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**Original Research Article**

# Comparative Assessment of Microbial Colony Counts before and after Havan Yagya: A Preliminary Observational Study Using the Settle Plate Method

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## Abstract

***Background:*** Havan Yagya is a traditional Vedic fumigation practice involving the combustion of medicinal herbs, wood, and clarified butter (ghee) in a consecrated fire. Ethnopharmacological evidence and emerging scientific literature suggest that the smoke generated during Havan possesses significant antimicrobial properties. ***Objective:*** The present preliminary study aimed to evaluate the effect of Havan Yagya on the observable microbial load settling from indoor room air using the passive settle plate method, and to quantify the percentage reduction in colony-forming units (CFU) at 30 minutes and 24 hours post-Havan. ***Methods:*** Sterile culture-media plates were exposed in a controlled indoor room environment before and after Havan under identical conditions. Plates were incubated aerobically at 37°C and visible colonies were enumerated. ***Results:*** A baseline average of 11.0 CFU/plate was recorded before Havan. At 30 minutes post-Havan, the average count decreased to 1.0 CFU/plate, representing a 90.90% reduction. At 24 hours post-Havan, the average count was 5.5 CFU/plate, corresponding to a 50% reduction from baseline. ***Conclusion:*** These findings indicate that Havan Yagya produces a marked short-term reduction in airborne microbial settling, with a sustained partial effect persisting for at least 24 hours. The results are consistent with published literature on medicinal smoke and traditional fumigation practices. Larger controlled trials are warranted to validate and extend these preliminary observations.

**Keywords:** Havan Yagya; Medicinal smoke; Airborne bacteria; Settle plate method; Colony-forming units; Indoor air quality; Ethnopharmacology; Antimicrobial fumigation; Traditional medicine.



## 1. INTRODUCTION

Indoor air quality (IAQ) is a critical determinant of human health, particularly in densely populated and enclosed environments. Airborne microorganisms, including bacteria and fungi, are ubiquitous components of indoor air and are implicated in a wide range of respiratory illnesses, allergic conditions, and healthcare-associated infections (HCAIs) [1]. The World Health Organization (WHO) has identified microbial contamination as a key element of indoor air pollution, noting its association with increased prevalence of respiratory symptoms, allergies, and perturbation of the immunological system [2]. Conventional approaches to indoor air disinfection include chemical fumigants, ultraviolet germicidal irradiation, and HEPA filtration. However, growing concerns about chemical residues, antibiotic resistance, and environmental sustainability have renewed interest in traditional, plant-based fumigation practices as complementary or alternative strategies for environmental decontamination [3]. Havan Yagya (also spelled Hawan or Yajna) is one of the oldest documented fumigation practices in human history, originating in the Vedic tradition of the Indian subcontinent. The ritual involves the systematic combustion of a carefully formulated mixture of medicinal herbs (havan samagri), aromatic wood (typically mango wood, *Mangifera indica*), and clarified butter (ghee) in a consecrated fire vessel (havan kund). The resulting smoke and volatile organic compounds (VOCs) are believed to purify the surrounding environment, a claim that is now receiving increasing scientific scrutiny [4].

The present study was undertaken as a preliminary observational investigation to assess the effect of Havan Yagya on the observable microbial settling from indoor room air, using the passive settle plate method. Microbial colony counts were recorded before Havan and at two post-Havan time points (30 minutes and 24 hours) to evaluate both the immediate and sustained antimicrobial effects.

## 2. BACKGROUND AND LITERATURE REVIEW

### 2.1 Scientific Evidence for Medicinal Smoke

The landmark study by Nautiyal, Chauhan, and Nene (2007), published in the *Journal of Ethnopharmacology*, provided the first comprehensive scientific validation of the antimicrobial properties of medicinal smoke generated from havan samagri. The authors demonstrated that a one-hour treatment with medicinal smoke caused over 94% reduction in airborne bacterial counts within a closed room. Remarkably, the bactericidal effect was maintained for up to 24 hours, and pathogenic bacteria including *Corynebacterium urealyticum*, *Enterobacter aerogenes*, *Staphylococcus lentus*, and *Pseudomonas syringae* were absent from the open room even after 30 days [4].

Rastogi *et al.,* (2022), in a study published in the *Interdisciplinary Journal of Yagya Research*, conducted a comprehensive temporal analysis of Hawan air quality, antibacterial activity, and phytochemical characterization. Using one-way ANOVA with post-hoc analysis, the authors reported a statistically significant persistent reduction of 88–90% ($p < 0.0001$) in viable bacterial count compared to control, sustained for ≥72 hours after Hawan. GC-MS analysis of Hawan medicinal fumes revealed an array of bioactive compounds with antimicrobial, antioxidant, and air-cleansing properties [5].

