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ALTERNATIVE NATURAL-SOURCES FUNGICIDE FOR PREVENTING THE FUNGAL GROWTH ON LEATHER SHOE: EXTRACTED FROM NEEM (Azadirachta Indica) LEAVES AND MAHAGONY (Swietenia Mahagony) FRUITS BARK

Md. Abdulla-Al-Mamun[1,2], **Rakibul Hasan**[2], **Md. Rubel Ahmed**[2] **and Rajon Kumar Raha**[2]

[1]Institute of Leather Engineering and Technology, Dhaka University, 44-50 Hazaribagh, Dhaka-1209
[2]Deparment of Leather Engineering, Faculty of Mechanical Engineering, Khulna University of Engineering & Technology, Khulna-9203, Bangladesh
almamunchem@gmail.com*, rakibul2k12@gmail.com, rubel.ahmed.kuet@gmail.com, and rajanraha@gmail.com

Abstract- The fungicide was made by a solvent extraction process and applied on the shoes by various way. Fungus was isolated from the shoe and cultured in 100 mm petri dishes with Sabouraud Dextrose Agar (SDA) media. Difference dosing of neem and mahagony extract was applied on the cultured fungus and measured the area of fungus by "Leaf Area Counter" software. Killing of fungus was optimized, and the minimum optimum dosing for neem leaves extract was found 0.6gm/5ml and for mahogany fruit bark extract 0.8gm/5ml respectively. The optimum doses of natural fungicide were then mixed with commercial shoe shiner and cultured the fungus in petri dishes with and without fungicides. Fungus was not able to grow in the fungicides containing shoe shiner, whereas fungus was grown in without fungicides containing shoe shiner within three days. We observed the fungicides-containing shoe-shiner treated dish until a month and found there is no fungus grown at all.
In conclusion, our extracted natural fungicides are very effective to kill the fungus and prevent the growth of fungus and it can be used commercially.

Keywords: Fungicide, Neem leaves extract, Mahagony fruit-bark extract, Leather shoe, Naturally fungus killing

1. INTRODUCTION

In rainy season or in atmospheric high content of moisture, fungus is grown on leather shoes. This fungus especially grew on the surface of shoe and damages the leather-surface by producing the stain. It is essential to prevent the fungal growth and remove the stains from the shoes for keeping the shining of shoe. Fungicide is one of the suitable substances to kill the fungus and to protect the shoe from fungal growth. There are many commercial grade of fungicides, among almost are manufactured by the conventional chemical process. However, most commercial available fungicides are highly toxic and expensive. Increasingly stringent environmental awareness, an alternative fungicides should have a high efficiency towards to fungi and should be less toxic, more environmentally friendly and cost effective. Searching, difference sources of natural fungicide, we found the neem leaves and mahagony fruits bark have been efficiently killed the fungus and prevented the fugal growth as well as the environmental friendly, cost effective, and it's an indigenous source.

Fungi have a wide diversification, and grown in an extensive range of habitats, including extreme environments such as deserts, high salt concentration, ionizing radiation, deep sea sediments, even some can survive in space [1, 2]. Most of the fungus grown in terrestrial environments, though several species live partly or solely in aquatic habitats. This organism spends part of its life cycle as a motile zoospore, enabling it to propel itself through water and enter its amphibian host [3].

Fungus is solely or partly aquatic habit and highly contagious, because fungus thrives in warm, sweaty, and dark places. Shoes, especially when worn for the entire day without any respite, or in rainy seasons are the perfect breeding ground easily to get fungus from used shoes or boots. It is necessary to remove or to protect the fungus, because its damage the shoe surface by staining, forming as well sometimes effected on the toe joint. In our present research, we extracted natural fungicides from Neem leaves and from Mahagony fruits bark for the first time application on shoes, which was dramatically kill the fungus and reduced to chances of becoming infected.

