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# Effect of Agnihotra Fumigation on Indoor Airborne Fungal Spore Load and In Vitro Antifungal Activity of Yagya Samagri Against Domiciliary Fungal Isolates

Mitali Das[1]

[1]Guest faculty, Department of Botany, Microbiology, Government Science College, Jabalpur, India

**Abstract.** Fungal spores are universal airborne components indoors and outdoors and are now generally recognized. Some fungi and their spores have a negative effect on human health and are the cause of allergies. Diseases caused by fungi that affect individuals include atopic dermatitis, skin infection, allergic conjunctivitis, asthma, and allergic bronchopulmonary mycosis. Agnihotra, a traditional fire ritual described in the Vedas, has been reported to purify air and water and reduce the pathogenicity of airborne microorganisms [1]. Due to lack of information about the beneficial effect of yagya people are more prone to synthetic chemical fresheners and disinfectants. This study was aimed to perform Agnihotra at home to reduce microbial concentration from air as well as use of some Indian herbs (Yagya Samagri) to produce fresh fragrance and to study the effect of herbal fume and extract on dominant fungal spores present in indoor air. The result from this experiment concluded that the fume generated from Agnihotra reduces the number of fungal spores present in the air. These findings suggest that Agnihotra fumes possess antimicrobial properties capable of reducing fungal spore concentrations, though further replicated studies are needed to confirm this effect. In addition, methanolic extracts of the Yagya samagri herbs, particularly Azadirachta indica, showed the strongest in vitro antifungal activity among the tested preparations, with a maximum zone of inhibition of 30 mm against Penicillium sp.

**Keywords.** Agnihotra, Yagya Samagri, fungal spores, antifungal activity.

*CORRESPONDENCE
Government Science College, Jabalpur, India Mobile no: 8770518049
Email: drmitali228@gmail.com

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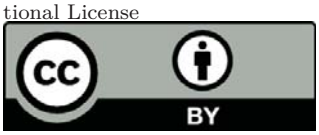

# INTRODUCTION

Indoor exposure to fungal spores is a risk factor for allergic disorders and various fungal infections. Exposure to these allergens is a risk factor for allergic respiratory diseases including rhinitis and asthma [27]. In ancient times, people used to do Agnihotra in their homes to avoid pollution. Agnihotra has also been associated with the generation of vital energy through resonance of far-infrared radiation during the ritual [1], though this present study focuses specifically on its effect on airborne fungal spore load and the antifungal properties of the herbs used as oblations. Agnihotra is a simple religious ritual described in ancient Vedas, which is performed to reduce pollution and spiritually purify the atmosphere. Agnihotra is a traditional domestic solemnity, performed to maintain harmony between living beings and nature, without harming and by giving respect. Agnihotra, the simplest forms of 'Yagya' performed at sunset/sunrise in which cow dung is burned in the copper pot by using cow ghee and brown rice as oblations along with chanting of mantras of sun and fire [1]. In this present research work the agnihotra was performed during sunset without chanting the mantras. The Agnihotra was performed using cow dung, mango tree wood, cow ghee, and Yagya samagri, including Neem (*Azadirachta indica*), sesame seeds (*Sesamum indicum*), Nagarmotha (*Cyperus rotundus*), and cardamom (*Elettaria cardamomum*); full collection and preparation details are given in the Methods section. Due to lack of information about beneficial effects of the yagya people are more prone to synthetic chemical fresheners and disinfectants. This study aimed to perform Agnihotra to reduce microbial concentration from air as well as use of some Indian herbs (Yagya Samagri) to produce fresh fragrance, to reduce the fungal spore load in indoor houses and to study antifungal activity of the extract of these herbs on fungal isolates, isolated from the indoor house before performing the agnihotra.