### 2.2 Ayurvedic Fumigation and Environmental Disinfection

Bhatwalkar *et al.,* (2019), in a study published in the *Journal of Ayurveda and Integrative Medicine* (Elsevier), validated the environmental disinfection efficiency of traditional Ayurvedic fumigation using natural plant products including garlic peel (*Allium sativum*), turmeric (*Curcuma longa*), carom seeds (*Trachyspermum ammi*), and loban resin. The study demonstrated significant reduction in airborne bacterial CFU/m³ and showed bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA) on inanimate surfaces, as confirmed by scanning electron microscopy (SEM) [3].

### 2.3 Physicochemical Properties of Agnihotra/Yagya Aerosols

Arya *et al.,* (2026), in a study published in the *Indian Journal of Traditional Knowledge* (CSIR-NIScPR), investigated the physicochemical properties of Agnihotra aerosol particles using advanced instrumentation. GC-MS analysis revealed more than 42 bioactive compounds in Agnihotra fumes. Real-time gas monitoring showed relative reductions of 25%, 20.76%, 15.45%, 23.22%, and 13.63% for CO, $NO_2$, $SO_2$, $O_3$, and $H_2S$ respectively. EDS and XRF analysis confirmed that no toxic elements were released during Agnihotra [6].

### 2.4 Antifungal Activity of Havan Fumes

Rana, Kumar, and Bithel (2025), in a study published in the *Environment Conservation Journal*, characterized havan fume extract through GC-MS and molecular docking, and evaluated antifungal activities against air-isolated fungi. The study reported 58.98% and 38.77% maximum reduction of fungal CFU count between pre-Havan and post-Havan exposed plates inside and outside the yagyashala, respectively. GC-MS analysis identified acetic acid (39.63%) and asarone (15.29%) as major bioactive constituents, with asarone demonstrating strong binding affinity (−6.3 kcal/mol) against the melanin biosynthesis enzyme of *Curvularia lunata* [7].

### 2.5 Yagya in the Lens of Modern Science

A comprehensive review by Shukla *et al.,* (2026), published in the *Proceedings of the Indian National Science Academy* (Springer Nature), synthesized biomedical, ecological, and interdisciplinary insights on Yajña. The review underscores the growing body of evidence supporting the environmental and health-related benefits of Vedic fire rituals, while calling for rigorous, standardized scientific investigations [8].

### 3. Objectives

The specific objectives of the present study were:

- To evaluate the effect of Havan Yagya on the observable microbial load settling from indoor room air using the passive settle plate method.
- To determine the percentage reduction in microbial colony counts at 30 minutes post-Havan compared to the pre-Havan baseline.
- To assess the persistence of the antimicrobial effect at 24 hours post-Havan.
- To contribute preliminary observational data to the growing body of scientific literature on the antimicrobial properties of traditional Vedic fumigation practices.

## 4. MATERIALS AND METHODS

### 4.1 Study Design

This study employed a pre-post observational design with the same room serving as its own control. Microbial settling was assessed at three time points: (i) before Havan (baseline), (ii) 30 minutes after completion of Havan, and (iii) 24 hours after completion of Havan. All observations were conducted under identical room conditions (same room, same ventilation status, same time of day) to minimize confounding variables.

**4.2 Havan Procedure**

Havan was performed according to standard Vedic protocol using a traditional copper havan kund. The havan samagri (oblation mixture) comprised a standardized blend of medicinal herbs, aromatic plant materials, and clarified butter (cow ghee). Mango wood (*Mangifera indica*) was used as the primary combustion substrate. The Havan was conducted for a standardized duration in the selected indoor room with doors and windows closed to maintain consistent environmental conditions during the ritual.

**4.3 Settle Plate Method**

The passive settle plate (exposed plate) method was employed for microbial air sampling, consistent with established protocols for environmental monitoring [9]. This method captures microorganisms that settle onto an agar surface under gravitational force and is widely used for qualitative and semi-quantitative assessment of airborne microbial contamination in indoor environments.

The procedure was as follows:

- **Media Preparation:** Sterile nutrient agar plates (90 mm Petri dishes) were prepared under aseptic conditions. Plates were pre-incubated at 37°C for 24 hours prior to use to confirm sterility (zero CFU growth required before proceeding).
- **Plate Placement:** Two sterile culture-media plates (Plate 1 and Plate 2) were placed at designated positions within the room, approximately 1 metre above floor level, at the pre-Havan baseline time point.
- **Exposure Duration:** Plates were exposed for a standardized period under identical room conditions at each of the three time points (before Havan, 30 minutes post-Havan, and 24 hours post-Havan).
- **Incubation:** After exposure, plates were covered and incubated aerobically at 37°C for 24–48 hours.
- **Colony Enumeration:** Visible microbial colonies were enumerated under a colony counter. Each visible colony was recorded as one colony-forming unit (CFU). Results were expressed as total CFU (Plate 1 + Plate 2) and average CFU per plate.

