Physico-Chemical Characterization and Antifungal Activity of Tunisian Marine Macroalgae Against *Botrytis cinerea*

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Challenges in modern agriculture

Global food security is threatened by interesting crises population growth that increase demands on agriculture systems , climate change and crop losses caused by phytosanitary problems.

Growing Population

The demographic growth implies a sharp rise in food demand.

Food production is estimated to increase by around 70 % by 2050 to feed the global population (FAO, 2025

Climate change

To meet this rising demand; agricultural systems have increasingly shifted toward industrialized production.

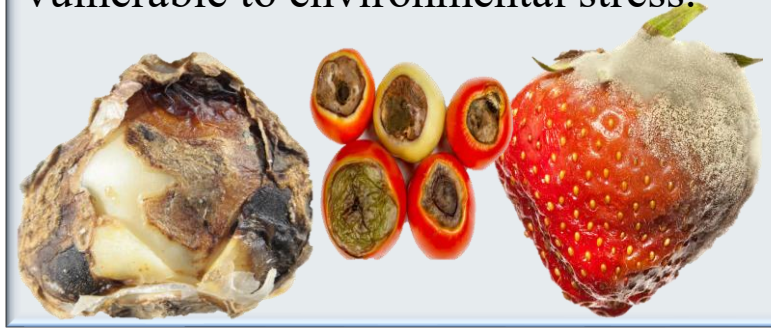
Rapid Food production

Environnemental Problems

Crop losses

Intensifying phytosanitary problems by altering temperature ,humidity, and weather extremes.

Increase crop losses and disrupt production systems that are already vulnerable to environmental stress.



Botrytis : The Gray Mold Threat

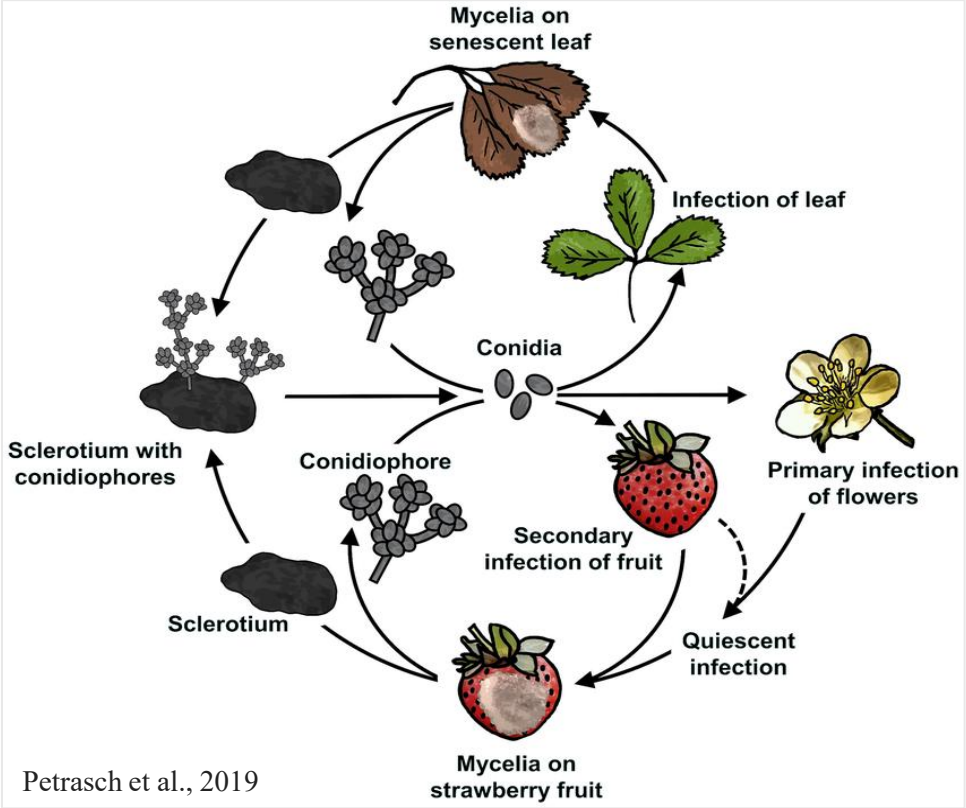
Definition : ***Botrytis cinerea***, commonly known as **gray mold**, is a widespread fungal plant pathogen

Plant Host : Grapes, Strawberries, Tomatoes, Lettuce Ornamental flowers (e.g., roses), Berry bushes (raspberry, blueberry), Onion

Postharvest Decay : Infections may remain latent in the field without visible symptoms. Symptoms develop after harvest during Storage, Transportation.