# METHODS AND MATERIAL

In this present research work the "Agnihotra" was performed at Jabalpur, Madhya Pradesh from January to May 2022. The Agnihotra was performed in an indoor environment of dwelling during sunset without chanting the mantras. The Agnihotra was performed with cow dung, mango tree wood, cow ghee and Yagya Samagri such as *Azadirachta indica* (Neem) leaves collected from trees. Other three herbs like *Sesamum indicum* (Sesame seeds), *Cyperus rotundus* (Nagarmotha), *Elettaria cardamomum* (cardamom) bought from the market. The aeromycological sampling was performed to isolate the fungal flora of the house before and after performing Agnihotra and to observe the effect of Agnihotra and Yagya samagri (herbs) on the fungal load of the indoor house environment by comparing the data. The fungal colonies isolated by the aeromycological sampling were used for further lab study, such as morphological studies of fungal isolates and to study the antifungal effects of Yagya Samagri (herbs) used during the process of Agnihotra.

## Sample collection

Aeromycological sampling was performed in indoor environments of the dwellings. Potato Dextrose Agar (PDA) Media and Gravity sedimentation method was used to perform air sampling [2, 3]. A total of four potato dextrose agar media plates were exposed to the air for 10 min. Two plates were exposed to the air before performing the agnihotra and two plates were exposed to the air after performing the agnihotra. The sampling was performed for five months. The sampling was performed twice in a day, that is:

(a) Air sampling to check the fungal load of indoor air before performing Agnihotra.

(b) Air sampling to check the fungal load of indoor air after performing Agnihotra.

All sampling was conducted at a single residential dwelling in Jabalpur, Madhya Pradesh.

## Preservation of Fungal Strain

All the isolated fungal species were obtained from the fresh colonies and grown on PDA plates. The isolated fungal cultures were preserved on the PDA slants at 4°C with proper labeling; each tube was labeled with the code number of the species and date of the storage.

## Morphological Identification of Fungal Isolates

The identification of fungi was based on Macroscopic (culture characteristics) and microscopic characteristics. The morphology of the fungal culture colonies or hypha, spores and the reproductive structure are observed. The methods used for the Morphological identification of fungi were Slide Culture Technique and Lacto phenol-Cotton Blue mount Technique [4, 5].

*Slide Culture Technique [4, 5]*

The moist chamber was prepared in petriplate and sterilized with a thin wetted cotton pad and a slide inside sterile Petri plate. One agar block sterile medium was placed inside the moist chamber over the

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slide. Fungal culture was inoculated by inoculating needle and 7 days older culture of respective fungi and cover slip placed over the medium. The moist chamber was incubated at 26-28°C for 7 days in the incubator. After sporulation microscopic analysis of the slide was done by using a compound microscope.

*Lacto phenol-Cotton Blue Technique [5]*

A drop of lacto phenol cotton blue staining solution was placed on a clean slide. Mix the fungal culture with the staining solution. Place a cover slip on top and without creating any bubbles. Press the cover slip gently to remove if there are any bubbles. Observe under microscope with 10X and 40X lenses.

## Yagya samagri (herbs) collection

Azadirachta indica (Neem) leaves were collected from trees. Other three herbs like Sesamum indicum (Sesame seeds), Cyperus rotundus (Nagarmotha), Elettaria cardamomum (Cardamom) bought from the market.

## Preparation of Herbs Extracts [6]

The herbs were shade dried and powdered by grinding. The powdered samples are hermetically sealed in separate polythene bags until the time of the extractions. The shade-dried herb material was used to prepare three extract types: water extract, boiled water extract, and methanol extract. To prepare the extracts, three separate 5 g portions of powder from each herb were weighed; each portion was mixed with either 15 ml of methanol or 15 ml of water (ratio 1:3, w/v). Two water and one methanol with herb samples were incubated overnight at 40°C for 24 hrs. After the incubation period the all three slurry was filtered through Whatman no. 1 filter paper in a beaker. The obtained liquid extract, one of the water samples, was stored in an air tight bottle to prepare water extract. Another water sample was boiled at 100°C to prepare boiled water extract and then extracted out to be stored in an air tight bottle. The methanol extract was also stored in an air tight bottle. After the extraction process, all the three extracts allowed it to evaporate at 40°C in a water bath for 2hrs.

