b	4.65 a	4.56 a	4.64 a	3.66 b	4.74 a	4.70 a	4.78 a	4.02 c	4.61 b	4.69 b	4.78 a	
**Average per site	4.52 a				4.42 b				4.47 b				4.52 a				

Table 2: The effects of sampling sites of *Sargassum vulgare*, types of organic extracts and tested concentrations on *Fusarium oxysporum* f. sp. *tuberosi* radial growth recorded after 4 days of incubation at 25°C as compared to control.

Values between parenthesis indicate the percentage (in %) of inhibition of *F. oxysporum* f. sp. *tuberosi* growth as compared to the untreated control

Concentration 0 mg/ml: (Control) PDA medium unamended with algal extracts but amended with 1 mL of DMSO; Types of organic extracts (PE: Petroleum Ether; MeOH: Methanol; $CHCL_3$: Chloroform; EtOAc: Ethyl Acetate)

* Average per site and per extract type for all concentrations combined

** Average per site for all extract types and concentrations combined

*** Average per concentration for all sampling sites and extract types combined

Values (per sampling site, per sampling site and per extract type, and per concentration) followed by the same letter are not significantly different according the Duncan's Multiple Range test at $P \leq 0.05$. LSD (Sampling sites × Extract types × Concentrations)=0.23 cm at P=0.05

Data detailed in Table 2 revealed that all tested organic extracts, whatever the alga sampling site, had inhibited pathogen mycelial growth in a concentration-dependent manner. For each sampling site, the lowest colony diameter was noted on PDA plates amended with petroleum ether extracts as compared to the other extracts.

In fact, using petroleum ether extract of *S. vulgare* sampled from Tunis, pathogen growth was suppressed by 18.99-46.59%, over control, depending on the tested concentrations. However, inhibitions induced by chloroformic, methanolic and ethyl acetate extracts were estimated at 17.80-23.74, 14.84-23.15 and 16.62-21.07%, respectively. Tested at 100 mg/mL, *S. vulgare* petroleum ether extract had suppressed FOT radial growth by 46.59%, relative to control, compared to 18.99% recorded at 1 mg/mL.

Pathogen radial growth was decreased by 20.47-44.81%, over control, when grown on PDA medium amended with *S. vulgare* petroleum ether extract collected from Monastir compared to 16.91-26.41, 17.80-25.82, and 16.02-26.41% recorded with chloroformic, methanolic and ethyl acetate extracts.

Also, petroleum ether extract of *S. vulgare* sampled from Mahdia1 was found to be the most effective against FOT where the inhibitions rates ranged between 18.99 and 51.04% for concentrations varying between 1 and 100 mg/mL. Chloroformic, ethyl acetate and methanolic extracts had also suppressed pathogen growth by 16.91-24.93, 15.73-21.66 and 15.13-21.07%, respectively, compared to control.

Petroleum ether extracts of *S. vulgare* removed from Mahdia2 had inhibited pathogen growth by 18.40 to 44.51%, relative to control, when applied at concentrations varying from 1 to 100 mg/mL. However, methanol, chloroform and ethyl acetate extracts had significantly limited FOT growth by 19.58-23.74, 18.69-20.77 and 14.84-22.55%, respectively, depending on the tested concentrations.

It should be noted that for all types of organic extracts and tested concentrations combined, extracts sampled from Monastir and Mahdia1 were found to be the most effective in reducing pathogen radial growth, followed by those from Mahdia2 and Tunis (Table 2).

Figure 3 shows that for all tested organic extracts, whatever their origin and concentrations used, FOT growth was lowest on PDA amended with petroleum ether extract when compared to the other extracts.

Disease-suppressive ability of *S. vulgare* aqueous extracts

Effects on rot lesion diameter: ANOVA analysis showed a significant (at $P \leq 0.01$) variation in the rot lesion diameter, depending on tested concentrations only whereas alga sampling sites had no significant effect neither individually nor in interaction with concentrations on this parameter.

