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Antifungal efficacy of neem leaves (*Azadirachta indica*) and mahagony fruit bark (*Swietenia mahagoni*) extracts on leather shoes

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Abstract

The natural fungicide has been successfully extracted from neem (*Azadirachta indica*) leaves and mahagony (*Swietenia Mahagoni*) fruit bark by a solvent extraction method. The antifungal efficacy was evaluated by the application of extract to the fungal-killing on cultured-fungus in Petri-plate and shoes. The fungus was isolated from the shoe and cultured in Sabouraud Dextrose Agar (SDA) plate of 100 mm size petri dish. Different dosing of neem and mahagony extract was applied on the fungus culture and measured the area of fungus killing as an antifungal efficacy of the extracts by "Leaf Area Counter" software. The maximum fungus killing efficacy was optimized. The optimum dosing of neem leaves and mahagony bark extracts were found 0.6 gm/5ml and 0.8 gm/5ml, respectively. After that, the optimum doses of natural fungicide were mixed with commercial shoe shiner and cultured the fungus in SDA plate with and without fungicides. Fungicides containing shoe shiner was an inhibitor to grow the fungus, whereas fungus was grown in fungicides-free shoe shiner within three days. We observed the fungicides-containing shoe-shiner treated dish for a period of one month and found that there was no fungus growth at all. The present findings indicated the possible use of neem and mahagony fruits-bark extract as a natural antifungal agent against post-harvest fungal infestation of shoe commodities and prevented the fungus contamination.

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Introduction

In rainy season or in an atmospheric high content of moisture, fungus grows on leather shoes. This fungus especially grows on the surface of the shoe and damage 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 substance to kill the fungus and to protect the shoe from fungal growth. There are many commercial grades of fungicides, mostly manufactured by the conventional chemical process. However, most commercial available fungicides are highly toxic and expensive. Increasingly stringent environmental awareness, an alternatives fungicides should have a high efficiency towards fungi, less toxic, more environmentally friendly and cost-effective. Searching, different sources of natural fungicide, we found that the neem leaves and mahagony fruit bark have been efficiently killed the fungus and prevent the fungal growth as well as environmentally friendly, cost-effective, and it's an indigenous source.

The 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 of its damage the shoe surface by staining, which forming sometimes effected on the toe joint-portion. In our present research, we extracted natural fungicides from neem leaves and mahagony fruit bark for the first time application on shoes, which dramatically killed the fungus and reduced to the chances of becoming infected.

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Globally, strict environmental legislation obliges adapt to alternative technologies in order to minimize the environmental impact. This obligation urges to search for new fungicides compounds, which 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 alternative to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health. Alternative strategies include the use of natural bio-pesticides from medicinal plants such as neem and mahagony to overcome contamination and pest resistance issues (Isman, 2006).

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 (Rangiah *et al*., 2016). Mahagony tree/fruit bark is a potential source of bioactive compounds such as antioxidant, antifungal and widely used in agriculture and medicine (Goun *et al.*, 2003). Application of neem leaves and mahagony fruits-bark extract used as an antifungal on shoe surface is the first time to the best of our knowledge.

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

Materials and methods

Chemicals

Methanol (Merck, Germany) and Sabouraud dextrose agar (HIMEDIA, India) were purchased from the supplier City Scientific Store, Khulna, Bangladesh.

Collection of neem and mahagony-fruits bark

The green neem leaves and brown mahagony fruit bark were collected from trees abundantly available at the Khulna University of Engineering & Technology (KUET), Khulna, Bangladesh. The plants were identified by the two co-authors Mr. Rakibul Hasan and Mr. Rubel Ahmed and collected approx. 5 Kg neem green leaf and approx. 3 Kg mahagony fruit-bark. The accession numbers of the herbarium sheet, for the identification of these two plants have been applied in National Herbarium, Bangladesh. The collected leaves and fruit-bark were washed with tap water at several times till the wash water contained no dirt particles and pigment followed by drying in sunlight. The dried leaves and fruits-bark were ground using domestic mixer grinder and different size fractions were collected. The collected powder was preserved in an intact container.

Methods of solvent extraction

The powdered leaves and fruit-bark were soaked initially in methanol for a period of 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 filtrate was dried at room temperature. Finally, the extract was collected and stored in an airtight container and keep it in a cool and dry place.

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, the fungus was grown on leather surface and then the fungus was isolated by using pincers. After that, collected fungus was cultured in a Petri dish containing nutrient medium and distributed the Petri dishes by using inoculation loop.