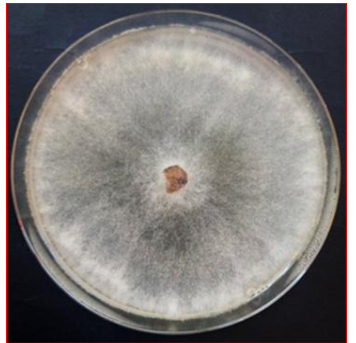
Losses in Agriculture : The infections caused by the fungal pathogen can vary, but it has been estimated that they cause between 10 and 70% of the losses pre- and postharvest (Orozco-Mosqueda et al. 2023)

Life cycle of Botrytis



Petrasch et al., 2019

Macroscopic observation



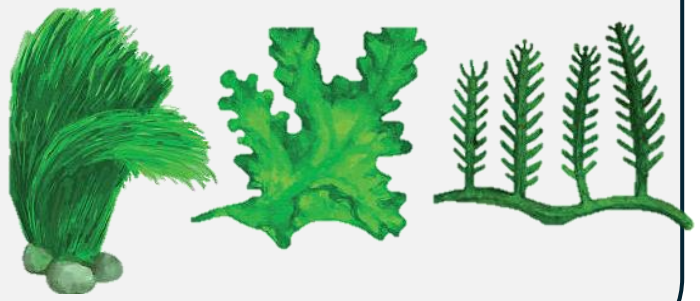
Microscopic observation

Classification of macroalgae and their main characteristic

Rhodophyta
(Red Algae)

Phaeophyta
(Brown Algae)

Clorophyta
(Green Algae)



Agriculture role

- Biostimulnant
- Biofertilizer
- Control agent

Producing valuable compounds

- Rich in vitamin and minerals
- Sources of agar and carrageenan

Uses in agriculture

- Liquid Extract
- Organic Extract
- Compost

Results

Sampling of seaweeds from the Tunisian coast

Algae collected from the coast of Tunisia				
Latin name	*Hypnea muciformis*	*Hypnea muciformis*	*Cladophora* sp.	*Ulva* sp.
Classification group	*Red Algae*	*Red Algae*	*Green Algae*	*Green Algae*
Region	*Mahdia*	*Sfax*	*Mahdia*	*Sfax*
Geograohic coordinates	35°09'17"N 11°03'18"E	34°45'30"N 11°13'39"E	35°09'17"N 11°03'18"E	34°31'20"N 10°30'15"E
Date of collect	13 May 2024	11 June 2024	13 May 2024	24 May 2024

Physicochemical Characterization of algal powder

Algae /Parametre	Color of the powder				
	L*(Lightness)	a*(Green ↔ Red	b*(Blue ↔ Yellow	C* (Chroma)	h*((Hue angle):
Ulva sp.	57.98±22.90a	1.66±0.06c	14.52±0.16a	14.61±0.16a	83.48±0.18b
Cladophora sp.	61.57±0.07a	3.92±0.006a	12.45±0.01b	12.61±0.15b	93.53±0.51a
Hypnea muciformis Sfax	55.98±0.13a	2.50±0.13b	14.40±0.14a	14.92±0.01a	74.76±0.03d
Hypnea muciformis Mahdia	60.89±0.43a	-0.88±0.113d	10.37±0.39c	10.67±0,39c	76.44±0.25c

-The colorimetric analysis showed that all algal powders had similar lightness (L*), indicating comparable brightness

-*Cladophora* sp. exhibited the highest a* and h° values, reflecting a strong green coloration

-*Ulva* sp. and *Hypnea muciformis* from Sfax showed higher b* and C* values, indicating more yellowish and saturated colors

-*Hypnea muciformis* from Mahdia presented lower a*, b*, and C* values, suggesting a less intense and duller coloration

These variations are likely related to differences in pigment composition and geographical origin

