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Full Length Research Paper

The preservative potentials of sweet orange seed oil on leather products in Nigeria

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Orange seed oil was extracted using the steam distillation method. The fungi isolated from the leather samples were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Paecilomyces* sp., *Penicillium* sp., *Rhizopus nigricans and Alternaria* sp. However, the fungal species vary from person to person. The orange seed oil was active against *Paecilomyces* sp., *Penicillium* sp. and *Rhizopus nigricans* at a minimum concentration of 2.0, 3.0 and 3.0%, respectively, whereas the other isolates were inhibited at higher concentrations. There was considerable reduction of the mycelia growth when 3% oil extract was introduced into the medium as against growth on only Czapek's Agar medium. There was no inhibition against *Alternaria* sp.

Key words: Sweet orange seed oil, *Aspergillus* sp., *Penicillium* sp., extract.

INTRODUCTION

Sweet orange tree (*Citrus sinensis*) belongs to the family *Rutaceae.* The orange tree is about 7.5 m or with great age, up to 15 m high. The fruit is globose, subglobose, oblate or somewhat oval. It is 6.5-9.5 cm wide. The outer rind is orange or yellow when ripe; the inner rind is white, spongy and non-aromatic. The pulp is yellow, orange or more or less red consisting of tightly packed membranous juice sacs enclosed in 10-14 wedge-shaped comparts which are separated as individual segments. Each segment may contain 2-4 irregular seeds, white externally and internally. The plant is found in China, India, South America, Mexico, Africa, Australia and other countries. Lesser qualities are produced in Puerto, Central America, New Zealand and West Africa where the fruit does not require an appealing color but is popular for its quality and sweetness.

It is widely used in juice making. The seeds and the pulp are used for animal feed but in some parts of Africa, the seeds are mainly discarded. Biodeterioration is an important factor impairing aesthetic, functional and other properties of many materials including leather (Orlita, 2004). Glucose, reduced chrome, vegetable tannages, natural fats and liquors which are added at several stages in leather manufacturing are retained in the finished leather and encourage the growth of fungi on the leather material (Muthusubramanian et al., 2008) which bring about deterioration. The case or challenge of fungal attack on leather and the leather products is very critical and has presented a nagging problem on finished products (Akpomie et al., 2006).

To produce protection on export of leather and for long term holding of leather, it is required that fungicides be effective against a wide range of fungal organisms that can bring about deterioration of the product. Fungicides like paranitrophen and pentachlorophenol were specified for use in the manufacture of leather (Adminis et al., 2001). Unfortunately, the toxic properties and poor degradability of these fungicides led to their non-acceptability as fungicides. The use of 2 -amino quinolone derivatives has been applied for the preservation of raw hides and skins and leather and found to be effective against many fungi. Its drawbacks however, are the costs implication and limited use for long-term preservation (Perumal et al., 2004). The organotins have been reported to possess great biocidal activities but have been illegalized in some countries due to increasing health and environment regulations and restrictions. Some of these chemicals when used in excess become recalcitrant in the environment and constitute environmental concern. One other important limitation in the use of chemical is the development of tolerance by some organisms due to mutability of genes responsible for resistance.

There is need to look into naturally occurring fungicides from plants which may be cost-effective with low human toxicity and minimum environmental impact because they are biodegradable. This study is therefore aimed at

Table 1. Occurrence of the fungal isolates from the samples (%).

Fungus	Number of occurrence (%)	Number of occurrence	Total number of samples
A. niger	100	12	12
A. fumigatus	83	10	12
A. flavus	67	8	12
Paecilomyces sp.	33	4	12
Penicillium sp.	83	10	12
Mucor mucedo	17	2	12
Rhizopus nigricans	80	10	12
Alternaria	100	12	12

extracting sweet orange oil and evaluating the antifungal activity against fungi isolated from leather and leather shoes.

MATERIALS AND METHODS

Isolation of organism

Microorganisms were isolated from leather materials obtained from Zaria and the open markets in Kano, Abraka and Lagos towns in Nigeria. The leather materials were swabbed with sterile cotton wool moistened with normal saline and subsequently shaken in 10 ml sterile distilled water in 50 ml flask. Thereafter, Czapek Agar plates were inoculated with a loopful of serially diluted samples. After incubation at room temperature (30 ± 2°C) for up to 5 days, emerging colonies were subcultured to fresh agar plates and identified by standard microbiological procedures (Morphology, color, spore shape, mycelia characteristics using the taxonomic keys of Ainsworth et al., 1973).

Extraction of oil

The seeds extracted from discarded orange fruits were washed with distilled water and stored in the refrigerators at 4°C for further use. The steam distillation method (Asthana et al., 1986) was used for the extraction of oil from sweet orange seeds.

