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Research Article

Combining Traditional Ayurveda and Modern Technology: A Study on the Impact of Electric Fumigation with Natural Substances on Microbial Load

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ABSTRACT

Airborne diseases and nosocomial infections contribute significantly to recurring illness, financial burden, morbidity, and mortality. Traditional fumigation using natural substances has been extensively employed to combat various pathogens, yet scientific validation of its efficacy remains limited. This study aims to evaluate the effectiveness of herbal fumigation in reducing microbial load. We conducted controlled experiments in specific areas, measuring the microbial load of bacteria and fungi before and after fumigation using an electric fumigation device with selected herbs. Results indicated that electric fumigation significantly reduced microbial colonies, achieving a 40% reduction in bacterial colonies and a 32.75% reduction in fungal spores in location A, and even greater reductions of 62.5 and 91% in location B, respectively. The electric device ensured uniform fumigation without requiring an ignition agent, enhancing the process's convenience and safety. The findings suggest that combining plant-based fumigation with modern electric devices offers a safe, non-toxic, cost-effective, and efficient method for community-level sanitization. This study provides scientific validation for the ethnopharmacological use of herbal fumigation and supports its integration into public health strategies, particularly in settings where conventional methods are inaccessible or costly.

Introduction

Many airborne infections are clinically significant including bacteria, viruses, and fungi. These organisms can be disseminated by sneezing, coughing, spraying liquids, spreading dust or any activity that produces aerosolized particles. Thin mists, dust, aerosols or liquids can disperse microorganisms transmitted airborne.[1] Aerosolized particles can be produced by a source of infection, such as the bodily secretions of an infected patient or an animal. During aerosolization, microorganisms smaller than 100 microns in size float in the air. Once microorganism-containing droplets have formed, they are spread via air currents across varied distances and can be inhaled by vulnerable hosts. Infected aerosolized particles frequently remain suspended in air currents and can travel long distances, which are silent but create significant risk factor in many human activities.[2,3] The issue of nosocomial infections and healthcare-associated infections could be considerably addressed by conventional fumigation using herbal products. Much research has been conducted on this subject in recent years, and assessing the degree of air microbial contamination in risky areas is considered to be an essential first step in prevention.[4] Extensive research is being conducted on the biological

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and ethnopharmacological significance of *Ayurvedic* medications, which has led to the identification of new bioactive compounds derived from plants.[5] Numerous laboratory-based research investigations have been carried out on plant materials as fumigants against insects of stored goods. In terms of antimicrobial flavor and health-promoting formulation for food application, the new rice hull-derived liquid smoke can be used effectively. It is anti-inflammatory and has the potential to supplement commonly used wood-derived liquid smokes.[5] According to one of the studies, formaldehyde fumigation is a fast and straightforward process to carry out in microbiological safety cabinets but it is fundamentally dangerous for human health. The fumigation by using holi sticks of apamarga (*Achyranthes aspera*) demonstrated a significant level of inhibition against *Streptococcus pyogenes.*[6]

Fumigation is the process by which positive results can be obtained in the field of disinfecting contaminated air and surface to fight against epidemiological diseases. An early example of 'antisepsis' of the air could be the sarshapa (*Brassica rapa L.*), ghee (clarified butter) and salt fumigant used in operating rooms (*Sushruta* 800–600 BC), which it was also employed to drive out evil spirits.[7] Nevertheless, in contrast to other medicinal practices, the efficacy of *Ayurvedic* fumigation has not been thoroughly examined in a way that is relevant to contemporary society.[8] Several comprehensive scientific investigations have been carried out to investigate the potential impact of medicinal smoke from the *havan samagri* on the different atmospheric bacteria and still, there is scope of exploration. According to one of the reviews, medicinal smoke is a usual method in 50 countries, which has multiple opportunities to study on different aspects of it applications.[9] In *Sushrutha Samhitha*, 'fumes' or fumigation procedures are classified into five categories to wit, habitual (*Prayogika*), unctuous (*Snehana/Snaihika*), expectorant (*Vairechana*), anti-cough (*Kasaghna*) and emetic (*Vamaniya*). Among them, unctuous and expectorant varieties are used for both preventive and curative treatment, while frequent fumigation or smoking is intended for the preservation of good health.[8, 10] According to the wide-ranging effects of fumigation, it has the potential to become a crucial component of hospital epidemiology and infection control efforts in the modern day. This could serve as the primary treatment for eliminating hazardous microorganisms, but a substantial amount of research is required to validate the scientific approach of fumigation for creating aseptic conditions. It is observed that the coarse powder of several dried herbs has reduced the microbial colony counts in air samples considerably in relation to Formalin.[8] The powder form of neem (*Azadirachta indica A. Juss.)* is used in many ways insect growth regulator, feeding deterrent, oviposition deterrent, sterilizer etc. Neem seed oil was extracted and used at different concentrations and exposed to *Lyctus africanus* Lesne larvae where positive results were notice. Neem seed oil has emerged as promising alternative of synthetic fumigants[11] *Tulasi* essential oil contains a valuable source of bioactive compounds that have antimicrobial activity against *S. aureus*, *E. coli* and *P. aeruginosa.*[12] Sarshap (*Brassica rapa L.*) has a distinct profile of glucosinolates that act as a biocide on certain infections, which can be useful in fumigation.[13] A study aimed to characterise and quantify the antimicrobial activity of black mustard (*B. nigra*) essential oil. It was applied directly into the liquid medium or exposed in the vapour phase to inhibit the growth of *Penicillium citrinum*, *Aspergillus niger*, and *A. ochraceus*. Vanatulasi (*Ocimum basilicum L.*) and its ingredients have the potential to improve quality of life of patients with nosocomial infections as both a preventative and curative treatment.[14] According to one of the *in-vitro* study, *Sarjarsa* (*Shorea robusta resin*) has a stronger and broader spectrum of antimicrobial activity against a number of pathogenic microorganisms.[15] If modern science approaches this age-old holistic treatment with an open mind and the intellectual enthusiasm, then it will be more advantageous on different platforms. In order to purify or disinfect the air and create a cleaner environment, this study reports on the scientific validation of our ancient knowledge of the antibacterial activity of medicinal smoke during the treatment of a room with the medicinal smoke, which is generated by burning cow dung cake, charcoal, cow ghee (clarified butter) and a mixture of medicinal herbs.