Physicochemical Characterization of algal powder

Algae /Parametre	Water activity	Dry matter content	Ash content (%MS)	Protein (%)
Ulva sp.	0.43±0.020a	91.1±4.53a	26.96± 5.75b	8.16±0.22b
Cladophora sp.	0.45±0.003a	88.96±0.95a	25.36±1.15b	8.386±0.24b
Hypnea muciformis Sfax	0.395±0.014a	88.73± 0.42a	28.3±0.9a	6.83±0.22c
Hypnea muciformis Mahdia	0.42 ± 0.02a	89.7±0.53a	29.33±0.71a	10.66±0.22a

All algal powders exhibited similar **water activity** values (0.39–0.45)

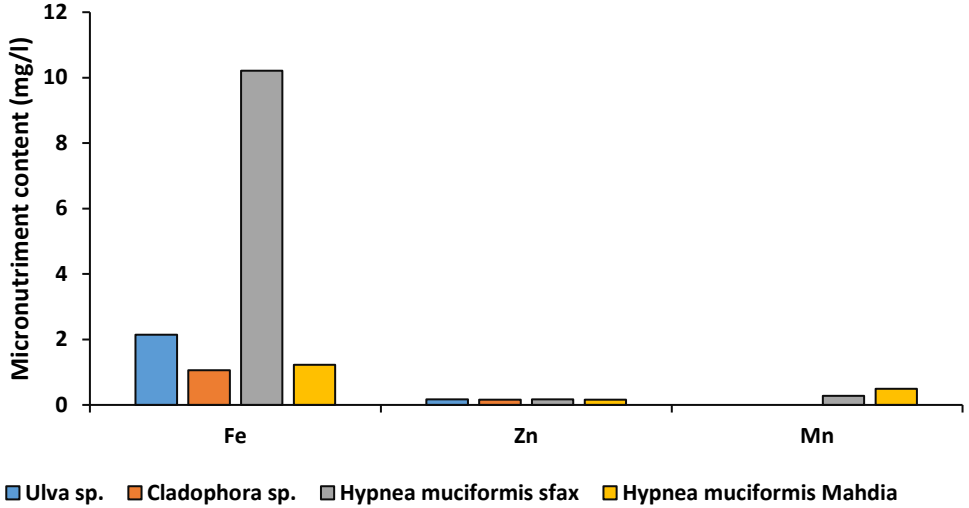
The **dry matter content** was high for all samples (88.73–91.10%)

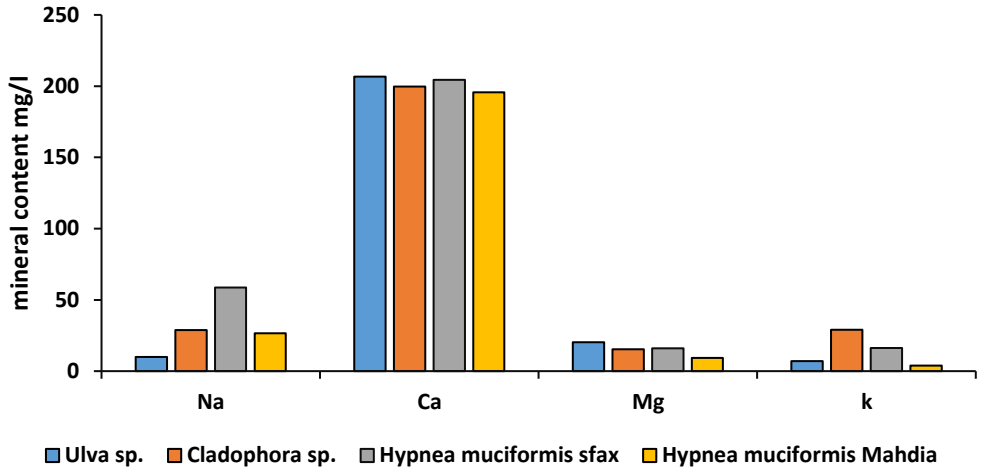
Ash content: both *Hypnea muciformis* presented the highest values (28–29%), indicating a high mineral content, while *Cladophora* sp. and *Ulva* sp. samples showed lower ash levels