Preparation of nutrient medium

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

Fungus culture, incubation with extract and counting

A typically isolated finished-leather-fungus was used in this study. The fungus was cultured in the SDA agar medium at 37 °C in an incubator (Frio cell, Germany). The fungus was plated in 100-mm Petri dishes and allowed to grow for two

days. The Petri-dishes were sterilized in an autoclave before culturing the fungus. Almost 80% fungus confluent was found within two days. The old culture medium was merged with the extract-containing (5 ml) different dosing medium and distributed uniformly over the whole surface area of Petri dishes and recultured for several hours in an incubator. The fungus was counted under a Leaf Area Measurement Software using the picture. The survival fungus was determined as the percentage of the area of live fungus against the control of dish fungus.

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

Results and discussion

Neem leaf and mahagony fruits-bark extraction

Total 320 g of dry neem leaf was soaked in 825 ml of methanol and found 16.63 g of neem extract. Moreover, 300 gm of dry mahagony fruit-bark was soaked in 750 ml of methanol and found 9.6 g of mahagony extract. This process was repeated three times.

Determination of optimum dosing

Fig. 1 shows the fungus area vs different 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 neither any extract nor any dosing. Fungus killing efficiency calculated based on control area of live fungus. Fig. 1 shows the 0.2 g/5ml and 0.4 g/5ml extract is not sufficient for fungus killing. But 0.6 g/5ml kill fungus effectively. From Fig. 1 it can be concluded that 0.6 g/5ml is the optimum concentration for effective fungus killing.

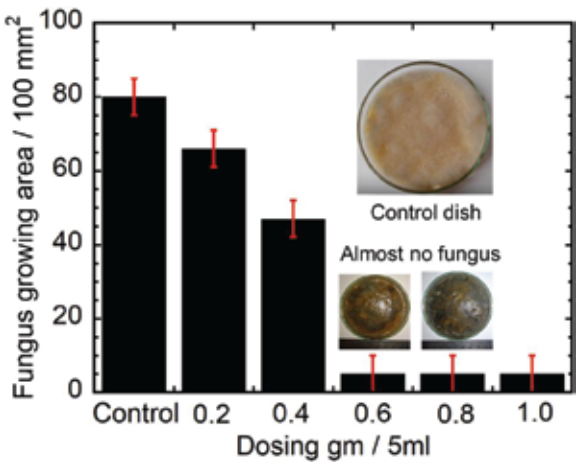

Fig. 1. Dosing graph for neem leaves extract with media until 48 hours. The inset shows the control and fungus killing treated with 0.6 g/5ml and 0.8 g/5ml, respectively.

The time optimization for further growth of fungus was observed until 97 hours of neem leaves of a treated dish. It was shown that 0.6 g/5ml and 0.8 g/5ml dosing of neem leave treated dish was not further grown of fungus. Whereas low concentration of 0.2 g/5ml and 0.4 g/5ml shown the fungus grown at 57 hours. Our results support that the 0.6 g/5 ml concentration neem leaves extract have the optimum hours for efficient fungus killing.

Similarly, the fungus area vs different dosing of 0.2, 0.4, 0.6, 0.8, and 1.0 g/5 ml of mahagony fruit-bark extract treating dish at 48 hours. The control dish shows without any extract and dosing. Mahagony fruit-bark 0.2, 0.4 and 0.6 g/5ml extracts are not efficient for fungus killing. But 0.8 and 1.0 g/5ml extracts kill fungus effectively. The doses were increased, the percentages of the viable fungus were decreased and finally, at a dose of 0.8 g/5ml, only a very few fungus were viable. These results indicate that mahagony fruits-bark shows significant potentiality against the viability and proliferation of fungus. It can be concluded that 0.8 gm / 5ml mahagony fruit-bark extract concentration is efficient for optimum fungus killing (Fig. is not shown).

The time optimization for further growth of fungus was observed until 97 hours. It was shown that 0.8 and 1.0 g/5ml dosing of mahagony fruit-bark treated dish were not shown any further grown. Whereas lower concentration of 0.2, 0.4 and 0.6 gm/5ml extracts showed the fungus grown at 40, 55, 62 hours respectively. This also supports that the 0.8 g/ 5ml concentration mahagony fruit-bark extract is optimum for efficient fungus killing. The higher concentration have been capable to longer time inhibition for further fungus growth due to efficacy of optimum concentration of extract. Because at optimum time 97 hours was not observed any further growth of fungus.

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.

