International Research Journal of Microbiology Vol. 2(3) pp. 104-108, March 2011
Available online@ http://www.interesjournals.org/IRJM


*Full Length Research Paper*

# Antifungal activity of Five Plant Essential Oils against wood decay fungi isolated from an old house at the Medina of Fez

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Accepted 6 April, 2011

**Oils from five medicinal plants were screened for their activities against wood rot fungi were isolated from an old house at the historic medina of Fez. The antimycotic activity was investigated by disc diffusion method and minimum inhibitory concentration (MIC) of oils by agar dilution method. The oils of *Origanum compactum, Eugenia caryophyllata* and *Ocimum basilicaum* have showed a maximum antimycotic activity against wood fungi as compared to control. In contrast, oil of *Thymus vulgaris* and *Melaleuca alternafolia* exhibited moderate activity against these fungi. Furthermore, mixed oils of *Origanum compactum* and *Ocimum basilicum* showed maximum activity as compared to control. Altogether, these results support the concept that plant oils may be used as efficient preservatives of wood in the historic monuments at the medina of Fez.**

**Key words:** Essential oil, Antifungal activity, wood decay fungi

## INTRODUCTION

Fez is the oldest and greater of Morocco's imperial cities. UNESCO has designated the entirety of the Fez Medina as a World Monument. In this medina, the wood used in the constructions is mainly the cedar. Decay of the wood of monuments in Fez, caused by many common white and brown rot fungi, has been well characterized in a precedent study (Zyani et al., 2009). The special properties of wood, including its appearance, low density, low thermal expansion and mechanical strength, have led to indoor and outdoor applications for the construction of these monuments. The durability of wood has often been recognized as one of disadvantages in this kind of construction. Find effective methods to prolong resistance have always been the interest of researchers from the timber industry. From the perspective of the environment respect, study of natural constituents found in very durable tree species and understanding their mechanisms are the most appropriate approaches to prolong the life of timber while protecting the environment (Chang et al., 2000).

The protection of wood is predominantly realized by chemicals compounds. These products are based on metals such as copper chromated arsenate (CCA). Since, researchers have focused for developing new methods for preserving wood against fungi, mold and insects (Kartal et al., 2004). The health impacts caused by mold in homes and buildings are a major concern for homeowners, builders and contractors. The health problems caused by mold exposure indoors of homes have caused loss of 2.8 billion dollars in 2002 (Hartwig et al., 2003). Chemical fungicides that are commonly used to control the growth of mold and fungal decay of wood are not suitable for many indoor applications. The searches for natural solutions that are user friendly and showing negligible toxicity to humans are increasingly

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**Table 1:** The five essential oils and their major components

| Botanical name | Density (g/ml) at 25°C | Major component(s) | % of Component(s) |
|---|---|---|---|
| Ocimum basilicum | 0,956 | linalool,<br>chavicol | 20,1<br>30,87 |
| Melaleuca alternafolia | 0,891 | terpinen-4-ol,<br>alpha-terpineol | 36,1<br>2,98 |
| Origanum compactum | 0,939 | carvacrol,<br>thymol | 51,2<br>43,3 |
| Thymus vulgaris | 0,917 | Thymol | 46,21 |

The table 1 shows the major components of the five essential oils tested, their major components and the **% of components**: *Origanum compactum*, *Eugenia caryophyllata*, *Ocimum basilicum*, *Melaleuca alternafolia* and *Thymus vulgaris.*

sought. The plant extracts such as essential oils and their derivatives are well known for their antimicrobial properties which are used in the pharmaceutical industry, health care (Adam et al., 1998; Moretti et al., 1998; Muanza et al., 1995; Rakotonirainy et al., 2005; Sridhar et al.,2003; Yang et al., 2008; Wang et al., 2005).

The aim of this study was to assess the antifungal activity of 15 essential oils against wood rot fungi. These fungi were isolated from an old house at the Medina of Fez. Initial screening showed that only 5 essential oils displayed substantial activity against decayed wood fungi. The 5 oils were then extensively characterized for their *in vitro* antifungal activity.

## Materials and methods

**Fungal strains:** In this study, four white rot fungi (*Thielavia hyalocarpa, Penicillium commune, Penicillium chrysogenum, Penicillium expansum*) and one brown rot fungi (*Cladosporium cladosporioides*) were used. These fungi were isolated from a damaged wood of an old house in the medina of Fez in our previous study (Zyani et al., 2009). These fungi were used in the antifungal assay as we have been previously described (Zouhar et al., 2009). They were maintained and grown on 2% malt agar at 25°C.