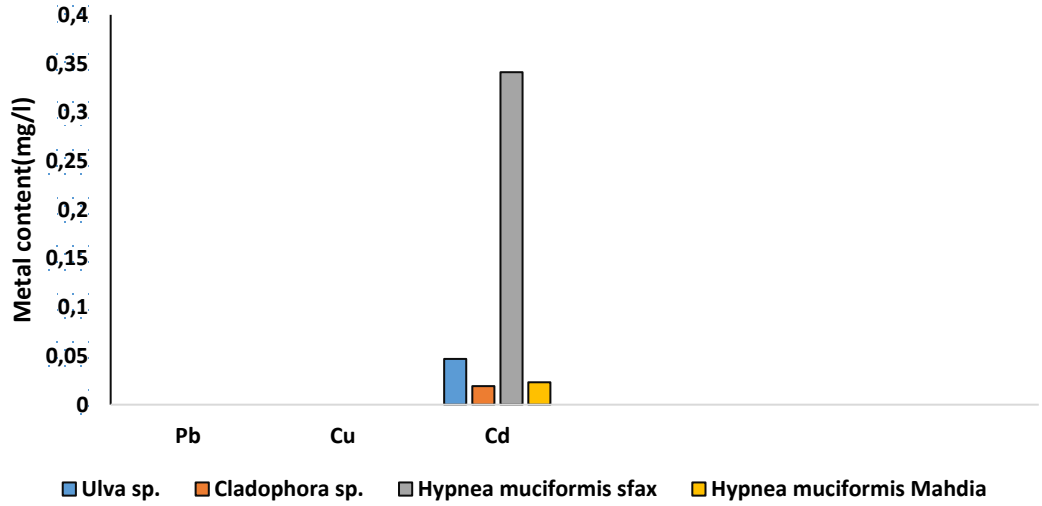
Protein content: all algae can be considered protein-rich, with Hypnea muciformis from Mahdia exhibiting the highest protein percentage, whereas Hypnea muciformis from Sfax showed the lowest value.

Physicochemical Characterization of algal powder

Mineral composition by atomic absorption







The results showed that the algae were rich in essential micronutrients (Fe, Zn, and Mn) as well as essential macronutrients (Na, Ca, Mg, and K). In contrast, no heavy metals such as Pb, Cu, or Cd were detected in the samples. These findings indicate that the algae have a high nutritional mineral value and are free from heavy metal contamination.

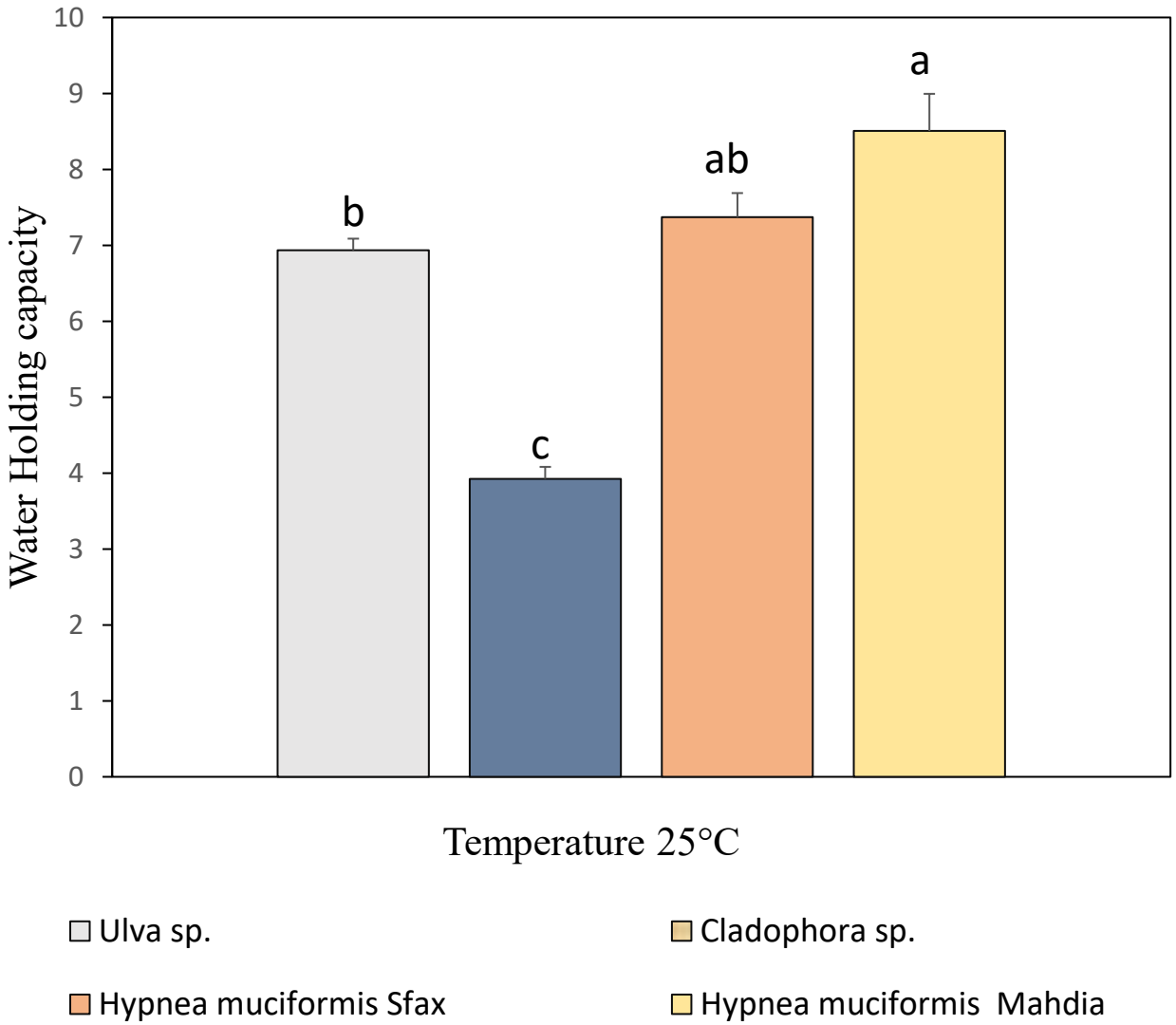
Physicochemical Characterization of algae powder

