

EFFICACY OF NATURAL PLANT PRODUCT FOR PREVENTIVE PRESERVATION OF DOCUMENTARY HERITAGE AGAINST *ASPERGILLUS FLAVUS*: A CASE STUDY

Sanjay Prasad GUPTA¹, Atul Kumar SRIVASTAVA^{2*}, Iliyas AHMED¹

¹ National Research Laboratory for Conservation of Cultural Property (NRLC), Ministry of Culture, Government of India, Lucknow, India

² Research and Development Department, Uttaranchal University, Dehradun, Uttarakhand-248007, India

Abstract

*Natural products have the antimicrobial property like plant extracts, essential oils, which obtained from the medicinal and aromatic plants. These natural products we used for the conservation of heritage property. Natural products have antimicrobial property and may be an alternative and useful source in the preventive conservation of documentary heritage without any negative impact on the environment and humans. Documentary heritages are susceptible to physical, chemical and biological damages. A biological damage seems to be more serious agent for the documentary heritages. Fungal spores are actively involved in the damaging of documentary heritages. In this work, the study of the antifungal activity of essential oil of Neem (*Azadirachta indica* L.), and various concentrations of Neem seed extracts applied against *Aspergillus flavus*, a fungal flora aligned with the bio-deterioration of documentary heritage. Seeds of medicinal plants were procured from the local market and extracts were obtained by soxhlet extraction apparatus. The antifungal activity was analyzed using well diffusion method against fungi isolated from the manuscript. It was observed that the aqueous Neem seed extract had shown good antifungal activity than extracts of various concentrations of seed extracts. This study has an important implication for the preventive conservation of manuscript by the use of the natural plant products in the prevention and control of bio-deterioration of documentary heritages.*

Keywords: Bio-deterioration; Bio-degradation; Documentary heritage, Essential oil; *A. flavus*; Old manuscript.

Introduction

The documentary heritage items like traditional books, handwritten paper manuscripts, old paintings and other such items urgent need to save like saving endangered species of plants and animals. These documentary heritage materials safely placed in museums, archives, libraries and other reservoirs and these are the priceless heritage of humans [1]. Documentation heritage preserves not only our ancient traditional precious knowledge but also preserve the authenticity of modern age manuscripts and these documented materials are the essential part of country's identity [2]. Thus, documented heritage preservation is necessary for the youth and institutions like librarians, archivist, scientists, scholars and other curators. From the ancient times conventional method used to prevent negative effects on documented property, but now many of the natural products uses for the conservation of documented property. Preservation of

* Corresponding author: atulsrivastav.micro@gmail.com

documented materials maintains the libraries and other archived documents for use in either physical form or any other usable forms [3]. Because of the unfavorable conditions, the stored documented materials, which are deposited in various type of reservoirs is affected by various kind of biological, physical, and chemical factors and is at risk of being lost in across the world [4]. Manuscripts are one of the important heritage materials, having valuable recorded thought of different society that must be transfer to the next generation plays significant role to strengthen the younger generation and society. The document heritages are mainly in the form of organic component that may be natural, synthetic, or semi synthetic [5]. Due to alteration of natural environment, several new kinds of microbes emerge, which are harmful to paper-based manuscript [6, 7]. The fungus is considered as a large heterogeneous group of infectious microorganisms affect plant and documentary heritage. Fungi secrete pigments and acid, which are able to alter the physiochemical property of documentary heritage and wooden materials present in exhibition halls, museum and library. Various kind of fungal spores present in the atmosphere, survive in dormant stage for a long period of time. Germination of fungal spores occurs when favorable temperature and humidity situation appears. 63-80% relative humidity with 20-35°C temperature is favorable environmental condition for the growth of fungal spores. In the fall and summer season, the highest concentration of fungal spore recorded in air and the most cultivable air borne fungi are *Aspergillus*, *Penicillium*, and *Cladosporium* [8].