Application of optimum dosing fungicides with commercial shoe shiner

The optimum fungicides (neem leaves 0.6 g and mahagony

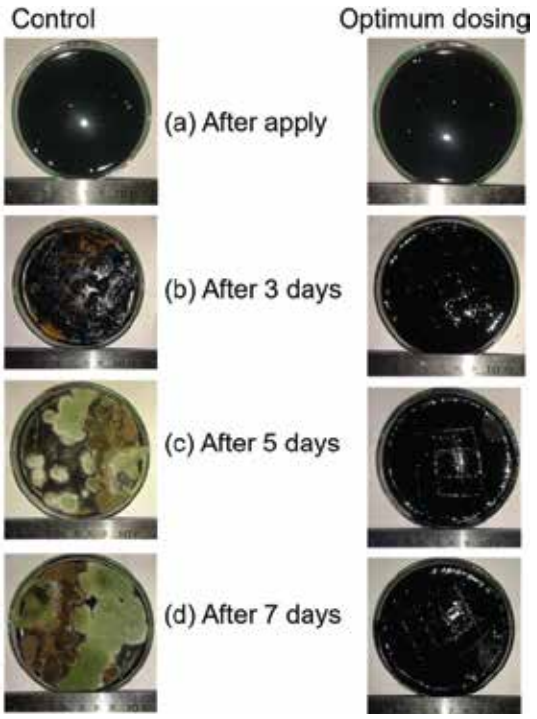

Fig. 2. Dosing for neem extracted with shoe shiner up to 7 days. The control (left) treated with only shoe shiner neither any extract nor any media. Optimum (right) treated with 0.6 gm neem extracted with shoe shiner

fruit bark to 0.8 gm) extract was mixed with 5 ml of commercial shoe shiner and applied on the fungus cultured Petri dishes. Fig. 2 shows the control and optimum neem extract with shoe shiner mixing treated dish photograph at several days' application. The control means (left side) neither any extract nor media, only with the shoe shiner applied photograph and right shows the optimum neem extract with shoe shiner treated dish. The figure clearly shows optimum dosing have the ability to inhibit the fungus growing and have the long-term protection capacity of viable fungus. Whereas in control fungus was grown within 3 days. It is clearly concluded from this figure that, only shoe shiner is not sufficient for long-term prevention for fungus. Besides, shoe shiner with neem leave extract-fungicides is effective for a long time prevention of fungus.

Fig. 3 shows the control and optimum mahagony fruits-bark extracted with shoe shiner mixing photograph of petri-dish at several days' application. The control means

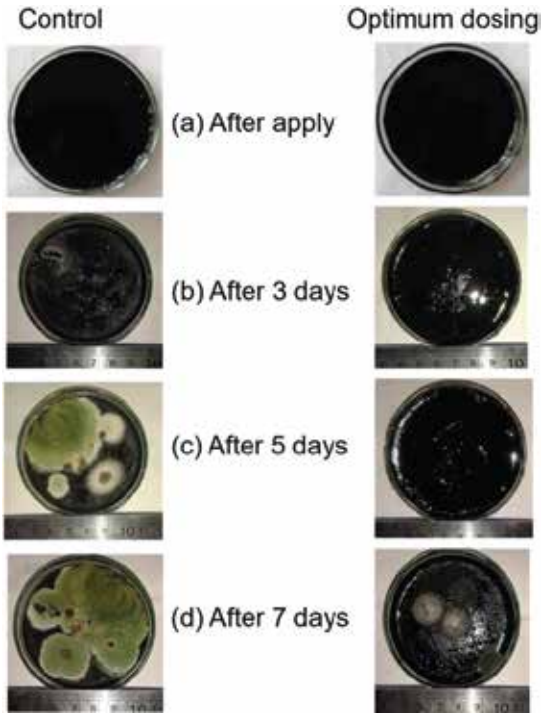

Fig. 3. Dosing for mahagony fruits-bark extracted with shoe shiner upto 7 days. The control (left) treated with only shoe shiner neither any extract nor any media. Optimum (right) treated with 0.8 gm mahagony fruits extracted with only shoe shiner

(left side) without any extract, only with shoe shiner applied photograph and right shows the optimum mahagony fruits extracted with shoe shiner treated dish. The figure clearly shows optimum dosing have inhibited the growth of fungus, whereas in control fungus was grown within 3 days. It is clearly concluded from this figure that, only shoe shiner is not sufficient for a long time prevention of fungus. After 7 days of optimum dosing shows growth of fungus. Shoe shiner with mahagony fruit-bark extract-fungicides is not so effective for the long-term prevention of fungus.