Previous research has extensively documented the antimicrobial properties of traditional fumigation methods using natural substances like neem (*A. indica*), Tulasi (*O. sanctum*), and sarshap (*B. rapa*). These studies have demonstrated the efficacy of medicinal smoke in reducing microbial load, highlighting its potential for controlling airborne pathogens. However, there has been limited scientific validation of these practices in modern contexts. Recent research has begun to explore the role of electric fumigation devices in enhancing the effectiveness of herbal fumigation, offering standardized and uniform fumigation that increases the reduction of microbial colonies. This study builds on these findings by empirically validating the impact of herbal fumigation using electric devices, providing a modern approach to integrating traditional methods into contemporary public health strategies.

In the current pharmaceutical landscape, the importance of developing cost-effective, safe, and accessible methods to combat airborne diseases and nosocomial infections is more critical than ever. The findings from our study offer significant pharmaceutical advantages by validating the use of herbal fumigation combined with modern electric devices. This approach not only provides an effective means of reducing microbial load in various environments but also presents a non-toxic and affordable alternative to conventional chemical fumigants, which often come with

safety and environmental concerns. The integration of this method into public health strategies could be particularly beneficial in resource-limited settings where access to conventional sanitization methods is restricted, thereby contributing to the broader goal of reducing the incidence of infection-related morbidity and mortality.

Materials and Methods

Materials of *Nimb-Sarshapadi Choorna*

The ingredients for the study were purchased from Durga Ayurvedic Pharmacy, Ahmedabad, Gujarat. The botanicals included Nimb (*A. indica* A. Juss.), belonging to the Meliaceae family, with leaves being the utilized part. Sarshapa (*B. rapa* L.) from the Cruciferae family was represented by its seeds. Tulasi (*O. tenuiflorum* L.) and vanatulasi (*O. basilicum* L.), both from the Lamiaceae family, contributed their leaves. Lastly, Sarjarsa (*S. robusta* Gaertn) from the Dipterocarpaceae family was included for its oleogum. These ingredients were selected for their reputed medicinal properties and relevance to the study's objectives.

Method

Comprehensive studies were conducted to evaluate the efficacy of traditional fumigation practices for environment disinfection.[16,17] The studies were conducted at two different pre-identified areas, i.e., a classroom and a female washroom had been chosen as location A and location B, respectively to measure the microbial load and to apply traditional fumigation to validate its effects on the same. The study based on each case was performed at intervals of 7 days. The whole study was performed three times and the average results have been highlighted. Throughout the entire process, no human movement was permitted.

Preparation of Culture Medium

The preparation of culture mediums for microbial cultivation involved the formulation of nutrient agar (NA) and potato dextrose agar (PDA) plates, utilizing specific ingredients at defined concentrations.[18] For nutrient agar plates, the following components were used per liter: 5.00 g of peptone, 1.50 g of HM peptone (beef extract), 1.50 g of yeast extract, 5.00 g of sodium chloride, and 15.00 g of agar. These ingredients collectively provide a rich source of nitrogen, vitamins, amino acids, and minerals, creating an optimal environment for the growth of a wide range of bacteria.

For the potato dextrose agar plates, the preparation included 200.00 g of potato infusion, 20.00 g of dextrose, and 15.00 g of agar per liter. The potato infusion supplies essential nutrients derived from plant tissues, while dextrose acts as a primary carbon source, promoting robust fungal growth. Agar in both mediums serves as a solidifying agent, facilitating the formation of a stable medium conducive to microbial colony development.[18]

Preparation of N-Agar and PDA Plates for Antibacterial and Antifungal Activity

Nimb (*A. indica A. Juss.*), *sarshapa* (*B. rapa L.*), tulasi (*O. sanctum L.*), vanatulasi (*O. basilicum L.*) and sarjarsa (*S. robusta Gaertn*)-these five herbs were used in equal quantities each of 10 gm to generate the fumes with the help of cow ghee along with charcoal and cow dung cake as an igniting agent. Washed and cleaned 20 petri plates were selected as a part of preparation. First, the plates were kept in incubator at a moderate temperature for few minutes to remove the moisture and make it suitable for microbiological study. The 9 cm diameter nutrient agar (N-Agar) plates for bacteria and potato dextrose agar (PDA) plates for fungi were prepared by performing following steps as per Fig. 1:[19]

- Take 7 gm of nutrient agar and 5 gm of agar-agar powder were mixed with 250 mL of distilled water in an appropriate size flask.
- Take 9.75 gm of potato dextrose agar and 5 gm of agar-agar powder were mixed with 250 mL of distilled water in an appropriate size flask.
- Both of these flasks were kept inside the autoclave apparatus at 121°C, 15 lbs pressure for one-hour duration to eliminate microbial contaminants.

