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Original Research Article

Medicinal Plants used in Kumaun Vedic Practices (*Agnihotra*) and their Influence on Aeromicroflora

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Abstract: This study investigated the antimicrobial efficacy of *Agnihotra* (*Havan*), an ancient Vedic ritual involving the combustion of medicinal plants, against pathogenic aeromicroflora in the Kumaun Himalayan region of India. Specifically, it examined the impact of aromatic smoke on airborne fungal spores and bacteria, while assessing seasonal variations across five distinct periods: summer, monsoon, autumn, winter, and spring. Experiments were conducted in both indoor and outdoor settings at the Department of Botany. Microbial samples were collected using two types of culture media (i) PDA for fungi and (ii) Nutrient Agar (NA) for bacteria under three conditions: (i) control (before *Agnihotra*), (ii) during *Agnihotra* (exposed to medicinal smoke) and (iii) immediately after *Agnihotra*. All petri dishes were incubated at 25±1°C to promote optimal microbial growth. The results revealed 14 distinct fungal species. Notably, some pathogenic species were completely absent in the smoke-exposed samples. These results indicate that the volatile compounds released during *Agnihotra* possess potent antimicrobial properties capable of suppressing a broad spectrum of airborne microorganisms, including several known human pathogens. Seasonal analysis confirmed the consistency of this antimicrobial effect throughout the year, although the degree of efficacy varied with environmental conditions. This study provides scientific validation for the antimicrobial potential of *Agnihotra* smoke, signifying its applicability in modern microbial control strategies. Such eco-friendly and culturally rooted practices hold promise for use in healthcare facilities, agricultural environments, and other settings where airborne pathogen reduction is critical.

Keywords: Aero-microflora, *Agnihotra,* culture media, Impact of medicinal smoke.

Introduction

Earth serves as a reservoir of diverse life forms, including a vast array of plants, animals, and airborne microorganisms such as fungi and bacteria. Meir (1930) was the first aerobiologist to coin the term "aerobiology" for the study of airborne spores, pollen and microorganisms.The term was further expanded by Jacob (1951) to encompass the dispersion of fungal spores, bacteria, insects and pollen grains. The history of aerobiology can be traced back to the Vedas, the oldest written texts of the Aryans, composed between 1500 and 3500 B.C. These texts describe the fundamental life-supporting system known as "Panchbhutas" (five elements), which includes Soil, Water, Energy, Space and Air. Beyond their spiritual significance, the Vedas emphasize air as the "breath of life" (Pran Vayu), prominence its essential role in sustaining human existence. The Atharvaveda notes that inhaling contaminated air can lead to respiratory tract diseases.

Additionally, the Shatapatha Brahmana emphasizes that the *Agnihotra* technique is beneficial for purifying the air. Microorganisms such as bacteria, fungi, and viruses that are associated with atmospheric particulate matter are collectively referred to as bioaerosols. Bioaerosols are reported to contribute up to 25% of atmospheric aerosols (Jaenicke, 2005). Additionally, chemical pollutants in the atmosphere play a significant role in human health, as they can accumulate in airborne particulate matter and lead to various adverse effects, particularly respiratory illnesses.

In addition to chemical pollutants, airborne microorganisms associated with atmospheric particles have garnered increasing attention in recent years. Evidence emphasizes their significant role in the atmospheric environment and their potential impacts on human health, agricultural productivity and ecosystem stability (Xu, *et al.,* 2017). The atmosphere presents a challenging environment for the growth of airborne microbes, yet it is characterized by a high richness and abundance of microorganisms. Airborne fungal propagules, including spores, are present year-round under specific climatic conditions; however, their prevalence in the environment fluctuates with climate change (Adhikari, *et al*., 2004).

Airborne fungal propagules are typically present throughout the year, but the seasonal patterns and diversity of airborne spores vary according to the climate type (Agrawal and Tiwari, 2019). Fungal densities in the air also vary across geographical regions and seasons, influenced by physical parameters such as wind direction, humidity, temperature, precipitation, and altitude (Harishankar, *et al*., 2016). Fungi, being ubiquitous, account for approximately 4–11% of ambient bioaerosols in both urban and rural environments (Womiloju, *et al*., 2003).

They pose significant health risks, impacting human health through a wide range of diseases, including IgE-mediated type I hypersensitivity, life-threatening primary and secondary infections in immunocompromised patients, allergic bronchopulmonary mycosis (ABPM), hypersensitivity pneumonitis, fungal sinusitis and toxic pneumonia (Oliveira *et al*., 2023). Additionally, fungi can produce various mycotoxins with neurotoxic, mutagenic, carcinogenic and teratogenic effects, leading to mycotoxicosis. Their small spore size (average: 2–10 μm; Simon-Nobbe, *et al*., 2008) facilitates dispersion in the atmosphere and deposition in the human respiratory tract. Fungi produce a vast number of spores that easily become airborne, making them a significant component of microbial aerosols. These fungi can cause serious diseases in humans, many of which can be fatal if left untreated (Hube, 2004). Indoor environments significantly impact health, with air quality being a crucial factor affecting human well-being and productivity. One major issue often overlooked is the presence of disease-causing microorganisms in indoor air (Osuolale, *et al*., 2019). The primary objectives of this experiment (study) are to prepare culture media to assess microbial growth and to implement *Havan* practices to evaluate their impact on aeromicroflora, specifically analyzing how *Havan* fumes influence microbial growth.

