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Antifungal potential of two *Cladophora* species (Green algae) against *Verticilium dalhiae Kleb.*

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ABSTRACT

Cladophora glomerata and *Cladophora albida*, two green macroalgae were collected from Moroccan water bodies. Its antifungal activities were assessed against *Verticilium dalhiae* Kleb. by using disc-diffusion method. The results showed a significant antifungal activity of both *C. glomerata* and *C. albida*. This work is the first report of the potentially antifungal activity of these two macroalgae against *V. dalhiae* isolated in Moroccan olive soils. The two studied macroalgae may constitute promising sources of bioactive metabolites that can be exploited as a biofungicide to control *V. dalhiae*.

Keywords: *C. glomerata, C. albida, V. dalhiae,* antifungal potential, olive, biocontrol

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INTRODUCTION

The olive (*Olea europaea* L.) is an important old domesticated tree. It has long been considered as the most typical Mediterranean tree. 98% of its global cultivation area was located in the Mediterranean basin allowing the production of 17.3 million tons of olives [1, 2]. Morocco, a Mediterranean country, known as one of the major olives producer and exporter worldwide. Unfortunately, olive tree is largely attacked by several varieties of pathogens which affect its health, yields and its oil quality [3]. The pathogenic fungi *V. dalhiae* causes defoliation and wilting of olive trees and death of young trees [4]. This pathogen infects also more than 160 plants species [5].

Synthetic Chemical Fungicides have been intensively used to control plant diseases [6]. Therefore, they have adverse effects on soil environment and human health and promote the development of pathogen resistance [7]. These last years, bioactive metabolites extracted from microbes and plants (eg; bacteria, actinomycetes, medicinal herbs) have emerged as safety and ecofriendly alternatives to synthetic fungicides [6].

Several macroalgae have demonstrated antibacterial, antiviral, anti-inflammatory, antifungal activities [8, 9]. These important potential bioactivities were demonstrated by various species of the genera *Gelidium, Gracilaria, Codium, Sargassum, Enteromorpha* [10, 11]. They produce a wide variety of bioactive compounds, which include lipopeptides, fatty acids, macrolides, amides, sesquiterpenes, terpenes and others that are involved in the antifungal activity against pathogens [11, 12]. The use of these types of organisms may constitute a real alternative control solution. Among these macroalgae, the species of the genus *Cladophora* which occur both in marine and in freshwater biotopes have shown an important potential of bioactive products [13]. However, there are few reports in the world on the use of *Cladophora* species to control *V. dalhiae* [14, 15, 16].

The aim of this study was to assess the antifungal activity of extracts of two macroalgae *C. glomerata and C. albida* against *V. dalhiae*. To our knowledge, this work is the first report in Morocco of the antifungal activity of these two macroalgae on *V. dalhiae*.

MATERIALS AND METHODS

Algal and fungal materials

Algal samples of *C. glomerata* and *C. albida* were collected in may 2016 from water channel at Haouz region, and Agadir sea beach (Morocco), respectively. The fungal species used in test was *V. dalhiae Kleb.* The strain was isolated from roots of the olive plant attacked by the verticiliose and conserved in the Polydisciplinary Faculty of Safi collection under VDK1 code number. Befor bioassay test, *V. dahliae* strain was grown in Sabouraud agar medium at 27°C for 72 h.

Extraction of algal biomass

Fresh biomasses were washed in the sea water to remove debris and epiphytic microbes, and then were rinsed several times in distilled water. The ethanol extraction was prepared according to the method described by Chowdhury et al. [17], 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 place.

The solutions were filtered through glass fiber papers, and then centrifuged at 5000×g at 15 min. The filtrate was concentrated using a rotary evaporator. Then, 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 assay

For concentrations preparation, 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.

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Antifungal activity was assessed using the disc diffusion technique in petri dishes. 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 *V. dalhiae* suspension. Plates were incubated at 27 °C for a period of 48-72 hrs, and the zones of inhibition that formed around the discs were measured (mm diameter). All the tests were performed in six repetitions. The antifungal activity was expressed in terms of antimicrobial index [18] and calculated according to the following formula:

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

Statistical analysis

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

RESULTS AND DISCUSSION

The antifungal activity of ethanol extracts of *C. glomerata* and *C. albida* collected from fresh and marine waters were assessed by agar well diffusion method. As shown in Table 1 and Fig 1, the extracts exhibited a significant antifungal effect on *V. dalhiae*. It shows that all tested concentrations exhibited strong inhibition against *V. dalhiae*.

Table 1. Antifungal activity of *C. glomerata* and *C. albida* extracts on *V. dalhae* .

