

3.2 Fungal genera and species isolated from different locations

Data illustrated in table (2) indicated that, a total of 2917.08 fungal colonies belonging to 7 genera were identified from indoor of EGBO of Egypt building. *Penicillium spp.* (20.22%), *Aspergillus flavus* (20.76%), *Trichoderma viride* (12.94%), *Alternaria tenuis* (10.79%) and *Aspergillus niger* (10.51%) were the main contaminating mould of all tested repositories. They were present throughout the year and they together constituted 54.42% of the total airspora.

Aspergillus flavus and *Penicillium spp.* were the dominant component in the indoor air with a total concentration of 605.48 and 589.74 cfu x103 /m3 with frequency occurrence of 20.76 and 20.22%, respectively. Its highest concentration was recorded in August (259.49 and 314.53cfu x103 /m3) and the lowest during winter in January (141.54 and 125.81 cfux103 /m3), respectively. The same trend was also observed, as general for the other detected moulds, as the highest concentration was showed during summer in August and the lowest during winter in January. Many reports dealing with the microbial levels in indoor air as those reported by Sahab et al.(2003), Deepake (2008) and Jankewicz et al. (2008). Shamsian et al. (2006) studied the fungal contaminations in historical manuscripts at Astan Quds museum library. They detected that, fungal genera of *Aspergillus*, *Penicillium* and *Mucor* could damage paper of manuscripts.

4. Selection of Cellulose Degrading Fungi

4.1 Growth on CMC Czapek's agar medium

Of the 53 fungal isolates screened for Cellulolytic activity on carboxymethyl cellulose (CMC) only 31(58.49%) had the ability to grow (Table, 3). Moreover, only 12 out off 31(25.8%) fungal isolates had a high ability to decompose CMC in Czepek's medium, including 4 isolates of *F. oxysporum* with radial growth between 82 and 88 mm, 2 isolates of *F. avenaceum* (Ø between 64 and 72 mm), 4 isolates of *T. viride* (Ø between 60 and 82 mm) and 2 isolates of *F. moniliforme* (Ø between 60 and 61mm). Moreover, 17of total 31 fungal isolates gave moderate growth (Ø between 25-54 mm).But only 2of 31 gave slight effect (Ø between 12-15 mm), respectively. These results are in agreement with that, recorded by El-Sayed (1980), Sahaba (1988) and Sahab et al. (2003).

4.2 Growth on cellulose-Czapek's agar medium:

Of the 28 fungal isolates screened for cellulolytic activity on cellulose-Czapek's medium (Table, 3) only 26 had the ability to grow. Only 10 (35.71%) fungal isolates showed maximum growth including 4 isolates belonging to *F. oxysporum* and 4 isolates of *T. viride* recorded radial growth between 70-73 mm with very good mycelium growth. While, the two isolates of *Stymphylium spp.* recorded radial growth between 78-80 mm with also very good appearance mycelium growth.

Table 3: *In-vitro* growth of fungal isolates selected from air of the GEBO building on modified Czapek's medium (CMC and Cellulose instead of sucrose)

