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# First assessment of the anti-phytopathogenic activity of two *Cladophora* species against *Fusarium oxysporum f.sp. albedinis*

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**ABSTRACT**

The anti-phytopathogenic of two macroalgae (*Cladophora glomerata* and *Cladophora albida),* collected from Moroccan water bodies, were investigated for their potential to control *Fusarium oxysporum f.sp. albedinis* (Foa), the causal agent of Bayoud on date palm. The disc-diffusion assay was used to assess the antifungal potential of the extracts against Foa. The obtained results showed that the extracts of both *C. glomerata* and *C. albida* exhibited high activities with (AI)= 94 and 89, respectively. This work is the first report of the potentially antifungal activity of these two macroalgae against Foa. They may have a great potential of bioactive metabolites which could be exploited for future as a biofungicide to control Foa.

**Keywords:** *C. glomerata, C. albida, F. oxysporum,* Antifungal potential, biocontrol

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## INTRODUCTION

The date palm (*Phoenix dactylifera L*) is an original domesticated tree in arid oases worldwide. Unfortunately, palm tree is attacked by various pathogens which affect its health, yield and its fruits quality [1]. The Bayoud, caused by *F. oxysporum f. sp. albedinis* (Foa), is the most destructive fungal disease of date palm (*Phoenix dactylifera L*) worldwide [2, 3]. The impact of this disease is most severe in North Africa particularly in Morocco where two-thirds of palm plantations (more than 10 million trees) were destroyed, causing considerable, ecological and socioeconomical damages. [4]

Chemical fungicides have been intensively used to control plant diseases [5]. However, they have adverse effects on soil environment, human health and development of pathogen resistance [6]. This has led to a growing need for biological control approaches using microbes and plants (eg; bacteria, actinomycetes, medicinal herbs) as an emerged safety and ecofriendly alternatives [7].

Macroalgae were well known as producer of a wide variety of bioactive compounds, which include lipopeptides, fatty acids, terpenes and others [8]. Therefore, they have demonstrated antibacterial, antiviral, anti-inflammatory, antifungal activities [9, 10]. Various genera such as Gracilaria, Caulerpa, Codium, Sargassum, Enteromorpha, Ulva have shown positive bioactivity [11].The genus Cladophora which occur in water biotopes have known by bioactive production [12]. Despite, the use of macroalgae in the biological control of plants infected by fungal pathogens remain limited [13, 14, 15]. However, there is no work in the world on the use of *Cladophora* species to control Foa.

Hence, this study deals on the assessment of the anti-phytopathogenic activity of *C. glomerata and Cladophora albida* extracts against Foa. This work is the first report of the antifungal activity of these two macroalgae on Foa.

## MATERIALS AND METHODS

### *Biological materials*

Algal samples of *C. glomerata* and *C. albida* were collected from water channel at Haouz region, and Agadir sea beach (Morocco) in may 2016, respectively. The fungal strains used in bioassay was Foa. The strain was isolated from the rachis of diseased palms detached from date palm tree presenting symptomatic attacks by the bayoud. The isolated strain was conserved in the FPS collection under FOAP1 code number. Before bioassay test, Foa strain was grown in Sabouraud agar medium at 27°C for 72 h.

### *Extract preparation*

Fresh biomasses were washed in the sea water to remove epiphytic microbes. Then, they were rinsed in distilled water. The ethanol extraction was prepared according to the method described by Chowdhury et al. [16], and slightly modified. Briefly, 10 g of each dried and powdered biomass were extracted with ethanol (75%) under agitation for two overnight at 25°C in a dark house. The obtained solutions were filtered through glass fiber papers, and then centrifuged at 5000×g /15 min. The filtrate was concentrated using a rotary evaporator. The dried precipitate was re-dissolved in DMSO (1%) to give 50 mg/mL extracts and preserved as stock solution at 4°C for further use.

### *Antifungal bioassay*

The stock solution was sterilized using glass fiber papers (Whatman GF/C, 0.22 µm pore size). 6 concentrations (0, 3.125, 6.25, 12.5, 25, 50 mg /ml) of both algal biomasses extracts were prepared.

Antifungal activity was assessed using the disc diffusion technique. Briefly, sterile filter paper discs, 6 mm in diameter, were loaded with 20µL of the different prepared concentrations and were air dried. Discs containing synthetic fungicide were used as positive control. The discs were placed on Sabouraud agar plates inoculated with 100 µL of the prepared Foa suspension. Plates were incubated at 27 °C for a period of 48-72 hrs, and the inhibition zones were measured (mm diameter). All tests were performed in six repetitions. The

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antifungal activity was expressed in terms of antimicrobial index [17] and calculated according to the following formula:

Antimicrobial Index (AI) = (Inhibition zone of sample/ Inhibition zone of the standard) x 100

***Statistical analysis***

The one way analysis of variances (ANOVA) with the Tukey´s test were used, using SPSS 10.0 Windows 2007, to asses significant differences between exposure concentrations and control test at p = 0.05.