All aqueous extracts had suppressed disease severity, as measured by lesion diameter, in a concentration-dependent manner. In fact, Figure 4 shows that the lesion diameter was reduced by 24.03-19.94%, relative to the untreated control; using aqueous extracts at 50-100 mg/ml compared to 15.89-7.42% achieved following tuber treatments with *S. vulgare* extracts at 1-25 mg/mL.

Effects on rot penetration: ANOVA analysis performed for

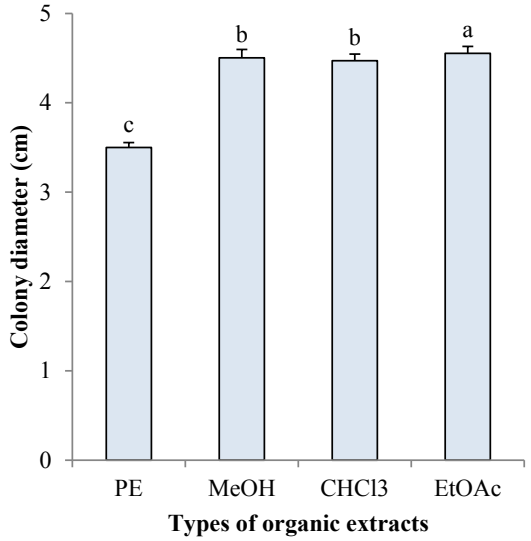

Figure 3: *Fusarium oxysporum* f. sp. *tuberosi* growth, noted after 4 days of incubation at 25°C, on PDA medium supplemented with different *Sargassum vulgare* organic extracts.

Types of organic extracts (PE: Petroleum Ether; MeOH: Methanol; $CHCL_3$: Chloroform; EtOAc: Ethyl Acetate). Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

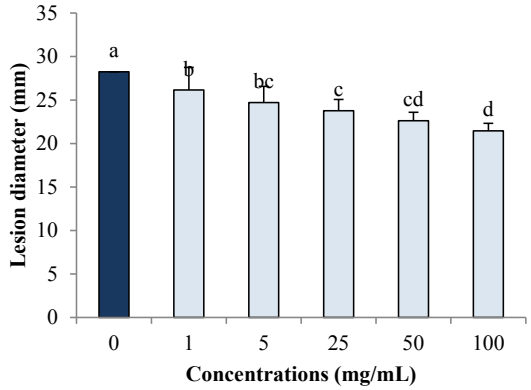

Figure 4: Disease-suppressive ability of *Sargassum vulgare* aqueous extracts, as measured by rot lesion diameter, depending on tested concentrations noted after 21 days of incubation at 25°C.

Concentration 0 mg/ml: (Control) Potato cv. Spunta tubers untreated with aqueous extract and inoculated with *Fusarium oxysporum* f. sp. *tuberosi*. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

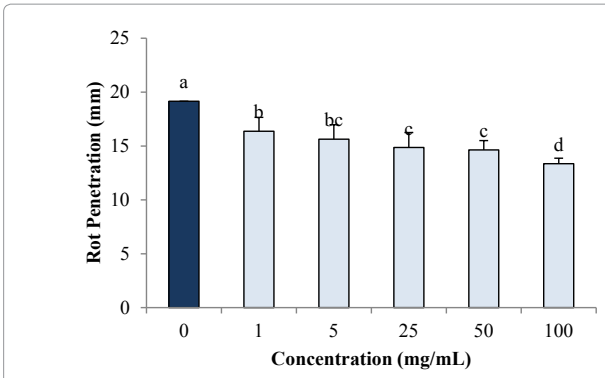

Figure 5: Disease-suppressive ability of *Sargassum vulgare* aqueous extracts, as measured by rot penetration, depending on tested concentrations noted after 21 days of incubation at 25°C.