Fatty acid	*Ulva* sp.	*Cladophora* sp.	*Hypnea muciformis* Sfax	*Hypnea muciformis* Mahdia
Tetradecanoïc Acid (C14:0)	1.44±0.08d	1.96±0.053c	3.726±0.037b	3.283±0.021a
Myristoleic Acid (C14 :1)	0.840±0.04a	0.603±0.021b	0.943±0.051a	0.863±0.021a
Palmitic Acid (C16 :0)	39.790±0.212a	37.590±0.119b	35.473±0.084c	36.160±0.163d
Palmitoleic acid(C16 :1)	6.873±0.159b	7.210±0.102b	6,106±0.042a	8.070±0.085c
Stearic acid (C18 :0)	1.040±0.073b	1.200±0.050ab	1.056±0.04b	1.227±0.021a
Oleic Acid (c18 :1)	42.053±0.071a	41.213±0.121b	42.043±0.042a	39.747±0.151c
Linoleic acid(c18 :2)	3.180±0.022c	4.560±0.121a	3.486±0.035b	2.977±0.093d
Linolenic acid (c18 :3	0.703±0.041c	0.923±0.031b	1.033±0.055b	1.290±0.045a
Arachidic acid(C20 :0)	0.910±0.050c	1.173±0.021b	1.94±0.06a	2.083±0.024a
Eicosenoic acid(C20 :1)	1.697±0.041c	1.953±0.060b	2.1666± 0.057a	2.313± 0.058a
Behenic acid C22:0	1.473±0.039c	1.617±0.062b	2.023±0.031a	1.987±0.033a

Fatty acids are analyzed after lipid extraction (soxhlet) followed by transesterification to fatty acid methyl esters (FAMEs): the four algae are rich in Palmitic acid and Oleic acid

Functional proprieties of algal powder

Water Holding Capacity (WHC)