**Essential oils*:*** A screening of 15 essential oils was carried out in liquid medium: *Origanum compactum, Eugenia caryophyllata, Ocimum basilicum, Melaleuca alternafolia ,Thymus vulgaris, Mentha spicata, Eucalyptus globulus, Lavandula hybrida, Citrus limon, Cedrus atlantica, Cupressus atlantica, Cupressus sempervirens, Juniperus communis, Rosmarinus officinalis and Allium sativum* (obtained from Sigma) . Five essential oils were selected for their important antifungal activity. Their major components are shown in Table 1.

**Antimycotic Assay by Disc Diffusion Technique:** The Oils were screened for their antifungal activity against five fungi by disc diffusion method. The fungal cultures were grown on 2% malt agar at 25°C. The mycelial fungus of 7 day culture was washed, suspends in normal saline solution and then filtered through glass wool aseptically. The colony forming units (CFU/ml) of each suspension of fungus was determined and inoculums were adjusted to 1.5 X $10_5$ CFU/ml. These suspensions were used for antifungal assay tests. An inoculum's of 0.1ml was applied on the surface of the Malt Agar plate and spread by using sterile glass spreader. The sterilized paper disc was placed on the other side of plate. An aliquot (1 µl) of the essential oil was added on the paper disc. Two concentrations of each oil and mixed oils, i.e., 0.1% (v/v), and 0.01% (v/v), were used for assay. The plate was sealed with a parafilm immediately after adding essential oil and incubated for 3 days at 25°C. Three replicates were used for each treatment. The radius of fungal mycelia was measured (tree times) and compared with that of untreated control.

**Agar Dilution Method:** The agar dilution method follow was approved by the NCCLS (Remmal et al., 1993; Bansod and Mehendra, 2008). In brief, a series of twofold dilutions of oil, ranging from 2% (v/v) to 0.01% (v/v), was prepared in Malt agar. Plates were dried at 25 °C for 30 min prior to inoculation with 1–2ml spots containing approximately 10 CFU of each microorganism. Malt agar which does not contain the essential oil was used as a positive growth control. Inoculated plates were incubated at 25°C for 48 h. Minimum inhibitory concentrations (MIC) and (IC50) were determined after 48 h for these fungi. The MIC was determined as the lowest concentration of oil able to inhibiting the visible growth of each microorganism on the agar plate and IC50 values was determined as the concentration that inhibited 50% of the mycelium of fungi growth were calculated by probate analysis.

**Vapor exposure treatment:** In the Petri dish test chamber, a small glass dish (4 cm diameter) containing individual test oil (3 ml) was placed next to the specimens

**Table 2:** Antifungal activity of the five oils of medicinal plants against fungi decay wood

| Fungi / Plants Oil | T. hyalocarpa | | P. commune | | P. chrysogenum | | P. expansum | | C. cladosporioides | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1% | 0.01% | 0.1% | 0.01% | 0.1% | 0.01% | 0.1% | 0.01% | 0.1% | 0.01% |
| O. basilicum | 22±0.5 | 10±0.5 | 21±0.5 | 14±0.5 | 18±0.5 | 6±0.5 | 20±0.5 | 8±0.5 | 17±0.5 | 5±0.5 |
| M. alternafolia | 12±0.5 | 2±0.5 | 14±0.5 | No Zone | No Zone | | No Zone | | 6±0.5 | No Zone |
| O. compactum | 20±0.5 | 12±0.5 | total inhibition | | 20±0.5 | 10±0.5 | 14±0.5 | 4 ±0.5 | 15±0.5 | 4 ±0.5 |
| T.vulgaris | 25±0.5 | 10±0.5 | 5±0.5 | 2±0.5 | 5±0.5 | No Zone | No Zone | | No Zone | |
| E. caryophyllata | total inhibition | | 25±0.5 | 15±0.5 | 12±0.5 | 2±0.5 | 10±0.5 | No Zone | total inhibition | |
| Mixed oils | total inhibition | | 24±0.5 | 18±0.5 | 20±0.5 | 16±0.5 | 21±0.5 | 10±0.5 | 12±0.5 | 10±0.5 |
| Paraffin oil (negative control) | No Zone | | No Zone | | No Zone | | No Zone | | No Zone | |

The table 2 represents the antifungal activity of the five oils of medicinal plants against *T. hyalocarpa, P. commune, P. chrysogenum, P. expansum and C. cladosporioides.* Two concentrations were used 0.1% and 0.01% for each essential oil. The paraffin oil was used as a negative control.
No zone: any inhibition
Each test was performed five times, and the data averaged (n = 5). Values of inhibition are significantly different at the level of $p<0.05$ according to Tukey's Test.

