RESEARCH PAPER | Biology | Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

Effect of *Chamaerops humilis* L. (Arecaceae) Essential Oils on The Inhibition of Some Fungal Strains Isolated From Wheat Silos

KEYWORDS: Activity antifungal, Chamaerops humilis L., essential oils, molds, yeasts.

HASNAOUI Okkacha	ADLI Djallal Edine Houari et	HALLA Noureddine
Département de Biologie, Faculté des Sciences, Université Dr Tahar Moulay – Saida	Département de Biologie, Faculté des Sciences, Université Dr Tahar Moulay – Saida	Département de Biologie, Faculté des Sciences, Université Dr Tahar Moulay – Saida

ABSTRACT *The effects of essential oils (E.O) are nowadays widely used in antibacterial and antifungal field. This work intends to evaluate the inhibitory effects of E.O extracts of leaves and fruits of the dwarf palm (Chamaerops humilis L.) on certain fungal strains from grain storage silos. 6 mold strains were extracted and identified (Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Rhizopus stolonifer, Penicillium viridicum and Alternaria). Antifungal tests reveal some inhibition of mycelial growth. It is total in the case of Aspergillus niger at a concentration of 2.5 µl/ml, and Aspergillus ochraceus and Rhizopus stolonifer at a concentration 12.5 µl/ml. Inhibitions larger than 50% were recorded for Penicillium viridicum et Alternaria sp at concentrations of 0.5 µl/ml. Analysis of the antimicrobial power against three yeast species (C. albicans) showed remarkable activity of this E.O.*

INTRODUCTION

Food safety is a concern increasingly important public health. In fact, the governments redouble their efforts to improve food safety and subsequently prevent or reduce foodborne illness. Indeed, grains are staple food for human and animal consumption. In Algeria, wheat occupies a very important place, not only for its wide consumption but also because of the existence of large grain regions in the north, including the highlands (Riba, et *al.*, 2008).

Therefore, cereals are the raw materials most exposed to fungal contamination. Fungal growth on these substrates may have several consequences: deterioration in the organoleptic properties, decreased nutritional value, appearance of diseases (allergies and fungal infections) or accumulation of toxic compounds (mycotoxins).

The extracts of medicinal plants represent very important sources in the preservation of food by their potent antimicrobial activities.

The dwarf palms its scientific name *Chamaerops humilis* L. (*Ch*) is a monocot in the Arecaceae family. The socio-economic role, ethno-pharmaceutical and ethnobotanical of *Ch*, has been reported by several authors (Halimi, 1997; Beloud; 2001; Benmehdi et *al.*, 2012; Hasnaoui et *al.*, (a; b); 2013). Many descriptive studies have been done on determining the role of traditional medicine for *Ch*. Leaves and fruits have medicinal properties (hypoglycemic, anti-inflammatory, anabolic, antiseptic, and diuretic (Benmehdi et *al.*, 2012; Hasnaoui et *al.*, (a; b), 2013).

This work aims to evaluate the antifungal power of E.O extracts of leaves and fruits of *Ch* on mold strains provided from grain storage silos. This type of approach is imperfectly known.

MATERIEL AND METHODS

1. PLANT MATERIAL

The parts used in this investigation are collected from chamaeropaies located in the mountains of Tlemcen (western Algeria). They were collected in August 2013.

2. EXTRACTION OF ESSENTIAL OILS

The extractions of essential oils (E.O) were carried out by three methods and tree solvents (hexane, water and chloroforme):

i- Hydro-distillation (E.D);
ii- Soxhlet (E.S);
iii- Maceration (E.M).

In E.D and E.S we used 30 grams (g) of ground plant material (Ashnagar et *al.*, 2007). The solvents used in this study depending of the process: it is water, hexane and chloroform (Table 1). As for the E.M were used 20 g of ground product in 60 ml of chloroform and 20 g in 50 ml of H_2O (Benmehdi et *al.*, 2012; Ashafa and Afolayan, 2009).