Materials and methods

Experiment design: To study the growth of airborne microbes the experimental workwas conducted in indoor and outdoor environment at Department of Botany, Soban Singh Jeena University Campus Almora (Uttarakhand). For this purpose, total ten *Havan* events were organized in five different seasons summer, rainy, autumn, winter, and spring respectively. Weather seasons were categorized as per Attri and Tyagi (2010) and Singh and Singh (2020). The observations were taken as (A)Pre *Havan* (control), (B) During *Havan* and (C) After *Havan.*

Preparation of culture medium: Bacterial culture was the first method developed to study the human microbiota (Lagier, *et al.,* 2018). To assess the impact of the growth of microorganisms, two types of culture medium were prepared (PDA and Nutrient agar medium) and used to isolate the microorganisms with all three conditions (control, during *Havan*, and after *Havan).* Waksman(1992) first uses different types of culture mediums for the identification of microorganisms.

Preparation of PDA Medium: Potato dextrose agar was used for cultivating fungi (Waksman, *et al.,* 1922). PDA is composed of dehydrated potato infusion and dextrose that encourage fungal growth. Agar was added as a solidifying agent, it has many uses, like for detection of yeasts and molds in dairy products and prepared foods (Tsudome, *et al.,* 2009).

Procedure of potato dextrose agar (PDA): For the preparation of potato dextrose agar (PDA), 250 g of potato infusion, 20 g of dextrose, 20 g of agar, and 1 liter of water were used. To prepare the potato infusion, 250 g of unpeeled, sliced potatoes were boiled in 1 liter of water for 10 minutes. The mixture was then strained, retaining the potato effluent. This effluent was mixed with dextrose, agar and water and boiled until fully dissolved. The solution was sterilized by autoclaving at 121°C for 15 minutes. After cooling, it was poured into petri dishes (Tsudome, *et al.,* 2009).

Preparation of nutrient medium: Nutrient agar is a general-purpose medium used for the cultivation of a wide variety of microbes, including bacteria and fungi, due to its rich nutrient content. It provides essential nutrients for bacterial growth. The composition of nutrient agar includes 20 g of yeast extract, a water-soluble substance that promotes bacterial growth (Tsudome, *et al.,* 2009).

Procedure of nutrient agar: Twenty gram of nutrient yeast powder was suspended in 1 liter of water and heat the mixture, stirring to fully dissolve all components. Sterilize the dissolved mixture by autoclaving at 121°C for 15 minutes. After autoclaving, allow the nutrient agar to cool but not solidify. Pour the nutrient agar into petri dishes and leave them on a sterile surface until the agar solidifies. Replace the lids on each petri dish and store the plates in a refrigerator. Nutrient agar is commonly used for the isolation and purification of microbial cultures (Sandle, 2015) (Figure 1).

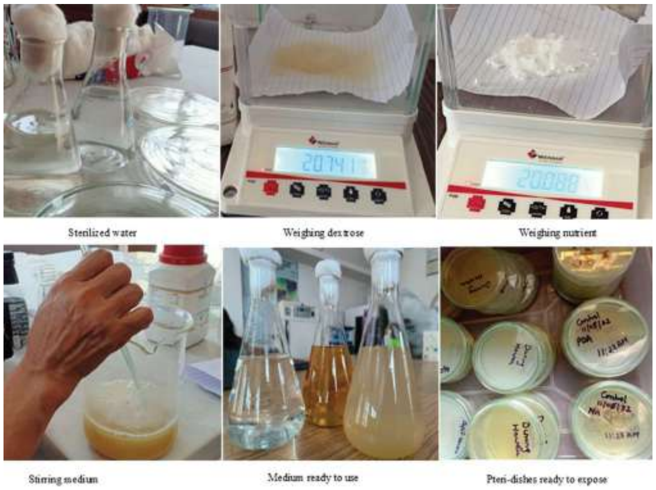

Sterilized water
Weighing dextrose
Weighing nutrient
Stirring medium
Medium ready to use
Pteri-dishes ready to expose

Fig. 1. Preparation of culture medium in laboratory condition.

Preparing and setting up *Agnihotra* (*Havan*): As part of the experiment, *Agnihotra* was conducted across five seasons- summer, rainy, autumn, winter and spring in two different environments: indoors and outdoors at Department of Botany SSJU Campus (Almora). The fire rituals were performed in the dark rooms of the department. The high moisture levels in these rooms created favorable conditions for airborne microbial flora to survive. The campus is situated in mountainous terrain at an altitude of 1604 m. Its landscape, dotted with large oak and cedar trees and other higher vegetation, contributes to the high moisture content levels that persist year-round. The buildings of the departments are nearly five decades old and the temperature fluctuates throughout the year in varying seasons. During the onset of rainy seasons, humidity and moisture content are very high. The rooms receive less sunlight, as the sunlight does not adequately reach the interior sides.

Using customized *Havan* materials such as camphor, sesame and barley seeds smeared with cow ghee were offered to the holy fire. Vedic texts revealed that the composition of *Havan* material consists of different kinds of woods including medicinal herbs, aromatic products, grains and millets. At the time of oblation in the sacred fire these plant ingredients produce aromatic fumes which influence the microbial diversity. In the Kumaun region, wood pieces from *Mangifera indica*, locally known as *Samidha*, are a significant source of bioactive compounds. These include pentagalloyl glucose (PGG), various polyphenolics and flavonoids (Chaube, *et al.*, 2020). During burning, it releases formic aldehyde, which kills aeromicroflora and purifies the surrounding (Viswanatha, *et al.*, 2013). Besides, *Cedrus deodara* wood also applied as *Havan Samidha* in the region which contains Cedrin,


Performing *Agnihotra* in During *Havan* condition in Indoor environment Performing *Agnihotra* in During *Havan* condition in Outdoor environment

Fig. 2. Performing *Agnihotra* practice in different environment.