| Fungal genera and species | Growth on CMC-Czapek's medium | | | | | | | | | | Growth on Cellulose -Czapek's medium | | | | | | | | | |
|----------------------------------|-------------------------------|---------------|--------------|---------|--------------|---------|--------------|---------|--------------|---------|--------------------------------------|------------|--------------|---------|--------------|---------|--------------|---------|--------------|---------|
| | No. of isolate | (+) ve growth | Isolate No.1 | | Isolate No.2 | | Isolate No.3 | | Isolate No.4 | | No. of isolate | (+) growth | Isolate No.1 | | Isolate No.2 | | Isolate No.3 | | Isolate No.4 | |
| | | | Ø mm | Density | Ø mm | Density | Ø mm | Density | Ø mm | Density | | | Ø mm | Density | Ø mm | Density | Ø mm | Density | Ø mm | Density |
| <i>Alternaria spp.</i> | 4 | 2 | 38 | + | 37 | + | - | - | - | - | 2 | 2 | 22 | + | 23 | + | - | - | - | - |
| <i>Aspergillus cheivaleri</i> | 1 | 1 | 25 | + | - | - | - | - | - | - | 1 | 1 | 18 | + | -- | - | - | - | - | - |
| <i>Aspergillus flavus</i> | 4 | 2 | 33 | + | 32 | + | - | - | - | - | | | | | | | | | | |
| <i>Aspergillus humicola</i> | 1 | 1 | 32 | + | - | - | - | - | - | - | 1 | 1 | 12 | + | - | - | - | - | - | - |
| <i>Aspergillus niger</i> | 4 | 3 | 43 | 2+ | 45 | 2+ | 40 | + | - | - | 1 | 1 | 22 | + | -- | - | - | - | - | |
| <i>Aspergillus parasiticus</i> | 4 | 2 | 45 | 2+ | 42 | 3+ | - | - | - | - | 2 | 2 | 37 | 2+ | 43 | 2+ | - | - | - | |
| <i>Aspergillus versicolor</i> | 4 | 2 | 42 | 3+ | 40 | 2+ | - | - | - | - | 2 | 2 | 36 | 2+ | 38 | 2+ | - | - | - | |
| <i>Aspergillus terreus</i> | 4 | 2 | 37 | 2+ | 34 | 2+ | - | - | - | - | 2 | 2 | 65 | 3+ | 59 | 3+ | - | - | - | |
| <i>Cladosporium sp.</i> | 1 | 1 | 15 | + | - | - | - | - | - | - | 1 | 1 | 18 | + | - | - | - | - | - | |
| <i>Fusarium avenaceum</i> | 4 | 2 | 64 | 3+ | 72 | 3+ | - | - | - | - | 2 | 2 | 56 | 2+ | 61 | 2+ | - | - | - | |
| <i>Fusarium moniliforme</i> | 4 | 2 | 60 | 2+ | 61 | 2+ | - | - | - | - | 2 | 2 | 50 | 3+ | 52 | 3+ | - | - | - | |
| <i>Fusarium oxysporum</i> | 4 | 4 | 88 | 4+ | 87 | 4+ | 80 | 3+ | 82 | 3+ | 4 | 4 | 73 | 3+ | 72 | 3+ | 70 | 3+ | 70 | 3+ |
| <i>Nigrospora sphaerita</i> | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Penicillium crysogenum</i> | 2 | 1 | 12 | + | - | - | - | - | - | - | 2 | - | - | - | - | - | - | - | - | |
| <i>Penicillium corylophyllum</i> | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Penicillium frequentance</i> | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Penicillium spp.</i> | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| <i>Stemphylium spp.</i> | 4 | 2 | 54 | 2+ | 46 | 2+ | - | - | - | - | 2 | 2 | 80 | 3+ | 78 | 3+ | - | - | - | |
| <i>Trichoderma viride</i> | 4 | 4 | 66 | 3+ | 60 | 3+ | 62 | 4+ | 82 | 3+ | 4 | 4 | 73 | 3+ | 72 | 3+ | 70 | 3+ | 70 | 3+ |
| Total | 53 | 31 | | | | | | | | | 28 | 26 | | | | | | | | |

*Each figure represents average of three replicates incubated at 27° C for 7 days

-= No growth, += weak growth, 2+= moderate growth, 3+= heavy growth, 4+= vigorous growth

