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Antifungal Activity of Basidiomycete Carpophores Fungi Against Mycotoxigenic Species

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***Abstract:** This study aims the determination of antifungal activity and the flavonoids content of extracts from carpophores of some wild and cultivated fungi.*
The work was conducted on Wild fungi (Fomes fomentarius, Hericiumerinaceus, Schizophyllum commune, Plerotus ostreatus were collected in Tunisia from Ain Drahem (Northern west), Tbainia (Northern west), Kef Rand (Northern east), and Tunis regions. Cultivated mushroom were Pleurotus ostreatus and Lentinus edodes (Shiitake) species from Ain Drahem. Cultivation of these last two species was realized on logs of Populus sp. and Quercus canariensis respectively. The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method.
The antifungal activity was tested against five fungal strains: Alternaria alternate, Penicillium olsonii, Ulocladium atrum, Phytophtora nicotianae, Aspergillus fumigates.
Results showed significant differences between the fungal species and between the two extracts studied. Methanol extracts showed the highest flavonoid amount. The most important value was reached by methanol extract of Wild Plerotus ostreatus (2.3 mg RE/ml).
Aqueous extract of studied mushrooms showed the most important antifungal activities.
Fomes fomentarius Ain Drahem and cultivated P. ostreatus aqueous extracts showed the highest inhibitory rate again Alternaria alternata species (70% and 63.33% respectively).
***Keywords:** Wild fugi, cultivated fungi, flavonoids, phytopathogene species, antifungal activity*

I. INTRODUCTION

Phytopathogenic fungi are species of parasitic micromycetes that cause fungal diseases in plants. Most fungi are saprotrophs. 10.000 fungal species are considered plant pathogens and are the main cause of disease in plants and are responsible for approximately 70% of crop disease [1].

The annual economic losses due to fungal diseases in world agriculture, before and after harvest, were estimated at billions euros, and the annual cost of fungicide treatments amounts to millions euros [2].

In addition the use of chemical fungicides in plant agriculture to control fungal diseases can lead to environmental contamination or may result in fungicide residues on food products [3].

Regarding these high costs, researchers began looking for an alternative by the exploitation of natural substances. The screening of medicinal plants is also another alternative that may produce chemical fungicides that are relatively non-toxic and cost-effective.

In this study, we investigate the antifungal activity and the flavonoids content of extracts from carpophores of some wild and cultivated fungi.

II. MATERIAL AND METHODS

A. Plant Material

Wild fungi (Fomes fomentarius, Hericium erinaceus, Schizophyllum commune, Plerotus ostreatus were collected in Tunisia from Ain Drahem (Northern west), Tbainia (Northern west), Kef Rand (Northern east), and Tunis regions. Cultivated mushroom were Pleurotus ostreatus and Lentinus edodes (Shiitake) species from Ain Drahem. Cultivation of these last two species was realized on logs of Populus sp. and Quercus canariensis respectively. Fruit bodies were cleaned with distilled water then dried at 40°C.

B. Extracts Preparation

20 g of mushroom samples was soaked in 200 ml of solvent (water or methanol 80%) for 24 hours with intermittent shaking. The extracts were filtered through Whatman filter paper into pill vials. The obtained filtrates were used for the experiments.

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C. Total Flavonoids Content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method [4]. 1 ml of diluted sample was mixed with 1 ml of 2% aluminum chloride methanolic solution. The mixture was allowed to stand for 15 min, and absorbance was measured at 430 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg rutin equivalent per mL of juice (mg RE/g).

D. Antifungal Activity

1) *Fungal Strains:* The antifungal activity of carpophores of some wild and cultivated fungi tested against five fungal strains: Alternaria alternate, Penicillium olsonii, Ulocladium atrum, Phytophtora nicotianae and Aspergillus fumigates. Fungal strains were isolated and identified by Dr. Awatef Slama, a mycologist, following conventional mycological methods.
2) *Antifungal test:* The preparation of the PDA medium was performed by adjusting with distilled water the aqueous extract of the broth of 200 g of potato to 1 liter and adding 20 g of agar and 20 g of glucose.

The culture was made on a PDA medium at the rate of 20 ml per Petri dish. 2 ml of juice were introduced into the 20 ml of PDA after having been mixed and homogenized with tween 0.1%. After cooling the medium, a 5 mm diameter disk of each fungal strain was placed in the center of the petri dish while placing the mycelial surface down. The dishes were incubated at 22°C for six days. The fungicidal effect was determined by calculating the growth diameter of the strain in question and comparing it to that of a negative control, i.e. a PDA medium without juice [5].

The results were calculated according to the method of Singh et al. [6] while calculating the percentage inhibition I according to the following formula: $I(\%) = [(dC-dE)/dC] \times 100$

Where: dC: witness diameter (mm)

dE: diameter in the presence of oil tested (mm)

E. Statistical Analysis

The statistical processing of the data was carried out using the SAS GLM (General Linear Models) procedure. An analysis of variance relative to the parameters studied was carried out.

Results are presented as the mean of three replicates ± standard deviation.