## Evaluation of Antimicrobial Activity of Yagya samagri (Herbs) [7]

Antimicrobial activity of herbs was tested through a well diffusion method. Well diffusion method is the most commonly employed method to evaluate the Antimicrobial activity of herbs of Azadirachta indica, Cyperus rotundus, Elettaria cardamomum, Sesamum indicum.

*Well Diffusion Method [8, 9]*

Agar well diffusion test is the primary method to determine the antimicrobial activity. It is qualitatively easy.

PDA media was poured into the Petri dish. After solidification wells were made using a sterile crock borer (6mm diameter) into agar plates containing inoculums. Fungal lawn was prepared on PDA media. Then 20 l of each extract was added to respective wells. Then the plates were incubated at 27°C for 48 hours. Antimicrobial activity was detected by measuring the zone of inhibition that appeared after the incubation period. Methanol alone served as the solvent (negative) control for the well-diffusion assay. A standard antifungal agent was not included as a positive control in this study.

# RESULTS

## Monitoring airborne fungal spores before Agnihotra

Concentration of fungal air spores was recorded during the five months (Jan-May 2022) before Agnihotra. A total of 152 spores were reported, where *Cladosporium herbarum* was prevalent in the indoor environment with a 67 spore count (44.07%), followed by *Alternaria tenuis* with a 26 spore count (17.1%), followed by *Aspergillus flavus* 13.15% (20 spores), *Penicillium* sp. (12 spore) 7.9% and *Aspergillus niger* (5 spore) 3.32%. (Table 1 and Fig. 1, 2)

## Monitoring airborne fungal spores after Agnihotra

Concentration of fungal air spores was recorded during the five months (Jan-May 2022) after Agnihotra. A total of 48 fungal spores were reported, where *Cladosporium herbarum* was prevalent in the indoor environment with a 14 spore count (29.16%), followed by *Aspergillus flavus* with a 12 spore count (25%), *Alternaria tenuis* with an 8 spore count (16.66%), *Penicillium* sp. with a 6 spore count (12.5%), *Aspergillus niger* with a 3 spore count (6.25%). (Table 2 and Fig. 1, 2)

| S.N. | Fungal Isolates | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | No. of spores | Percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | Chaetomium sp. | 0 | 2 | 0 | 0 | 0 | 2 | 1.31 |
| 2 | Alternaria tenuis | 11 | 8 | 5 | 1 | 1 | 26 | 17.1 |
| 3 | Aspergillus niger | 1 | 1 | 1 | 1 | 1 | 5 | 3.32 |
| 4 | Aspergillus flavus | 10 | 5 | 3 | 1 | 1 | 20 | 13.15 |
| 5 | Cladosporium herbarum | 30 | 20 | 12 | 5 | 0 | 67 | 44.07 |
| 6 | Curvularia sp. | 0 | 2 | 0 | 0 | 0 | 2 | 1.31 |
| 7 | Helminthosporium sp. | 0 | 1 | 0 | 0 | 0 | 1 | 0.66 |
| 8 | Nigrospora sp. | 1 | 0 | 0 | 0 | 0 | 1 | 0.66 |
| 9 | Penicillium sp. | 6 | 4 | 2 | 0 | 0 | 12 | 7.9 |
| 10 | Phoma sp. | 0 | 2 | 0 | 0 | 0 | 2 | 1.31 |
| 11 | Stemphylium sp. | 0 | 1 | 0 | 0 | 0 | 1 | 0.66 |
| 12 | Yeast | 0 | 0 | 5 | 2 | 1 | 8 | 5.26 |
| 13 | Scytalidium sp. | - | 0 | 1 | 0 | 0 | 1 | 0.66 |
| 14 | Verticillium | 0 | 0 | 1 | 0 | 0 | 1 | 0.66 |
| 15 | Drechslera sp. | 0 | 0 | 1 | 0 | 0 | 1 | 0.66 |
| 16 | Unidentified | - | - | 2 | - | - | 2 | 1.31 |
| | *Total Spore Count* | | | | | | 152 | 100 |