Fig. 3. Observations on growth of microbial colonies in different culture medium in indoor environment.

Himachalol, Himachalene and Atlantone that possess anti-insecticidal properties (Bisht, *et al.*, 2021). For each *Havan* event in every season, the prepared culture plates were exposed for 3 to 4 minutes. The petri dishes were exposed within the fumigation zone between 50-70 cm² area. The exposed plates were incubated at ±25°C for 3 to 4 days. The sample plates were labeled as 'control,' 'during *Havan*,' and 'after *Havan*.' Firstly, the set of control plates were exposed before the oblation of *Havan* materials began. After it, 'during *Havan*' sample plates were exposed in the presence of fumes when oblation to sacred fire. At last, 'after *Havan*'sample plates were exposed when flames were over and only the aromatic smell of *Havan* was present in the surroundings (Figure 2). Observations on the growth and number of microbial colonies were recorded at intervals of 24, 48 and 72 hours following the *Havan* (Figure 3 & 4).

Identification of microbes: Identification was done with the help of cotton blue, lactophenol, glycerine and fungal species identified by the compound microscope (Nagamani, *et al.*, 2006).

Data analysis: Data analysis was performed using SPSS software. Pearson's two-tailed correlation test was applied, and the average number of colonies was calculated using the mean and occurrence, along with the standard error.

Result

During the study, it was observed that a total of 14 distinct fungal species *Alternaria alternata*, *Alternaria triticina*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Chaetomium* sp., *Cladosporium* sp., *Drechslera*sp, *Rhizopus nigricans*, Sterile mycelium, *Mucor* sp., *Curvularia* sp., *Penicillium* sp and *Fusarium* sp.were present across all seasons

Fig. 4. Observation on growth of microbial colonies in different culture medium in outdoor environment.

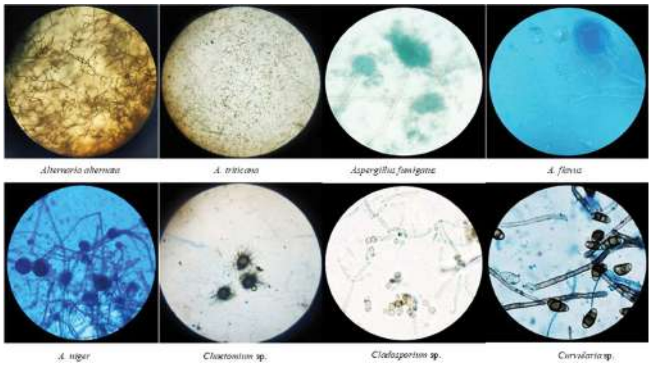


Fig. 5A. Illustration of different fungal species identified during the study.

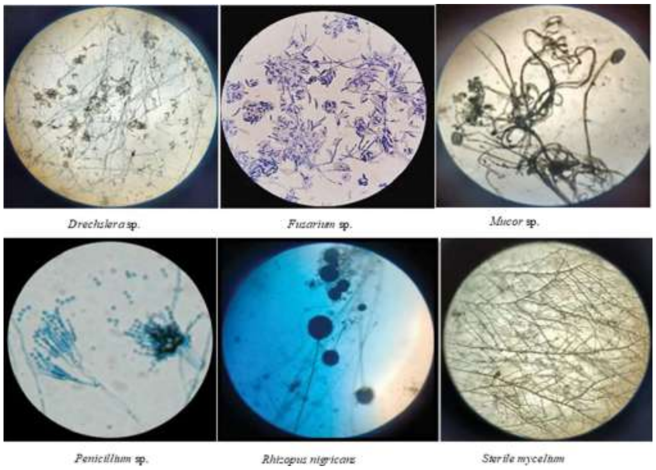


Fig. 5B. Illustration of different fungal species identified during the study.

(Figure 5A & 5B). These species exhibited diverse colony morphologies, indicating variations in their growth patterns. In addition to the fungal colonies, the presence of some bacterial colonies was also noted, although fungal growth predominated in the samples. The seasonal distribution of these microbes displays that environmental factor may have influenced the composition and abundance of microbial communities. All these fungi are ubiquitous in both indoor and outdoor environments. While some species are beneficial to humans, others can cause serious diseases that significantly impact health. In the rainy season, the study recorded a total of 218 fungal colonies in the indoor environment across all three conditions. Under control conditions, there were 152 colonies, which reduced to 64 during the *Havan*, and further dropped to just 2 colonies after the *Havan*. Of these, 122 colonies were observed on PDA and 96 on Nutrient Agar (NA). In the outdoor environment, a total of 246 fungal colonies were recorded. Under control conditions, 176 colonies were noted, which reduced to 67 during the *Havan*, and only 3 were observed after the *Havan*. On PDA, 134 colonies were observed, while NA recorded 112 colonies (Figure 6).These results indicate the significant antifungal impact of *Havan* fumes

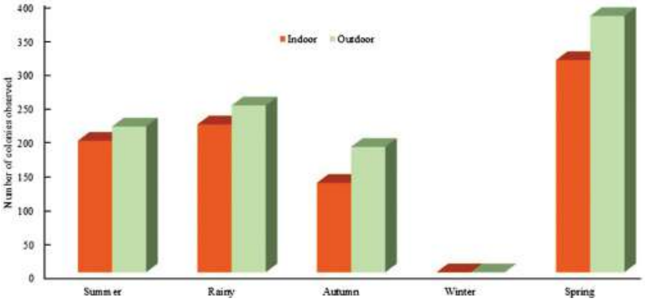


Fig. 6. Number of fungal colonies observed in different seasons.

