**Inhibitory activity of clove, moringa and neem essential oils on the growth of some molds isolated from foods**

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This study aimed to assess the antifungal activity of some essential oils on some mold strains isolated from foods. The essential oils were extracted from cloves, moringa and neem. The assayed mold strains were: *Penicillium* spp., *Rhizopus* spp., *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Sporotricum* spp., *Alternaria* spp. and *Aspergillus fumigatus*. The results obtained showed that clove (*Syzygium aromaticum*) essential oil showed prominent antimold activity on all assayed mold strains, whereas moringa (*Moringa oleifera*) and neem (*Azadirachta indica*) essential oils showed no activity against all of the assayed mold strains.

**Key words:** Antimold activity, essential oils, cloves, moringa, neem, fungi.

**INTRODUCTION**

The use of plants and their products for the treatment of a variety of fungal infections is an old practice. This is because many plants contain a variety of compounds that have promising potentials for antimicrobial activity. According to Naqui et al. (1994), some plants contain compounds that are able to inhibit microbial growth. The association between molds and our food is interesting. Molds in addition to negatively affecting the aesthetic value of the food, render it unsafe for consumption, due to their ability to produce extracellular toxic metabolites from the mycotoxins in the food.

Essential oils, also called volatile oils, are oils which frequently occur in oil glands of plants. They are usually odoriferous. Well known examples are eucalyptus oil, and oil from orange peel (Pandey, 2007). They are not oils in strict sense but often share with oils a poor solubility in water (Wikipedia, 2012). Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Kordahi et al., 2005). They mostly contribute to the odoriferous constituents or ‘essences’ of the aromatic plants that are used abundantly in enhancing the aroma of some spices (Martinez et al., 2008). Essential oils are secreted either directly by the plant protoplasm or by the hydrolysis of some glycosides. Plant structures associated with the secretion of essential oils include: glandular hairs (Lamiaceae, for example, *Lavandula angustifolia*), oil tubes (or vittae) (Apiaceae, for example, *Foeniculum vulgare* and *Pimpinella anisum* (Aniseed)), modified parenchymal cells (Piperaceae, for example, *Piper nigrum* - Black pepper), and schizogenous or lysigenum passages (Rutaceae, for example, *Pinus palustris* - Pine oil). Chemically, a single volatile oil comprises more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odour (Firn, 2010).

According to Faid et al. (2005), some other oils have been used in food preservation. It is therefore reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential (Darokar et al., 1998). The aim of this work is therefore to evaluate the bioactivity of cloves, moringa and neem essential oils to some molds.

**MATERIALS AND METHODS**

**The plant materials**

The cloves were obtained from Kurmi market in Kano, the moringa seeds were obtained from Sharada in Kano, while the neem seeds were obtained from the trees in Bayero University, Kano (BUK). All plant materials were
confirmed in the herbarium section of Plant Science Department of BUK.

**Extraction of essential oils**

The essential oils were extracted from clove (*Syzygium aromaticum*) seeds, moringa (*Moringa oleifera*) seeds and neem (*Azadirachta indica*) seeds using the Soxhlet extraction process described by Lekgari (2010). Essential oils from clove seeds, moringa seeds, neem seeds, guava leaves and black pepper seeds were extracted using the Soxhlet Extraction Process described by Lekgari (2010) and William (2007) as follows:

A quantity (300 ml) of petroleum ether was poured into a round bottom flask equipped with a Soxhlet apparatus and condenser. Six pieces of anti-bumping granules were then added and 10 g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Soxhlet extraction was carried out at 40-60°C. When the solvent was boiling, the vapour rose through the vertical tube into the condenser at the top. The liquid condensate dripped into the filter paper thimble in the centre which contained the solid sample to be extracted. The extract sipped through pores of the thimble and filled the siphon table, where it flowed back down into the round bottom flask quick fit. This was allowed to continue for 30 min. It was then removed from the tube, dried in the oven, cooled in the dessicator and weighed again to determine the amount of oil extracted. Further extraction was carried out at 30 min interval. The experiment was repeated by placing 5 g of the sample into the thimble again, the weight of oil extracted was determined for each 30 min interval. At the end of the extraction, the resulting mixture containing the oil was left for three days for the solvent to evaporate and the oil was left behind.

**Mold strains**

The mold strains were isolated from farinaceous food samples (rice, maize and millet grains). The procedure described by Navi et al. (1999) for isolation of molds from food grains was followed. *A. niger*, *A. flavus*, *A. ochraceus*, *Penicillium spp*, *Rhizopus spp*, *Trichosporon spp*, and *Alternaria spp* were isolated, identified and used as test microorganisms in the antimold assays.