Table 1: Total counts and percentages of indoor airborne fungal isolates recorded before performing Agnihotra (January–May 2022)

| S.N. | Fungal Isolates | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | No. of spores | Percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | Alternaria tenuis | 5 | 3 | 0 | 0 | 0 | 8 | 16.66 |
| 2 | Aspergillus niger | 1 | 1 | 1 | 0 | 0 | 3 | 6.25 |
| 3 | Aspergillus flavus | 6 | 4 | 2 | 0 | 0 | 12 | 25 |
| 4 | Cladosporium herbarum | 5 | 3 | 2 | 2 | 2 | 14 | 29.16 |
| 5 | Penicillium sp. | 2 | 1 | 1 | 1 | 1 | 6 | 12.5 |
| 6 | Yeast | 1 | 1 | 1 | 1 | 1 | 5 | 10.43 |
| | *Total Spore Count* | | | | | | 48 | 100 |

Table 2: Total counts and percentages of indoor airborne fungal isolates recorded before performing Agnihotra (January–May 2022).

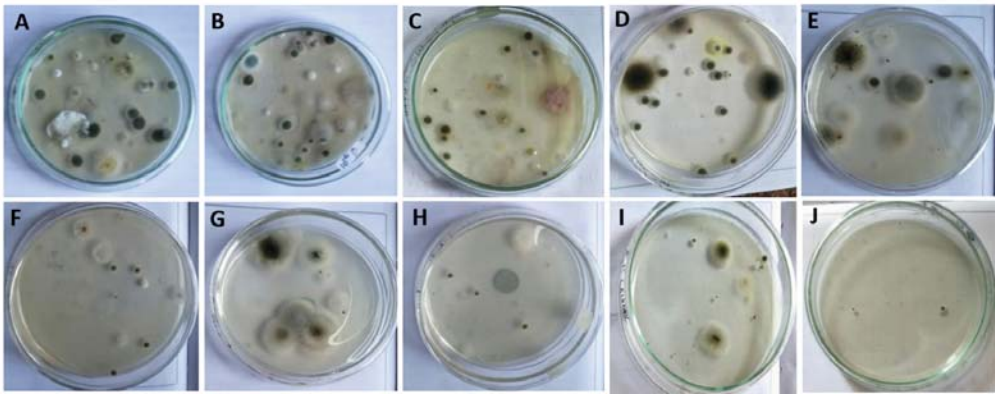


Figure 1: (A) 1st month sampling before Agnihotra (B) 1st month sampling after Agnihotra (C) 2nd month sampling before Agnihotra (D) 2nd month sampling after Agnihotra (E) 3rd month sampling before Agnihotra (F) 3rd month sampling after Agnihotra (G) 4th month sampling before Agnihotra (H) 4th month sampling after Agnihotra (I) 5th month sampling before Agnihotra (J) 5th month sampling after Agnihotra.

## Antifungal activity of water extract of herbs (Yagya samagri)

Total four different water extract of plant material (Yagya samagri) were Sesamum Indicum, Azadirachta Indica, Cyperus rotundus, Elettaria Cardamomum used to test their antifungal activity against five different fungal isolates, those are Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Cladosporium herbarum, Penicillium sp. Azadirachta indica and Cyperus rotundus showed the