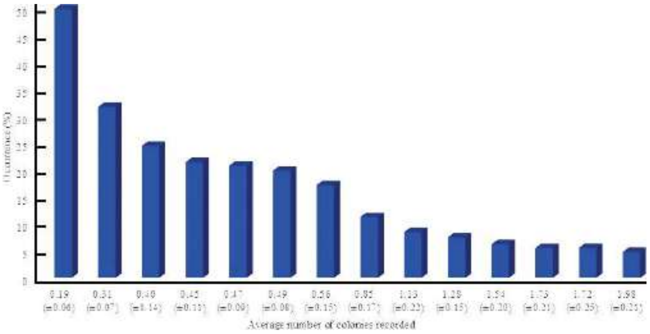
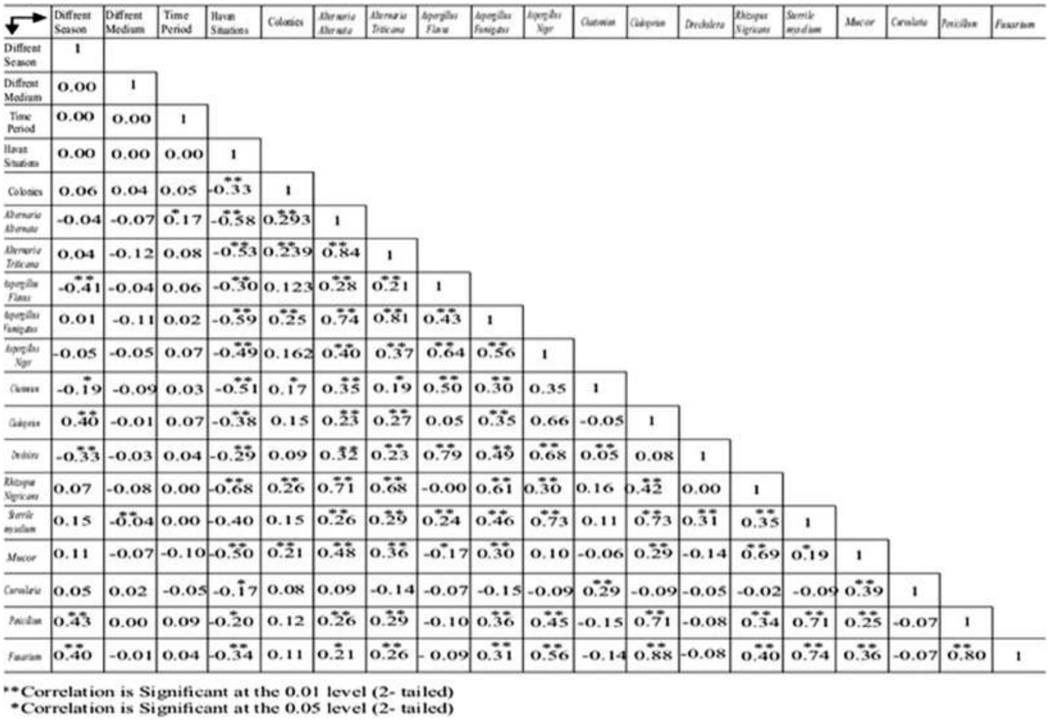


Fig. 7. Number of colonies of different fungal species occurred during the study.

during the rainy season, where high humidity generally favours fungal growth. In the autumn season, the indoor environment had a total of 132 fungal colonies across all three conditions. Under control conditions, 102 colonies were recorded, which reduced to 25 during the *Havan* and further decreased to 5 after the *Havan*. PDA accounted for 80 colonies, while NA recorded 52 colonies (Figure 6). Similarly, in the outdoor environment, a total of 185 fungal colonies were observed. Of these, 134 colonies were present under control conditions, which dropped to 44 during the *Havan* and 7 after the *Havan*. PDA recorded 93 colonies and NA had 92 colonies (Figure 6). These results suggest that the *Havan* fumes effectively inhibited fungal growth even during the autumn season, despite relatively moderate environmental conditions.

In the winter season, no fungal growth was observed in either the indoor or outdoor environments. This absence of growth can be attributed to the combination of low temperatures, reduced moisture levels and other unfavorable environmental factors, which are known to inhibit fungal