3. YIELD CALCULATION

The yield of essential oil (YEO) is defined as the ratio between the mass of E.O obtained after extraction (M') and the mass of the plant material used (M) (Bssaibis, et *al.*, 2009). The yield is expressed in percentage and is given by the following formula:

YEO (%) = M'/M x 100

With: YEO: EO yield of dry matter;

M': EO mass in grams from the dry plant material;

M: mass of dry plant material used in gram

4. MYCOLOGICAL ANALYZES

4.1. Fungal isolation

Dilution plating was used as isolation technique (Pitt and Hocking, 2009). 10 g of the sample were added to 90 ml of 0.1% peptone water. This mixture was then shaken on a rotary shaker for approximately 15 min and diluted 10, 10 and 10 fold. Aliquots composing of 0.1 ml of each dilution were spread (in triplicate) on the surface of the dichloran Rose-Bengal chloramphenicol agar (DRBC), Czapek dextrose agar (CDA) and potatoes dextrose agar (PDA). All plates were incubated for 5 to 7 days at 28°C in the dark and under normal atmosphere. The identification of fungal

Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

strain is realized on the basis of morphological characteristics, under the microscope, and single spore method by colony characteristics after their culture on different culture media (Barnett and Hunter, 1972).

4.2. Molds identification

The identification was performed based on the study of macroscopic and microscopic characters of isolates grown on malt extract agar medium and Czapek-yeast extract agar with single spore method by colony characteristics after their culture on different culture media (Barnett and Hunter, 1972).

5. ANTIFUNGAL ACTIVITY OF E.O

5.1. Against the mold strains

In our study we tested the antifungal power of E.O by the method of direct contact on 6 mold species isolated (*Aspergillus flavus, A. niger, A. ochraceus, Alternaria. S*pp, *Rhizopus stolonifer* and *Penicilium viridicatum).* E.O leaves of *C.h* are tested with the following concentrations: 0.05; 0.2; 0.5; 2.5; 5 and 12.5 µl/ml. These concentrations are achieved by the addition of 1, 4, 10, 50, 100 and 250 of the E.O µl in 20 ml of tepid PDA medium in a test tube (C.L.S.I, 2002). After shaking the tubes the medium is poured into glass Petri dishes (9 cm). The inoculation is done by depositing of a mycelium of a pre-culture of 3 to 7 days. A Petri dishes containing 20 ml PDA without E.O was inoculated as a control. For each concentration of 3 tests were performed. After incubation for 7 days at a temperature of 28 ± 4 ° C; by taking into account of the growth of mycelium; the antifungal index is calculated by the following formula: **Antifungal index = (1-Da/Db) x 100**

With:

Da: diameter of the zone of growth of the test.

Db: diameter of the zone of growth of the control

5.2. Against the yeasts

The method of micro-dilutions in liquid medium (C.L.S.I, 2002) has been used on three reference strains of yeast to determine the antifungal activity of E.O; these are: *Candida albicans* ATCC 10231 and *Candida albicans* 2679, *Candida albicans* IPP444. They are maintained by subculturing on Sabouraud agar and stored at 4 ° C.

The MIC of E.O against *C.albicans* was determined by adapting approved methods of the National Committee for Clinical Laboratory Standards (M27-A). MICs were determined in RPMI 1640 medium. The starting inoculum was approximately 0.5×10^3 to 2.5×10^3 CFU/ml. Microtiter trays were incubated at 35°C in a moist, dark chamber, and the MICs were recorded after 24 and 48 h of incubation.

5.3- Effect of E.O on spore germination

The boxes that show no growth after tested with E.O, the disk has undergone a scraping mycelium in sterile saline within the carton and its growth was inhibited in order to loosen the conidiospores. The resulting solution was filtered to remove mycelia residue, and then transferred to a sterile sealed vial. Then, a few seconds of stirring was carried out by adding a surfactant droplets "Tween80." 1 ml of the resulting mixture was deposited on a box containing PDA medium in order to make a light display. This technique aims to detect inhibitory activity in the germination of spores (Doumbouya et *al.*, 2012).