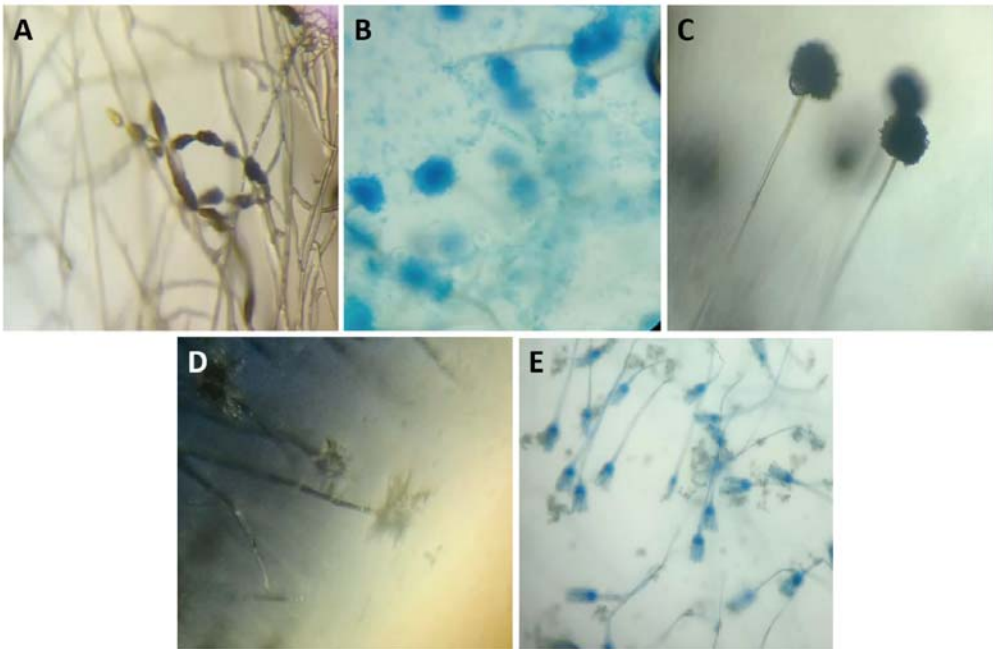


Figure 2: Microscopic view of different fungi: (A) Alternaria tenuis (B) Aspergillus sp. (C) Aspergillus niger (D) Cladosporium herbarum (E) Penicillium species. (These photographs are captured by Mitali Das and Nandani Patel by use of Samsung mobile camera set on the eye piece of compound microscope).

maximum inhibitory effect with 15mm zone of inhibition against Penicillium sp. and Aspergillus flavus followed by Elettaria Cardamomum and Sesamum indicum, which each showed a 10mm zone of inhibition against Aspergillus niger. (Table 3 and Fig. 3)

| S.No. | Fungal Isolates | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter |
|---|---|---|---|---|---|
| S.No. | Fungal Isolates | Azadirachta indica | Elettaria cardamomum | Cyperus rotundus | Sesamum indicum |
| 1. | Alternaria tenuis | 0 mm | 0 mm | 0 mm | 0 mm |
| 2. | Aspergillus flavus | 10 mm | 0 mm | 15 mm | -ve |
| 3. | Aspergillus niger | 0 mm | 10 mm | 10 mm | 10 mm |
| 4. | Cladosporium herbarum | 0 mm | 0 mm | 0 mm | 0 mm |
| 5. | Penicillium sp. | 15 mm | 0 mm | 0 mm | 0 mm |

Table 3: Microscopic view of different fungi: (A) *Alternaria tenuis* (B) *Aspergillus* sp. (C) *Aspergillus niger* (D) *Cladosporium herbarum* (E) *Penicillium* species. [These photographs are captured by Dr. Mitali Das and Nandani Patel by use of Samsung mobile camera set on the eye piece of compound microscope].

## Antifungal activity of boiling water extract of herbs (Yagya samagri)

Total four different boiled water extract of herbs (Yagya samagri), those were Sesamum Indicum, Azadirachta Indica, Cyperus rotundus and Elettaria cardamomum, used to test their antifungal activity against five different fungal isolates, those are Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Cladosporium herbarum, Penicillium sp., where Cyperus rotundus showed the maximum inhibitory effect with 20mm zone of inhibition against Aspergillus sp. and Azadirachta indica showed