Concentration 0 mg/ml: (Control) Potato cv. Spunta tubers untreated with organic extract and inoculated with *Fusarium oxysporum* f. sp. *tuberosi*. Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

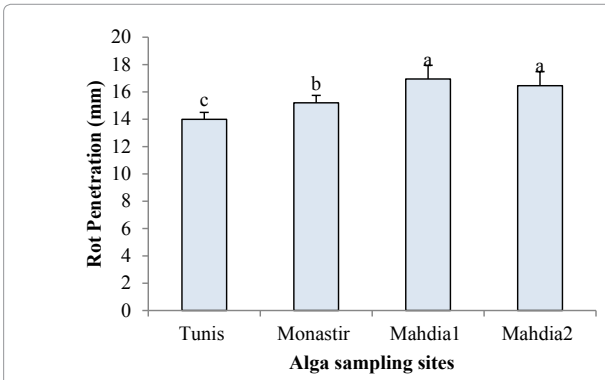

Figure 6: Effect of aqueous extracts of *Sargassum vulgare* sampled from different sites on Fusarium dry rot severity, as measured by rot penetration, recorded after 21 days of incubation at 25°C.

Bars sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$)

rot penetration revealed a significant (at $P \leq 0.01$) variation in this parameter depending on alga sampling sites and tested concentrations but no significant interaction was noted between the two fixed factors.

Data presented in Figure 5 show that all aqueous extracts had suppressed disease severity, as measured by rot penetration, in a concentration-dependent manner. Indeed, aqueous extract of *S. vulgare* was found to be more active when used at 100 mg/mL where rot penetration was reduced by 30.27%, compared to 23.60, 22.39, 18.40, and 14.52% noted at concentrations 50, 25, 5 and 1 mg/ml, respectively.

As given in Figure 6 and for all concentrations combined, tubers treated with aqueous extracts of *S. vulgare* sampled from Tunis showed the lowest Fusarium dry rot severity as compared to those from Monastir, Mahdia2 and Mahdia1.

Disease-suppressive ability of *S. vulgare* organic extracts

Effects on lesion diameter: Lesion diameter of Fusarium dry rot, noted after 21 days of incubation at 25°C, varied significantly (at $P \leq 0.05$) depending on types of *S. vulgare* organic extracts (petroleum ether, methanol, chloroforme and ethyl acetate) and tested concentrations (1-100 mg/mL).

Sampling sites had no significant effect on this parameter when considered individually. However, a significant interaction was noted between sampling sites and types of organic extract tested and between extracts and tested concentrations.

Figure 7 shows that for the sampling site of Tunis, all tested *S. vulgare* organic extracts had the same effect on the lesion diameter of Fusarium dry rot. However, potato tubers treated with methanolic and chloroformic extracts sampled from Monastir (20.51 and 23.88 mm) and Mahdia2 (24.38 and 22.64 mm), respectively, showed the lowest dry rot severity. Concerning effects of alga sampled from Mahdia1, methanolic and chloroformic extracts induced the highest decrease in

the lesion diameter as compared to petroleum ether and ethyl acetate extracts.

All organic extracts tested decreased Fusarium dry rot severity in a concentration-dependent manner. In fact, Figure 8 shows that for all sampling sites combined, the rot lesion diameter was lowered by 45.48-50.48%, relative to control, on potato tubers already inoculated by pathogen and treated by 50-100 mg/mL of *S. vulgare* petroleum ether extract compared to 12.32% recorded at 1 mg/mL. It is also the case of the methanolic extract which was more effective when applied at 100 mg/mL by decreasing the lesion diameter by 53.04%, relative to pathogen-inoculated and untreated control, compared to 30.91-34.26% recorded at 1-5 mg/mL. Tuber treatments with chloroformic extract, whatever alga origin, had suppressed dry rot severity by 23.65% when used at 1 mg/mL compared to 51.29-55.93% obtained at a concentrations ranging between 50 and 100 mg/mL. Also, *S. vulgare* ethyl acetate, applied at these two high concentrations, was found to be more effective where the lesion diameter was lowered by 35.44-37.68% relative to 20.29% noted at 1 mg/mL (Figure 8).