	Diffrent Season	Diffrent Medium	Time Period	Havan Situations	Colonies	*Alternaria Alternata*	*Alternaria Triticana*	*Aspergillus Flavus*	*Aspergillus Fumigatus*	*Aspergillus Niger*	*Chaetomium*	*Cladosporium*	*Drechslera*	*Rhizopus Nigricans*	*Sterile mycelium*	*Mucor*	*Curvularia*	*Penicillium*	*Fusarium*
Diffrent Season	1																		
Diffrent Medium	0.00	1																	
Time Period	0.00	0.00	1																
Havan Situations	0.00	0.00	0.00	1															
Colonies	0.06	0.04	0.05	-0.33**	1														
Alternaria Alternata	-0.04	-0.07	0.17*	-0.58**	0.293**	1													
Alternaria Triticana	0.04	-0.12	0.08	-0.53**	0.239**	0.84**	1												
Aspergillus Flavus	-0.41**	-0.04	0.06	-0.30**	0.123	0.28**	0.21**	1											
Aspergillus Fumigatus	0.01	-0.11	0.02	-0.59**	0.25**	0.74**	0.81**	0.43**	1										
Aspergillus Niger	-0.05	-0.05	0.07	-0.49**	0.162	0.40**	0.37**	0.64**	0.56**	1									
Chaetomium	-0.19*	-0.09	0.03	-0.51**	0.17*	0.35**	0.19*	0.50**	0.30**	0.35	1								
Cladosporium	0.40**	-0.01	0.07	-0.38**	0.15	0.23**	0.27**	0.05	0.35**	0.66	-0.05	1							
Drechslera	-0.33**	-0.03	0.04	-0.29**	0.09	0.32**	0.23**	0.79**	0.49**	0.68**	0.05**	0.08	1						
Rhizopus Nigricans	0.07	-0.08	0.00	-0.68**	0.26**	0.71**	0.68**	-0.00	0.61**	0.30**	0.16	0.42**	0.00	1					
Sterile mycelium	0.15	-0.04**	0.00	-0.40	0.15	0.26**	0.29**	0.24**	0.46**	0.73**	0.11	0.73**	0.31**	0.35**	1				
Mucor	0.11	-0.07	-0.10	-0.50**	0.21**	0.48**	0.36**	-0.17*	0.30**	0.10	-0.06	0.29**	-0.14	0.69**	0.19*	1			
Curvularia	0.05	0.02	-0.05	-0.17*	0.08	0.09	-0.14	-0.07	-0.15	-0.09	0.29**	-0.09	-0.05	-0.02	-0.09	0.39**	1		
Penicillium	0.43**	0.00	0.09	-0.20*	0.12	0.26**	0.29**	-0.10	0.36**	0.45**	-0.15	0.71**	-0.08	0.34**	0.71**	0.25**	-0.07	1	
Fusarium	0.40**	-0.01	0.04	-0.34**	0.11	0.21*	0.26**	-0.09	0.31**	0.56**	-0.14	0.88**	-0.08	0.40**	0.74**	0.36**	-0.07	0.80**	1

**Correlation is Significant at the 0.01 level (2- tailed)
*Correlation is Significant at the 0.05 level (2- tailed)

Fig. 8. Pearson correlation across different season, culture medium, *Havan condition*, observation time, fungal species along with their number of colonies recorded.

activity. These findings are consistent with previous studies, which indicates that fungal growth is significantly limited in winter due to harsh environmental conditions (Pietikäinen, *et al.*, 2005; Tung, *et al.*, 2015; Nara, 2009; Kreyling, 2010). In spring season, Indoor environment has total 313 colonies in all three conditions representing 253 in Control, 40 During *Havan* and 20 colonies After *Havan* respectively. Highest number of colonies were observed in NA (164) followed by lower (149) in PDA (Figure 6). Outdoor environment has total 378 colonies in all three conditions; Control (295), During *Havan* (66) and After *Havan* (17). In which PDA shows 188 and NA were 190 colonies present (Figure 6). Overall, the data across the seasons demonstrate the potential of *Havan* fumes as an effective natural antifungal agent. The observed reduction in fungal colonies during and after the *Havan* underscores its role in creating a less favorable environment for fungal growth, especially in conditions typically conducive to their proliferation. The bioactive compounds released, such as essential oils, disrupt the growth and survival of various microbes. Additionally, the aromatic fumes may alter the diversity of aeromicroflora by exerting selective pressure on various species. As a result, this Vedic *Agnihotra* practice of the region can enhance the quality of both indoor and outdoor environments. Across all the seasons, *Drechslera* sp. showed its highest occurrence (50%) followed by *Aspergillus flavus* (31.81%), *Curvularia* sp. (24.56%), *Penicillium* sp. (21.53%), Sterile mycelium (20.89%) and *Chaetomium* sp. (20%) respectively. Whereas *Fusarium* sp. and *Cladosporium* sp. were contributed only 17.28% and 11.38% of their occurrence. However, rest of the species shows less than 10% of their occurrence. Similarly, average number of colonies of the fungal species were also observed. Fungal species *A. alternata, R. nigricans, Mucor sp., A. triticina, A. fumigatus* and *A. niger* exhibits more than one colony per petri-plate (Table 1). Remaining species shows less than one colony per petri-plate. Standard error (SE) was also calculated for the presence of fungal species on sampled petri- plates and ranged between ±0.06 to ±0.25 (Table 1). The study also exhibits the diversity and variability in the occurrence of fungal species across different seasons (Table1; Figure 7). Pearson correlation was calculated to assess the relationship between different season, culture medium, *Havan* situation, observation time, fungal species and the number of colonies they formed. The analysis revealed that most fungal species exhibited a strong positive correlation, meaning that as the number of colonies increased, the presence of these species also became more pronounced. These results collectively show varying degrees of correlation among fungal species and environmental factors, with *Havan* fumes demonstrating a notable inhibitory effect. Pearson correlation analysis was conducted at the 0.01 and 0.05 significance levels. The results show a positive correlation with most fungal species, indicating a positive effect of *Havan* smoke on these species (Figure 8). Fungal colonies with different seasons, different medium, time period values are (.064, .042, .051) which show very low positive correlation. Colonies count with *Havan* situations value (-.334**) which show negligible or no correlation and the varying conditions. *Alternaria alternata* value with different season, different mediums (-.046, -.079) which shows low negative correlation, and with time period (.173*) which show low positive correlation. In *Havan* situation it shows (-.580**) a moderate negative correlation, with colonies its value (.293) which show negligible or no correlation. For colonies under *Havan* situations, the correlation value was -0.334 (significant at the $p<0.01$ level), indicating a negligible or no correlation. This suggests that *Havan* fumes have a weak inverse relationship with fungal colony counts, pointing to their limited but noticeable impact on reducing fungal growth. In the case of *A. alternata*, the correlation values with different seasons and mediums were -0.046 and -0.079, respectively showing a low negative correlation. These findings imply that changes in seasons and mediums slightly inhibit the presence of *A. alternata*. The correlation value with time period was 0.173 (significant at the $p<0.05$ level), indicating a low positive correlation. This suggests that the presence of *A. alternata* tends to increase slightly over time. Under *Havan* situations, the correlation value for *A. alternata* was -0.580 (significant at the $p<0.01$ level), indicating a moderate negative correlation. This shows the notable inhibitory effect of *Havan* fumes on