20mm zone of inhibition against Penicillium sp. and Sesamum indicum showed 15mm zone of inhibition against Cladosporium herbarum. (Table 4 and Fig. 4)

| S.No. | Fungal Isolates | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter | Zone of Inhibition in Diameter |
|---|---|---|---|---|---|
| S.No. | Fungal Isolates | Azadirachta indica | Elettaria cardamomum | Cyperus rotundus | Sesamum indicum |
| 1. | Alternaria tenuis | 0 mm | 0 mm | 0 mm | 0 mm |
| 2. | Aspergillus flavus | 10 mm | 0 mm | 20 mm | 0 mm |
| 3. | Aspergillus niger | 0 mm | 10 mm | 10 mm | 15 mm |
| 4. | Cladosporium herbarum | 10 mm | 0 mm | 0 mm | 0 mm |
| 5. | Penicillium sp. | 20 mm | 0 mm | 10 mm | 0 mm |

Table 4: Sensitivity of fungal isolates against different Herb (Yagya samagri) water extract. Size of well: 6mm; Quantity of extract: 20µl; Water as negative control. -ve = no measurable zone of inhibition observed.

### Antifungal activity of Methanol Extract of herbs (Yagya samagri)

Total four different water extract of herbs (Yagya samagri), those were Sesamum Indicum, Azadirachta Indica, Cyperus rotundus and Elettaria cardamomum, used to test their antifungal activity against five different fungal isolates, those are Alternaria tenuis, Aspergillus flavus, Aspergillus niger, Cladosporium herbarum, Penicillium sp., where Azadirachta indica showed the maximum inhibitory effect with 30mm zone of inhibition against Penicillium sp., followed by Cyperus rotundus which showed 25mm zone of inhibition against Alternaria tenuis and Penicillium sp., and Elettaria cardamomum, Sesamum indicum showed 25mm zone of inhibition against Aspergillus niger and Cladosporium herbarum. Azadirachta Indica, Cyperus rotundus and Elettaria cardamomum showed 20mm zone of inhibition against Cladosporium herbarum. (Table 5 and Fig. 5)

| S.N. | Fungal Isolates | Zone of Inhibition in Diameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Azadirachta indica | | Elettaria cardamomum | | Cyperus rotundus | | Sesamum indicum | |
| | | Plant methanol extract | Methanol control | Plant methanol extract | Methanol control | Plant methanol extract | Methanol control | Plant methanol extract | Methanol control |
| 1. | Alternaria tenuis | 14mm | 10mm | 15mm | 10mm | 25mm | -ve | 15mm | 10mm |
| 2. | Aspergillus flavus | 25mm | 14mm | 10mm | 5mm | 10mm | -ve | 15mm | 10mm |
| 3. | Aspergillus niger | 20mm | 14mm | 15mm | 10mm | 20mm | -ve | 20mm | 15mm |
| 4. | Cladosporium herbarum | 20mm | 10mm | 20mm | 10mm | 20mm | 10mm | 15mm | 10mm |
| 5. | Penicillium sp. | 30mm | 20mm | 15mm | 10mm | 25mm | 20mm | 20mm | 10mm |

Table 5: Sensitivity of fungal isolates against different boiled water herbal (Yagya samagri) extract. Size of well: 6mm; Quantity of extract: 20µl; Water as negative control.

## DISCUSSION

The frequency of sensitization to molds Alternaria, Cladosporium herbarum increased significantly with increasing asthma severity for either severe vs mild asthma [10]. The most frequently occurring indoor and outdoor fungal types were *Penicillium*, *Aspergillus* sp. and Cladosporium sp. These three types of fungi were observed in 70% or more of the homes [11]. Chadeganipour et al. 2010, during the study in Isfahan, identified *Cladosporium* spp, *Penicillium* spp, *Alternaria* spp and Aspergillus spp. as the most abundant air borne fungi [12]. Verma et al. 2011 reported that prevalence of allergy was more common in the age group 20-40 years [13]. Chaubey