Effects on rot penetration: Rot penetration, noted after 21 days of incubation at 25°C, varied significantly (at $P \leq 0.05$) depending on *S. vulgare* sampling sites (Tunis, Monastir, Mahdia1 and Mahdia2), types of organic extracts and tested concentrations. Moreover, significant interactions were noted between sampling sites and types of organic extracts and between extracts and tested concentrations.

Data presented in Figure 9 indicates that potato tubers treated with methanolic and chloroformic extracts of *S. vulgare* sampled from sites of Monastir (11.60 and 13.21 mm) and Mahdia1 (12.45 and 12.48 mm) showed the lowest disease severity as compared to those treated with the other extracts (13.70-15.52 mm). Chloroformic extract of alga sampled from Tunis was the most effective in decreasing rot penetration as compared to the other treatments. However, for *S. vulgare* samples collected from Mahdia2, tuber treatments using petroleum ether and chloroformic extracts led to the lowest rot penetration records relative to the other extracts.

Figure 10 shows that all tested organic extracts had reduced rot penetration in a concentration-dependent manner. In fact, this parameter was lowered by 57.45% on potato tubers treated by 100 mg/mL *S. vulgare* petroleum ether extract, whatever its sampling site, compared to 26.77% noted at 1 mg/mL. Tuber treatment with methanolic extract had decreased rot penetration by 49.91-53.92%,

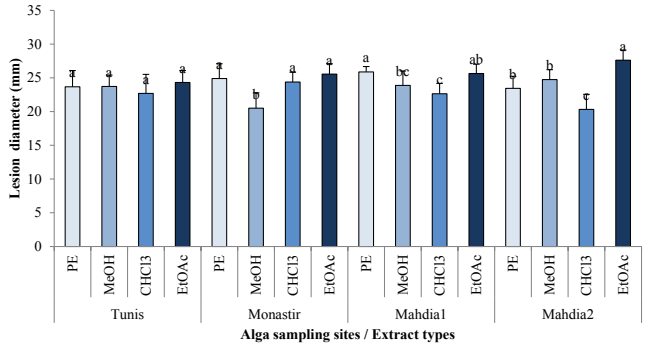

Figure 7: Disease-suppressive ability of *Sargassum vulgare* organic extracts, as measured by rot lesion diameter, depending on alga sampling sites noted after 21 days of incubation at 25°C.

Types of organic extracts (PE: Petroleum Ether; MeOH: Methanol; $CHCL_3$: Chloroform; EtOAc: Ethyl Acetate). For each sampling site, organic extracts sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$). LSD (Alga sampling sites × Extract types)=2.14 mm at P=0.05.

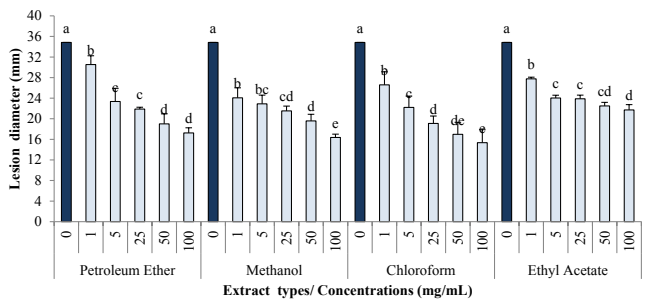

Figure 8: Disease-suppressive ability of *Sargassum vulgare* organic extracts, as measured by rot lesion diameter, depending on tested concentrations noted after 21 days of incubation at 25°C.