Concentrations (mg/ml)	*C. glomerata*		*C. albida*	
	ZI (mm)	AI	ZI (mm)	AI
50	12,42 ± 0,33	93	12,82 ± 0,39	84
25	12,61± 0,4	96	12,54 ± 0,3	94
12,5	11,92 ± 0,25	91	11,87 ± 0,2	92
6,25	11,53 ± 0,56	88	11,07 ± 0,13	87
3,125	9,18 ± 0,26	70	9,75 ± 0,3	71
Control +	13,13 ± 0,35	-	13,67 ± 0,33	-
Control - (DW)	0	-	0	-

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

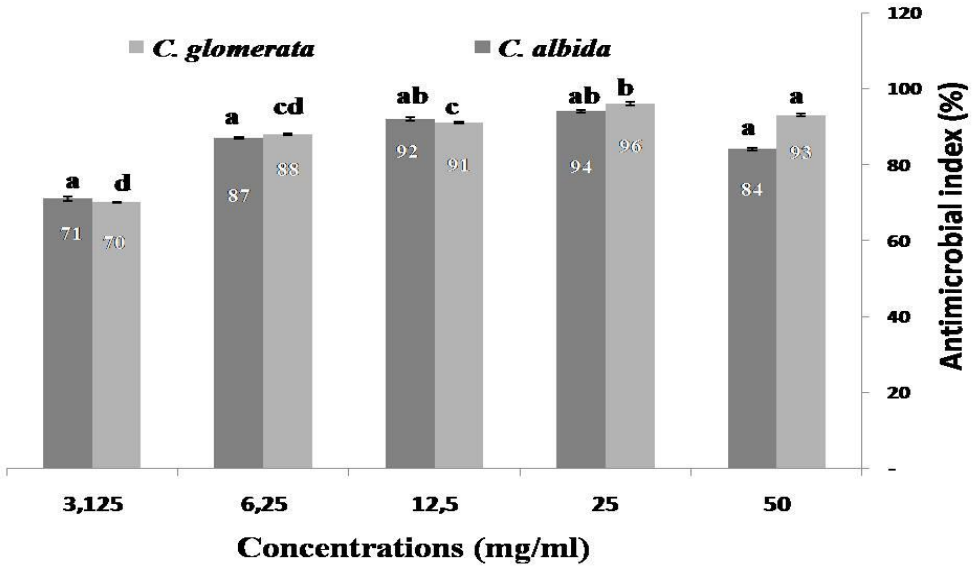

Fig. 1. Antimicrobial index of *C. glomerata* and *C. albida* extracts on mycelium growth of *V. dalhae.* (n=6, the data are the mean, and the error bar indicates the SD of the replicates. Different lowercase letters indicate significant differences (p 0.05).

Compared to the positive control, the zones of inhibition (ZI) were exceeded 9 mm only at 3.25 mg/ml concentration for both the two algae extracts. Thus, the maximums ZI were exceeded 12 mm at 50 mg/ml concentrations (Table 1). Moreover, these results were translated and supported by high AI which the ratios were exceeded 80 % at the concentration (6,25 mg/ml). The effective concentrations were ranged from (6.25-50 mg/ml (Table 1, Fig 1). Furthermore, it was observed that both the two macroalgae show almost similar potential activity on *V. dalhiae* , which *C. glomerata* appears slightly more inhibitor than *C. albida*, especially under the two high concentrations.

The extracts of both *C. glomerata* and *C. albida* on *V. dalhiae* showed an interesting antifungal activity. These results are in accordance with those obtained by several authors that found that ethanolic extracts of *Cladophora* species exhibited strong antifungal activity against fungal strains. Mudassir (1995) [19] demonstrated that *Cladophora fracta* ethanol extracts exhibited strong antifungal activity against ten fungal strains. Also, Ghazal *et al.* [14] confirmed the antifungal effect of ethanol extract of *Cladophora albida* on several fungal species which among them *Verticillium* strains. Also Mahadik & Jadhav [16] demonstrated the antifungal activity of the *Cladophora Crispata* extracts on several pathogenic fungi.

Macroalgae produce a wide variety of bioactive compounds. Previous phytochemical studies have demonstrated that the main allelochemicals of *Cladofora* species are Sterols, terpenoids [20], fatty acids,tannins and others polyphenols [10, 13]. The presence in high concentrations of these specific bioactive products may be responsible on the antifungal potential of both *C. glomerata* and *C. albida* assessed in this study.

CONCLUSION

This study concludes that ethanol extracts of the two macro algae *C. glomerata* and *C. albida* showed potentiality fungicidal activity against *V. dalhiae*. Further researches should be made to identify and purify natural product from these macroalgae against *V. dalhiae* which can act as an effective natural biofungicide.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest. The manuscript is original and is not published or communicated for publication elsewhere either in part or full

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