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ORIGINAL ARTICLE

Antifungal Activity of Essential Oils against Local Wood Degrading Cellulolytic Filamentous Fungi

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ABSTRACT
Fungal growth on wood is a critical economic concern for the building industry. An ideal compound for wood protection for interior applications must be nontoxic, hypoallergenic and able to provide long term efficacy under high humidity. The objective of this study was to investigate the inhibitory effects of natural plant extracts, such as essential oils, on wood. Five essential oils (ajwain oil, clove oil, garlic oil, neem oil and olive oil) were evaluated for their ability to inhibit weight loss by soil wood blocks tests. In this study neem, ajwain and clove oil was found most effective against all the test fungi (Aspergillus niger 101-6, A. flavus102-4, Paecilomyces variotii103-7, Penicillium sp.104-4, Trichoderma sp.106-1, Grifola sp.107-1 and Trichoderma viride 108-1). A perusal of the data showed that most of the essential oils tested here posses antifungal ingredients in them. In another experiment soil wood block test and dip state method, neem and clove oil also inhibited fungal growth on the wood surface after 16 weeks. These findings support the potential use of essential oils for natural wood protection against decay mold fungi infestation for surface treatment or fumigation of wood products.
Key Words: *Essential oil, Antifungal activity, Wood decay fungi.*

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INTRODUCTION

Wood is the most important natural renewable sources of energy and there for has a major future role as an environmentally cost effective alternative to burning fossile fules. The major role of wood is not only the provision of energy but also the provision of energy sufficient material for our buildings and many others products. Wood is the fifth most important product of the world trade. Resource analysis have led to the conclusion that wood and fibre needs over the next 40 years can only be met by logging 20-40% of the total present standing timber inventory in the natural forest.

We know that microbial biodegradation causes heavy losses of wood forest and their products. Necessary steps have been taken to overcome the problem of microbial hazards by improving suitable measures to check the biodegradability. The term wood preservative defines that are wood preservation is the process of preserving wood from the wood destroying agents like insects and fungus so that the life span of the wood can be extended. It refers to the treatment of wood with chemicals to impart resistance to degradation and deterioration by living organisms. The poisonous nature of wood preservatives presents some risk that they might also be hazardous to the people, livestock and greenhouses plant. Preservative effectiveness is influenced by the type of preservative chemical, the method of application, the extent of penetration into the wood and the retention of the preservative in the wood after treatment. The effectiveness of a wood preservative depends on the depth of its penetration into the wood. The depth of penetration depends on the wood species, the proportion of sapwood to heartwood and the treatment process used.

These chemical fungicides are not environmentally suitable for many indoor applications. Recent restrictions, internationally are limiting the use of chemical fungicides for wood preservations, primarily due to increased disposal problems as treated wood is taken out of service. The current trend is therefore to seek alternatives to synthetic chemicals with attention focused on the use of natural products of plant

origin, which are not only effective, but are also biodegradable. It has been estimated that less than 1-10% of the large diversity of 250-500 plant species on the earth have been studied chemically and pharmacologically for their medicinal properties [1].

The searches for natural solutions that are user friendly and showing negligible toxicity to humans are increasingly 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 [2].

The aim of this study was to evaluate the ability of five commercially available essential oils for fungal inhibition on wood. The five oils were then extensively characterized for their in vitro antifungal activity.

MATERIALS AND METHODS

Fungal strains

In this study, seven local wood degrading cellulolytic filamentous fungi *Aspergillus niger101-6, Aspergillus flavus102-4*, *Paecilomyces variotii 103-7, Penicillium sp.*104-4, *Trichoderma sp. 106-1, Grifola sp*. *107-1* and *Trichoderma viridi 108-1* were used. These fungi were isolated from degraded wood samples and these fungi were used in the antifungal assay. They were maintained and grow on 2% PDA at 25°C.

Sources of essential oils

The essential oils are secondary metabolite plant extracts. The variety of uses for essential oils and their components are very broad and determined by their chemical, physical and sensory properties. The essential oils used in this study are listed in Table 1. These extracts were selected due to their commercial availability and association with broad function groups. All essential oil products were purchased from health shops and have99.7% purity (information provided by essential oil extraction companies to health shop).