Concentration 0 mg/ml: (Control) Potato cv. Spunta tubers inoculated with *Fusarium oxysporum* f. sp. *tuberosi* and untreated. For each extract type, concentrations sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$). LSD (Extract types × Concentrations)=2.29 mm at P=0.05

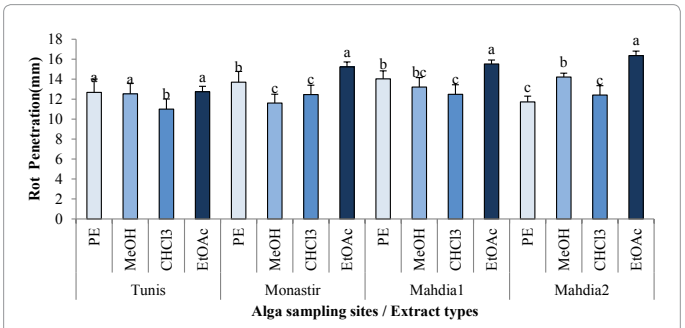

Figure 9: Disease-suppressive ability of *Sargassum vulgare* organic extracts, as measured by rot penetration, depending on alga sampling sites noted after 21 days of incubation at 25°C.

Types of organic extracts (PE: Petroleum Ether; MeOH: Methanol; $CHCL_3$: Chloroform; EtOAc: Ethyl Acetate). For each sampling site, organic extracts sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$). LSD (Alga sampling sites × Extract types)=1.25 mm at P=0.05

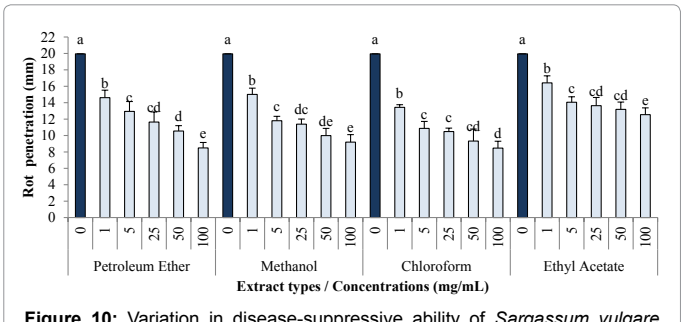

Figure 10: Variation in disease-suppressive ability of *Sargassum vulgare* organic extracts, as measured by dry rot penetration, depending on types of organic extracts and tested concentrations noted after 21 days of incubation at 25°C.

Concentration 0 mg/ml: (Control) Potato cv. Spunta tubers inoculated with *Fusarium oxysporum* f. sp. *tuberosi* and untreated. For each extract type, concentrations sharing the same letter are not significantly different according to Duncan's Multiple Range test (at $P \leq 0.05$). LSD (Extract types × Concentrations)=1.40 mm at P=0.05

relative to control, when applied at 100 mg/mL compared to 24.78% obtained at the concentration 1 mg/mL. *S. vulgare* chloroformic extract, applied at 50-100 mg/mL, was effective in suppressing Fusarium dry rot severity by 53.28-57.54% compared to 32.68% obtained at 1 mg/mL. Using *S. vulgare* ethyl acetate extract at 100 mg/mL, rot penetration was 37.11% lower than control compared to 17.72% noted at 1 mg/mL.

Discussion

Today, the challenges faced by the agriculture sector are immense. Environment friendly agriculture for the achievement of quality and healthy food is in high demand. Efforts are underway for a biological and sustainable agriculture. In Tunisia, important post-harvest potato tuber losses were induced during storage by Fusarium dry rot [2,3,24]. For this reason, there is a pressing need to develop biological alternative methods to avoid over-reliance on chemical pesticides that can damage environment and human health. The present study was undertaken to investigate the *in vitro* and *in vivo* ability of extracts from the brown alga *S. vulgare*, collected from four geographical sites (Tunis, Monastir, Mahdia1 and Mahdia2) to inhibit the *in vitro* growth of FOT and to suppress potato dry rot severity.