All windows and doors of the both the locations were closed to create an enclosed space. Processed and labelled N-Agar and PDA plates were kept at 4 corners and centre area of classroom for 30 minutes to check the microbial load then after sealed it properly. This step was repeated for all above listed methods of fumigation where five plant-based herbs were used as a working medium. After 30 minutes of fumigation, new petri plates were exposed to pre-determined places in the same classroom on same day for 30 minutes and after that sealed plates were kept in the incubator for 24 hours to observe the colony growth of bacteria and fungi with fumigation in different ways as per shown in Fig. 2.[20,21]

Results and Discussion

After the incubation process, N-agar and PDA plates were kept under the digital colony counter apparatus to count the no. of colonies formed in both the cases, i.e., pre-fumigation (normal air flora) and after fumigation as observed in Table 1.

The data presented in Table 1 highlights the comparative effectiveness of different fumigation methods in reducing microbial load, specifically bacterial colonies on N-agar plates and fungal spores on PDA plates, at location A. The

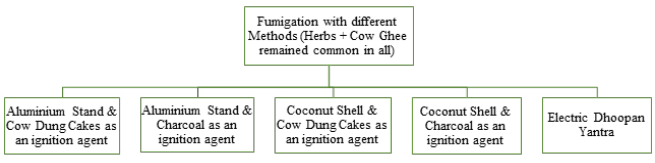


Fig. 1: Methods of fumigation used as different cases to perform the study

Table 1: Result displayed by digital colony counter for N-agar and PDA plates (Location A)

Case	*Fumigation method*	*Total colonies in N-Agar plate*	*%Reduction in bacterial colonies*	*Total colonies in PDA plate*	*%Reduction in fungal spores*
1	Microbial load of enclosed classroom (Pre-Fumigation)	58	13.79	32	21.90
	Microbial load by using aluminum stand and cow dung cakes (Post-Fumigation)	50		25	
2	Microbial load of enclosed classroom (Pre-Fumigation)	153	24.83	57	21.00
	Microbial load by using aluminum stand and charcoal (Post-Fumigation)	115		45	
3	Microbial load of enclosed classroom (Pre-Fumigation)	137	16.05	47	14.90
	Microbial load by using coconut shell and cow dung cakes (Post-Fumigation)	115		40	
4	Microbial load of enclosed classroom (Pre-Fumigation)	108	12.00	25	16.00
	Microbial load by using coconut shell and charcoal (Post-Fumigation)	95		21	
5	Microbial load of enclosed classroom (Pre-Fumigation)	125	40.00	58	32.75
	Fumigation by automatic electric device (Post-Fumigation)	75		39	

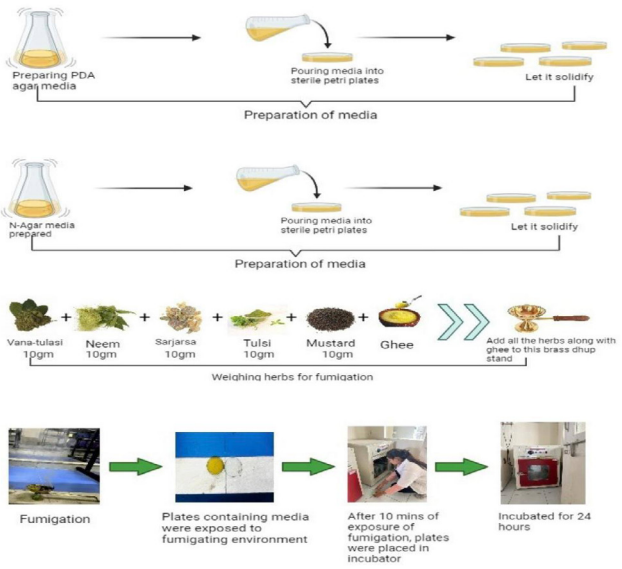


Fig. 2: Steps of the preparation for the experiments

results demonstrate that fumigation with an automatic electric device is the most effective method, achieving a significant 40% reduction in bacterial colonies and a 32.75% reduction in fungal spores. This superior performance can be attributed to the precise and uniform distribution of antimicrobial agents by the electric device, ensuring comprehensive coverage and effective microbial control. In contrast, traditional methods, such as using an aluminum stand with cow dung cakes or charcoal, show relatively lower reductions. For instance, the aluminum stand with cow dung cakes achieved only a 13.79% reduction in bacterial colonies and a 21.90% reduction in fungal spores. The use of charcoal slightly improved outcomes with a 24.83% reduction in bacterial colonies and 21.00% in fungal spores. The moderate effectiveness of these traditional methods can be linked to the antimicrobial properties of the materials used—cow dung and charcoal—which release volatile antimicrobial compounds during combustion. However, their effectiveness is limited by inconsistent distribution and potential variations in the concentration of active compounds.

The combination of coconut shells and cow dung cakes or charcoal also demonstrated modest effectiveness, with a 16.05% reduction in bacterial colonies and 14.90% in fungal spores for the former and 12.00 and 16.00%, respectively, for the latter. Coconut shells, known for releasing phenolic compounds during combustion, provide additional antimicrobial action, but the results suggest that this effect may not be as potent or uniformly distributed as the electric device's output. The findings underscore the importance of using advanced fumigation technologies like the automatic electric device, which not only enhances the efficacy of microbial reduction but also offers a more reliable and consistent approach compared to traditional methods.