Globally, strict environmental legislation obliges adapt to alternative technologies inorder to minimize the

environmental impact. This includes the search for new fungicide compounds that are environmentally friendly. Contamination of synthetic chemical insecticides is also becoming a major problem. There is an urgent need for effective biodegradable pesticides with greater selectivity. Botanical insecticides have long been touted as an attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health. Alternative strategies include use of natural bio-pesticides from medicinal plants such as neem and mahagony to overcome contamination and pest resistance issues [4].

The botanicals (plant extracts) of neem and mahogany have enormous potential to influence modern agrochemical research. Neem, a natural pest control agent, belongs to the family of Meliaceae. It is also known as a "village pharmacy", due to its unique multifunctional-antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer, anti-malarial, antifungal and anticancer properties [5]. Mahagony tree/fruits bark is a potential source of bioactive compounds such as an antioxidant, antifungal and widely used in agriculture and medicine [6]. Application of neem and mahagony fruits extract used as an antifungal on shoes surface is the first time to our best knowledge.

The work is focused on the searching of new fungicide with higher efficiency against the fungi of leather shoes. Those fungicide should be less toxic with lower environmental and health impact than the conventional fungicide. The conventional synthetic antifungal might be hazardous due to contaminate with air, food as well as direct contact with human foot. In this research, we extracted environment friendly fungicide from neem leave and mahagony fruits bark by simple solvent extraction method and applied for the first time on fungus containing leather shoes for fungus killing.

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals Methanol, (Merk Germany), as for solvent, and Sabouraud dextrose agar (HIMEDIA, India) for medium preparation was purchase from supplier City Scientific Store, Khulna, Bangladesh.

2.2 Collection of Neem and Mahagony-fruits Bark

The green neem leaves and brown mahagony fruits bark were collected from trees abundantly available at the Khulna University of Engineering & Technology, Khulna, Bangladesh. The collected leaves and fruits were washed with tap water several times till the wash water contained no dirt particles and pigment followed by drying in sunlight. The dried leaves and fruits were ground using domestic mixer grinder and different size fractions were collected. The collected powder was preserved in an intact plastic container.

2.3 Methods of Solvent Extraction

The powdered leave and fruits-bark were soaked initially in methanol solvent having in plastic container for 7 days and 3 days respectively. Additional solvent is then poured on top of the materials and allowed to filter for percolate extraction by Whiteman filter paper. The additional filtration of the extract was repeated for two times, due to the percolation of extract.

The solvent-extract solution drying by air at normal room temperature condition. Where the methanol was vaporized in normal atmospheric temperature and extract was collected and stored in an air tight plastic container and keep it in a cool and dry place.

2.4 Isolation of Fungi from Contaminated Leather

A piece of the finished leather sample was wetted and place it in a closed cap pot to produce the artificial warm humid environment. After three days, fungus was grown on leather surface and then isolated the fungus by using a pincers. Collected fungus were than cultured in a Petridis containing nutrient medium and distributed the petri dishes by using inoculation loop.

2.5 Preparation of Nutrient Medium

Fungus nutrient medium was prepared by using Sabouraud dextrose agar (SDA). 26 gm of SDA was suspended in 400 ml of sterilized distilled water in a bottle. After that heated with frequent agitation and boil for one minutes to completely dissolve the medium. The medium containing bottle was then sterilized at 121 °C for 15 minutes in an autoclave (HIRAYAMA, Japan). Then cool the medium to room temperature and pour into the petri dishes.

2.6 Fungus Culture, Incubation with Extract and Counting

A typical isolated finished-leather-fungus was used in this study. The fungus were cultured in the SDA agar medium at 37 °C under in an incubator (Friocell, Germany). The fungus were plated in 100-mm Petri dishes and were allowed to grow for two days. The petri-dishes were sterilize in an autoclave before culturing the fungus. Within two days almost 80% fungus confluent was found. The old culture medium was merged with the extract-containing (5 ml) different dosing medium and distributed uniformly through the whole surface area of petri dishes. After that recultured with keep-up for several hours in an incubator. The fungus were counted under a Leaf Area Measurement Softwear with using taking picture. The fungus survival was determined as the percentage of the area of live fungus against the control dish fungus.