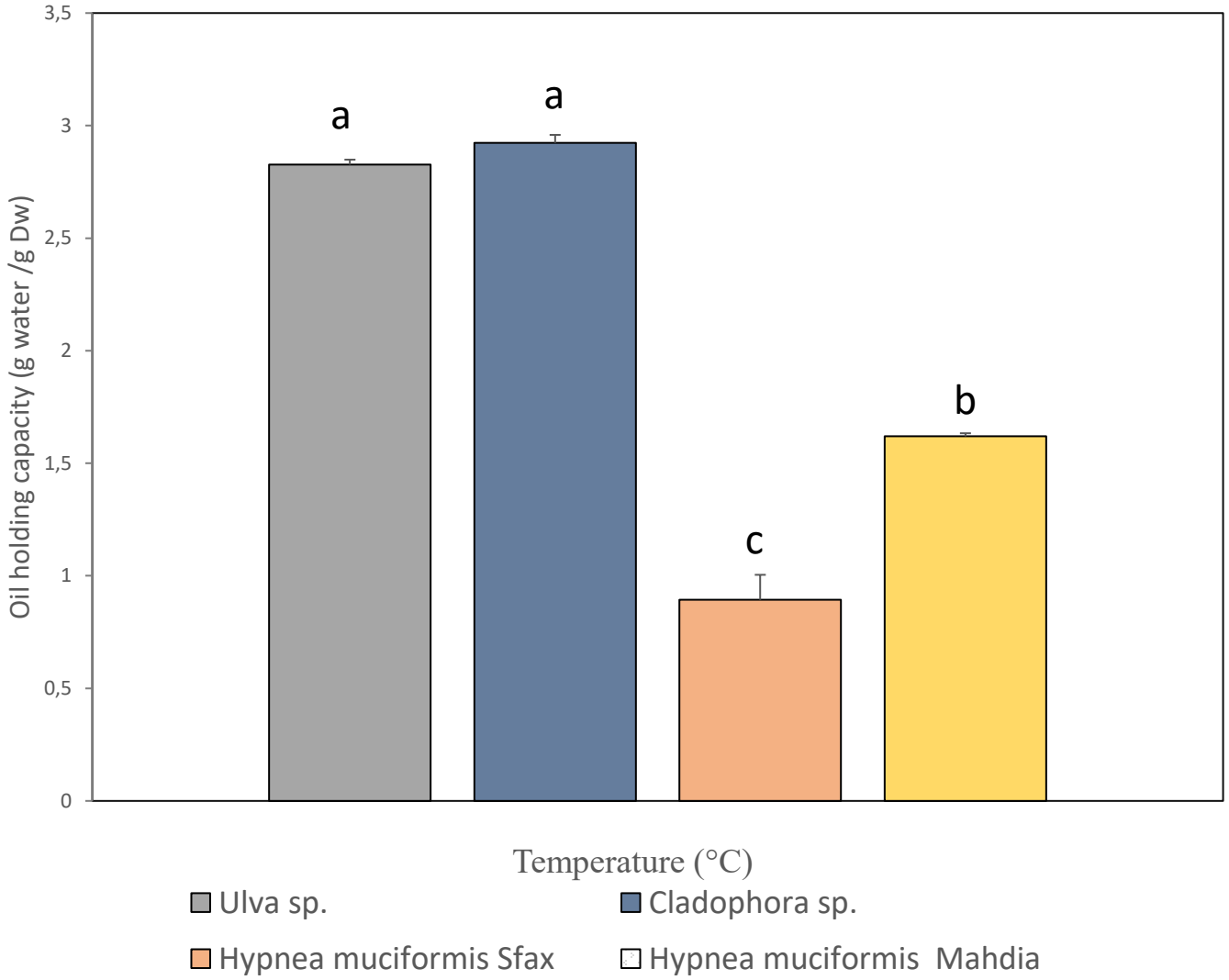


Water Holding Capacity (WHC) is the ability of a substance to retain water in its structure

The highest water-holding capacity was observed for *Hypnea muciformis* from Mahdia, while *Cladophora* sp. exhibited the lowest water-holding capacity among the tested algae.

Functional proprieties of algal powder

Oil holding capacity



Oil holding capacity (OHC) revealed the capacity of algae to interact with lipids

The oil-holding capacity was higher in *Cladophora* sp. and *Ulva* sp. compared to the other tested algae.

Mycelial Growth (cm)	
Treatment	***Botrytis cinerea***
Control	**8.18 ± 0.25 a**
***Ulva* sp. 0.5%**	**7.50 ± 0.11 bc**
***Ulva* sp. 1%**	**7.02 ± 0.031 cd**
***Ulva* sp. 2%**	**6.35 ± 0.20 e**
***Ulva* sp. 5%**	**7.39 ± 0.17 c**
***Cladophora* sp. 0.5%**	**8.10 ± 0.16 ab**
***Cladophora* sp. 1%**	**7.40 ± 0.36 c**
***Cladophora* sp. 2%**	**7.24 ± 0.20 c**
***Cladophora* sp. 5%**	**7.04 ± 0.08 cd**
***Cladophora* sp. 10%**	**5.12 ± 0.32 f**
***Hypnea muciformis* /Sfax 0.5%**	**5.22 ± 0.22 f**
***Hypnea* muciformis /Sfax 1%**	**5.12 ± 0.15 f**
***Hypnea* muciformis/Sfax 2%**	**4.22 ± 0.22 g**
***Hypnea muciformis* /Sfax 5%**	**3.72 ± 0.22 g**
***Hypnea muciformis* /sfax 10%**	**1.65 ± 0.18 i**
***Hypnea muciformis* / Mahdia 0.5%**	**6.51 ± 0.21 de**
***Hypnea muciformis*/ Mahdia 1%**	**5.00 ± 0.24 f**
***Hypnea* muciformis /Mahdia 2%**	**4.19 ± 0.14 g**
***Hypnea* muciformis /Mahdia 5%**	**2.62 ± 0.34 h**