Fig. 3 indicates that the automatic electric fumigation device is the most effective method, showing the lowest microbial counts with 39 colonies on PDA and 75 on N-agar. This is followed by the combination of coconut shell and

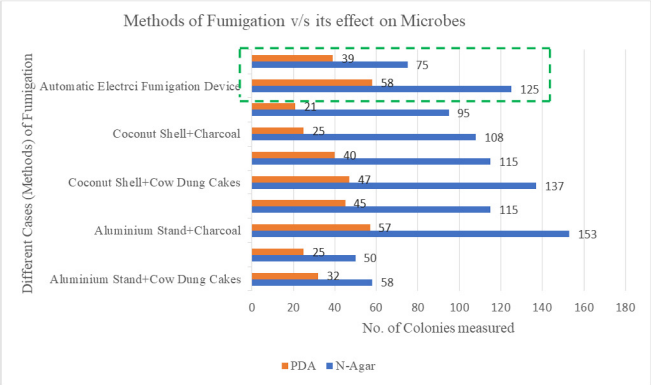


Fig. 3: Methods of fumigation v/s its effect on N-agar and PDA plate colonies

charcoal, which also demonstrates a significant reduction, particularly on N-Agar (95 colonies). Traditional methods such as coconut shell with cow dung cakes and another method. Aluminum stand with cow dung cakes show higher colony counts, suggesting these methods are less effective, though still reducing microbial load compared to pre-fumigation levels. The highest number of colonies is observed in the aluminum stand with cow dung cakes method on N-Agar (153 colonies), indicating it is the least effective among those tested. Overall, the data highlights that modern methods, especially the electric fumigation device, provide superior microbial control compared to traditional methods. The study aimed to evaluate the efficacy of various fumigation methods in reducing microbial load within an enclosed classroom environment. Post-incubation, the number of colonies formed on N-Agar and PDA plates were quantified using a digital colony counter, comparing pre-fumigation (normal air flora) and post-fumigation conditions. This approach allowed for a precise assessment of bacterial and fungal load reductions attributed to different fumigation techniques.

Case 1: Aluminium Stand and Cow Dung Cakes

During the first case, the pre-fumigation average microbial load recorded was 58 bacterial colonies on N-agar and 32 fungal colonies on PDA plates. Post-fumigation with an aluminum stand and cow dung cakes, the bacterial colonies decreased to 50, showing a reduction of 13.79%, while fungal colonies reduced to 25, indicating a 21.90% reduction in fungal spores. The observed reduction in microbial load could be attributed to the bioactive compounds released during the combustion of cow dung cakes, known for their antimicrobial properties.[22]

Case 2: Aluminium Stand and Charcoal

The second case's data showed a more significant reduction with the use of an aluminum stand and charcoal. The pre-fumigation bacterial count was 153, which reduced to 115 post-fumigation, showing a 24.83% reduction. Similarly, the fungal count decreased from 57 to 45, a 21.00% reduction. Charcoal fumigation likely produced volatile compounds capable of disrupting microbial cell membranes, enhancing the reduction efficiency.[23]

Case 3: Coconut Shell and Cow Dung Cakes

In the third case, the pre-fumigation bacterial colonies were 137, which reduced to 115 post-fumigation, marking a 16.05% decrease. The fungal colonies decreased from 47 to 40, showing a 14.90% reduction. The combined use of coconut shells and cow dung cakes likely created a synergistic effect, releasing antimicrobial compounds that contributed to the microbial load reduction.[24,25]

Case 4: Coconut Shell and Charcoal

Fumigation with coconut shells and charcoal in the fourth case resulted in a pre-fumigation bacterial count of 108, which reduced to 95 post-fumigation, a 12.00% reduction. Fungal colonies decreased from 25 to 21, indicating a 16.00% reduction. The coconut shell's pyrolytic products combined with charcoal's volatile compounds possibly enhanced the antimicrobial efficacy, albeit to a lesser extent compared to other days.[25,26]

Case 5: Fumigation with Automatic Electric Device

Fifth Case, using an automatic electric fumigation device, showed the most significant reductions. The pre-fumigation bacterial colonies were 125, which notably reduced to 75 post-fumigation, indicating a 40% reduction. Fungal spores reduced from 58 to 39, showing a 32.75% reduction which is highlighted by dashed lines in Fig. 3. The automated device likely provided a consistent and controlled release of antimicrobial agents, maximizing the reduction in microbial load.

This study investigated the effectiveness of various fumigation methods for reducing microbial loads in an enclosed space. The type of material being burned (cow dung cakes, coconut shells), the method of combustion (automatic electric device vs burning materials), and the initial level of contamination all significantly impacted the reduction in microbes. Notably, the controlled release from the electric device provided the most consistent results, suggesting that precise control over exposure duration is crucial. Additionally, environmental factors like humidity, temperature, and air circulation influenced how well the released agents dispersed and interacted with microbes, ultimately affecting the overall effectiveness of the fumigation method. The device eliminates the potential hazards associated with manual fumigation, such as fire risks and exposure to harmful fumes. The compact and user-friendly design of the device makes it convenient to operate in various settings is key features of automatic electric fumigation device. The electric fumigation device depicted in Fig. 4 incorporates several notable features. The device comprises a cylindrical chamber equipped with an electric heating element and a fan for efficient distribution of fumigants. The inclusion of adjustable

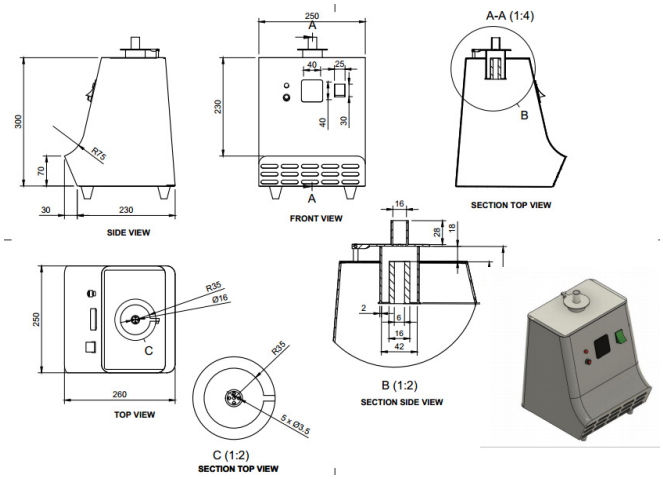


Fig. 4: Design and development of electric fumigation device

settings for fumigation duration and intensity allows for customization based on specific requirements.