Challenge test

Czapek's Agar Medium was incorporated with 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 (%v/v) oil extract. The plates with each concentration was inoculated with the isolates and incubated at room temperature for 5 days. The minimum concentration that prevented the growth was taken as the minimum inhibitory concentration (MIC).

The leather shoe samples were polished with the extract. Two small pieces of each of sterilized and unsterilized leather materials were dipped in the 5% extract agitated and in an orbital shaker for 4 h and each of the samples was left in the open and observed daily for fungal growth.

RESULTS AND DISCUSSION

Table 1 presents the percentage occurrence of the different fungal isolates. The isolates from all the samples were identical and included *Aspergillus niger*, *A. fumigatus*, *A. flavus*, *Paecilomyces, Penicillium* sp., *Mucor mucedo*, *Rhizopus nigricans* and *Alternarias* sp. Many representatives of the isolated fungi utilized for their growth and development, the constituents of tanning processes, hence they are found on the finished leathers and their products where they cause fermentation of the material due to the effect of tannase enzymes. The fungi present varied from person to person which revealed that fungi present depended mainly upon the materials used in the processing, the surroundings in which the leathers were got and the footwear worn (Kanat and Ranmanathan, 2007).

A. niger and *Alternaria* sp. had the highest frequency of occurrence (100%). They occurred in all the samples. *M. mucedo* occurred least. The temperature of growth (30°C) favored the growth of most of the organisms. This is evidenced in the work of Rakdonirainy and Lavedine (2005) that tropical and subtropical climates favor the growth and occurrence of many fungi in soil and decomposing organic matter. The variation in temperature might also account for why some fungi occurred more than others.

Table 2 shows the percentage growth of the fungal isolates with and without the oil extract was in the medium. The mycelia diameters were quite high for *A. fumigatus* and *R. nigricans*. There was 100% reduction for *Paecilomyces* and *Penicillium* and 29 and 64% for *A. niger* and *A. flavus*, respectively. The fungal mycelia might have been better reduced with increased concentration of the oil extract.

The response of the isolated organisms to the oil extract as evidenced in Table 3 suggests that the oil has a great potential as a natural substance against deterioration or spoilage of the leather materials. All the organisms except *Alternaria* sp. were inhibited by the oil extract but there were variations in the concentration at which the inhibitory actions were effected. The MIC ranged from 2.0 mg/ml for *Paecilomyces* sp. to 5.0 mg/ml for *M. mucedo.*

The treated samples treated with the oil extract showed positive results with the oil extract. There was no visible growth on the sterilized samples dipped in the oil extract.

Table 2. Diameter of mycelia of fungi with (+) (2.5%) and without (-) oil extract in the medium.

Fungus	Reduction of mycelia (%)	With oil extract (+)	Without oil extract (-)
A. niger	29	10.50	7.42
A. fumigatus	64	14.60	5.23
A. flavus	29	12.20	8.64
Paecilomyces sp.	100	16.10	0
Penicillium sp.	100	12.25	0
Mucor mucedo	-	8.70	9.82
Rhizopus nigricans	79	6.25	1.30
Alternaria	11	11.72	10.38

Table 3. Minimum inhibitory concentration (MIC) of the sweet orange seed oil on the fungal isolates (% v/v).

Isolates	MIC (% v/v)
A. niger	4.0
A. fumigatus	3.5
A. flavus	4.5
Paecilomyces sp.	2.0
Penicillium sp.	3.0
Mucor mucedo	5.0
Rhizopus nigricans	3.0
Alternaria	5.0

Table 4. Macroscopic observation for fungal growth.

SAMPLES	Zaria	Kano	Lagos	Leather shoe A	Leather shoe B	Leather C
Sterilized in oil extract	-	-	-	ND	ND	ND
Unsterilized in oil extract	+	++	-	-	-	-
Untreated samples	+++	+++	++	+++	++	+++

ND = Not determined; - = no growth; + = sparse growth; ++ = moderate growth; and +++ = heavy growth

The unsterilized samples in oil extract showed little growth on the Zaria samples and moderate growth on Kano samples. There was no visible growth on the other samples; the untreated samples had heavy growths (Table 4). The orange seed oil may possess chemicals that are constituents of a wide variety of plants and have great anti-microbial potentials that could have been responsible for the inhibition of the growth of most of the fungi on the treated leather materials. Phenolic acids and essential oil extracts of pepper fruit have antifungal activity against some fungi (Ejechi and Akpomedaye, 2004).

Conclusion and Recommendation

Most of the chemicals presently used as fungicides in the tanning industry poses environmental and safety hazards. The sweet orange oil tested possesses a great potential in the prevention of spoilage of leather and leather products by the organisms isolated. Orange seed oil is a natural organic substance which can be biodegraded and so, poses little or no environmental hazards.

It is therefore recommended that the oil be incorporated in some steps in leather processing, in shoe polishes and other shoe treatment agents so that it serves as a preservative against fungi that can cause deterioration of these products.

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