Direct Contact of the product(Hot Extract)

In the direct contact assay against *Botrytis cinerea,* the algal extracts exhibited a dose-dependent inhibitory effect. Among the tested extracts, *Ulva* showed the highest inhibition at 2%, *Cladophora* sp. at 10%, *Hypnea muciformis* at 10%, and *Hypnea Mahdia* at 5%. Notably, *Hypnea Mahdia* and *Hypnea Sfax* demonstrated the highest levels of inhibition against *B. cinerea* overall.

Mycelial Growth (cm)	
Treatment	***Botrytis cinerea***
control	6.00 ± 0.00 a
Ulva sp. 0.25%	1,56 ± 0.21 efg
Ulva sp. 0,5%	1.26 ± 0.10 g
Ulva sp. 1%	1.43 ± 0.30 fg
Ulva sp. 2%	0.00 ± 0.00 h
Ulva sp. 5%	0.00 ± 0.00 h
Cladophora sp. 0.25%	2.38 ± 0.09 b
Cladophora sp. 0,5%	2.45 ± 0.33 b
Cladophora sp. 1%	1.99 ± 0.13 cde
Cladophora sp. 2%	1.84 ± 0.12 de
Cladophora .sp 5%	0.00 ± 0.00 h
Hypnea muciformis/ Sfax 0.25%	2.18 ± 0.15 bcd
Hypnea muciformis /Sfax 0,5%	1.84 ± 0.09 de
Hypnea muciformis /Sfax 1%	1.34 ± 0.17 fg
Hypnea muciformis/ Sfax 2%	0.00 ± 0.00 h
Hypnea muciformis/ Sfax 5%	00 .00 ± 00.00 h
Hypnea muciformis /Mahdia 0.25%	2.24 ± 0.18 bc
Hypnea muciformis /Mahdia 0,5%	2.17 ± 0.08 bcd
Hypnea muciformis / Mahdia 1%	1.68 ± 0.16 ef
Hypnea muciformis / Mahdia 2%	00.00 ± 00.00 h
Hypnea muciformis /Mahdia 5%	00.00 ± 00.00 h

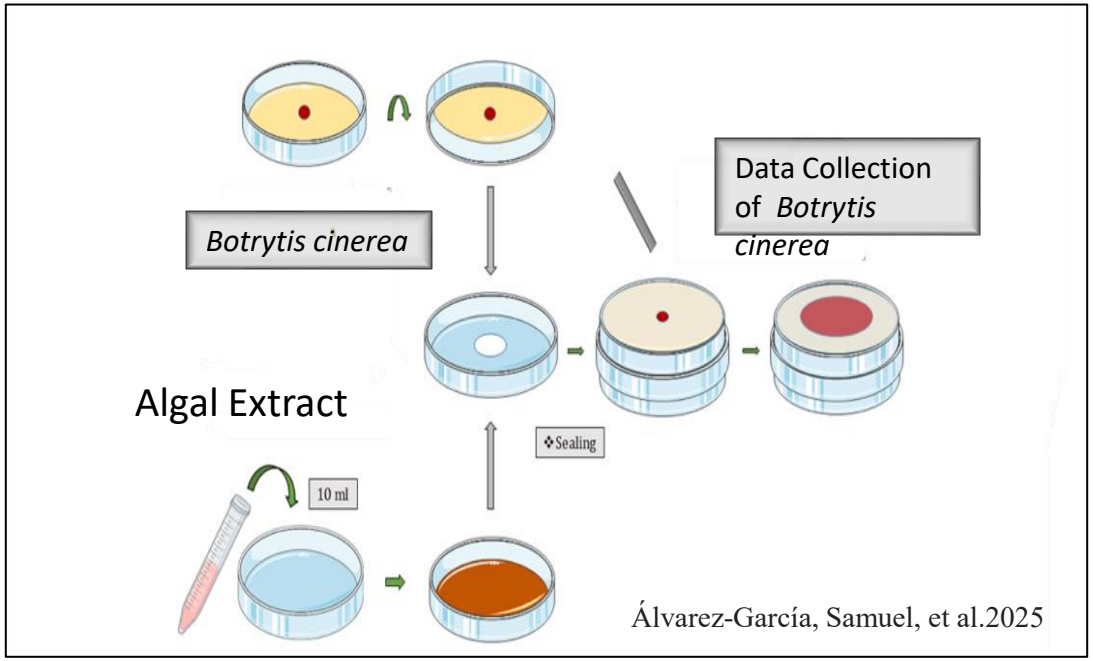
Direct Contact of the product (Cold Extract)