**4.4 Calculation of Percentage Reduction**

Percentage reduction in microbial colony count was calculated using the following formula:

**Percentage Reduction (%) = [(CFU_before - CFU_after) / CFU_before] x 100**

**Percentage reduction at 30 minutes:** [(11.0 - 1.0) / 11.0] x 100 = 90.90%

**Percentage reduction at 24 hours:** [(11.0 - 5.5) / 11.0] x 100 = 50.00%

## 5. RESULTS

**5.1 Microbial Colony Counts**

The results of the settle plate observations are summarized in Table 1. Before Havan, the combined colony count from Plate 1 and Plate 2 was 22 CFU, yielding a baseline average of 11.0 CFU per plate. At 30 minutes post-Havan, the total count decreased markedly to 2 CFU, with an average of 1.0 CFU per plate, representing a 90.90% reduction from baseline. At 24 hours post-Havan, the total count was 11 CFU, with an average of 5.5 CFU per plate, corresponding to a 50.00% reduction from baseline.

**Table 1: Comparative Microbial Colony Count Before and After Havan Yagya**

| Observation Time Point | Total CFU (Plate 1 + Plate 2) | Average CFU per Plate | Percentage Reduction (%) |
|---|---|---|---|
| **Before Havan (Baseline)** | 22 | 11.0 | — |
| **After Havan (30 minutes)** | 2 | 1.0 | **90.90%** |
| **After Havan (24 hours)** | 11 | 5.5 | **50.00%** |

*CFU = Colony-Forming Units. Values represent observable settling colonies under the specific experimental conditions.*

**5.2 Summary of Findings**

The key findings of the study are summarized as follows:

- **Immediate effect (30 minutes post-Havan):** A 90.90% reduction in average CFU per plate (from 11.0 to 1.0 CFU/plate), indicating a strong and rapid antimicrobial effect of Havan smoke on airborne microbial settling.
- **Sustained effect (24 hours post-Havan):** A 50.00% reduction in average CFU per plate (from 11.0 to 5.5 CFU/plate), demonstrating a persistent, though attenuated, antimicrobial effect at 24 hours.
- **Temporal pattern:** The data indicate a biphasic pattern: a pronounced immediate reduction followed by partial recovery of microbial settling over 24 hours, consistent with the gradual dissipation of antimicrobial volatile compounds from the room air.

## 6. DISCUSSION

The present study demonstrates that Havan Yagya produces a substantial reduction in observable airborne microbial settling in an indoor environment. The 90.90% reduction at 30 minutes post-Havan is strikingly consistent with the findings of Nautiyal *et al.,* (2007), who reported over 94% reduction in airborne bacterial counts following one hour of medicinal smoke treatment [4]. Similarly, the 50% reduction persisting at 24 hours aligns with the sustained antimicrobial effects reported by Rastogi *et al.,* (2022), who documented 88–90% reduction for up to 72 hours [5].

### 6.1 Proposed Mechanisms of Antimicrobial Action

The antimicrobial effects observed in this study are likely attributable to the complex mixture of bioactive volatile organic compounds (VOCs) released during the combustion of havan samagri and ghee. GC-MS analyses of Havan/Agnihotra fumes have identified over 42 bioactive compounds [6], including:

- **Acetic acid (39.63%):** A well-established antimicrobial agent that disrupts bacterial cell membranes and inhibits metabolic processes.
- **Asarone (15.29%):** A phenylpropanoid compound with demonstrated antifungal activity, confirmed by molecular docking studies showing strong binding affinity (-6.3 kcal/mol) against fungal virulence factors.
- **Terpenes and phenolic compounds:** Released from aromatic herbs in havan samagri, these compounds are known to disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with quorum sensing.
- **Ghee combustion products:** The pyrolysis of clarified butter generates short-chain fatty acids and other bioactive molecules that contribute to the overall antimicrobial milieu.

The partial recovery of microbial settling at 24 hours (50% reduction vs. 90.90% at 30 minutes) is consistent with the gradual dissipation of these volatile antimicrobial compounds from the enclosed room environment. This temporal pattern suggests that the antimicrobial effect is primarily mediated by airborne VOCs rather than permanent structural changes to the room environment.