A. alternata. Additionally, the correlation value of *A. alternata* with overall fungal colonies was 0.293, signifying a negligible or no correlation, signifying that the relationship between the presence of *A. alternata* and the total fungal colony count is weak and inconsistent (Figure 8). These results collectively suggest that while the impact of seasons, mediums, and time periods on fungal growth is generally minimal, *Havan* fumes show a moderate inhibitory effect, particularly on *A. alternata*. This underscores the potential role of *Havan* in fungal management and exhibits areas for further study. The analysis of *A. triticina* revealed a correlation value of 0.041 with different seasons, indicating negligible or no correlation. When analysed with different mediums, the correlation value was -0.125, showing a low negative correlation. Regarding time period and fungal colonies, the correlation values were 0.080 and 0.239 (significant at $p<0.01$), respectively, indicating a low positive correlation. Under *Havan* conditions, *A. triticina* exhibited a correlation value of -0.530 (significant at $p<0.01$), indicating a moderate negative correlation, suggesting a substantial inhibitory effect of *Havan* fumes on its growth. Furthermore, the correlation between *A. triticina* and *A. alternata* was found to be 0.842 (significant at $p<0.01$), reflecting a very high positive correlation, implying a strong association between these two species. The analysis of correlations for *Aspergillus flavus*, *A. fumigatus* and *A. niger* across various factors revealed as- with different seasons: -0.411 ($p<0.01$), indicating a moderate negative correlation, suggesting its growth decreases significantly with seasonal changes; with different mediums, time period, colonies, *A. alternata* and *A. triticina*- correlation values of -0.089, 0.064, 0.123, 0.287 ($p<0.01$), and 0.216 ($p<0.01$) indicate negligible or no correlation; with *Havan* situations: -0.308 ($p<0.01$), showing a low negative correlation, suggesting a slight inhibitory effect of *Havan* fumes. *A. fumigatus*: with different seasons, time period, and fungal colonies: correlation values of 0.012, 0.022 and 0.256 ($p<0.01$), respectively, indicate a low positive correlation, suggesting a minimal relationship with these factors; with different mediums: -0.113, indicating a very low negative correlation; with *Havan* situations: -0.596 ($p<0.01$), showing a moderate negative correlation, displaying a significant inhibitory effect of *Havan* fumes; with *A. alternata* and *A. triticina*: correlation values of 0.749 ($p<0.01$) and 0.817 ($p<0.01$) indicate a highly positive correlation, suggesting strong associations between these species. *A. niger*: with different seasons, different mediums, and *Havan* situations: correlation values of -0.053, -0.055, and -0.493 ($p<0.01$), respectively, indicate very low to moderate negative correlations, with the most notable inhibitory effect observed under *Havan* situations; with time period, *A. alternata*, and *A. triticina*: correlation values of 0.074, 0.408 ($p<0.01$) and 0.372 ($p<0.01$) indicate low positive correlations, suggesting slight relationships with these factors; with fungal colonies: 0.162, reflecting negligible or no correlation. Inter-species correlations present *A. flavus* and *A. fumigatus*: correlation values of 0.646 ($p<0.01$) and 0.569 ($p<0.01$) indicate moderate positive correlations, suggesting a consistent association between these two species. These results collectively show varying degrees of correlation among fungal species and environmental factors, with *Havan* fumes demonstrating a notable inhibitory effect, particularly on *A. fumigatus* and *A. niger*. The strong inter-species correlations between *A. flavus*, *A. fumigatus*, and other species highlight potential ecological associations or shared environmental preferences.

The analysis of *Chaetomium sp.* revealed the correlations as with different seasons: -0.196 ($p<0.05$), indicating a low negative correlation, suggesting a slight decrease in its growth across seasons; with different mediums: -0.094, showing a negligible or very weak negative correlation; with time period, fungal colonies and *A. triticina*: Correlation values of 0.033, 0.178 ($p<0.05$) and 0.196 ($p<0.05$), respectively, indicate negligible or no significant relationship; with *Havan* situations: -0.510 ($p<0.01$), indicating a moderate negative correlation, highlighting the inhibitory effect of *Havan* fumes on *Chaetomium sp.*; with *A. flavus*: 0.506 ($p<0.01$), reflecting a moderate positive correlation, indicating a significant association between these two species; with *A. alternata*, *A. fumigatus*, and *A. niger*: Correlation values of 0.351 ($p<0.01$ for *A. alternata*), 0.301 ($p<0.01$ for *A. fumigatus*), and 0.351 for *A. niger*suggest a low positive correlation, indicating weak but consistent relationships. These results suggest that *Chaetomium sp.* shows varying levels of correlation with environmental factors and other fungal species, with a notable inhibitory effect observed under *Havan* conditions and a significant positive association with *A. flavus*.