Table 4: Effect of essential oils on percent inhibition of mycelia growth of *Fusarium oxysporum* and *Trichoderma viride*

| Tested | <i>Fusarium oxysporum</i> | | | | | | <i>Trichoderma viride</i> | | | | | |
|--------------|---------------------------|-------|------|-------|-----|---------------------|---------------------------|-------|-------|-------|-----|---------------------|
| | fungi | 0.05 | 0.1 | 0.2 | 0.4 | Mean of inhibition% | 0 | 0.05 | 0.1 | 0.2 | 0.4 | Mean of inhibition% |
| Rosemary | 0 | 36.9 | 48.4 | 52.5 | 100 | 47.6 | 0 | 50.6 | 41.1 | 74.3 | 100 | 53.2 |
| | h | g | fg | efg | a | | f | bcda | de | abcd | a | |
| Anise | 0 | 57 | 75.9 | 83.3 | 100 | 63.2 | 0 | 16.3 | 52.5 | 90.6 | 100 | 51.9 |
| | h | defg | bcd | ab | a | | f | cde | bcde | ab | a | |
| Tea tree oil | 0 | 71.9 | 74.4 | 80.4 | 100 | 65.3 | 0 | 47 | 81.4 | 69.9 | 100 | 59.7 |
| | h | bcde | bcde | abc | a | | f | cde | abcd | abcd | a | |
| Rocket | 0 | 58.9 | 58.2 | 64.5 | 100 | 56.3 | 0 | 81.8 | 83.3 | 86.7 | 100 | 70.4 |
| | h | cdefg | defg | bcdef | a | | f | abc | abc | abc | a | |
| Fennel | 0 | 52.3 | 53.7 | 64.1 | 100 | 54 | 0 | 40.8 | 64.4 | 82.2 | 100 | 57.5 |
| | h | efg | efg | dcdef | a | | f | de | abcd | abc | a | |
| Mean | 0 | 55.4 | 62.1 | 68.95 | 100 | | 0 | 47.31 | 64.55 | 80.56 | 100 | |

On the other hand, the six isolates which belonging to *A. terreus*, *F. avenaceum* and *F. moniliforme* recorded linear growth between 50-65 mm with moderate and heavy mycelium growth.

Moreover, the other 10 fungal isolates showed slight growth with moderate, and even no mycelium growth. It is well known that, the majority of fungal isolates obtained from the air of archives, libraries and museums exhibited cellulolytic activity produce acid, excrete pigments on the paper and contribute to the formation of biofilms, which accelerate the deterioration of the different document substrates (Florian, 2004 and Borrego et al., 2012).

5. Antifungal activity of essential oils

The antifungal activities of five essential oils on percentage inhibition of mycelial growth of *F. oxysporum* and *T. viride* are shown in Table (4). These essential oils were tested by agar diffusion plate method caused significant reduction in the growth of above mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium. Results showed that, all tested essential oil possess a remarkable antifungal activity against the two tested fungi compared to control.

All tested materials were found to highly effective and gave 100% reduction in the growth of the two fungi at the higher concentration of 0.4%. The Tea tree essential oil was most effective against *F. oxysporum* responsible for 65.3 mean % inhibition followed by Anise essential oil responsible for 63.2 mean% inhibition without significant difference. While, Rocket essential oil was the most effective against *T. viride* responsible for 70.4 mean % inhibition. On the other hand, Rosemary and Anise showed less effect on the growth of *F. oxysporum* and *T. viride* respectively, as the mean percentage inhibition were 47.6 and 51.9% respectively. The plant extracts such as essential oils are well known for their antimicrobial properties (Sridhar et al., 2003, Wang et al., 2005, Yang and Clausen, 2008, and Zyani et al., 2011).

Our data on the antifungal properties of oils suggested that, these oils should be examined and further evaluate its potential as a natural fungicide against deteriorated fungi.

6. Conclusion

To avoid bio-deterioration of old manuscripts, it is recommended to store the valuable documents in a suitable environment, ideally with a relative humidity of 44-55% and constant temperature below 20 °C without use of chemicals. This study also demonstrated the in-vitro antifungal activity of essential oils against paper decay fungi and potential use oils preservative for the control of paper decay by many fungi. However, for the development essential oils as alternative of synthetic fungicides, further studies are required to evaluate toxicity and the effectiveness of treatment.

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