The direct contact assay of the cold extract demonstrated Total inhibition of *Botrytis* growth. Specifically, *Ulva* sp. Showed total inhibition at concentrations of 2% and 5%, while *Cladophora* sp. achieved complete inhibition at 5%. *Hypnea muciformis* collected from Sfax and Mahdia both showed total inhibitory effects at 2% and 5% concentrations.

These results indicate that the cold extracts of these macroalgae possess potent antifungal activity against *Botrytis*.

Mycelial Growth (cm)	
Treatment	***Botrytis cinerea***
control	8.26 ± 0.10 a
Ulva sp. 0.5%	7.24 ± 0.20 ab
Ulva sp. 1%	7.16 ± 0.13 bc
Ulva sp. 2%	6.20 ± 0.36 cdefgh
Ulva sp. 5%	6.12 ± 0.388 defgh
Ulva sp. 10%	6.642 ± 0.353 bcdef
Ulva sp. 20%	5.8 ± 0.44 efjhig
Cladophora sp. 0.5%	7.66 ± 1.11 bcde
Cladophora sp. 1%	6.8 ± 0.24 bcde
Cladophora sp. 2%	6.18 ± 0.288 cdefgh
Cladophora sp. 5%	5.592 ± 0.288
Cladophora sp. 10%	6.1 ± 0.48 bcdefg
Cladophora sp. 20%	6.702 ± 0.32 bcdef
Hypnea muciformis/ Sfax 0.5%	7.04 ± 0.048 bcd
Hypnea mucifoormis /Sfax 1%	5.75 ± 0.6 fghij
Hypnea muciformis /Sfax 2%	5.9 ± 0.48 efghi
Hypnea muciformis/ Sfax 5%	6.44 ± 0.288 bcdefg
Hypnea muciformis/ Sfax 10%	5.66 ± 0.48 defgh
Hypnea muciformis /Sfax 20%	6.06 ± 0.37 efhij
Hypnea muciformis /Mahdia 0.5%	5.36 ± 0.312 hij
Hypnea muciformis /Mahdia 1%	5.26 ± 0.168 hij
Hypnea muciformis / Mahdia 2%	5.78 ± 0.384 efghig
Hypnea muciformis / Mahdia 5%	5.062 ± 0.0456 ij
Hypnea muciformis /Mahdia 10%	5.402 ± 0.3984 hij
Hypnea muciformis / Mahdia 20%	4.842 ± 0.530 j

Volatile Organic compound of the product



Volatile organic compounds (VOCs) produced from the cold algal extracts exhibited antifungal activity against ***Botrytis cinerea*****.** The inhibitory effect increased with algal concentration, **indicating a concentration-dependent response**. Among the tested concentrations, *Ulva* sp., *Cladophora* sp., *Hypnea muciformis* from Mahdia exhibited maximal inhibition at 20%, and *Hypnea muciformis* from Sfax at 10%. These findings suggest that VOCs from these macroalgae possess significant potential as antifungal agents.

Conclusion

Summary:

- **The studied algae are rich in proteins, minerals, and essential fatty acids, making them valuable as natural fertilizers and growth enhancers in agriculture.**
- **Color and composition variations reflect species and geographical origin.**
- **All samples are free from heavy metals, making them safe and beneficial for agricultural use.**
- **They exhibit high water- and oil-holding capacities, supporting food formulation potential.**
- **Cold and hot extracts: show an effect on Botrytis growth depending on the concentration**
- **Volatile compounds also produced by the algae, show an inhibitory effect against *Botrytis cinerea***


Thank you for your attention