This study obviously demonstrated the ability of *S. vulgare* aqueous extracts to control the FOT with varying levels depending on alga sampling sites and extract tested concentrations. Pathogen radial growth was inhibited in a concentration-dependent manner. In fact, tested at 100 mg/mL, FOT radial growth was decreased by 28.99%, compared to control, whatever sampling sites, compared to 20.33% noted at 1 mg/mL. These results are in accordance with our previous findings [24] where the *Pythium aphanidermatum* mycelial growth was also reduced by up to 28% using *S. vulgare* aqueous extracts at 40-50 mg/mL. In a recent study and in line with our findings, mycelial growth of *V. dahliae* was significantly reduced using *S. vulgare* hot water extract at two doses 50 g/L and 25 g/L, by 43 and 35%, respectively, compared to control [27]. Furthermore, *F. oxysporum* mycelial growth was inhibited by 70 and 55% using aqueous extract of the brown alga *Padina tetrastromatic* at 3 and 4 mg/mL, respectively [28]. *Halopitys incurvus* and *Stypocaulon scoparium* aqueous extracts applied at 5% (v/v) were effective in reducing the mycelial growth of *F. oxysporum* f. sp. *albedinis* by 60% over control [29]. In contrast, Chbani et al. [30] demonstrated that *S. vulgare* aqueous extract applied at 200 mg/mL did not display any antifungal activity toward *Penicillium digitatum*. In the same way, *S. wightii* extract, applied at 5 mg/mL, was totally inactive against *F. oxysporum* and *F. solani* [31]. Moreover, the screening of the antifungal activity of aqueous extracts from *S. tennerrimum*, *S. swartzii*, *Dictyota dichotoma*, *Halimda tuna*, *Jania capillacea*, *Scinaia shameelii* and *Coelarthum muelleri*, showed that they were all totally inactive against *F. oxysporum* and *F. solani* when applied at 2-4 mg/mL [28]. Also, Thinakumar and Sivakumar did not detect any antifungal activity against *P. aphanidermatum* using *S. wightii* and *S. ilicifolium* water extracts at 100 mg/mL [32].

The present study showed that FOT radial growth was strongly affected by the type of *S. vulgare* organic extract, alga sampling site and tested concentration. Organic solvents were more efficient in extracting compounds with high antifungal potential compared to water based methods [33]. According to our experiments, *S. vulgare* petroleum ether extract, tested at 100 mg/mL, displayed an important antifungal activity leading to more than 43% pathogen inhibition for those extracted from samples collected from Tunis, Mahdia2 and Monastir. For those extracted from Mahdia1 samples and applied at the same concentration, FOT mycelial growth was reduced by over 51.04%. In another study [34], a moderate antifungal activity was noted with chloroformic, ethanolic and ethyl acetate *S. vulgare* extracts against *F. oxysporum* while acetonic one was totally inactive. Cyclohexane extracts of *S. vulgare*, *Cystoseira barbata*, *Dictyopteris membranacea*, *Dictyota dichotoma* and *Colpomenia sinuosa*, applied at 50 mg/mL displayed an important suppressive effect against *F. oxysporum* mycelial growth [34]. In addition, a total suppression of *F. moniliforme* growth was recorded using 50 mg/mL of *S. vulgare* methanolic and chloroformic extracts [35]. Ammar et al. [24] demonstrated that mycelial growth of *P. aphanidermatum* was slightly reduced (12.63%) using *S. vulgare* petroleum ether extract, compared to the methanolic one (70.72%). In addition, treatment using *S. vulgare* methanolic extract at 0.5-1 g/L was effective in suppressing mycelial growth of *V. dahliae* by more than 85% [27]. Furthermore, methoxybifurcarenone, isolated from the brown alga *Cystoseira tamariscifolia*, displayed an interesting antifungal activity toward various tomato pathogenic fungi including *F. oxysperium* f. sp. *lycopersici* [36]. Chbani et al. [30] found that *P. digitatum* growth was not affected when treated by 200 mg/mL of *S. vulgare* dichloromethanol extract. In the same way, *S. swartzii*, *S. ilicifolium* and *S. lanceolatum* ethanolic extracts, tested at 2 mg/mL, were found to be inactive against *F. oxysporum* and *F. solani* [37]. In addition, Lavanya and Veerappan demonstrated that acetone, methanol, diethyl ether, chloroform, hexane, ethyl acetate and water extracts of *S. wightii*, *Turbinaria conoides*, *Caulerpa scapelliformis* have no antifungal activity toward *F. oxysporum*. Padmakumar and Ayyakkannu found that extracts of *S. ilicifolium*, *S. myriocystum*, *S. tenerrimum*, *S. wightii* and *S. plagiophyllum*, tested at 0.4 g/L, were inactive against *F. oxysporum* [31,38].