An automatic electric fumigation device has been designed and developed by using stainless steel. The assembly consists of a cartridge heater of 60 mm length and 8 mm diameter with a 350-Watt capacity, which can work with 550˚C temperature limit. A portable air pump was used inside the device to supply the oxygen inside the heating barrel. A heating barrel can hold dry powder of herbs with a capacity of 30 g. An RTD temperature sensor is used to measure the initial temperature of fumes. Users can set the required initial temperature of fumes and according to that, the controller will control the heater to maintain a constant temperature.[27,28]

Experiment at Location- A

This study conducted a comparative analysis of microbial colonies before and after fumigation using N-agar and PDA plates to assess the efficacy of different fumigation methods in reducing bacterial and fungal loads in an enclosed classroom environment. The results were statistically analysed using Wilcoxon's Signed rank test to determine the significance of the reductions observed as per Table 2.[29,30]

Bacterial Load Reduction on N-Agar Plates

The pre-fumigation bacterial colony count on N-agar plates had a mean of 29.72, with a standard deviation (S.D.) of 20.75 and a median value of 27. Post-fumigation, the mean bacterial colony count reduced to 20.84, with an S.D. of 8.65 and a median of 22. The Wilcoxon's Signed rank test yielded a Z-value of -1.981 and a *p-value* of 0.048, indicating a statistically significant reduction at the 5% significance level.

This significant reduction in bacterial colonies can be attributed to the antimicrobial properties of the fumigation agents used, which likely disrupted bacterial cell membranes, inhibited their growth, or caused bacterial death. Factors influencing this reduction include the type and concentration of antimicrobial agents released during fumigation, the duration of exposure, and the initial bacterial load.[31] The variability in colony counts, as reflected by the S.D., suggests differing effectiveness across samples, possibly due to variations in environmental conditions such as temperature and humidity within the enclosed space.

Fungal Load Reduction on PDA Plates

For fungal colonies on PDA plates, the pre-fumigation mean count was 9.36, with an S.D. of 5.20, and a median value of 8. Post-fumigation, the mean fungal colony count decreased to 6.96, with an S.D. of 4.17 and a median of 6. The Wilcoxon's Signed rank test resulted in a Z value of -3.653 and a *p-value* of 0.001, indicating a highly significant reduction at the 1% significance level.

The substantial decrease in fungal colonies post-fumigation suggests that the fumigation agents were highly effective in inhibiting fungal spore germination and growth. Factors contributing to this significant reduction include the specific antifungal properties of the fumigation agents, the method of application, and the concentration of antifungal volatiles in the air.[31,32] The relatively lower S.D. post-fumigation implies a more consistent reduction across samples compared to the bacterial results.

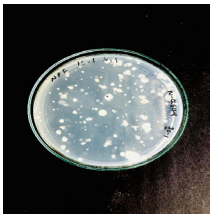
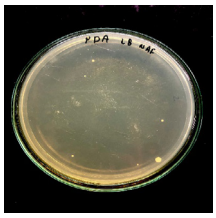
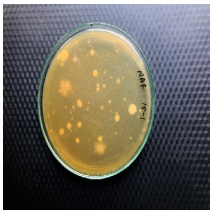
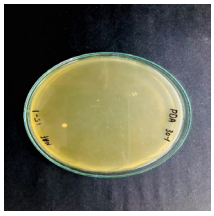
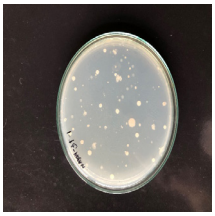
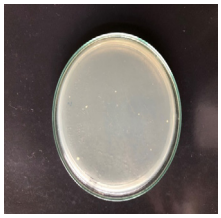
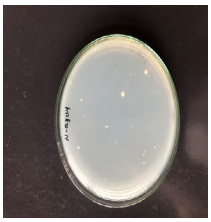
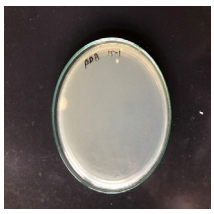
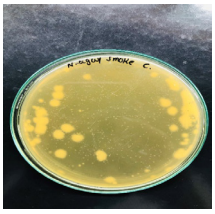
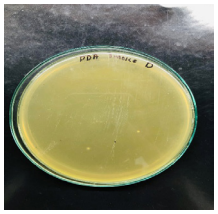
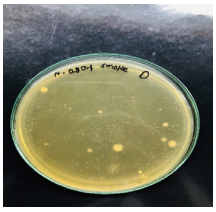
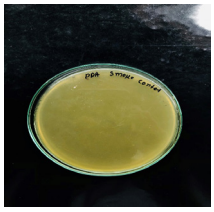
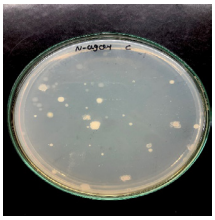
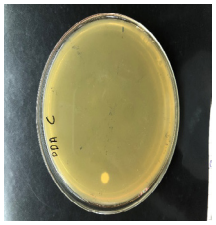
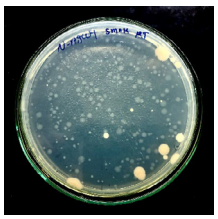
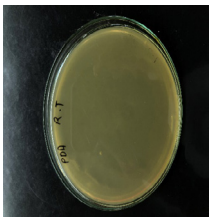
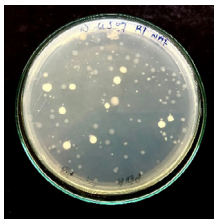
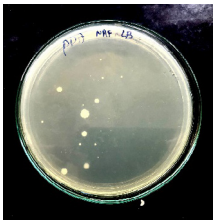
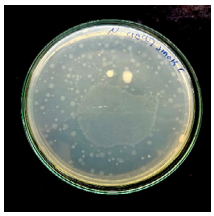
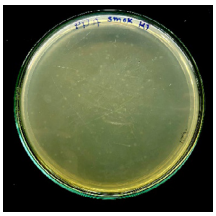
This study explored the science behind successful fumigation for reducing microbial loads in a closed room.