### 6.2 Comparison with Published Literature

**Table 2: Comparison of Present Study Findings with Published Literature**

| Study | Method | Reduction Reported | Duration | Journal |
|---|---|---|---|---|
| Present Study (Chug, 2026) | Settle plate, 37°C incubation | **90.90% (30 min); 50% (24 h)** | 24 hours | Present |
| Nautiyal *et al.*, (2007) | Biolog microplate, Microlog DB | >94% (60 min); maintained 24 h | 30 days | J Ethnopharmacol |
| Rastogi *et al.*, (2022) | Active air sampling, GC-MS, ANOVA | 88-90% ($p<0.0001$); 72+ h | 72+ hours | IJYR |
| Bhatwalkar *et al.*, (2019) | Active air sampling, SEM | Significant CFU/m3 reduction; MRSA surface disinfection | Not specified | J Ayurveda Integr Med |
| Rana *et al.*, (2025) | Settle plate, GC-MS, molecular docking | 58.98% (inside); 38.77% (outside) | Post-Havan | Environ Conserv J |

*IJYR = Interdisciplinary Journal of Yagya Research; J Ayurveda Integr Med = Journal of Ayurveda and Integrative Medicine; Environ Conserv J = Environment Conservation Journal.*

### 6.3 Public Health Implications

The findings of this study have potential implications for public health, particularly in the context of healthcare-associated infections (HCAIs). HCAIs represent a major global health burden, with the WHO estimating that hundreds of millions of patients are affected annually. Environmental decontamination is a critical component of infection prevention and control strategies [2]. The demonstrated ability of Havan Yagya to reduce airborne microbial settling by over 90% in the short term, and by 50% over 24 hours, suggests potential utility as a complementary environmental decontamination strategy, particularly in resource-limited settings where conventional disinfection technologies may be inaccessible.

Furthermore, the eco-friendly nature of Havan - using natural, biodegradable materials without synthetic chemicals - aligns with growing global interest in sustainable and green approaches to infection control. The absence of toxic elements in Agnihotra aerosols, as confirmed by EDS and XRF analysis [6], further supports the safety profile of this traditional practice.

### 7. Limitations and Future Directions

The present study has several important limitations that must be acknowledged:

- **Preliminary design:** This is a preliminary observational study with a small sample size (two settle plates per time point). The findings should be interpreted as hypothesis-generating rather than definitive.
- **Settle plate method limitations:** The passive settle plate method captures only microorganisms that settle under gravity and does not quantify the total airborne microbial load. Active air sampling methods (e.g., impaction samplers) would provide more comprehensive data on airborne CFU/m3 [9].
- **Absence of microbial identification:** The study enumerated total colony counts without identifying the specific microbial species present. Future studies should incorporate Gram staining, biochemical characterization, or 16S rRNA sequencing to identify the microbial taxa affected by Havan.
- **Lack of control group:** The study used a pre-post design without a concurrent control room. A

 

parallel control room (without Havan) would allow for more rigorous attribution of observed changes to the Havan intervention.
- **Absence of statistical analysis:** Given the preliminary nature of the study, formal statistical testing was not performed. Future studies with larger sample sizes should employ appropriate statistical methods (e.g., paired t-test, ANOVA) to assess the significance of observed reductions.
- **Havan samagri composition not characterized:** The specific composition of the havan samagri used was not documented or chemically characterized. Future studies should include GC-MS analysis of the fumes to identify the bioactive compounds responsible for the observed antimicrobial effects.

**Future Directions:**

Future research should address these limitations through: (i) randomized controlled trials with larger sample sizes and concurrent control groups; (ii) active air sampling to quantify total airborne CFU/m3; (iii) microbial identification using molecular methods; (iv) GC-MS characterization of havan fumes; (v) assessment of effects on specific pathogenic organisms of clinical relevance (e.g., MRSA, *Mycobacterium tuberculosis*, SARS-CoV-2); and (vi) evaluation of the safety profile of prolonged Havan smoke exposure for human occupants.

## 8. CONCLUSION

The present preliminary study provides observational evidence that Havan Yagya produces a substantial reduction in observable airborne microbial settling in an indoor environment. A 90.90% reduction in average CFU per plate was recorded at 30 minutes post-Havan, with a 50.00% reduction persisting at 24 hours. These findings are consistent with and corroborated by a growing body of peer-reviewed scientific literature demonstrating the antimicrobial properties of medicinal smoke and traditional Vedic fumigation practices [4], [5], [3]. While the preliminary nature of this study necessitates caution in interpretation, the results support the scientific plausibility of the traditional claim that Havan Yagya purifies the indoor environment. The practice represents a potentially valuable, eco-friendly, and culturally accessible approach to environmental decontamination that merits rigorous scientific investigation. Larger, well-controlled studies incorporating active air sampling, microbial identification, and chemical characterization of fumes are strongly recommended to validate and extend these findings.

**9. Conflict of Interest**

The author declares no conflict of interest. This study received no external funding. The Havan was performed as part of a routine traditional practice, and no commercial interests influenced the design, conduct, or reporting of this study.

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