Our investigation showed that *S. vulgare* aqueous and organic extracts used as tuber treatment for dry rot control had significantly reduced disease severity (estimated based on rot lesion diameter and penetration) compared to the pathogen-inoculated and untreated control. Results from the present study clearly showed that preventive treatment by aqueous extracts, at 100 mg/mL, was effective in reducing lesion diameter and rot penetration by 24.03 and 30.27% compared to the protection recorded at 1 mg/mL (7.42 and 14.52%, respectively). For the second severity parameter, extracts from alga issued from Tunis were the most active leading to the highest decreases in disease severity compared to the other sites. Also, Ammar et al. [24] found that *S. vulgare* aqueous extract was effective in reducing penetration of *P. aphanidermatum* by more than 32%. Sbaihat et al. [39] also found that *S. fusiforme* water extract had significantly limited incidence and severity of three tomato diseases incited by *Oidium* spp., *Phytophtora infestans* and *Botrytis cinerea*. Furthermore, cucumber plants grown under greenhouse condition and sprayed and/or drenched with 0.5 and 1% (v/v) of *Ascophyllum nodosum* extracts showed decreased incidence of Fusarium wilt caused by *F. oxysporum* f. sp. *cucumerinum* [40]. In a recent study, Guesmi [27] found that *S. vulgare* methanolic extract, tested at 0.5 and 1 g/L, suppressed leaf damage index (96%) and the vascular discoloration extent (100%) caused by *Verticillium dahliae* infection on tomato and eggplant plants, compared to the untreated controls. Soil amended with *S. tenerrimum, S. swartzii* and *S. wightii* powder 0.5 and 1% (w/w) combined with biocontrol agents had significantly suppressed root rot infection caused by *M. phaseolina* and *F. solani* on sunflower, whereas a total suppression of *F. solani* infection was noted using *Pseudomonas lilacinus* combined with *S. swartzii* [41]. Chbani et al. [30] noted the failure of aqueous and dichloromethanolic extracts of *S. vulgare*, applied at 200 mg/mL, to prevent infection of citrus fruits by *P. digitatum*.

Treatments of pathogen-inoculated potato tuber with *S. vulgare* organic extracts was found to be efficient in controlling potato dry rot severity depending on alga sampling sites, types of extracts and tested concentrations. In fact, under extremely favorable conditions for

FOT infection and development, *S. vulgare* organic extract decreased Fusarium dry rot severity in a concentration-dependent manner. Tuber treatment with chloroformic and methanolic extracts exhibited the highest disease-suppressive effects relative to pathogen-inoculated and untreated control. In fact using these extracts at 100 mg/mL, lesion diameter and rot penetration were lowered by more than 53 and 55%, respectively. In line with our investigation, methanolic extract of *S. vulgare* applied at the lowest concentration was sufficient for getting an effective protection against potato leak where rot penetration was decreased by up to 82% [24]. Also, Ibraheem et al. [42] found that root rotting of *Solanum melongena* caused by *F. solani* was inhibited, by over 56% versus control, using *S. latifolium* methanol extract at 0.1 mg/mL.