RESULTS

1. The yield of essential oils (YEO)

The YEO is 5% for leaves while it is only 2.7% for fruits using E.S. The YEO obtained in E.M is variable depending on the medium maceration; it is 1.5% for the leaves and 0.4% only for the fruit; using chloroform as the middle of maceration; as to the extraction in H_2O the results are low; they are respectively 0.3 % for the leaves, and 0.2% for the fruits. The Extraction by distillation give only traces in two parts used (Table 1).

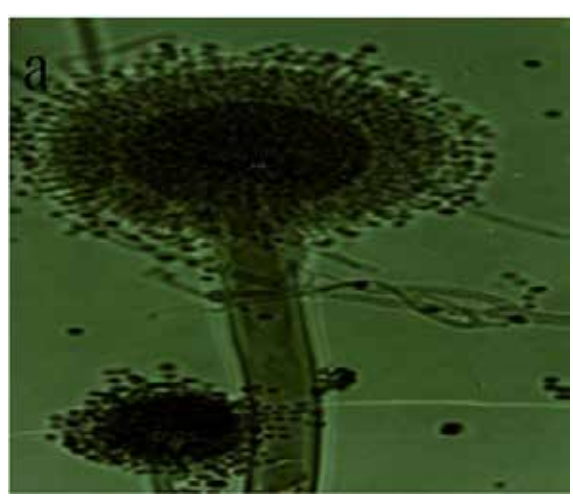
Table 1: Change YEO depending on the method used

	Extraction method.	Amount of the Plant material (g)	The solvent	The extraction time (h)	The yield %
Leaves	E.S	30	Hexane	6h	5
	E.D	30	Water	2h	Traces
	E.M	20	Chloroforme	15min	1.5
	E.M	20	Water	15min	0.3
Fruit	E.S	30	Hexane	6h	2.7
	E.D	30	Water	2h	Traces
	E.M	20	Chloroforme	15min	0.4
	E.M	20	Water	15min	0.2

The results obtained in YEO have different values for the two parts of *Ch*; this depends for a large part of the extraction method used. In our case the E.S provides an important YEO compared to other methods (E.D and E.M). Quantitatively the leaves are richer in E.O as fruit. The extraction in chloroform gives a less important result with 1.5% for leaves and 0.4% for fruits.

2. Identification of mold strains

The different microscopic and macroscopic aspects of both fungal strains searched are demonstrated in Figures 1 and 2. The aspects of fungal colonies of the same strains by single spore method on different culture media are shown in Table 2.



Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

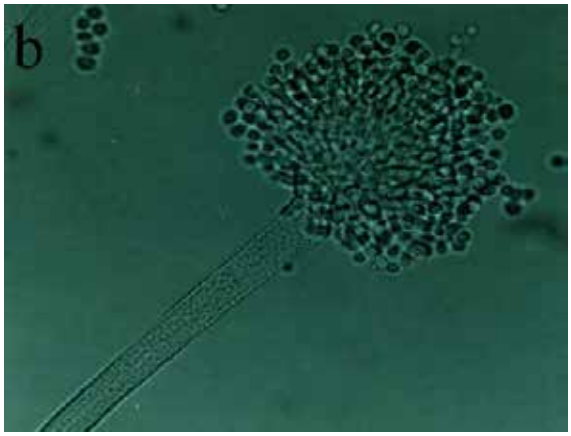
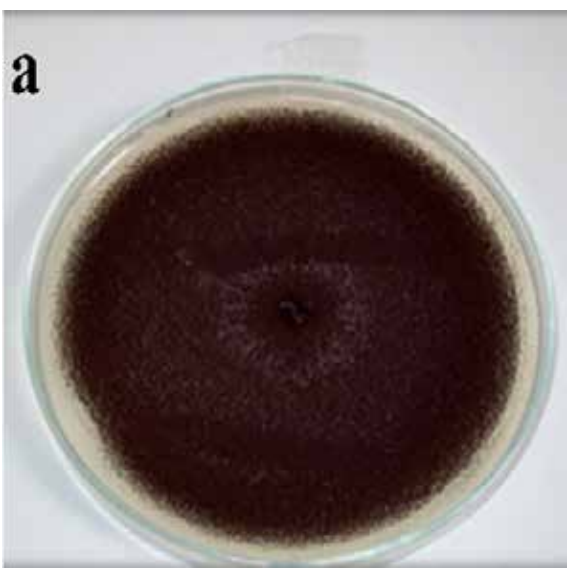