24 hr prior to inoculating with spores of mold fungi. The test oil remained in the Petri dish chamber for the entire test period (20 weeks). Vegetable oil served as a control. Specimens were sprayed with 1ml of mixed or individual mold spore inoculum 24 hr post-treatment. Petri dish test chambers were incubated and specimens were rated (Yang and Clausen, 2007).

**Statistical analysis**: Statistical analysis of the data obtained in the present study was carried out in a completely randomized design layout with three replicates using Statgraphics plus 2.0. Where a significant difference between means was verified based on ANOVA, the comparison of means of different treatments was performed using Tukey's test at $p = 0.05$.

## RESULTS

### Antifungal activity of Five Plants Essential Oils

The antifungal activities of five plant oils obtained by the disc diffusion method are shown in Table 2. The maximum antifungal activity was shown by *Origanum compactum*, *Eugenia caryophyllata* and *Ocimum basilicum* ($p<0.05$). The oils of *Melaleuca alternafolia* and *Thymus vulgaris* showed low activity against all fungi ($p<0.05$). Subsequently we have determinate the MIC and IC50 values of these five oils against the fungal mycelium by the agar dilution method. The MIC of these 5 plant oils is shown in Table 3. *Origanum compactum* and *Eugenia caryophyllata* had lowest MIC of (0.01%v/v) against *Thielavia hyalocarpa and Cladosporium cladosporioides*. In addition *Ocimum basilicum* inhibited *Thielavia hyalocarpa* at low concentration, but the others fungi had moderate MIC. However *Melaleuca alternafolia* and *Thymus vulgaris* failed to inhibit the five fungi at the highest concentration, which was 2% (v/v). Mixed oils had lowest MIC of 0.01% (v/v) against *T.hyalocarpa and C.cladosporidies*. The lowest minimum inhibitory concentrations were 0.01 % (v/v) of mixed oils against *T.hyalocarpa and C.cladosporidies*. The effectiveness of *O.compactum* and *E.caryophyllata* as an antifungal agent was also reported (Zouhar et al., 2009 and Ezzaoui et al., 2007).

### Fungi response fungi to vapor exposure of essential oils

The most effective fungi inhibitor was *O.compactum* and *E.caryophyllata* oil vapor; it retarded growth of all seven fungi for at least 20 weeks. *O.basilicum* inhibited *T.hyalocarpa* and *C.cladosporidies* for 20 weeks, and *P.commune, P. expansum* and *P.chrysogenum* for 15 weeks. *Melaleuca alternafolia* vapor retarded *T.hyalocarpa and C.cladosporidies* for 12 weeks and *P.commune, P. expansum and P.chrysogenum* for 6 weeks. *Thymus vulgaris* retarded *T. hyalocarpa* for 15 weeks and *C.cladosporidies* 10 weeks but was ineffective against *P.commune, P.expansum and P.chrysogenum*. These finding suggest that components carvacrol, eugenol, eugenyl acetate linalool, chavicol may play a role in preventing spore germination for 3 essential oils *O.compactum*, *E.caryophyllata* and *O. basilicum*.

**Table3:** Minimum inhibitory concentration (MIC) and (IC50 values) of selected essential oils (%v/v) against fungi decay wood using agar diffusion method