The analysis of *Cladosporium sp.* revealed the correlations as: with different seasons and *A. fumigatus*: correlation values of 0.408 ($p<0.01$) and 0.354 ($p<0.01$) indicate a low positive correlation, suggesting a weak but consistent association with these factors; with different mediums and *Havan* situations: correlation values of -0.016 and -0.386 ($p<0.01$) show a negative correlation, with *Havan* situations exhibiting a moderate inhibitory effect on *Cladosporium sp.*; with time period, fungal colonies, *A. alternata*, *A. triticina*, *A. flavus*, and *Chaetomium sp.*: correlation values of 0.072, 0.153, 0.237 ($p<0.01$), 0.051 and -0.050, respectively, suggest negligible or no significant correlation with these factors or species; with *A. niger*: A correlation value of 0.661 indicates a moderate positive correlation, reflecting a noticeable association between these two species. These findings suggest that *Cladosporium sp.* has weak to moderate positive correlations with specific factors, such as seasons and certain fungal species like *A. niger*, but exhibits a moderate negative correlation with *Havan* situations, indicating its susceptibility to inhibitory effects under such conditions.

Drechslera sp. exhibited the correlations as: low negative correlation with seasons (-0.336, $p<0.01$); no or negligible correlation with different mediums, time period, *Havan* situations, *A. triticina* and *Cladosporium sp.* (values: -0.037, 0.045, -0.292 ($p<0.01$), 0.097, 0.236 ($p<0.01$), 0.087); low positive correlation with *A. alternata* (0.325, $p<0.01$) and *A. fumigatus* (0.498, $p<0.01$); high positive correlation with *A. flavus* (0.797, $p<0.01$); moderate positive correlation with *A. niger* (0.686, $p<0.01$) and *Chaetomium sp.* (0.500, $p<0.01$). The results present varying levels of association, with notable positive correlations observed with *A. flavus*, *A. niger* and *Chaetomium sp.*

Rhizopus nigricans exhibited the correlations as: no or negligible correlation with different seasons, mediums, time periods, colonies, *A. flavus*, *Chaetomium sp.*, and *Drechslera sp.* (values: 0.78, -0.086, 0.003, 0.260 ($p<0.01$), -0.006, 0.163, 0.006); moderate negative correlation with *Havan* situations (-0.683, $p<0.01$), indicating significant inhibition; moderate positive correlation with *A. triticina* (0.682, $p<0.01$) and *A. fumigatus* (0.618, $p<0.01$); low positive correlation with *A. niger*(0.306) and *Cladosporium sp.* (0.424, $p<0.01$); very high positive correlation with *A. alternata* (0.714, $p<0.01$), suggesting a strong association. The results indicate the inhibitory effect of *Havan* fumes and a strong positive correlation with *A. alternata*, along with moderate associations with other species.

Sterile mycelium correlations are as: no or negligible correlation with different seasons, mediums, time period, colonies, *A. alternata*, *A. triticina*, *A. flavus* and *Chaetomium sp.* (values: 0.154, -0.042, 0.007, 0.153, 0.269 ($p<0.01$), 0.297 ($p<0.01$), 0.242 ($p<0.01$), 0.114); low negative correlation with *Havan* situations (-0.407); low positive correlation with *A. fumigatus* (0.465, $p<0.01$), *Drechslera sp.* (0.311, $p<0.01$) and *R. nigricans* (0.352, $p<0.01$); high positive correlation with *A. niger* (0.734, $p<0.01$) and *Cladosporium sp.* (0.737, $p<0.01$). The results show minimal correlations with most factors but show strong positive associations with *A. niger*and *Cladosporium sp.*

Mucor sp. exhibited the correlations as: no or negligible correlation with different seasons, mediums, time period, colonies, *A. flavus*, *A. niger*, *Chaetomium sp.*, *Cladosporium sp.*, *Drechslera sp.*, *Sterile mycelium* (values: 0.114, 0.073, -0.104, 0.215, 0.489, -0.176, 0.109, -0.063, 0.291, -0.146, 0.195); moderate negative correlation with Havan situations (-0.505, $p<0.01$); moderate positive correlation with *R. nigricans* (0.694, $p<0.01$); low positive correlation with *A. alternata* (0.489), *A. triticina* (0.367, $p<0.01$) and *A. fumigatus* (0.305). The results show a moderate negative correlation with *Havan* fumes and positive associations with *R. nigricans* and certain other fungal species.

Curvularia sp. exhibited the correlations as: no or negligible correlation with different seasons, mediums, time period, *Havan* situations, colonies, *A. alternata*, *A. triticina*, *A. flavus*, *A. fumigatus*, *A. niger*, *Chaetomium sp.*, *Cladosporium sp.*, *Drechslera sp.*, *R. nigricans*, *Sterile mycelium* (values: 0.050, 0.028, 0.059, -0.177, 0.087, 0.090, -0.145, -0.075, -0.155, -0.098, 0.292, -0.092, -0.059, 0.026, -0.093); low positive correlation with *Mucor* (0.393, $p<0.01$). The results suggest minimal correlations with most factors, except for a low positive correlation with *Mucor*.

Penicillium sp. exhibited the correlations as: no or negligible correlation with different mediums, time period, *Havan* situation, colonies, *A. alternata*, *A. triticina*, *A. flavus*, *Chaetomium sp.*, *Drechslera sp.*, *Mucor sp.*, *Curvularia sp.* (values: 0.005, 0.092, -0.208, 0.123, 0.263, 0.299, -0.106, -0.083, 0.258, -0.074); low positive correlation with different seasons, *A. fumigatus*, *A. niger*, *R. nigricans* (values: 0.435, 0.365, 0.452, 0.341, $p<0.01$); high positive correlation with *Cladosporium sp.* (0.711, $p<0.01$) and *Sterile mycelium* (0.718, $p<0.01$). The results indicate minimal correlations with most factors, with notable positive associations with *Cladosporium sp.* and *Sterile mycelium*.

