

# A Study of the Inhibitory Action of Neem in Powder and Oil Form for Controlling the Growth of Microorganisms on Paper-based Materials in a Library

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## Abstract

**Background:** The major part of a library's stock is made up of paper-based materials namely books, these paper-based materials being organic, tends, to not only attract insects but also promote the growth of microorganisms. Neem is readily available in India and Libraries have been using neem leaves, to protect their collections from ancient times, even in the present times, many of the libraries use neem leaves as an organic means of controlling pests by placing stalks of neem leaves among the books. Thus, the insecticidal properties of neem are well known; however, its effectiveness in controlling the growth of microorganisms needs to be clarified, this study was conducted to validate the effectiveness of neem in controlling microorganisms that exist on library books.

**Methods:** Neem is readily available in India, in powder as well as oil form. Neem powder and neem oil would also be able to cover more surface area, hence these two forms of neem were considered for the study. The ditch plate method was performed to check the inhibitory actions of neem oil (Neem oil-methanol solution) and Neem powder (1.0 grams dissolved in methanol) on the organisms caused by the diffusion.

**Findings and conclusions:** Neem in powder and oil form was used in the experiment and its antibacterial properties were ascertained.

**Keywords:** Natural inhibitors; Organic inhibitors; Control of microorganisms; Neem

(Estimated date: 6 BCE) and Charak Samhita (Estimated date: 100-200 BCE). It is highly therapeutic, without any side effects on man. Neem has many medicinal properties, and every part of the neem tree leaves, flowers, seeds, fruits, roots, and bark are used for some or the other medicinal purpose, hence it is called "Sarvaroga Nivarini" in Sanskrit which means "The curer of all ailments"[3].

In India, since ancient times neem leaves were used as an insecticide, they were placed in cupboards or between clothes to prevent them from being eaten up by insects. Neem powder is also used as an organic source to repel insects from crops and grains. Neem leaves are believed to have antiulcer, antimalarial, antifungal, antibacterial, antioxidant, antiviral, anticarcinogenic, anti-inflammatory, properties to name a few [4]. Neem oil contains a complex mixture of volatile compounds. The bioactive compounds found in neem leaves such as Nimbodin, Sodium Nibidate, Nimbin, Nibolide, Meli tetraolene, Odoratone, Zafaral, Meliacin anhydride, 6 $\alpha$ -O-acetyl-7-deacetyl nimocinol, Meliacinol, Nimonol, Nimonoactone, Nimonoide, Isomeledenin, etc. are antifungal, antibacterial, insecticidal, and antifeedant. This makes the neem leaves and oil useful as an inhibitory agent for controlling the growth of microorganisms.

As books form a major portion of a library's collection there is a need to preserve and maintain them, hence regular pest control is undertaken by almost all the libraries to ensure the safety of their collection. Books are organic and can turn into a breeding ground for spores that may exist in the environment, especially in the presence of high humidity, favourable temperature, and atmospheric pollutants. Also, various studies have been conducted to establish the presence of microorganisms on books; these studies have also shown that chances of transmission of microorganism increases when books are circulated [5-7]. Regular pest control treatments can eliminate pests, but these pest control treatments may not be able to eliminate microorganisms that grow on paper, further such chemical treatments can have adverse effects on the environment and health of the library staff as well as the library users.

The Regional Conservation Laboratory (RCL), a wing of the National Conservation Laboratory of the Union Ministry of Culture, India, in their research observed that special papers

## Introduction

Azadirachta indica or Neem tree as it is commonly known as a medium-sized tree believed to have originated in Upper Myanmar [1] and was later introduced in India. Neem is ancient to the Indian subcontinent and its remains were traced back to the Mohenjo-Daro archeological site [2]. Its use can be traced back to the earliest Indian medical scriptures Shushrut Samhita

impregnated with the aromas of the neem leaves can act as an effective agent in preserving manuscripts for a longer time [8]. As Neem is available in several forms a study was undertaken using the powder and oil form to test the effectiveness of neem in controlling microorganisms that may be present on paper-based material. Neem powder is prepared by drying neem leaves till they are crisp and then they are crushed to a fine powder, Neem oil is obtained by extracting the kernels from the seeds, these are then pressed in hand or industrial expellers. This neem oil is used in soaps, waxes, cosmetics, and lubricants; it is also used as an insecticide [9].

In powder form, neem could easily be dusted on the pages of the books, however as neem oil cannot be directly applied on paper-based materials due to staining and damaging of paper, neem in its oil form could be mixed with a volatile substance and placed among the book stacks. In Mumbai city, books are used maximum in academic libraries, hence, for this study books belonging to an academic library in Mumbai city were identified and microbiological assays were conducted to study the effectiveness of neem in its powder and oil form in controlling the growth of microorganisms.