Table 1: Oils of Indian medicinal plants used for antifungal activity against wood decaying cellulolytic filamentous fungi.

S.No.	Local Name	Botanical Name	Plant part used	Family	Medicinal use
1	Garlic oil	Allium sativum	Bulb	Liliaceae	Antifungal, Antibacterial, expectorant
2	Neem oil	Azadirachta indica	Leaf & Seeds	Meliaceae	Antifungal, Antiviral
3	Clove oil	Eugenia caryophyllata	Dry flower	Myrtaceae	Antifungal, Antibacterial
4	Olive oil	Olea europea	Fruit	Oleaceae	Antifungal, Antibacterial
5	Ajwain oil	Trachyspermum captivum	Dried fruits	Umbelliferae	Antifungal, Antibacterial, Antispasmodic throat infection

Antimycotic activity of some essential oils against selected isolated cellulolytic filamentous fungi

Growth inhibition measurments on culture medium

In the present study essential oils of five plants (Table 1) were tested against isolated seven cellulolytic filamentous fungi. The agar diffusion plate method [3] was used to test oils for the antifungal properties against seven fungal species viz, *Aspergillus niger101-6, Aspergillus flavus102-7, Paecilomyces variotii 103-7*, *Penicillium sp.104-4, Trichoderma sp. 106-1*, *Grifola sp*. *107-1* and *Trichoderma viridi 108-1*. Required amount of oils were dissolved in 0.1% Tween 80 (for enhance solubility of oils) and thoroughly mixed with melted potato dextrose agar to provide 0.1%, 0.15% and 0.5% concentration. About 10 ml treated (with oil) or untreated (without oil) medium were poured into petri plate (70 mm diameter). Untreated medium was used as a control. Seven days old fungal cultures, maintained in the laboratory, were placed in the center by sterile cork borer (6 mm diameter disc) of each petri plate. There were three replicates of each treatment. The inoculated petri plates were incubated 28±2°C for five days. After incubation the colony diameter was measured in millimetre scale. The fungitoxicity of the tested oils in terms of Antifungal Index (AI) i.e. percentage inhibition of mycelial growth was calculated by using the formula-

$$\text{Antifungal Index (\%)} = \frac{Dc - Dt}{Dc} \times 100$$

Where Dc = average increase in mycelial growth in control
Dt = average increase in mycelial growth in treatment [4].

Evaluation of the antifungal activity of essential oil under soil wood block test by dip state treatment

Soil block culture bottles were prepared according to "American Wood Preserves Association" [5] E-10-06 with a modification of local available timber wood block size to 1×1×1cm. In soil block bottles, local timber wood blocks were inoculated with isolated locally available cellulolytic filamentous fungi (*Aspergillus niger101-6, Aspergillus flavus102-4, Paecilomyces variotii 103-7*, *Penicillium sp.104-4, Trichoderma sp. 106-1*, *Grifola sp*. *107-1* and *Trichoderma viridi 108-1*). Bottles were incubated at 28±2˚C temperature, 80% RH for 3 weeks until the fungi completely colonized each isolates. Pre-weighed local timber wood blocks, conditioned at 28±2˚C temperature and 80% RH were dip treated for 15 seconds in individual full strength (100%) essential oils, before inoculation with spores of the test fungi. Specimens were inoculated with 1 ml of individual fungal spores 24 hours post-treatment. Specimens were evaluated for mold growth at 4, 6, 10, 12 and 16 weeks and rated on a scale of 0 to 5 with 0 indicating no growth and 5 indicating heavy mold growth. Specimens rating ceased when test oils failed to substantially inhibit mold growth. Following incubation, surface mycelial growth was rated visually by percentage of surface coverage before mycelia were brushed off each block. Blocks were oven dried at 60˚C for 24 hrs and reconditioned at 28±2˚C temperature, 80% RH for 2 weeks. Block weights were measured and average percent weight loss was calculated. Three replicates were used in each test.