Extract application of a typical fungus content leather

The optimum of neem leaves extract was mixed with commercial shoe shiner and applied on fungus containing leather. Fig. 4 clearly shows the clean leather (right) after treatment of fungicides containing shoe shiner. It can be concluded from this figure, inhibition of fungus and stain removing capacity of our natural fungicides is very effective and it can be used commercially. The main purpose of this research is to provide a process of antifungal treatment of

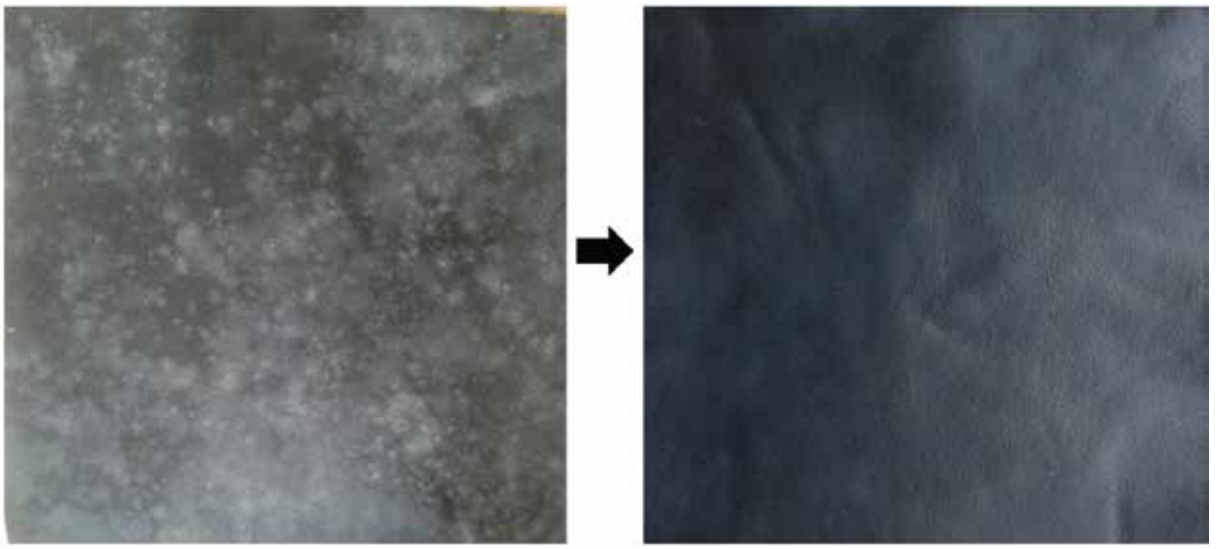

Fig. 4. Fungicide-treated on leather for neem leave extracted with shoe shiner. Left shows the typical fungus containing leather and right shows neem extract spray-treated leather.

footwear. We have successfully done it by the final application on shoe with the shoe polish.

We chose natural fungicides of neem leaves and mahagony fruit bark, because it is naturally available, environmentally friendly and have no effect in eco-system. Our research shows neem leaves and mahagony fruit bark have enormous potential to kill the fungus. Conventionally, synthetic 2-(Thiocyanomethylthio) benzothiazole (TCMTB) and the mixture of phenolic compound as a biocide used in the leather, pulp-paper, and water-treatment industries. TCMTB might be entering 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 (Nawrocki *et al*., 2005). 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 agroecosystem health (Cuados *et al*., 2012).

Plants and other organisms have chemical defense that give them an 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 (Govindachari *et al*., 1998). The Phytochemical constitutes of mahogany fruit bark might be effective for fungus killing (Falah *et al.*, 2010).

Minimum inhibitory concentration (MIC) is defined as the lowest concentration (expressed as g/mL) of an antimicrobial that will inhibit the visible growth of a microorganism such as a fungus after an incubation period. Several dilutions at concentrations ranging from 0.2 g/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 /5 ml MIC for neem leave extract and 0.8 g/5 ml for mahagony fruits bark extract are responsible for preventing the fungus growth. According to results, the neem leave has better antifungal activity than mahagony fruit bark extract. Therefore, mahagony bark extract needs more concentration.

Conclusions

Fungicide is used to protect the shoe from fungal attack. As the chemical-fungicide is harmful to both environment and human health, so, a natural fungicide should solve the problem of environmental pollution occurred by conventional chemically derived fungicide. Neem leaves extract and mahagony fruit bark extract both have fungicidal activity. These extracts can be used as the replacement of conventional chemically derived fungicide, which is harmful to the environment. Neem leaves extract shows the better fungicidal activity than mahagony fruit bark extract. We found the optimum dose for fungus killing is 0.6 g/5ml and

0.8 g/5ml of neem and mahagony fruit bark, respectively. Based on our results, we conclude that our extraction method and fungicide applications on shoes is suitable for practical application. It can also be used commercially by means of cost-effective and environmentally friendly way.

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Notes

The authors declare that no conflict of interest

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