## Methodology

### Selection of sample

As the study involved studying the inhibitory action of neem powder and oil on the microorganisms, books that would have a microbial load needed to be identified. It was decided that books that have been recently printed and acquired by the library would have undergone chemical treatment during its manufacturing and printing process, as the chances of microbial growth would be minimal in the recently acquired books. A sample of five books bearing different accession numbers according to the year of the purchase was selected randomly as follows Acc. No. 1964 (Year 1960); Acc. No. 6469 (Year 1962); Acc. No. 10926 (Year 1970); Acc. No. 30994 (Year 1984); Acc. No. 67749 (Year 2011). The newest being a book acquired in 2011 and the oldest being a book acquired in 1960.

### Experimental procedure followed to identify the microorganisms

Small pieces of paper from each of the books were taken and each paper was dipped in 23 ml of Nutrient Broth (consisting of a mixture of Peptone-5 g/L, Meat extract-1 g/L, Yeast extract-2 g/L, Sodium chloride-5 g/L, at a pH of  $7.0 \pm 0.2$ ) and incubated at 37°C.

### Stage one

**Preparation of the agar plate:** Nutrient Agar (NA) Plate is prepared by pouring nutrient agar which has been autoclaved in sterile Petri dishes with the help of a pipette. The Petri dishes were kept until the agar is set to form a gel. A loopful of each culture is taken and streaked on the individual plates and left overnight. All instruments are cleaned with alcohol and the entire procedure is done in sterile conditions so that there is no external contamination.

### Stage two

**Ditch-plate method:** The ditch plate method was performed to check the inhibitory actions of neem oil (neem oil-methanol solution) and neem powder (1.0 grams dissolved in methanol) on the organisms caused by the diffusion.

This method consists of preparing a ditch in an agar plate on which the organisms are grown. To ensure even dispersion, the neem oil and neem powder were dissolved in methanol. The inhibitors were then introduced in the ditches and the plates were incubated for 24 hours at 37°C. Triplicates were maintained for each set. The Zones of inhibition were studied and measured in mm.

## Results and Discussion

**Table 1** Represents Microbial load on books treated with neem oil and neem powder.

Cultures	Neem oil			Neem powder		
	1	2	3	1	2	3
1964	30	28	27	33	33	32
6469	-	28	-	32	36	35
10926	-	-	-	-	-	-
30994	-	-	-	-	-	-
67749	30	27	27	33	35	30

**Table 1:** Microbial load on books treated with neem oil and neem powder. **Note:** Hyphen indicates no zones of inhibition.

Table 2 indicates that these findings are summarised statistically and standard deviation and the coefficient of variation is calculated.

Count	Sum	Average	STD Dev	C.V
7	197	28.1429	1.34519	0.0478
9	299	33.2222	1.85592	0.05586

**Table 2:** Statistical summary of Microbial load on books treated with neem oil and neem powder.

The F-test to test the equality of variances is summarized below in **Table 3**.

Pairs	F Calculated	F-Tabulated	Conclusion
(1,2)	1.90351	f. 05(8,6)=4.14680 4	As F Tabulated is higher than F Calculated one must accept the null hypothesis that the Variances of samples (1) and (2) do not differ significantly.

**Table 3:** F-test results the equality of variances.

## Findings

Based on the above observations and analysis it can be inferred that

Data is heteroscedastic.

t-test to examine the equality of effects of Neem Oil and Neem Powder yields  $p\text{-value}=1.40731E-05 \ll 0.05$ . Hence there is a significant difference in the effects of the two. This implies that Neem Powder is more effective than Neem oil.

Thus,

- Neem powder is more effective in inhibiting the growth of microorganisms as compared to neem oil.
- Standard deviation being the least, neem oil is the most consistent compared to neem powder.

Hence it can be stated that both neem powder and neem oil are both effective in controlling the growth of microorganisms, however, neem powder is more effective as compared to neem oil.

Thus, neem powder can be dusted on the book pages to prevent the growth of microorganisms, however, this powder will stick to the hands of the users, and hence the excess powder needs to be brushed or wiped with a soft cloth to prevent it from sticking to the hands of the person reading the books.

## Conclusion

The traditional knowledge about Neem when tested scientifically revealed that neem can control the growth of microorganisms on paper-based materials in the library without the adverse effects of synthetic inhibitors. The current study showed that even though both neem powder and oil are effective in controlling the growth of microorganisms, neem

powder is a better inhibitor. Further research is required to study the apt duration for which the neem powder should be kept in contact with the paper-based materials and the duration after which it needs to be reapplied to maintain its effectiveness.

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