Different amount of extract with 5 ml commercial shoe shiner (Bata) was also applied on the fungus-cultured petri dishes as follow the before procedure. The optimum dosing was done on the typical fungus containing finished leather and observing the performance of prevention capacity for few days.

3. RESULTS

3.1 Neem leaf and Mahagony Fruits-bark Extraction

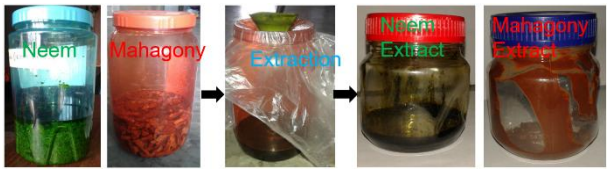

Fig. 1: Extraction process of neem leaf and mahagony fruits-bark by methanol-solvent

Fig. 1 shows the extraction process of neem leaf and mahagony fruits bark by methanol solvent. Total 320 gm of dry neem leaf was soaked in 825 ml of methanol and found 16.63 gm of neem extract. The extract production efficiency is about 5%. Moreover, at 300 gm of dry mahagony fruits-bark was soaked in 750 ml of methanol and found 9.6 gm mahagony extract. Production efficiency about 3.2%. Solvent extraction process is used for the separate compounds and percolate of perfumes, vegetable oil, or biodiesel etc. The selection of a suitable extraction method depends mainly on the work to be carried out, and whether or not the metabolites of interest are known. Plant constituents are usually contained inside the cells. The solvent used for extraction must therefore diffuse into the cell to dissolve the desired compounds and the solution must pass the cell wall in the opposite direction and mix with the surrounding liquid [7,8]. Extraction method is more efficient when done repeatedly with the amount of solvent. We repeatedly three times deepening the neem and mahagony fruits bark by exchanging and filtering of solvent.

3.2 Determination of optimum dosing

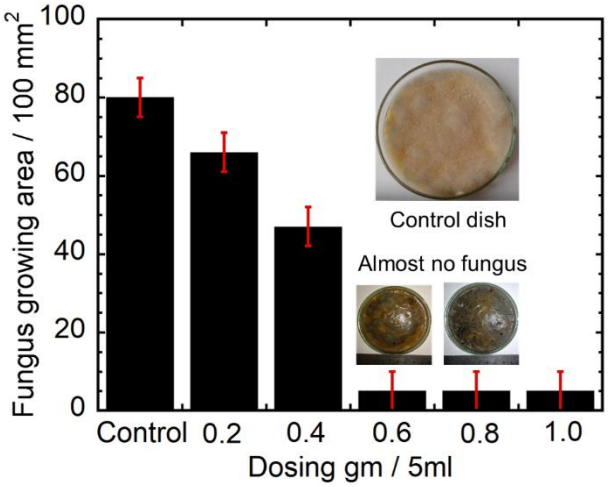

Fig. 2: Dosing graph for neem leaves extracted with media until 48 hours. Inset shows the control and fungus killing treated with 0.6 gm/5ml and 0.8 gm/5ml respectively.

Fig.2 shows the fungus area vs difference dosing of 0.2, 0.4, 0.6, 0.8, and 1.0 gm /5 ml neem extract treating dish at 48 hours. The control dish shows without any extracted neither any dosing. Fungus killing efficiency calculated based on control area of live fungus. Fig.2 shows the 0.2 and 0.4 gm extracted is not sufficient for fungus killing. But 0.6 affectedly kill the all fungus and found 0.8 and 1.0 gm same as steady state. From this Fig.2 it can be conclude 0.6 gm/5ml are optimum concentration for effective fungus killing.

The time optimization for further grow of fungus was observed until 97 hours of neem leaves of treated dish. It was shown that, 0.6 and 0.8 gm dosing of neem leaves treated dish was not further grown of fungus. Whereas low concentration of 0.2 and 0.4 gm extracted shown the fungus grown at 57 hours respectively. This also supporting that the 0.6 gm/5 ml concentration neem leave extract have the optimum for efficient fungus killing.