Fusarium sp. exhibited the correlations as: no or negligible correlation with different mediums, time period, colonies, *A. alternata*, *A. triticina*, *A. flavus*, *Chaetomium sp.*, *Drechslera sp.*, *Curvularia sp.* (values: -0.019, 0.047, -0.341, 0.119, 0.213, 0.261, -0.098, 0.147, -0.081, -0.072); low positive correlation with different seasons, *A. fumigatus*, *R. nigricans*, *Mucor sp.* (values: 0.405, 0.312, 0.409, 0.360, $p<0.01$); low negative correlation with Havan situations (-0.341); moderate positive correlation with *A. niger* (0.562, $p<0.01$); high positive correlation with *Cladosporium sp.*, *Sterile mycelium*, *Penicillium sp.* (values: 0.884, 0.746, 0.746, $p<0.01$).

Discussion

These findings suggest that the coexistence and interactions of fungal species in a specific environment are influenced by various factors, including competition for resources, symbiotic relationships, and environmental conditions. The study also emphasizes the dual impact of *Agnihotra* (both scientific and spiritual) on daily life. Fungal colonies were more abundant outdoors than indoors, displaying the effectiveness of medicinal fumes in reducing indoor microbial presence. It was found that the aromatic scents of the herbal components burned in the sacred fire fostered a positive atmosphere, promoting peace and a healthy environment. It suggests the need for further research into the chemical constituents of these ingredients, which not only create a favourable ambiance but also contribute to eliminating airborne microorganisms in the vicinity. Notably, most fungal species were effectively eradicated by the smoke from *Agnihotra*, enhancing the effectiveness of the research. The aromatic components of the sacred fire contributed to a peaceful and healthy environment, improved agricultural productivity, and demonstrated air purification potential. According to evidence from referenced studies, *Aspergillus flavus*, *A. fumigatus*, *Cladosporium* sp., and *Curvularia* sp. exhibited more than 60% positive reactions in skin prick tests across various experimental cases (Gosh, *et al.,* 2011). *Homa* therapy (*Havan*/*Agnihotra*), an ancient practice, aims also to restore harmony in nature and mitigate air pollution through *Agnihotra* practices. Present study indicates that *Agnihotra* creates a distinct energy field in the

Table 1. Fungal species identified during the study.

Fungal species	Occurrence (%)	Average number of colonies observed (± SE)
Drechslera sp.	50.00	0.19 (±0.06)
Aspergillus flavus	31.81	0.31 (±0.07)
A. niger	8.58	1.13 (±0.22)
A. fumigatus	7.56	1.28 (±0.15)
Curvularia sp.	24.56	0.40 (±0.14)
Penicillium sp.	21.53	0.45 (±0.11)
Sterile mycelium	20.89	0.47 (±0.09)
Chaetomium sp.	20.00	0.49 (±0.08)
Fusarium sp.	17.28	0.56 (±0.15)
Cladosporium sp.	11.38	0.85 (±0.17)
Alternaria triticina	6.3	1.54 (±0.20)
A. alternata	4.91	1.98 (±0.21)
Rhizopus nigricans	5.62	1.73 (±0.21)
Mucor sp.	5.64	1.72 (±0.25)

atmosphere, contributing to environmental purification. This energy field has been observed to aid in air quality improvement and also plays a role in purifying water. Experiments conducted by Berk and Sharma (2014) demonstrate that the energy generated during *Agnihotra* can influence water properties, making it cleaner and more suitable for use. Such findings emphasize the potential of *Homa* therapy as an eco-friendly method to address modern environmental challenges while promoting a balanced ecosystem. The similar types of observations were observed by earlier scientists and aerobiologist on pollen and fungal spores (Choudhary and Singh, 1991; Pawar, 1991; Peerally and Rao, 2003; Jothish, *et al.,* 2004; Kunjam 2007; Shukla, 2011; Saluja, *et al.,* 2011; Arora and Jain, 2003; Sawane, 2010; Kulkarni, 2011). Additionally, smoke-based practices like *Agnihotra* offer rapid brain delivery, efficient absorption, and low production costs, making them a valuable traditional practice with modern relevance. The impact of *Havan* fumes is influenced by various factors. One key element is the fragrance, as different combinations of herbs and medicinal plants release distinct aromatic compounds that can affect the surrounding air quality. The specific chemical compounds released during combustion, such as essential oils and other bioactive substances, also play a critical role, with some having strong antimicrobial properties. The intensity of the flame affects the rate and volume of these emissions, potentially altering their concentration in the air. Finally, the environment in which the *Havan* is performed whether indoors or outdoors, in a controlled or open space can significantly influence the dispersion and effectiveness of the fumes. These effects include a significant reduction in bacterial and fungal counts in the surrounding environment, as the antimicrobial properties of the fumes create a hostile environment for many microorganisms. This study emphasizes the significant impact of *Havan* fumes on environmental feasibility and the management of various curable diseases. In today's world, challenges related to microorganisms are a pressing concern, not only in India but globally and these issues could potentially be addressed through *Havan* methods. Despite India being the birthplace of Vedic culture, there has been limited research published on this topic, indicating a need for further exploration.

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