Figure 1. Identification of the genus by micro-culture method, a) *Aspergillus niger*; **b)***Aspergillus flavus*



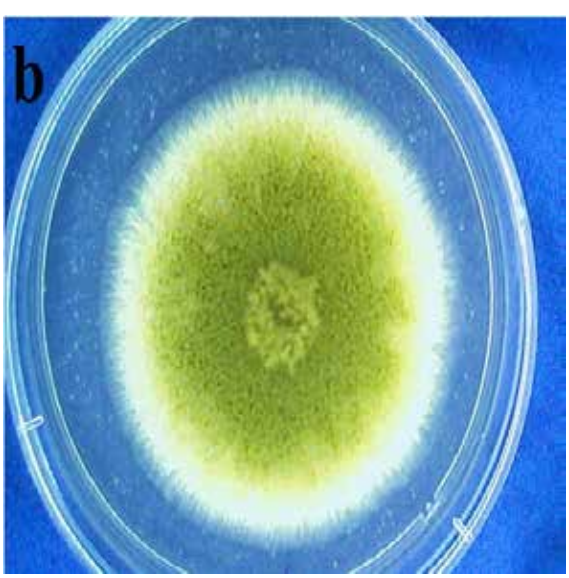


Figure 2. Identification of fungal species by Single Spore method.
a) Colonies of *Aspergillus niger* **on PDA medium; b) colonies of** *Aspergillus flavus* **on PDA medium.**

Table 2. Identification of *Aspergillus niger* **and** *Penicillium viridicatum* **by single spore method.**

Genera species	Medium	Reading (Color)
Aspergillus niger	MEA 25°C	Black
	CYA 37°C	Black grayish
Aspergillus flavus	MEA 25°C	Pistachio green
	CYA 37°C	Dark brown
	G 25N	Greenish yellow
	AFAP	Orange back

3. Test of the antifungal activity

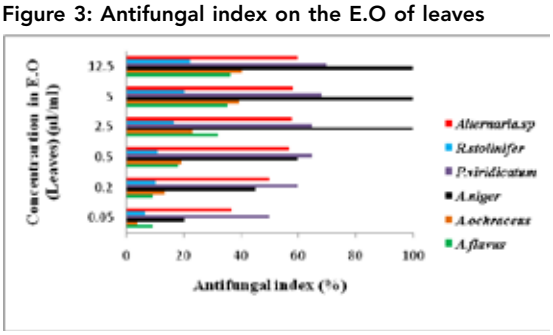
3.1. The activity of E.O on the molds

The antifungal activity of the E.O of the leaves on the molds was evaluated by the direct contact method. The results obtained are reported in Figure 3.

The analysis of the results for fungal growth under the action of different concentrations of E.O *Ch* (Figure 3) allowed us to see that E.O has a different antifungal action on molds species tested at concentrations ranging from 0.05 µl/ml to 12.5 µl/ml.

Indeed, at a concentration of 2.5 µl/ml E.O has inhibited the growth of *A. niger* could; while the growth of *P. viridicatum, A. flavus, A. ochraceus, Alternaria spp* and *R. stolonifer* are not fully inhibited. It was also observed that the inhibition of fungal growth rate is proportional to the concentration of the E.O.