III. RESULTS AND DISCUSSION

A. Total Flavonoids Content

Results of total flavonoid content are summarized in table 1.

TABLE1. TOTAL FLAVONOID CONTENT OF CARPOPHORES FUNGI EXTRACTS (MG RE/ML)

Fungal species	Methanol extract	Aqueous extract
Wild Plerotus ostreatus (Kef Rand)	2.30±0.1	1.92±0.01
Schizophyllum commune (Tunis)	1.66±0.2	1.44±0.15
Cultivated P. ostreatus	1.40±0.05	1.26±0.3
Cultivated Lentinus edodes	2.18±0.3	1.86±0.1
Hericium erinaceus Ain Drahem	1.89±0.02	1.64±0.2
Fomes fomentarius kef rand	1.19±0.01	1.06±0.05
Fomes fomentarius Tbainia	1.31±0.2	1.33±0.03
Hericium erinaceus Tbainia	2.10±0.1	1.61±0.12

Significant differences were observed between the fungal species and between the two extracts studied. Methanol extracts showed the highest flavonoid amount. The most important value was reached by methanol extract of Wild Plerotus ostreatus (2.3 mg RE/ml). In the literature it was reported that the extractive capability of flavonoid components from plant material is considerably depended on the type of solvent. The highest yield of flavonoids are generally obtained using methanol [7, 8, 9]. This is in accordance with our findings.

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Villares [10] reported that the total flavonoid content of mushrooms ranges from 0.4 to 17 mg /g DM. This concentration may slightly differ depending on the mushroom species, as well as genetic and environmental factors.

B. Antifungal Activity

Results of antifungal activity are presented in table2.

Globally, aqueous extract of studied mushrooms showed the most important antifungal activities. In fact, the solvent used to extract secondary metabolites with antifungal properties is an important factor and depends on polarity [11]. Differences in inhibition values of different extracts could be related to the difference on interactions type of phenolic compounds with membrane proteins of phytopathogens species and to the responses of these pathogens to phenols and flavonoids contents [12, 13].

Fomes fomentarius Ain Drahem and cultivated P. ostreatus aqueous extracts showed the highest inhibitory rate again Alternaria alternata species (70% and 63.33% respectively) followed by cultivated L. edodes, Fomes fomentarius and Hericium erinaceus Tbainia aqueous extracts. Ulocladium atrum phytopathogen species was mostly inhibited with methanolic extracts of Fomes fomentarius Kef Rand (62.5%), Hericium erinaceus Tbainia (57.81%) and Shizophyllum commune (53.13%). Aqueous extracts of Fomes formentarius Kef Rand and Hericium erinaceus Tbainia also made inhibition of this pathogen growth.

Aqueous extracts were also inhibitorier than methanolic one against Phytophtora nicotiana species with similar inhibition rates (approximately 63%) with all Wild species. Methanol extract has higher inhibition than aqueous one for Fomus fomentarius Kef Rand (63.64 % and 52.17% respectively).

TABLE2. ANTIFUNGAL ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS OF STUDIED MUSHROOM AGAINST PHYTOPATHOGEN FUNGI: A. ALTERNARIA, ULOCLADIUM ATRUM, PHYTOPHTORA NICOTIANA AND A. FUMIGATUS

Tested species	solvent	Alternaria alternata	Ulocladium atrum	Phytophtora nicotianae	Aspergillus fumigatus
Wild P.ostreatus	Methanol	14.49±0.03	31.25±0.33	12.12±0.03	32.69±0.02
	Water	31.11±0.4	28.26±0.05	63.04±0.45	25.93±0.2
Schizophyllum commune	Methanol	18.84±0.02	53.13±0.8	Nd	32.69±0.35
	Water	25.56±0.10	30.43±1.3	63.04±0.50	40.74±0.25
Cultivated P. ostreatus	Methanol	36.23±0.12	23.44±0.03	Nd	11.54±0.05
	Water	63.33±0.30	4.35±0.50	47.83±1.30	31.48±0.06
Cultivated L. edodes	Methanol	37.68±0.33	42.19±0.32	36.36±0.55	19.23±0.33
	Water	51.11±0.05	45.65±1.1	63.04±0.32	22.22±1.2
Hericium erinaceus Ain Drahem	Methanol	33.33±0.5	26.56±0.33	Nd	42.31±1.33
	Water	57.78±0.33	34.78±1.2	71.74±0.33	66.67±0.7
Fomes fomentarius Kef Rand	Methanol	53.62±0.60	62.50±0.4	63.64±1.2	34.62±0.6
	Water	54.44±0.65	58.70±0.8	52.17±0.9	61.11±1.3
Fomes fomentarius Ain Drahem	Methanol	33.33±0.42	51.56±0.05	3.03±0.05	23.08±0.33
	Water	70.00±0.1±0.05	36.96±0.02	63.04±0.02	64.81±1.1
Hericium erinaceus Tbainia	Methanol	17.39±0.01	57.81±0.5	33.33±0.04	15.38±0.8
	Water	56.67±0.50	52.17±1.1	45.65±0.36	16.67±0.7

Nd: non determined activity

Aspergillus fumigatus pathogen fungus was inhibited mostly with Hericum erinaceus Ain Drahem, F. fomentarius Kef Rand and Ain Drahem aqueous extracts (66.67%, 61.11% and 64.81% respectively). All tested extracts showed an important effect against pathogenic studied fungi. Only three methanolic extracts (of S. commune, cultivated P. ostreatus and H. erinaceus Ain Drahem species) were deprived of any registered effect against P. nicotiana species.