RESULTS AND DISCUSSION

Antimycotic activity of some essential oils against selected isolated cellulolytic filamentous fungi

Growth inhibition measurments on culture medium

The initial screening (Figure 1) showed that antifungal activity of Garlic, Neem, Clove, Olive and Ajwain oils were determined against seven fungi viz. *Aspergillus niger101-6, Aspergillus flavus 102-4*, *Paecilomyces variotii 103-7*, *Penicillium sp.104-4, Trichoderma sp. 106-1, Grifola sp*. *107-1* and *Trichoderma viridi 108-1*. 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. Result showed that neem, clove and ajwain oils possess a remarkable antifungal activity against all tested fungi. At the concentration of 0.5%, these essential oils showed highest inhibition of the growth of all tested fungi.

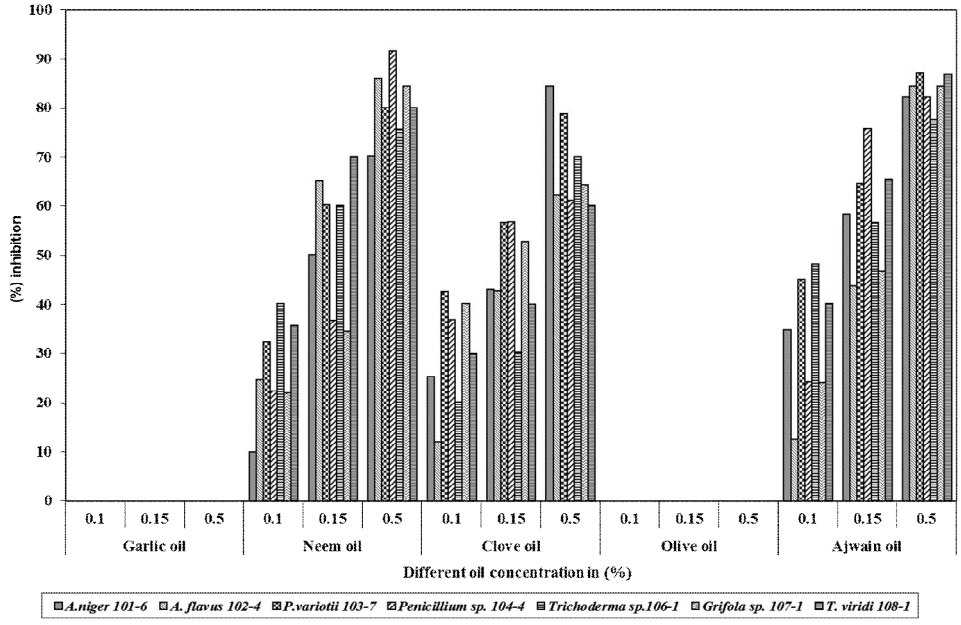

Fig. 1: Antifungal Index (%) of various concentration of different essential oils against wood degrading cellulolytic filamentous fungi.

The neem oil was found most effective at 0.5% concentration against all the test fungal isolates. It showed 70.22±0.05%, 86.02±2.45%, 80.01±0.06%, 91.70±2.58%, 75.65±0.01%, 84.40±2.44% and 80.05±0.06% growth inhibition (Antifungal Index) against, *Aspergillus niger101-6, Aspergillus flavus102-4, Paecilomyces*

variotii 103-7, Penicillium sp.104-4, *Trichoderma sp. 106-1, Grifola sp*. *107-1* and *Trichoderma viridi 108-1* respectively.

Ajwain oil was the second most effective oil with 82.20±1.46%, 84.41±2.44%, 87.11±1.28%, 82.25±1.46%, 77.79±1.71%, 84.45±2.44% and 86.80±1.09% growth inhibition against, *Aspergillus niger101-6, Aspergillus flavus102-4, Paecilomyces variotii 103-7*, *Penicillium sp.104-4, Trichoderma sp. 106-1, Grifola sp*. *107-1* and *Trichoderma viridi 108-1* respectively.