Table 2: Comparison of no. of colonies for pre and post fumigation (Location A)

Types of microbes	*Total no. of colonies*	*Pre-fumigation*	*Post-fumigation*	*Wilcoxon's signed rank test*		
				Z-value	*p-value*	*Significance*
N-agar plates (Bacterial)	Mean	29.72	20.84	-1.981	0.048*	Significant
	Median	27	22			
	S.D.	20.75	8.65			
PDA plates (Fungal)	Mean	9.36	6.96	-3.653	0.001**	Highly Significant
	Median	8	6			
	S.D.	5.20	4.17			

*Significant level at 5%, **Significant level at 1%

Table 3: Bacterial and fungal colonies at pre-fumigation and post-fumigation with N-agar and PDA media

Case	*Experimental conditions*	*N-Agar*	*PDA*
Case-1	Microbial load of enclosed classroom (Pre-Fumigation)		
	Microbial load by using Aluminium stand and Cow Dung Cakes (Post-Fumigation)		
Case-2	Microbial load of enclosed classroom (Pre-Fumigation)		
	Microbial load by using Aluminium stand and Charcoal (Post-Fumigation)		
Case-3	Microbial load of enclosed classroom (Pre-Fumigation)		
	Microbial load by using Coconut Shell and Cow Dung Cakes (Post-Fumigation)		
Case-4	Microbial load of enclosed classroom (Pre-Fumigation)		
	Microbial load by using Coconut Shell and Charcoal (Post-Fumigation)		
Case-5	Microbial load of enclosed classroom (Pre-Fumigation)		
	Fumigation by Modern Method (Automatic Electric Device)		

Several factors played a key role: the type of fumigant used (like cow dung cakes with broad-spectrum activity), the concentration and even spread of the agent (controlled methods like electric devices were most effective), the duration of exposure (longer being better), the initial level of contamination (higher starting counts showed greater reduction), and even environmental conditions (temperature, humidity, and ventilation) all impacted how well the fumigation worked. Overall, the study confirms that fumigation significantly reduces microbes in classrooms, with effectiveness depending on the chosen method and the environment. This highlights the importance of selecting appropriate fumigation strategies to maximize microbial reduction and improve air quality in enclosed spaces.[33] Pre-fumigation assessments consistently show high microbial loads, indicating significant contamination in the classroom environment. as shown on Table 3.

Case-1

Post-fumigation with an aluminum stand and cow dung cakes, a noticeable reduction in both bacterial and fungal colonies is observed on N-Agar and PDA plates. This suggests that traditional fumigation methods using cow dung, known for its antimicrobial properties, are effective in reducing microbial load. The mechanism here likely involves the release of volatile antimicrobial compounds during the combustion of cow dung, which disrupts microbial cells.[34]

Case-2

The use of aluminum stands and charcoal for fumigation results in a similar reduction in microbial load, with

Table 4: Result displayed by Digital Colony Counter for N-Agar and PDA plates (Location B)

No. of Plates	*Day*	*Total colonies in N-Agar Plate*	*%Reduction in Bacterial Colonies*	*Total colonies in PDA Plate*	*%Reduction in Fungal Spores*
Microbial load of Washroom (Pre-Fumigation)	1	624	63.47	45	91.00
	2	585		52	
	3	595		34	
Fumigation by Automatic Electric Device (Post-Fumigation)	1	238		04	
	2	211		04	
	3	210		04	

fewer colonies present on both types of agar compared to pre-fumigation levels. Charcoal, when heated, releases activated carbon that adsorbs and neutralizes microbes and toxins in the air, thereby reducing microbial contamination.[35]

Case-3

Fumigation using coconut shells and cow dung cakes shows effective microbial reduction, with decreased colony counts on both media. Coconut shells, when burned, release phenolic compounds with known antimicrobial properties, which, combined with the effects of cow dung, further enhance the fumigation process.[36]

Case-4

The combination of coconut shells and charcoal for fumigation also leads to a significant decrease in microbial load. This suggests a synergistic effect, where the phenolic compounds from the coconut shells and the adsorptive properties of charcoal work together to reduce airborne microbial contaminants effectively.[37]

Case-5

Fumigation with a modern automatic electric device results in the most significant reduction in microbial load across both N-Agar and PDA plates. This method likely uses advanced technologies to disperse disinfectants or antimicrobial agents evenly throughout the environment, leading to superior microbial control.[27]

The study investigated the efficacy of different fumigation methods in reducing microbial load. Cow dung cakes released antimicrobial compounds like phenols and aldehydes, while charcoal adsorbed microbes and toxins. Coconut shells offered additional phenolic compounds, and the combination of coconut shells and charcoal created a synergistic effect. The modern electric fumigation device provided uniform dispersion of antimicrobial agents, ensuring maximum microbial contact.