Figure 3: Antifungal index on the E.O of leaves



3.2. The activity of the EO (MIC) on the yeasts

From the results obtained (Table 3), the E.O exerted an inhibitory effect on the: *C. albicans* 10231 and *C. albicans* 2679 with MIC about 250 mg / ml and an inhibition against *C. albicans* IP444 with MIC of 500 mg / ml.

Table 3: Results of the MIC of *Ch* **E.O against** *Candida albicans***.**

	1	2	3	4	5	6	7	8	9	10	11	12
Candida albicans 2679	-	-	+	+	+	+	+	+	+	+	+	+
Candida albicans 10231	-	-	+	+	+	+	+	+	+	+	+	+
Candida albicans IP444	-	+	+	+	+	+	+	+	+	+	+	+
CMI (mg/ml)	500	250	125	62.5	31.25	15.62	7.81	3.9	1.95	0.97	0.48	-

Plus (+) = Growth (-) = No growth

Volume : 4 | Issue : 11 | November 2014 | ISSN - 2249-555X

4 - Effect of EO on spore germination

After testing the E.O against the germination of spores of *A. niger* following inhibition of growth to 2.5 µl / ml, no growth of these strains were noted. That E.O was effective action on the germination of spores of strain aroused.

DISCUSSION

The E.O is used in alternative medicine for a long time for their inhibitory properties against many bacteria and molds. The antibacterial and antifungal capacity of these substances of vegetable origin has been reported by numerous studies *In vitro* (Doumbouya et *al.*, 2012)

The results of our research show the inhibitory effect of E.O on the growth of some fungal strains and can be applied to stop the growth of fungi in stored food.

Numerically the inhibition values obtained ranged from 3.9% to 100%. We note that the degree of inhibition depends on the concentration of E.O used and nature of the mold and / or of the yeast tested. Mycelia of *Aspergillus niger* are inhibited 100% from a concentration of 2.5 µl/ ml; those of *A.ochraceus and P. stolonifer are* inhibited to 100% at a concentration of 12.5 µl/ml. Our results confirm those of other researchers who worked on the inhibition of fungal strains by E.O (Pawar and Thaker, 2006; Bansod and Rai, 2008).

Unlike the action of E.O on the molds where we found MIC of 0.05 µl/ml may have an effect on the growth of the molds. The MIC on the yeasts was evaluated at 250 mg / ml in our case. These results highlight the difference in the action of E.O on the growth of molds and yeasts. The antifungal effect is proportional to the concentration of E.O in the medium (Degryse et *al.*, 2008).

The level of the antifungal activity is proportional to the concentration of E.O. Generally, the reduced growth of the mycelia in the presence of E.O compared to the control could be explained by the presence of terpenes compounds in *Ch* (Tatsadjieu**,** 2003). The chemical characters of some terpenes are particularly active against microbial cells, because they are soluble in aquous media and cause significant damage to the cell walls of microorganisms (Ferdousi et *al.*, 2010) Indeed, analyzes of different phytochemicals aerial parts of *Ch* show of their wealth in terpenes (Benmehdi et *al.*, 2012).

In the other hand, it has contributed to test the antifungal activity of E.O in the germination of spores of strain *A. niger*. In fact, E.O was made effective since there was no germination giving rise thereafter to fungal forms. Several studies have tried in vivo and in vitro using the E.O as substances which replace the synthetic fungicides such as Carbendazimes that interfere with the formation and / or functioning of microtubules and block the cell division mycelial hyphal elongation (Ferdousi et *al.*, 2010; Doumbouya et *al.*, 2012)).

CONCLUSION

The YEO obtained in our contribution show the link between the extraction process and richness E.O every part of the plant. In our case the leaves are richer in E.O as fruit.

The extracted leaves E.O of *Ch* has an inhibitory effect on all strains tested. The antifungal activities of E.O on *Ch* mold strains from storage silos shows positive inhibitory responses. These responses can be 100% in the case of *Aspergillus flavus, Aspergillus niger* and *Rhizopus stolonifer*, in other cases the inhibition up to 70% is going to *Penicillium viridicatum*.