Clove oil was also effective against all the test fungi. Clove oil exhibited 84.41±2.44%, 62.20±0.96%, 78.80±2.45%, 61.15±0.96%, 70.18±0.19%, 64.41±2.85% and 60.09±1.91% growth inhibition against, *Aspergillus niger101-6, Aspergillus flavus102-4*, *Paecilomyces variotii 103-7, Penicillium sp.*104-4, *Trichoderma sp. 106-1, Grifola sp*. *107-1* and *Trichoderma viridi 108-1*.

A perusal of the data showed that most of the essential oils tested here possess antifungal ingredients in them. However the oils of garlic and olive shows could not inhibit the growth of all tested fungi. The presence of antimicrobial activity in the oils obtained from the plants may be due to the presence of the component like aliphatic acids and aldehydes.

In this study, pure active component of the extracts rather than the essential oils itself were used. Reason because many factors affect the consituents of essential oils, depending on the method of extraction, ecology and genotype. The same species of plant can give rise to essential oils with different compositions and different bioactivities [6]. Therefore, it appears more judicious to use a commercially available pure component which is more stable, consistent and relatively cheaper than an essential oils itself.

Essential oils have a long history of safe use as an antimicrobial agent in the food industry. For example, a study performed by Guynot *et al.,* [7] showed that cinnamon leaf, clove, bay, lemongrass and thyme essential oils completely inhibited the growth of fungi commonly causing the deterioration of baking products.

Neem oil showed greater suppression in the growth of all tested fungi. According to Niaz and Kazmi [8] neem oil was quite effective for *Aspergillus* sp. Vir and Sharma [9] found antifungal activity in neem oil against *Alternaria alternata* and *Aspergillus* sp. Sinniah *et al*., [10] also studied the toxicity of neem oil on *Aspergillus* sp.

More recently Wagh *et al.,* [11] reported the antifungal properties of oils obtained from *Trigonella foenum-graecum* and *Pongamia pinnata.* Both the oils showed high degree of antimycotic activity against *A.niger* and *A.fumigatus*. Chemical analysis of oils performed by gas chromatography (GC) and gas chromatography/ mass spectrometry (GC-MS) showed the presence of metalinic acid, palmitic acid, linoleic acid, oleic acid and stearic acid. The presence of antimicrobial ingredients in higher plants has been reported have various workers [11]. The active principle of plant parts can be anyone like alkaloid, glycocides, saponins, phytosterol, phenolic compounds, tannins, proteins, aminoacids or fixed oils. The exact mechanism of action of these active principles of plants is still not known. The growth inhibitory effect of essential oils may be due to their direct effect or supperssion of enzymatic activity of test fungi.

Evaluation of the antifungal activity of essential oil under soil wood block test by dip state treatment

Essential oil evaluated in this study was selected for their previously reported anti-microbial properties in pharmaceutical food and packaging applications. Antifungal effect of essential oils on wood against selected seven isolated local common airborn cellulolytic filamentous mold fungi were treated by dip method and soil wood weight loss method and the result presented as the average weight loss (%) and rating of three specimens in Figure 2 and 3. In this study essential oils were selected from various referenced literature for their antifungal properties.

In the soil block test, mycelial growth on the wood surface was first evaluated visually. All specimens treated with full strength essential oils showed no growth. Untreated control specimens showed extensive growth for all test fungi and weight loss occured in wood blocks (Figure 2) and these figure also show no weight loss occured in specimens treated with full strength essential oils but slightly weight loss in *Grifola sp. 107-1* treated with neem oil wood block (1.0%) and 1.5% weight loss (*A .flavus 102-4*) with ajwain oil.

Soil block culture method demonstrated that essential oils inhibited the growth of test fungi. In order to evaluate essential oils' long term efficacy, an experiment to study the shelf life of the treatment is in progress. Wood treated with essential oils will be stored for 6 and 12 months before challenge with test fungi.

In another experiment, antifungal effects of essential oils on wood against selected seven common isolates mold fungi were assessed by dip stake method and the result are presented as the average ratings of three specimens in Figure 3. Specimens were initially rated after 4 week incubation. Rating continued periodically through 16 weeks incubation.