It is clearly recorded that the selections of solvent play a vital role in the extraction of seaweed active compounds. Numerous studies have also demonstrated the effectiveness of organic extracts in extracting secondary metabolites with antifungal activities against various soil borne plant pathogens compared to water-based procedures [24,33]. In fact, Pérez et al. [43] reported that the yield of extractable antimicrobials from the different seaweed species is solvent-dependent. Lee et al. [44] found that *S. thumbergi* water extract (used at 4 mg/ml) did not show any antimicrobial activity contrarily to the ethanolic extract which was found to be very active. Abundant studies have confirmed that alcoholic solutions and/or hydrophilic solvent mixtures provided better activity, i.e., methanol extracts were more active than those extracted using lipophilic solvents [45]. In fact, Roy-Angel and Maheswari showed that the highest antifungal activity was recorded in methanolic extracts compared to the chloroformic and acetonic ones [46]. In contrast, Kumar et al. [47] demonstrated that *S. cinereum* chloroformic extract was more effective against phytopathogenic fungi they tested than the ethanolic and acetonic ones. In a recent study, Vimala and Poonghuzhali [48] found that hexane extract of *Hydroclathrus clathratus* (used at 5 mg/mL) exhibited a strong antifungal activity against *F. oxysporum* compared to methanol, ethyl acetate and aqueous extracts. Phytochemical screening of hexane extract showed presence of flavonoids, tannins, phenols, terpenoids, coumarins and others [48].

Variation in the production of secondary metabolites may be the cause of ecological parameters induced by climatic factors such as, temperature, salinity, light, dissolved oxygen and nutrients or related to the biology and physiology of the algae itself [49]. In fact, light, temperature, mineral salts and water movement are the key environmental parameters in determining the fertility of the algae. Light and temperature are the main causes of seasonal variation and spatial algal flora distribution. They have influence on the growth of algae and on their morphological characteristics [49]. In addition, Lima-Filho et al. [33] reported that this important variation was due to differences in species used, time and place of sample collection, differences in extraction protocols, solubility of bioactive metabolites (which can be soluble in one solvent but not soluble in the others) and also, the variable susceptibilities of targeted fungal pathogens and tested concentrations [24,33].

Numerous studies confirmed that seaweeds can be considered as a source of bioactive compounds with several biological activities. Production of terpenes and phenols by some seaweed species has the potential to inhibit the growth of various fungal plant pathogens [50]. These bioactive polyphenol compounds, acting singly or in combination, interfere with the life process of fungi by binding their protein molecules, acting as chelating agents, altering structural component synthesis, weakening or destroying the permeability barrier of the cell membrane and changing the physiological status of cells. Ara et al. [41] found that fractions containing fatty acid esters from an extract of *Spatoglossum asperum* are able to inhibit the growth of *M. phaseolina*, *R. solani* and *F. solani*. Chanthini et al. [51] correlated the direct antifungal activity displayed by some red and green algae against *Alternaria solani* with their phenolic content. Also, Ammar et al. [24] showed the presence of phenolic acids and flavonoid compounds in the methanolic extracts of *S. vulgare* using HPLC-DAD chemical profiling which showed a strong antifungal potential against *P. aphanidermatum* infecting potato tubers.

Conclusion

This research revealed for the first time the *in vitro* and *in vivo* inhibitory activity of aqueous and organic extracts from *S. vulgare* collected from Tunisian coastal sites, against FOT causing potato dry rot. This study is an additional demonstration of the ability of *S. vulgare* to synthesize antifungal compounds active against this pathogen. Further research is necessary for successful characterization of biologically active compounds using various chemical analysis techniques. The importance of this work is high as it is the first report on the ability of seaweed extracts to suppress severity of this economically important potato disease and probably other potato pathogens.

S. vulgare extracts, selected based on their ability to suppress Fusarium dry rot disease, will be further evaluated for their wilt-suppressive potential on inoculated potato plants.

Acknowledgement

This work was funded by the Ministry of Higher Education and Scientific Research in Tunisia through the budget assigned to UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, The Regional Center of Research on Horticulture and Organic Agriculture of Chott-Mariem, University of Sousse, Tunisia

Authors would like to thank all technical staff of the laboratory of Phytopathology of the Regional Center of Research on Horticulture and Organic Agriculture of Chott-Mariem, Tunisia, for their valuable technical assistance.

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This article was originally published in a special issue, **Advances in Microbiology and Biotechnology** handled by Editor(s). Dr. Gamil Sayed Gamil Zeedan, , National Research Center, Egypt