**Screening for antimold activity**

Diffusion plate procedure using filter paper discs was used for the determination of antimold activity of essential oils (Cheesbrough, 2000; Serban et al., 2011). For this, 1 ml of mold suspension (approximately 10^6 spores) prepared with sterile 0.85% physiological saline solution (and standardized using Mc Farland) was uniformly spread on the sterile Saboraud agar (SDA) contained in Petri dishes using sterile swab sticks. Sterile filter paper discs (Whatman diameter 6 mm) were soaked with 0.02 ml of each essential oil and placed on the centre of the Saboraud agar Petri dishes inoculated with the mold suspension. The incubation time was 5-7 days at 25°C. At the end of the incubation period, the inhibition halo diameters were measured and expressed in millimeters. When the inhibition halo observed was equal or higher than 10 mm diameter, it was considered as positive antimold activity (Lima et al., 1993).

**Determination of the minimum inhibitory concentration (MIC)**

Essential oil that showed activity against the test organisms was evaluated for its MIC and it was carried out by the plate diffusion procedure using filter paper discs as described above (Serban et al., 2011). The different concentration solutions used in the MIC assay were: 7.5 µl/disc (0.75 ml of essential oil and 0.25 ml of DMSO plus 100 filter paper discs), 5.0 µl/disc (0.5 ml of essential oil and 0.5 ml of DMSO plus 100 filter paper discs), and 2.5 µl/disc (0.25 ml of essential oil and 0.75 ml of DMSO plus 100 filter paper discs). Control was carried out with discs impregnated with DMSO only by the diffusion technique using filter paper discs. The incubation period was 5-7 days at 25°C. The lowest concentration able to develop inhibition halos equal or higher than 10 mm diameter was considered as MIC.

**RESULTS AND DISCUSSION**

Bioassay of essential oils against the isolated mold strains showed varying results. Clove essential oil showed a considerable result with inhibitory activity on all assayed mold strains at various concentrations. Other essential oils (neem and moringa) showed absence of any inhibitory activity on all assayed mold strains.

Plants are natural reservoir of medicinal agents almost free from the side effects normally caused by synthetic chemicals (Fennel et al., 2004). Essential oils are volatile substances contained in plant organs. Screening results for essential oils antifungal activity are shown in Table 1. From all essential oils evaluated, only one (*S. aromaticum* essential oil) showed inhibitory activity on all assayed mold strains. In other words, all the mold strains were sensitive to the essential oils of cloves. This work agrees with the work of Pinto et al. (2009), who also showed that clove essential oil has wide spectrum antifungal activity. Bonso and Rai (2008) also reported clove’s essential oil to have activity against *A. fumigatus* and *A. niger*.

The result from this work showed *Moringa oleifera* seed oil to have no activity against the test molds. *Azadirachta indica* essential oil also did not show any antimold activity. Contrary to our results, some researchers like Sai Ram et al. (2000) have shown that this essential oil has a high antibacterial activity. On the other hand, in the
Table 1. Inhibition zone diameters (mm).

<table>
<thead>
<tr>
<th>Mold strains</th>
<th>S. aromaticum</th>
<th>M. oleifera</th>
<th>A. indica</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria spp</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rhizopus spp</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. niger</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. flavus</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. ocraceus</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sporotrichum spp</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Minimum inhibitory concentration values for S. aromaticum (µl/disc).

<table>
<thead>
<tr>
<th>Mold strains</th>
<th>S. aromaticum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria spp</td>
<td>2.5</td>
</tr>
<tr>
<td>Rhizopus spp</td>
<td>2.5</td>
</tr>
<tr>
<td>A. niger</td>
<td>2.5</td>
</tr>
<tr>
<td>A. flavus</td>
<td>2.5</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>2.5</td>
</tr>
<tr>
<td>A. ocraceus</td>
<td>2.5</td>
</tr>
<tr>
<td>Sporotrichum spp</td>
<td>2.5</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>5.0</td>
</tr>
</tbody>
</table>

MIC determination assay, S. aromaticum essential oil showed activity to all the test concentrations on all assayed mold strains except for Penicillium spp where the MIC was 5.0% (Table 2). Absence of inhibitory activity of Moringa oleifera and Azadirachta indica essential oils on the assayed mold strains indicated that the oils were not active against the tested molds which may be due to membrane efflux systems or due to insensitivity of the target sites (Morrissey and Osbourn, 1999).

The bioactivity of the essential oil from cloves is indicative of its promising potentials for the development of antimicrobial agents, which would have a great acceptability particularly in developing countries. The World Health Organization estimates that herbal medicine is still the main stay of about 75-80% of the world population, mainly in the developing countries for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side-effects (Kamboj, 2000; Yadav and Dixit, 2008).

Conclusion

According to the results, one of the essential oils tested showed some in vitro inhibitory activity against molds, and that plant derivative products could be used as alternatives to control microbial contaminants of foods such as molds, and could be able to become useful tools for application in foods conservation systems.

REFERENCES