Fig.3 shows the fungus area vs difference dosing of 0.2, 0.4, 0.6, 0.8, and 1.0 gm/5 ml of mahagony fruits-bark extract treating dish at 48 hours. The control dish shows without any extracted neither any dosing. Fungus killing efficiency calculated based on control area of live fungus. Mahagony fruits-bark shows the 0.2, 0.4 and 0.6 gm extracted is not efficient for fungus killing. But 0.8gm affectedly kill the all fungus and found 1.0 gm same as steady state. The doses were increased, the percentages of viable fungus were decreased and finally at a dose of 0.8 gm/5ml only a very few fungus were viable. These results indicates that Mahagony fruits-bark shows significant potentiality against the viability and proliferation of fungus. From this Fig.3 it can be conclude 0.8 gm / 5ml mahagony fruits-bark extract concentration are efficient for optimum fungus killing.

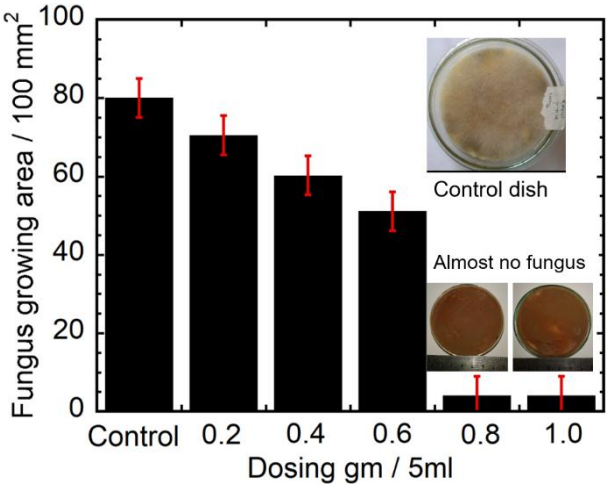

Fig. 3: Dosing graph for Mahagony fruits-bark extracted with media until 48 hours. Inset shows the control and fungus killing treated with 0.8 gm / 5ml and 1.0 gm / 5ml respectively.

The time optimization for further grow of fungus was observed until 97 hours. It was shown that, 0.8 and 1.0 gm dosing of mahagony fruits-bark treated dish was not shown any further grown. Whereas low concentration of 0.2, 0.4 and 0.6 gm extracted shown the fungus grown at 62 hours respectively. This also supporting that the 0.8 gm/ 5ml concentration mahagony fruits-bark extract are optimum for efficient fungus killing.

The proliferation fungus is inversely proportional related with the amount of extract dosing. If the extract

concentration is increased, fungus protection and killing potentiality also proportionally increased.

3.3 Application of optimum dosing fungicides with commercial shoe shiner

The optimum fungicides (neem leaves 0.6 gm and mahagony fruits bark 0.8 gm) extract was mixing 5 ml of commercial shoe shiner and applied on the fungus cultured petri dishes. In Fig. 4 shows the control and optimum neem extracted with shoe shiner mixing treated dish photograph at several days' application. The control means (left side) neither any extract nor media, only with shoe shiner applied photograph and right shows the optimum neem extracted with shoe shiner treated dish. All shoe shiner and extract applicability taken picture within the 7 days. The figure clearly shows, optimum dosing have ability to protected the fungus growing and have the long-term protection capacity of viable fungus. Whereas in control within 3 days fungus was grown with shoe shiner neither any extract. It is clearly conclude from this figure that, only shoe shiner is not sufficient for long-term preventing the fungus. But shoe shiner with neem leave extract-fungicides are effective for long time preventing the fungus. Observed a month, found any fungus grown at all.