## RESULTS AND DISCUSSION

The main purpose of this work was to assess the antifungal activity of ethanol extracts of *C. glomerata* and *C. albida* against Foa. by agar well diffusion method. As shown in Table 1 and Fig 1, both the macroalgae extracts act negatively and exhibit a significant antifungal effect on *Foa.*

**Table 1: Antifungal activity of *C. glomerata* and *C. albida* extracts on *FOA* .**

| Concentrations (mg/ml) | *C. glomerata* | | *C. albida* | |
|---|---|---|---|---|
| | ZI (mm) | AI | ZI (mm) | AI |
| 50 | 12,82 ± 0,39 | 94 | 12,10 ± 0,53 | 89 |
| 25 | 12,54± 0,3 | 92 | 11,85 ± 0,26 | 87 |
| 12,5 | 11,87 ± 0,2 | 87 | 11,40 ± 0,14 | 83 |
| 6,25 | 11,07 ± 0,13 | 81 | 10,79 ± 0,23 | 79 |
| 3,125 | 9,75 ± 0,3 | 71 | 9,83 ± 0,29 | 72 |
| Control + | 13,67 ± 0,33 | - | 13,67 ± 0,33 | - |
| Control - (DW) | 0 | - | 0 | - |

ZI were expressed as mean ± standard deviation of six replicates; DW : sterilized distillated water

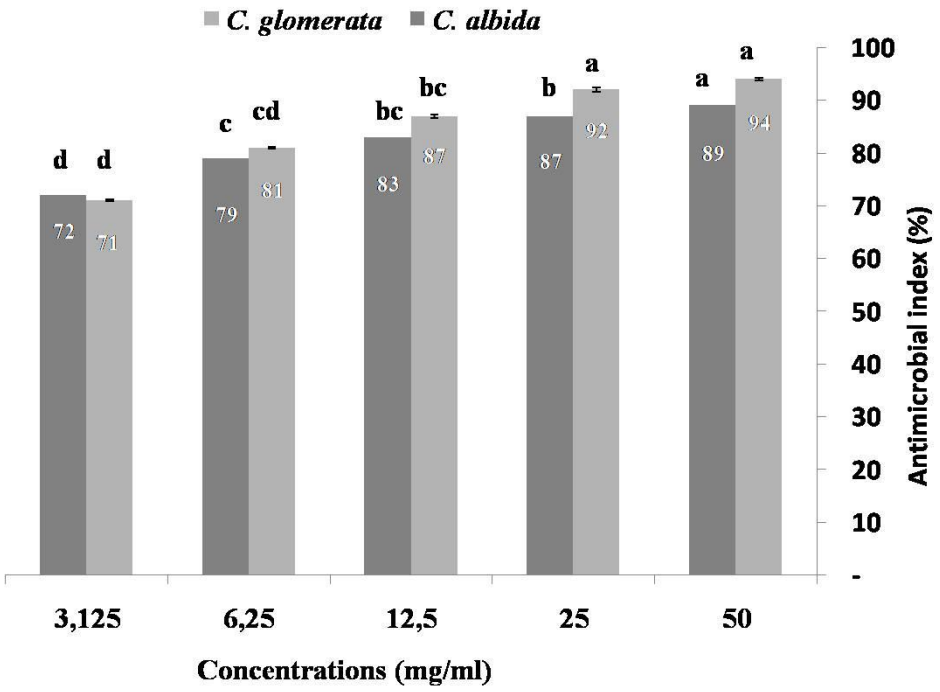


**Fig 1: Antimicrobial index of *C. glomerata* and *C. albida* extracts on mycelium growth of *Foa* . (n=6, the data are the mean, and the error bar indicates the SD of the replicates. Different lowercase letters indicate significant differences (p 0.05).**

For both the two macroalgae extracts, the zone of inhibitions (ZI) were ranged from 9.75 ± 0.3mm to 12.82 ± 0.39mm. They exceeded 9 mm at 3.25 mg/ml concentration. Besides, the maximums ZI were exceeded 12 mm at 50 mg/ml concentrations (Table 1). Moreover, these results were confirmed by high AI (more than 79 % at the concentration (6,25 mg/ml)). The effective and significant concentrations were ranged between (3.125-50 mg/ml) (Table 1, Fig 1). Furthermore, it was observed that *C. glomerata* appears slightly more inhibitor than *C. albida*, especially under the three high concentrations.

From the obtained results, it was found that the extracts of both *C. glomerata* and *C. albida* on Foa showed a considerable antifungal activity. Although other biocontrol approaches on Foa have used antagonistic bacteria [3, 4] there is no work has been done until now to demonstrate the antiactivity of macroalgae on Foa. So, our results will be original for further studies on biocontrol of Foa.

Nevertheless, previous studies using ethanolic extracts of *Cladophora* species against others *fusarium* strains have been carried out: *Cladophora albida* [13], *Cladophora fracta* [18], *Cladophora Crispata* [15], *Cladophora callicoma* [19].

In order to explain the antifungal inhibition mechanism, it is well known that macroalgae produce various bioactive metabolites. Phytochemical characterizations have demonstrated that the main allele chemicals of *Cladofora* species are Sterols, terpenoids fatty acids, tannins and others polyphenols [12, 20, 21]. This diversity in allelochemicals products would be responsible for the demonstrated antifungal effects of *C. glomerata* and *C. albida* assessed in this study.

## CONCLUSION

The obtained results in this work confirmed that the two macroalgae *C. glomerata* and *C. albida* demonstrated an anti-phytopathogenic effect against Foa. This work adds an original data for further studies on biocontrol of Foa. Further profound studies should be done in order to identify the allelochemicals that may act as an effective natural biofungicide.

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