Aspergillus flavus is a saprophytic soil born fungus found in many types of decaying materials with cosmopolitan distributed, belongs to the family trichocomaceae. However, it is also recognized as a plant and human pathogens [9]. *A. flavus* is a thermo tolerant fungus and survive in hot and humid condition so it can simply tolerate a heating condition in documentary storage heritage that other fungus cannot [10]. In libraries, fungal growth is known as mold or mildew and appearance as brown or black vegetative growth in the surface of manuscript, leather, and textiles. These microbes' intake cellulose and other nutrients from the documentary heritage material like paper, glues, leather, binding threads, pastes etc., that make stain on the paper and weaken the objects with discoloration.

For the conservation of documentary heritage, the chemical used is must be non-toxic and non-destructive. In the past decades 'thymol chamber' was largely used for the sanitization purposes of conserved material by conservator by thymol vapor. But today thymol vapor is not continuing for the sanitization purpose because of its hazardous nature to environment and human's health [11]. Besides this ethylene oxide also used for the fumigation purposes by the European conservator. For preceding the ethylene oxide fumigation activity, we need a chamber which is not feasible at all time in all cases. Apart from this it is hazardous and dangerous for the human as well as environment [12]. We all are familiar that excessive use of chemical pesticides harms the environment and harm the beauty of nature. Wherever excess amount of chemicals used, nature bear load in the physical damages like raising temperature, water, air and soil pollution, so we need to alert and reduce the use of chemical pesticide for preventing the damaging of natural environment.

Many of the plant extract, and essential oils which extracted from the medicinal and aromatic plants have been reported as antimicrobial activity and may work as non-hazardous fumigant [21]. In the modern era, researchers show their interest on the natural products that have antimicrobial potential and eco-friendly in nature [22, 23]. However, in the field of conservation of heritage materials, for the removal of microbes which destruct the documentary items, conservators are rarely used these natural products against the pests [13-15].

There is a well-known phrase that "prevention is better than cure" we must follow this to save our environment. Prevent the damages of cultural heritage by physical, chemical or biological means; we have to develop the strong conception for the preservation of

documentary heritage and other cultural documents. In the modern world the ideas of conservation grow gradually and becoming the central issues for the curators. Thus, it is necessary to take forward step to preventive preservation and good and lively condition of all the cultural heritage documents. It comprises all the methods of good housekeeping, caretaking, dusting, periodical supervision, and prevention of any possibility of damage by physical, chemical, biological and other factors. Preventive conservation plays a vital role and has assumed much importance in our country because a large number of institutions do not have proper preservation facilities. In fact, if diagnosis in time is followed by proper preventive measures many problems can be solved.

This work aimed to evaluate the efficacy of medicinal plant products like plant extracts and essential oil of Neem seed for preventive preservation of manuscript against specific fungus associated with the biodeterioration and biodegradation of documentary heritage.

Material and methods

Extraction of plant material (Neem seed) by Soxhlet apparatus

Seeds of medicinal plants were purchased from the local market from Lucknow, India, and evaluated for their antifungal activity through the disc diffusion assay technique. Purchased seed were washed with tap water followed by sterile distilled water, then dried in shade for few days and form a powder by crushing in grinder mixture. These crushed materials now convert into powder form and then the Neem seed powder were extracted sequentially in distilled water solvent with the help of Soxhlet apparatus. The evaporation of water takes much time approximate 8 hours per day for three days. Resulting extracts were evaporated and concentrated to dryness using the rotary vacuum evaporator at 50°C. All process of Neem seed extract preparation and evaporation of solvent through rotary vacuum evaporator done in lab (Fig. 1).



Fig. 1. Preparation of Neem seed extract (A); Evaporation of solvent through rotary vacuum evaporator (B)

Collection of various samples for experiments

Samples of the manuscript, handmade paper, cotton cloth, and canvas cloth were procured from the local market of Lucknow, Uttar Pradesh, India. All the purchased samples were required to wet by spraying sterilized distilled water as per design experimental procedure. Then after, samples were kept into the covered glass chamber for further study as per Table 1.