The study highlights the varying effectiveness of traditional and modern fumigation methods, with the modern electric device showing the highest efficacy.[38] The mechanisms of microbial reduction vary depending on the materials used, involving physical adsorption, chemical disruption of microbial cells, and the release of antimicrobial compounds. These findings underscore the potential of both traditional and modern fumigation methods in maintaining hygiene in enclosed spaces.[16, 39]

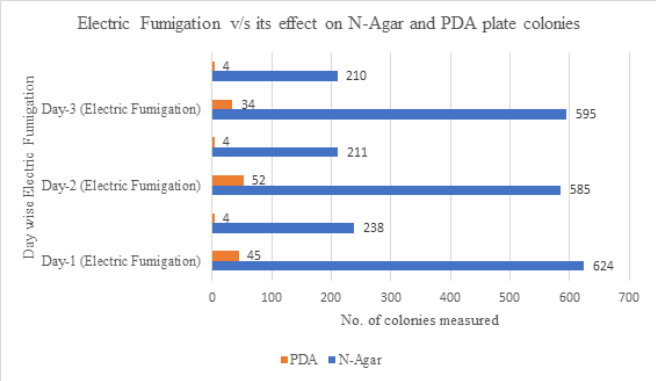


Fig. 5: Effect of electric fumigation (Location B)

Experiment at Location-B

To validate the effect of electric fumigation, another study was designed to identify the microbial load in location B (highly contaminated room) and fumigation was applied for 3 days at the interval of a week. Only an electric fumigation device was used here. The results have been analyzed by performing the same steps as previously described, which are listed in Fig. 5 and Table 4. Further results are mentioned in the supplementary document.

From Table 4 and Fig. 5, it is being interpreted that the impact of electric fumigation on microbial load reduction in a washroom environment by quantifying the number of bacterial and fungal colonies on N-agar and PDA plates, respectively. The results highlight the effectiveness of electric fumigation in reducing microbial colonies, demonstrating its potential as a method for maintaining hygiene in enclosed spaces.[40]

Pre-Fumigation Microbial Load

Before fumigation, the washroom environment exhibited a high microbial load. The total number of bacterial colonies on N-Agar plates was 624, 585, and 595 on days 1, 2, and 3, respectively. Fungal colonies on PDA plates were recorded as 45, 52, and 34 on the same days. These high initial

Table 5: Comparison of no. of colonies for pre and post fumigation (Location B)

Types of microbes	*Total no. of colonies*	*Before fumigation*	*After fumigation*	*Wilcoxon's signed rank test*		
				Z-value	*p-value*	*Significance*
N-Agar Plates (Bacterial)	Mean	120.26	43.93	-3.408	0.001*	Highly Significant
	Median	120.00	42.00			
	S.D.	31.70	25.13			
PDA Plates (Fungal)	Mean	8.73	0.80	-3.416	0.001**	Highly Significant
	Median	7.00	0.00			
	S.D.	4.44	1.26			

*Significant level at 0.1%, **Significant level at 0.1%

counts underscore the significant presence of microbial contaminants in the washroom environment, emphasizing the need for effective fumigation strategies.

Post-Fumigation Microbial Load

Following fumigation with an automatic electric device, there was a substantial reduction in microbial colonies. The bacterial colonies on N-agar plates decreased to 238, 211, and 210 on days 1, 2, and 3, respectively, showing a reduction percentage of 63.47% on the first day. Fungal colonies on PDA plates dropped dramatically to 4 on each day, indicating a 91% reduction in fungal spores on the first day. This pronounced decrease in microbial load post-fumigation highlights the efficacy of the electric fumigation method.

This study investigated the effectiveness of electric fumigation for reducing microbial loads in a washroom. The observed reduction in bacterial colonies on N-agar plates after fumigation can be linked to the bactericidal effects of the herbal compounds released during the fumigation process. Essential oils and bioactive compounds, such as terpenes, phenols, and aldehydes, are known to disrupt bacterial cell membranes, leading to leakage of cellular contents and eventual cell death. These compounds may also interfere with essential bacterial processes, such as protein synthesis and enzyme function, by denaturing proteins and altering the pH of the surrounding environment. The electric fumigation device plays a crucial role in this process by ensuring a uniform and controlled release of these active components, maximizing their contact with airborne bacteria and surfaces within the environment. The significant drop in fungal colonies on PDA plates post-fumigation suggests that the fumigation process effectively targets fungal spores. Fungal spores are highly resistant structures, but the bioactive compounds in herbal fumigants, particularly those with antifungal properties like eugenol, thymol, and carvacrol, can penetrate the spore's outer layers and disrupt cellular integrity. These compounds may inhibit the germination of spores by interfering with the synthesis of ergosterol, an essential component of fungal cell membranes.

Additionally, the electric fumigation device ensures that these volatile compounds are evenly distributed in the air and on surfaces, leading to consistent exposure and enhanced antifungal activity. The effectiveness of the electric fumigation device lies in its ability to generate consistent and uniform fumes without the need for an ignition agent. This method ensures that the bioactive compounds are evenly dispersed throughout the environment, maintaining a stable concentration of fumigants over time. The controlled release mechanism allows for sustained antimicrobial action, which is critical for achieving the observed reductions in microbial load. Furthermore, the absence of an open flame reduces the risk of fire hazards, making this method safer for use in enclosed spaces. The results suggest that the fumigation process impacts both airborne and surface-bound microorganisms. The reduction in bacterial and fungal colonies indicates that the fumigants are effective in both air disinfection and surface decontamination. This dual action is essential in environments like washrooms, where microorganisms can be spread through aerosols and deposited on various surfaces. The automatic dispenser ensured consistent release of the antimicrobial agents, providing uniform exposure to microbes. The high initial level of contamination allowed for a clearer demonstration of the reduction effect. Additionally, stable environmental conditions within the washroom likely aided the consistent reduction observed. The antimicrobial properties of the agents themselves, designed to disrupt microbial functions and cause cell death, played a key role in significantly reducing both bacterial and fungal colonies.[40-42] These findings suggest that electric fumigation is a reliable and practical method for improving hygiene and reducing microbial risks in enclosed spaces.