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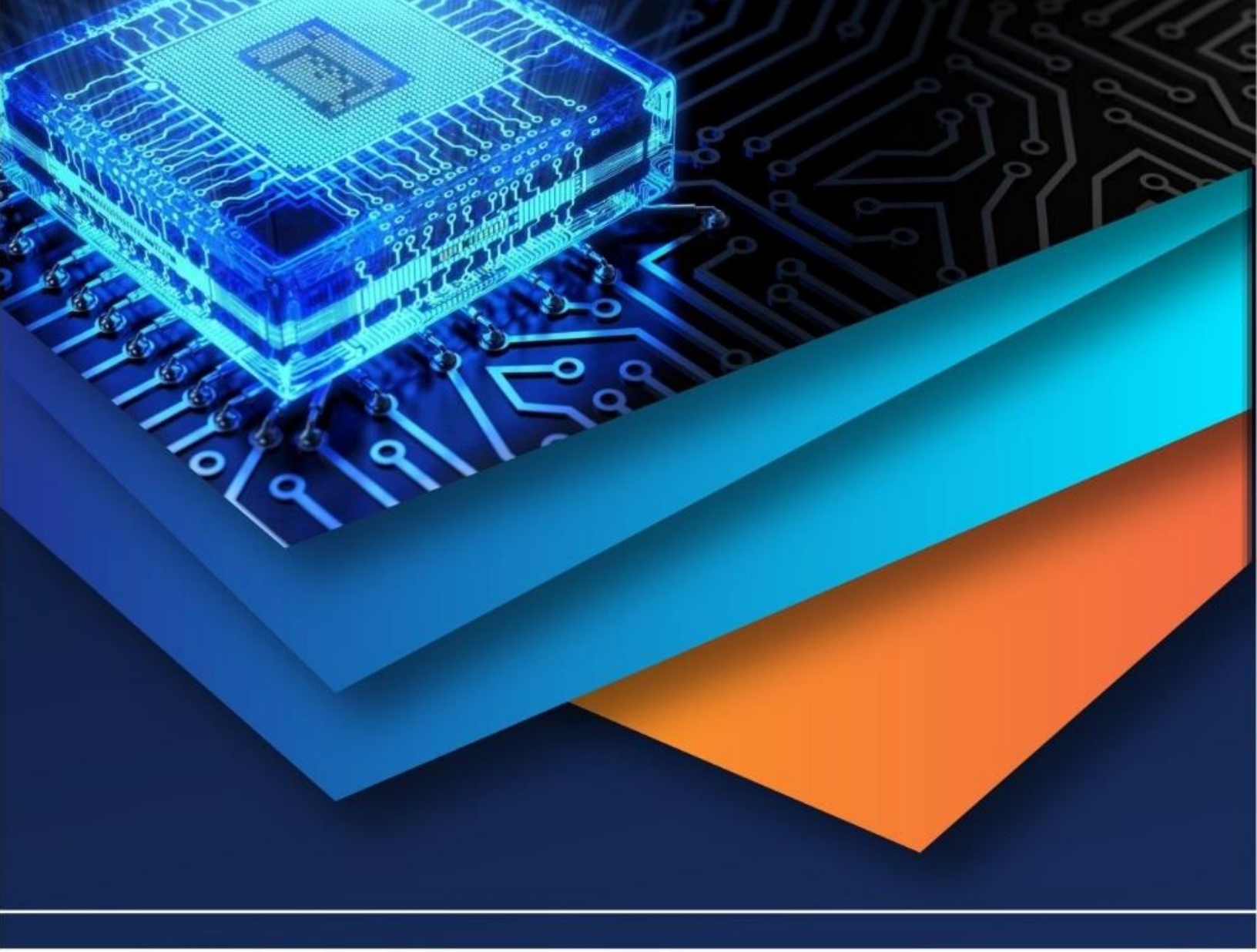
A. alternate and Aspergillus fumigatus were known as mycotoxigenic fungi. Mycotoxigenic fungi are pathogens that damage the quality of agricultural crop and impact negatively on food safety [14]. Mycotoxins, which occur by ingestion leads to various diseases, such as mycotoxicoses and mycoses that may eventually cause the death [15] some of these mycotoxins could repress the immune system [16] and thus establish a great threat for human health.

IV. CONCLUSIONS

Results found here could be valorized in biopesticide new synthetize based on aqueous extracts of carpophores mushrooms. Such valorization could minimize chemical pesticide danger and overcome a new approach on mushroom production, medicinal and agricultural use.

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