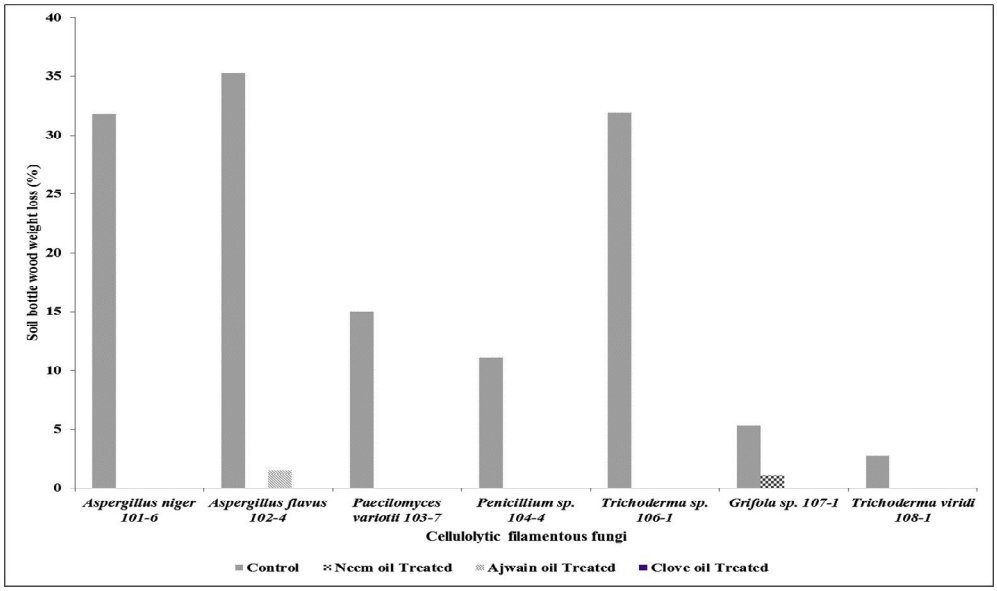

Fig. 2: Essential oil (full strength 100%) inhibit fungal growth by soil bottle wood weight loss study.