The comparison of microbial colonies before and after fumigation in location B, as shown in Tables 4 and 5, provides significant insights into the efficacy of the fumigation process. The data was analyzed using Wilcoxon's Signed Rank Test, revealing highly significant reductions in both bacterial and fungal colonies on N-agar and PDA plates, respectively.[29,30]

The microbial load of a washroom location-B was assessed using N-Agar and PDA plates both before and after fumigation. Pre-fumigation results on N-Agar showed

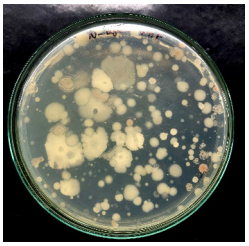
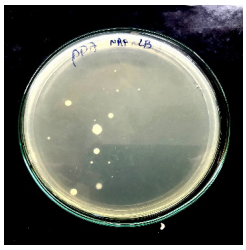
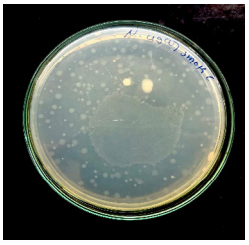
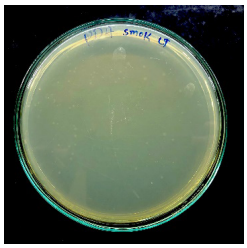
Conditions of experiment	*N-Agar*	*PDA*
Microbial load of Washroom (Pre-Fumigation)		
Microbial load by using Electric Fumigation Device (Post-Fumigation)		

Fig. 6: Microbial load on N-agar and PDA plates before and after electric fumigation

a high microbial load, with dense bacterial colonies indicative of substantial contamination. The PDA plate, selective for fungi, also revealed the presence of several fungal colonies, highlighting the washroom's susceptibility to microbial growth in a moist environment. Post-fumigation, there was a notable reduction in microbial load on both media. The N-Agar plate demonstrated significantly fewer bacterial colonies, indicating the effectiveness of the electric fumigation device in reducing bacterial contamination.[39] Similarly, the PDA plate showed a marked decrease in fungal colonies, suggesting successful eradication of fungal spores as pe Fig. 6. These results underscore the importance of fumigation in maintaining hygiene in high-use areas like washrooms, effectively reducing the microbial load and minimizing the risk of pathogen transmission.[43]

Bacterial Load Reduction on N-Agar Plates

Before fumigation, the mean bacterial colony count on N-Agar plates was 120.26, with a standard deviation (S.D.) of 31.70 and a median value of 120. After fumigation, the mean count significantly decreased to 43.93, with an S.D. of 25.13 and a median of 42. The Wilcoxon's Signed rank rest yielded a Z-value of -3.408 and a *p-value* of 0.001, indicating a highly significant reduction at the 0.1% significance level. This substantial reduction in bacterial colonies can be attributed to the effectiveness of the fumigation method employed. The significant drop in the mean and median values demonstrates the fumigation's ability to target and reduce bacterial populations effectively. Factors contributing to this reduction include the nature and concentration of the fumigants used, their antimicrobial properties, and the duration of exposure. The variation in colony reduction, suggests that while the fumigation was generally effective, environmental factors such as air circulation and surface types may have influenced the degree of bacterial reduction.[42]

Fungal Load Reduction on PDA Plates

For fungal colonies on PDA plates, the pre-fumigation mean count was 8.73, with an S.D. of 4.44, and a median value of 7. Post-fumigation, the mean fungal colony count decreased dramatically to 0.80, with an S.D. of 1.26, and a median of 0. The Wilcoxon's Signed Rank Test resulted in a Z-value of -3.416 and a *p-value* of 0.001, indicating a highly significant reduction at the 0.1% significance level. The marked decrease in fungal colonies post-fumigation highlights the effectiveness of the fumigation method in eliminating fungal spores. The significant reduction in both mean and median values, coupled with the low post-fumigation S.D., indicates a consistent and robust reduction in fungal spores across the samples. This consistency suggests that the fumigant's antifungal properties were highly effective in this context, likely disrupting fungal cell walls and preventing spore germination. The low post-fumigation median further supports the efficacy, indicating that the majority of samples had minimal to no fungal colonies remaining.[42]

The study demonstrates that fumigation significantly reduces bacterial and fungal colonies in an enclosed environment, with highly significant results confirmed by Wilcoxon's Signed rank test. The substantial reductions observed underscore the importance of using effective fumigation agents and maintaining optimal environmental conditions to maximize microbial load reduction. These findings contribute valuable insights into improving hygiene and reducing microbial contamination in enclosed spaces, supporting the use of fumigation as a reliable method for microbial control.

Conclusion

The study comprehensively concludes that electric fumigation with nimb (*A. indica A. Juss.*), *sarshapa* (*B. rapa L.*), tulasi (*O. sanctum L.*), vanatulasi (*O. basilicum L.*), and sarjarsa (*S. robusta Gaertn*) significantly reduce the microbial load of the air. These herbs are inexpensive, safe, non-toxic and readily available on the market. An electric fumigation device produces fumes uniformly and doesn't require an ignition agent. It can reduce bacterial colonies by 40% and fungi colonies by 32.75% within location A, while 62.5% and 91% reductions were noticed within location B, respectively. The combination of electric fumigation and aforementioned plant products indicate positive results which promote evidence-based research in *Ayurveda.*

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Compliance with Ethical Standards

There is no participation of human and animals or their biological materials; therefore, no ethical approval is required.

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