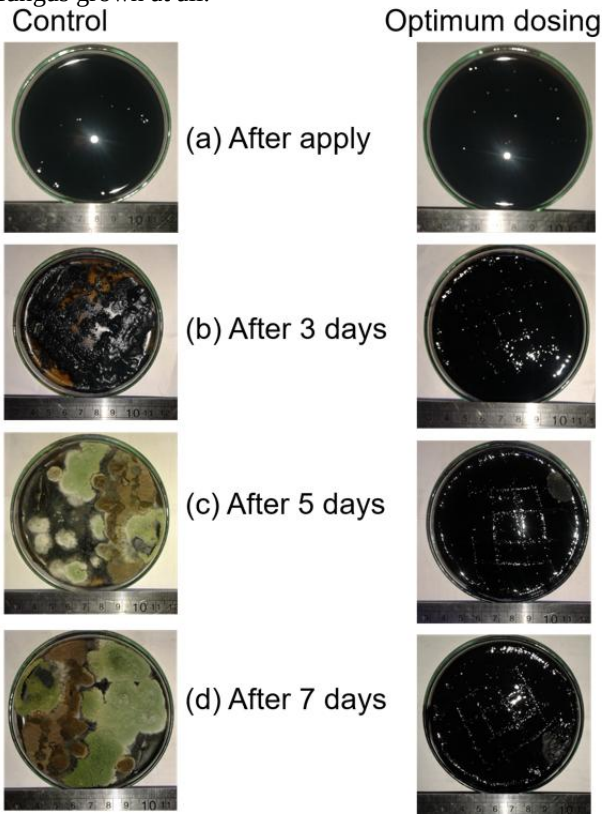

Fig. 4: Dosing graph for neem extracted with shoe shiner upto 7 days. The control (left) treated with only shoe shiner neither any extract nor any media. Optimum (right) treated with 0.6 gm neem extracted with shoe shiner.

In Fig. 5 shows the control and optimum mahagony fruits-bark extracted with shoe shiner mixing dish photograph at several days' application. The control means (left side) without any extract, only with shoe shiner applied photograph and right shows the optimum mahagony fruits extracted with shoe shiner treated dish. All shoe shiner and extract applicability taken picture within the 7 days. The figure clearly shows optimum dosing have protected the fungus growing and long-term not viable the fungus with extracted, whereas in control within 3 days fungus was grown with shoe shiner neither any extract. It is clearly conclude from this figure that, only shoe shiner is not sufficient for long time preventing the fungus. The optimum dosing after 7 days also shows some fungus. Shoe shiner with mahagony fruits-bark extract-fungicides is not so effective for long time preventing the fungus.

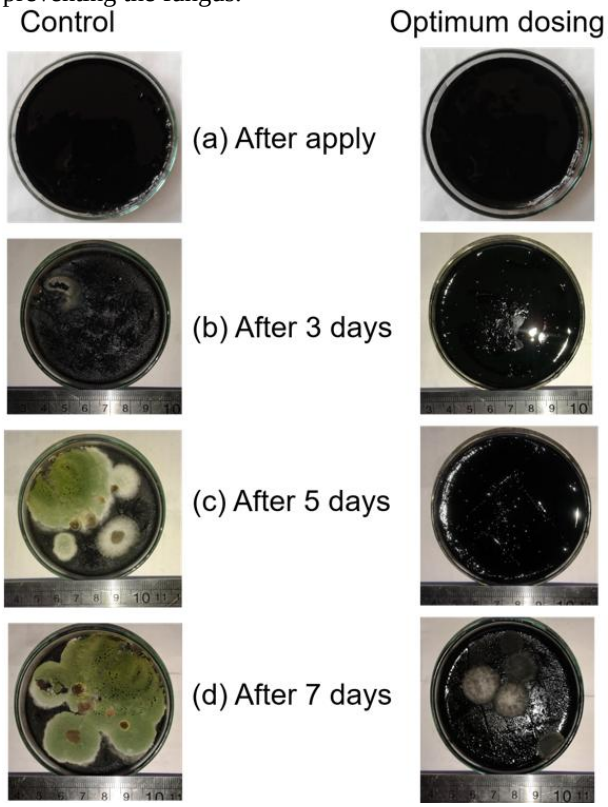

Fig. 5: Dosing graph for mahagony fruits-bark extracted with shoe shiner upto 7 days. The control (left) treated with only shoe shiner neither any extract nor any media. Optimum (right) treated with 0.8 gm mahagony fruits extracted with only shoe shiner.