Table 1. Efficacy of Neem oil and Neem seed extracts against the *Aspergillus flavus* fungal species placed in a closed glass chamber

Sample No.	List of materials	The concentration of Neem extract with pH 6.8/oil 6.7					
		10%	20%	40%	60%	80%	100%
1	* Manuscript	-	-	-	-	-	-
2	** Handmade paper	-	-	-	-	-	-
	Manuscript wrapped in Handmade paper	-	-	-	-	-	-
4	Manuscript wrapped in Handmade paper and #canvas cloth	-	-	-	-	-	-
5	Wet ##Cotton cloth	-	-	-	-	-	-
6	PDA plates wrapped in canvas cloth impregnated with seed extract	+	+	+	+	+	+
7	PDA plate wrapped in canvas cloth impregnated with Neem oil	NA	NA	NA	NA	NA	-
8	PDA plate with various concentration of Neem seed extract through well diffusion agar method	+	+	+	+	+	+

Manuscript:** Simple Xerox machine-made paper; *Handmade paper:** A paper made from lokta fiber by beating and pulping process; #**Canvas cloth:** Cloth made from hemp fiber; ##**Cotton cloth:** A natural, soft fiber that grows with the seeds of the cotton plant; (-): Absence of fungal growth; (+): Presence of fungal growth; (NA): Not Applicable

Isolation and identification of fungi

Samples were collected from old manuscript of The National Research Laboratory for Conservation of Cultural Property (NRLC) for the isolation and identification of fungal species. For the isolation of fungus, Potato Dextrose Agar (PDA) (HiMedia) media is used. The fungal samples were collected from the surface of the manuscript through a cotton swab and prepare the fungal spore suspension with autoclaved distilled water. 100µL of suspension of the sample is spread on PDA plates and incubated for four days at 28°C in BOD incubator. After four days fungal colony was counted with colony meter. On PDA plate the fungal colony was appeared as powdery masses, granular, flat often with radial grooves, black in center and light white at edge (Fig. 2).

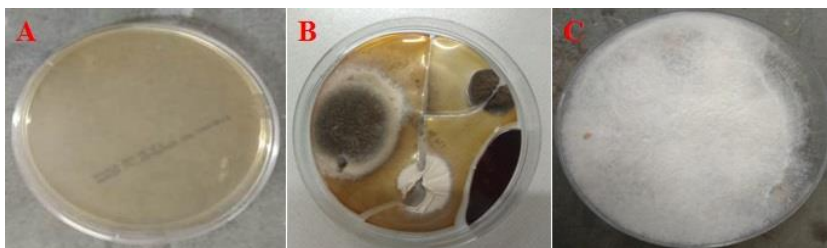


Fig. 2. Petridis with PDA media (A); Fungal growth in PDA media (B); Pure culture of *A. flavus* (C).

Agar-well diffusion method

The assay was conducted by the agar well diffusion method. About 15 to 20mL of potato dextrose agar medium was poured in the sterilized Petri dishes and allowed to solidify. The fungal lawn was prepared using pure culture strain. 1.0mL of fungal strain was spread over the medium using a sterilized glass spreader. Well was a formed using 200µL tip in already spreader with fungal spore culture media. Required concentrations of serially diluted extracts (10, 20, 40, 60, 80 and 100mg/mL as per Table 1) were added to the wells. The prepared plates were left for diffusion of extracts into media for one hour in the refrigerator and then placed at room temperature in covered glass chambered for 7 days. The disc diffusion method (MIC) was followed by taking antibiotic streptomycin sulfate for the inhibition of microorganisms. The plates were examined and observed the growth of fungal species. Dimethyl sulfoxide (DMSO)

with Streptomycin Sulphate was used as a negative control. The experiments were conducted in triplicates.

Fungal species and growth condition

The experiments were carried out with fungal species, i.e. *A. flavus* isolated from old manuscript and indoor environments of repositories of our laboratory and maintained on PDA plate. The antifungal activity of the Neem seed oils and Neem extract were evaluated by hole-plate diffusion methods [17, 18] as seen in figure 3. A suspension of the fungal species was used for an experiment and spread into the PDA plates in laminar air flow. Each experiment was done in triplicate.