| Plant oils | *T. hyalocarpa* IC 50 / MIC | *P. commune* IC 50 / MIC | *P. chrysogenum* IC 50 / MIC | *P.expansum* IC 50 / MIC | *C. cladosporioides* IC 50 / MIC |
|---|---|---|---|---|---|
| **Ocimum basilicum** | 0,3 / 0.4 | 0,8 / 1.5 | 0,6 / 1 | 1 / 1.4 | 0,6 / 1.5 |
| **Melaleuca alternafolia** | 0,3 / 0.4 | 1,2 / 2 | 1 / 1.4 | nd / >2 | 0,8 / 1.6 |
| **Origanum compactum** | nd / 0.01 | 0,2 / 0.4 | 0,1 / 0.2 | 0,08 / 0.1 | nd/ 0.01 |
| **Thymus vulgaris** | 0,08 / 0.1 | 1,5 / 2 | nd / >2 | nd / >2 | 0,5 / 1 |
| ***Eugenia* caryophyllata** | nd / 0.01 | 0,6 / 0.4 | 0,3 / 0.4 | 0,2 / 0.4 | nd/ 0.01 |
| **Mixed oils ( O.basilicum and Origanum compactum** | nd / 0.01 | 0,4 / 1 | 0,2 / 0.4 | 0,2 / 0.5 | 0,05 / 0.1 |

In table 3 Minimum inhibitory concentration and IC50 values (the concentration that inhibited 50% of the mycelium of fungi growth ) was determined for each oil against different fungi causing wood decay using agar diffusion method. Each test was performed three times, and the data averaged (n = 3). Values of MIC are significantly different at the level of $p<0.05$ according to the Tukey's Test.

## DISCUSSION

To develop environment-friendly alternatives to synthetic fungicides for the control of wood rot fungi, the interest of essential oils has been increased. In this study, we investigated the antifungal activities. For this objective, five essential oils were used as volatile compounds against fungi decay wood. This was done by exposure to vapor phases of the oils. As the results show, the essential oils of *O. compactum* and *E. caryophyllata* are active on all fungus. However *M. alternafolia* and *T. vulgaris* are active only against one fungus. The essential oil of *O. compactum* and *E. caryophyllata* showed the broadest antifungal spectrum in this study. The inhibitory effect against wood decay fungi and the antibacterial activities of *O. compactum* and *E. caryophyllata* oil were reported previously by several studies (Ezzaouia et al., 2007; Hoffman et al., 2004; Hoffman, 1987; Mau et al., 2001).

In this study, *O. basilicum* oil shows the maximum antifungal spectrum as *O. compactum* oil. Our data are in agreement with Oyewale et al., (1988) who reported that the oil of *O. basilicum* possessed a wide spectrum of fungicidal activity. Beside it insecticidal and nematicidal activities, antifungal activity of *O. basilicum* against several species of *Aspergillus* and *Penicillium* was also reported (Isman, 2000; Pandey et al., 2000; Yang and Clausen, 2007).

*T. vulgaris* oil inhibits mycelial growth of two wood decay fungi such as *T. hyalocarpa* and *C. cladosporidies*, but was not active on *P. commune*, *P. expansum* and *P. chrysogenum*. The essential oil of *T. vulgaris* inhibits various fungi involved in food spoilage; mycotoxin producing and postharvest pathogenic fungi (Nguefack et al., 2004; Reddy et al., 1997) reported that *T. vulgaris* oils controlled decay of strawberry fruits caused by *Botrytis*controlled decay of strawberry fruits caused by *Botrytis cinerea* up to 74-76% (Muanza et al., 1994). The major compound of essential oil of *T. vulgaris* used in this study was thymol, identical to those of *thymol chemotype* of *T. vulgaris* (Giordani et al., 2004).

The best results were obtained with mixed oils; all fungi (*T. hyalocarpa, C. cladosporidies, P. commune, P .expansum and P. chrysogenum*) were inhibited at lowest MICs. These results support the notion that plant essential oils may have a role as preservatives of wood.

This study demonstrated the in vitro antifungal activities of essential oils against wood decay fungi and potential use of essential oils as antifungal preservatives for the control of wood decayed by many fungi *T. hyalocarpa, P. commune, P. chrysogenum, P. expansum and C. cladosporioides*. However, for the development of essential oils as alternatives of synthetic fungicides, further studies are required to evaluate toxicity and the effectiveness of treatment for long term of essential oils on wood. In conclusion, the five essential oils had an antifungal activity against *T.hyalocarpa*, *P.commune, P.chrysogenum, P.expansum and C.cladosporioides*. *O.compactum, E.caryophyllata* and mixed oils showed maximum activity as compared to control. The positive results obtained in *vitro* suggest the use of these essential oils as a means of struggle against wood decay and consequently as a method of preservation of the wood.

## ACKNOWLEDGEMENT

We thank Biomatec-US Manuscript Review Committee for helpful comments and discussions and Dr Abdelilah Soussi Gounni (Manitoba Research Chair).

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