3.3 Extract application of a typical fungus content leather

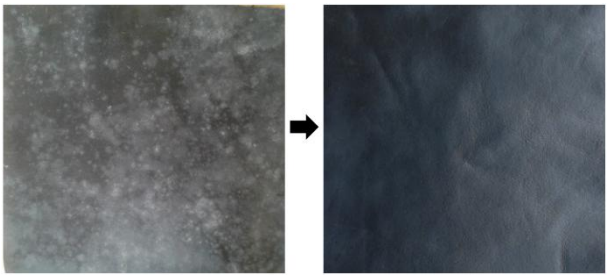

Fig. 6: Dosing graph for neem leave extracted with shoe shiner. Left shows the typical fungus containing leather and right shows neem extract spray-treated leather.

The optimum of neem leave-extract mixed with commercial shoe shiner and applied on fungus containing leather. Fig. 6 clearly shows the clean leather (right) after treatment of fungicides containing shoe shiner. It can be conclude from this figure, fungus killed and stain removing capacity of our synthesized natural fungicides is very effectible and it can be used commercially. The main purpose of this research is to provide a processes of antifungal treatment of footwear. We successfully done it by final application on shoe with the shoe polish.

4. DISCUSSION

We chose natural fungicides of neem leaves and mahagony fruits, because it is naturally available, environmental friendly and not any effect in eco-system. Our research shows neem leaves and mahagony fruits bark have enormous potential to kill the fungus. Conventionally synthetic 2-(Thiocyanomethylthio) benzothiazole (TCMTB) and mixture of phenolic compound as a biocide used in the leather, pulp-paper, and water-treatment industries. TCMTB might be enter aquatic ecosystems during its manufacture and uses. TCMTB is environmentally unstable; therefore, it is important to evaluate the toxicity of the more persistent degradation products [9]. Many of the first fungicides developed were inorganic compounds based on sulfur or metal ions such as copper, tin, cadmium and mercury that are toxic to fungi. Copper and sulfur are still widely used. Most other fungicides used today are organic compounds and thus contain carbon. The term "organic" as used here is based on chemistry terminology and differs from "organic" used to describe a system of agriculture that strives to be holistic and to enhance agro ecosystem health [10].

Plants and other organisms have chemical defenses that give them advantage against microorganisms such as fungi. Quercetin and β-sitosterol were the first polyphenolic flavonoids purified from neem fresh leaves and were known to have antibacterial and antifungal properties [11]. The Phytochemical constitutes of mahogany fruits-bark is might be effective for fungus killing [12].

Minimum inhibitory concentration (MIC) is defined as the lowest concentration (expressed as gm/mL) of an antimicrobial that will inhibit the visible growth of a microorganism such as fungus after an incubation period. Several dilutions at concentrations ranging from 0.2 gm/mL to 1.0 g/mL of the fungicides were prepared and the dilution that inhibits fungal growth was taken as the minimum inhibitory concentration. We found 0.6 gm /5ml MIC for neem leave extract and 0.8 gm/5ml for mahagony fruits bark extract are responsible for preventing the fungus growth. Those of results the neem leave is better than mahagony fruits bark extract. Therefore, mahagony bark extract need more concentration and also found the fungus grown after 7 days treated dish.

5. CONCLUSIONS

Fungicide is used to protect the shoe from fungal attack. As the chemical-fungicide is harmful for both environment and human health. So, natural fungicide should solve the problem of environmental pollution occurred by conventional chemically derived fungicide. As neem leaves extract and mahagony fruit bark extract both have fungicidal activity. This extract can be used as a replacement of conventional chemically derived fungicide, which is harmful for environment. Among neem leaves and mahagony fruit bark extract, neem leaves extract shows the better fungicidal activity than mahagony fruit bark extract. We found optimum dose for fungus killing is 0.6 gm/5ml and 0.8 gm/5ml of neem and mahagony fruits-bark respectively, which also shows the preventing capability of further fungal growth. Based on our results, we conclude that our extraction method and fungicide applications on shoes is a suitable for practical application. It can also be used commercially by means of cost-effective and environmental friendly way.

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