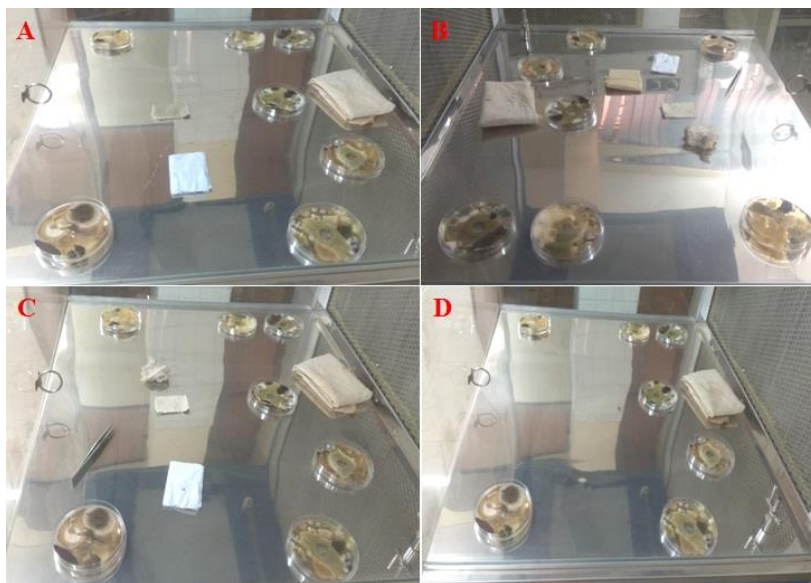


Fig. 3. Fungus not grown on manuscript (A); Fungus not grown on cloth (B); Fungus not grown on handmade paper (C); Fungal grown in various concentration solution of Neem seed extraction with PDA media well in agar as well as Petridish with PDA wrapped in canvas cloth impregnated with Neem seed extract (D)

Result and discussion

The isolated fungus was identified as *A. flavus* by its morphological characteristics. At a concentration of 10, 20, 40, 60, 80 and 100% aqueous extracts of Neem seed were recorded not effective for *A. flavus* species because same types of fungal growth observed in Petri dish with PDA media wrapped in canvas cloth impregnated with seed extract and Petri dish with PDA media with various concentration of Neem seed extract through disc diffusion method by well in agar in both observation fungal growth was recorded in both condition (Table1), while no any effect recorded on moist manuscript, moist handmade paper, manuscript wrapped in handmade paper, manuscript wrapped in handmade paper and canvas cloth and moist cotton cloth. At a 100% concentration, the Neem seed oil was taken in this study as PDA media in Petri dish wrapped in canvas cloth impregnated with Neem oil and recorded effectively (Fig. 3&4). Canvas cloth made from hemp and coated with Neem seed extract was not effective against the growth of fungal species recorded. The pH value of the extract is noted to be 6.8, and pH of Neem oil noted to be 6.7, which are near to neutral, and it can be used as coating material on cloth as well as paper.