These very positive results can find an industrial application in the field of preservation of certain foodstuffs and may be used in organic food as a preservative.

REFERENCE Ashafa, A. and Afolayan, A., (2009). Screening the root extracts from Biden pilosa L.var. radiate (Asteraceae) for antimicrobial potentials. J. of Med. Plants Research, 3 (8), 568-572. | Ashnagar, A., Gharib, N. and Haidari, N. H. (2007). Isolation and Identification of Anthralin From the Roots of Rhubarb Plant(Rheum palmatum). E-Journal of Chemistry.4, (4) 546-549. | Beloued, A. (2001). medicinal plants in Algeria. University publications office, Algiers, ISBN: 9961.0.0304.4, pp: 277. | Benmehdi, H., Hasnaoui,O., Benali,O. and Salhi, F. (2012). Phytochemical investigation of leaves and fruits extracts of Chamaerops humilis L.- J. Mater. Environ. Sci. 3 (2) 320-237. | Bansod, S. and Rai, M. (2008). Antifungal Activity of Essential Oils from Indian Medicinal Plants Against Human Pathogenic Aspergillus fumigatus and Aspergillus niger. World Journal of Medical Sciences 3 (2): 81-88. | Barnett, HL., Hunter, BB. (1972). Illustrated genera of Imperfect fungi.3th Ed. Minnesota, USA: Burgess Publishing Company. | Bssaibis, F., Gmira, N. and Meziane, M. (2009). Antibacterial Activity of Dittrichia viscosa (L.) W. Greuter. Rev. Microbiol. Ind. San et Environn. Vol 3, N°1, p : 44-55 | C.L.S.I- (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard M27-A2, 2nd ed. PA, 22 (15). | Degryse A. C., Delfa I et Voinier M. A. (2008). Risks and potential benefits of essential oils. School of Advanced Studies in Public Health 94, 8-11. | Doumbouya M., Abo kouabenan. L., Nicaise A., Camara B., Kanko K., Aidara D. and Kone D. (2012). Activités comparées in vitro de deux fongicides de synthèse et de deux huiles essentielles sur des champignons telluriques des cultures maraîchères en Côte d'Ivoire. J. Appl. Biosci. P 3523-3530. | Ferdousi, Begum M.S.T. Ferdowsi M. and Shahidul Alam M.D. (2010). Inhibition of spore germin ation and mycelial growth of three fruit rot pathogens using some chemical fungicides and botanical extracts. J. Life Earth Sci., Vol. 5: 23-27 | Halimi, A. (1997). Guide to medicinal plants in Algeria. Report of the ministry of agriculture and maritime fisheries, pp: 207. | Hasnaoui, O., Adli, D. E. and Sennour, R. (2013). Antibacterial Activity of Essential Oils of Chamaerops humilis (Arecaceae) on Some Pathogenic Bacteria ; RJPBCS 4(4); 626 -633 | Hasnaoui, O, Benali, O., Bouazza, M. and Benmehdi, H. (2013). Ethnobotanical approaches and phytochemical analysis of Chamaerops humilis L. (Arecaceae) in the area of Tlemcen (western Algeria); RJPBCS 4(2): 910-918- | Pawar, VC and Thaker, VS. (2006). In vitro efficacy of 75 essential oils against Aspergillus niger. Mycoses; 49 (4): 316-23. | Pitt, JI., Hocking, AD. (2009). Fungi and Food Spoilage. Blackie Academic and Professional, London. | Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A., Sabaou, N. (2008). Mycoflora and ochratoxin A producing strains of Aspergillus in Algerian wheat, International Journal of Food Microbiology 122: pp 85–92. | Tatsadjieu, N.L. (2003). Study of the inhibitory activity of essential oils of some spices and aromatic plants of Cameroon on the growth and toxicological genesis of molds of the genus Aspergillus. Thesis Doct. E.N.S. of Agricultural Science, 176 p. | |