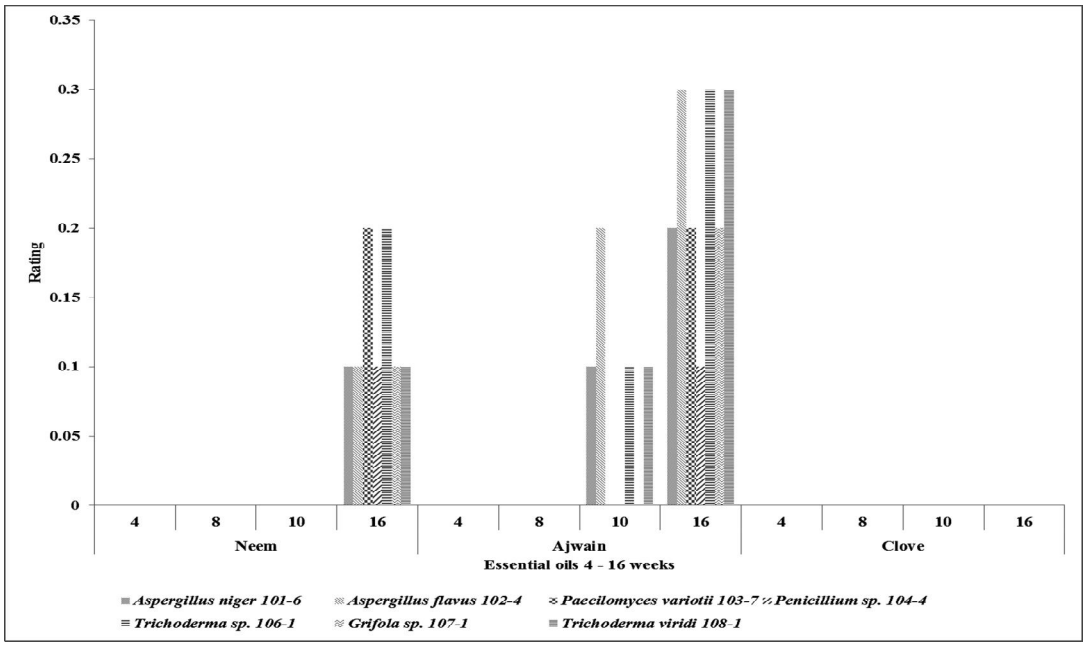

Fig. 3: Cellulolytic fungi inhibition of neem oil, ajwain oil and clove oil by dip stake method.

Result of the dip stake method showed that neem oil completely inhibited all test fungi for at 4-10 weeks (rated zero) but showed 0.1 and 0.2 mold coverage at 16 week for *Aspergillus niger101-6, Aspergillus flavus102-4*, *Penicillium sp.104-4*, *Grifola sp*. *107-1* and *Trichoderma viridi 108-1* and *Paecilomyces variotii 103-7* and *Trichoderma sp. 106-1* respectively. Ajwain oil inhibit growth of *Aspergillus niger101-6*, *Aspergillus flavus102-4, Trichoderma sp. 106-1* and *Trichoderma viridi 108-1* test fungi for up to 8 weeks but only *Paecilomyces variotii 103-7, Penicillium sp.104-4* and *Grifola sp*. *107-1* fungi for up to 10 weeks.
In case of clove oil all test fungi completely inhibited for 4-16 weeks and completely inhibited of mycelial growth on wood specimens. When comparing data obtained in different studies, most publications provide generalizations about whether or not plant oil possesses activity against fungi. However not all details is provided about the extent or spectrum of this activity. Some publications also show the relative activity of plant oils and extracts by comparing results from different oils tested against the same organism(s). Comparison of the data obtained in this study with previously published results is problematic. First, the composition of plant oils and extracts is known to vary according to local climatic and environmental conditions. Furthermore, some oils with the same common name may be derived from different plant species [12]. Secondly, the method used to assess antimicrobial activity and the choice of

test organism(s), varies between publications [13]. A method frequently used to screen plant extracts for antimicrobial activity is the agar disc diffusion technique [14].

The application of this method was only to the generation of preliminary, qualitative data only, as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium [13]. Agar and dilution methods are also commonly used. The results obtained by each of these methods may differ because variation of many factors between assays [15]. These include differences in microbial growth, exposure of micro- organisms to plant oil, the solubility of oil or oil components and the use and quantity of an emulsifier.

Eugenol is the main component of clove oil [16]. Which is the strongest inhibitor of enzyme processes and derivative compounds as methyle or acetyleugenol could change this property. Antimicrobial activity of this oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in deactivation of enzymes in fungi [17]. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to the irrelative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. In addition, Cowan [18] found that more highly oxidized phenols are more antimicrobial.

In general, the mechanisms thought to be responsible for phenolic toxicity of plant extracts to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins. Phenolic compounds possessing a C_3 side chain at a lower level of oxidation and containing no oxygen are classified as essential oils and are often cited as antimicrobial as well [19]. Many authors emphasized that antimicrobial effects of essential oil constituents are dependent on their hydrophobicity and partition in the microbial plasmatic membrane. The effect of specific ions due to their addition in/on plasmatic membrane had a great effect on the proton motive force, intracellular ATP content and overall activity of microbial cells including turgor pressure control, solute transport and metabolism regulation [20]. The fungicidal effect of eugenol (clove oil) resulted from an extensive lesion of the cell membrane. Clove oil and eugenol also caused a considerable reduction in the quantity of ergosterol, a specific fungal cell membrane component [21].

Considering their attribute and broad-spectrum activities, successful development of these compounds as antifungal would not only provide a potent tool for control of wood degradation, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other wood diseases.

CONCLUSION

These natural antimycotic compounds are useful to inhibit mold and decay fungi on wood in service or during storage of building materials, such as framing lumber, millwork, or truss systems.

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