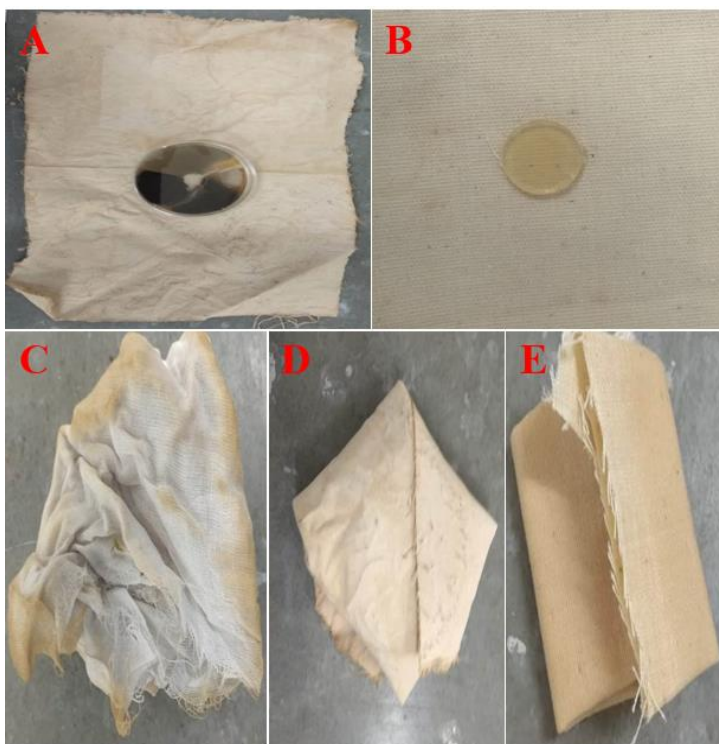


Fig. 3. Fungus grown in Petridis with PDA media wrapped in canvas cloth impregnated with Neem seed extract (A); PDA placed in canvas cloth impregnated with Neem oil (B); Moist cotton cloth (C); Petridis with PDA media wrapped in canvas cloth impregnated with Neem seed extract (D); PDA wrapped in canvas cloth impregnated with Neem oil (E)

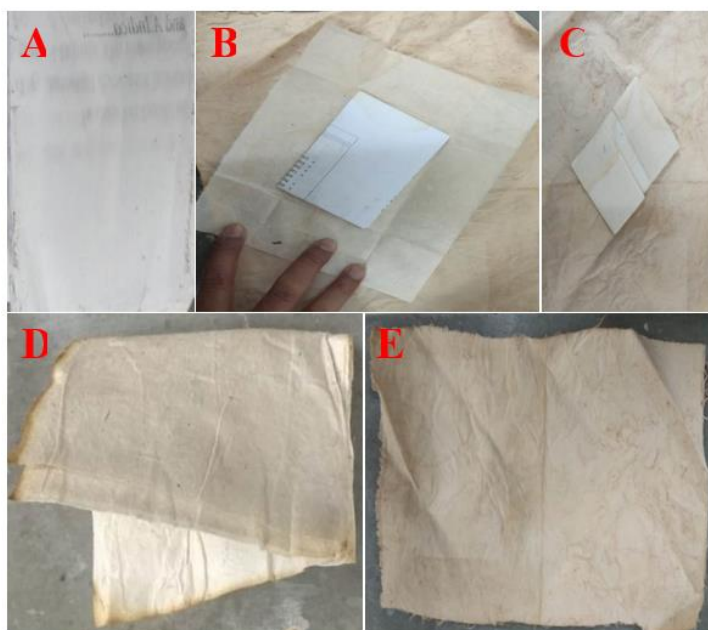


Fig. 4. Moist manuscript sample (A); Manuscript placed in handmade paper (B); Manuscript placed in handmade paper and canvas cloth (C); Moist handmade paper (D); Canvas cloth impregnated with Neem seed extract (E)

Essential oils played a significant role against fungal spores and reduce the growth of fungal culture, without any negative impact [19]. The microbes which is harmful for the documentary heritages, isolated from documents, libraries, museum, and environment of archives have to be reduced by the use of essential oil. Many of the scientist and researchers evaluate the study of antimicrobial activity of different essential oil and give remarkable reason behind this [13-15]. Besides the essential oil, leaves, bark and other parts of medicinal plants are also used for the conservation of cultural heritage against dermatophytes and other filamentous fungi. Many researchers already reported that plant metabolites and plant-based pesticides or biocides appear to be one of the better alternatives as they are known to have minimal environmental impact and eco-friendly to conservator/scientist involved in this field as well as stone components in contrast to synthetic chemicals used as pesticides/biocides [20].

Conclusion

This study showed that the Neem oil and Neem seed extract have fungicidal activity, but the essential oil of Neem seeds found to be more effective than Neem seed extracts against isolated fungus *A. flavus*. After studying its effects on the chemical, molecular, structural, and aesthetic characteristics of the paper, this study may have an important implication for preventive preservation of organic documentary heritage by herbal biocide to control the biodeterioration and biodegradation. Thus, essential oil of Neem seed is used for preventive action against fungal species especially *A. flavus* in the documented storage heritages.

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