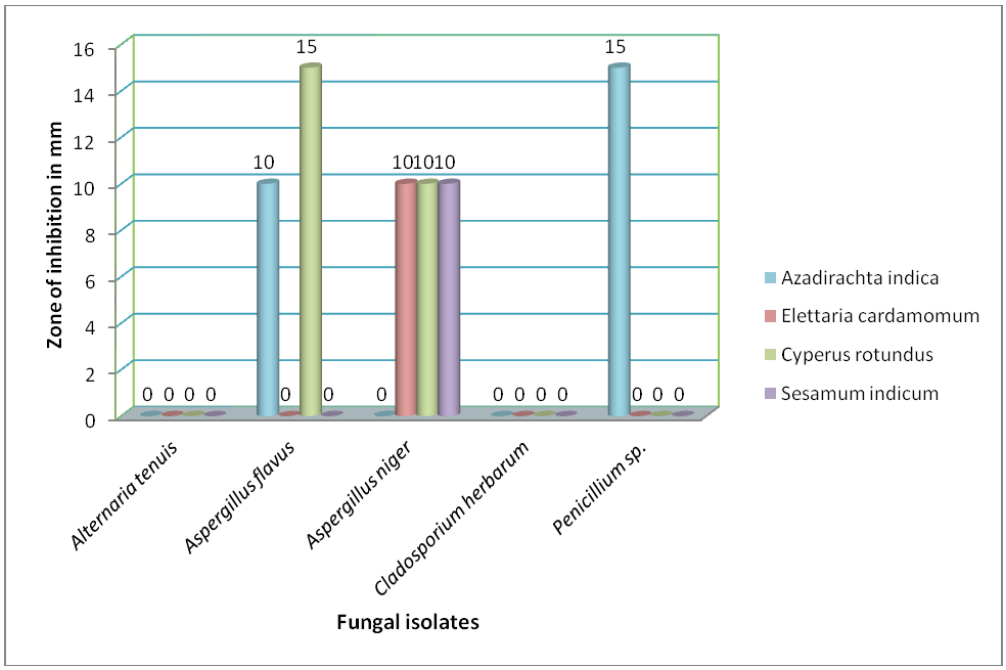


Figure 3: Sensitivity of fungal isolates against different water extract of Herb (Yagya samagri).

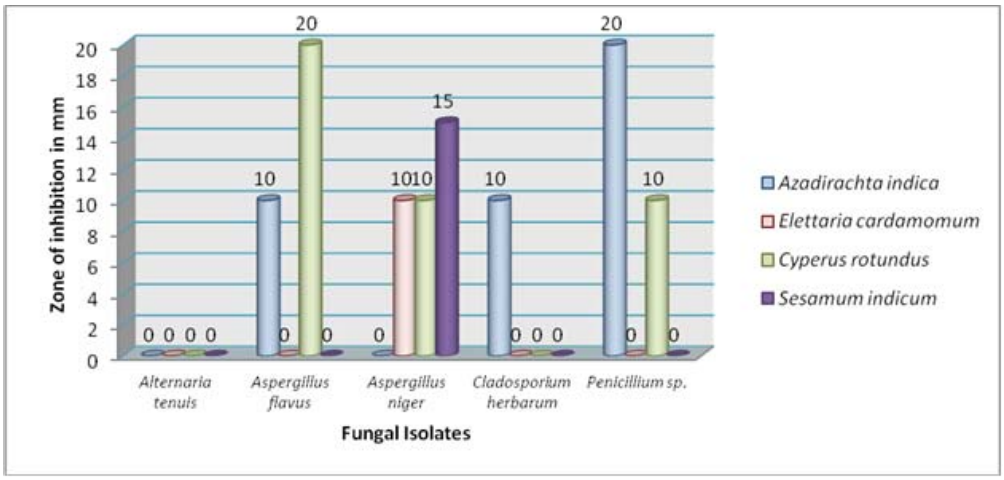


Figure 4: Sensitivity of fungal isolates against different boiled water extract of Herb (Yagya samagri).

et al. 2020 also performed laboratory tests directed by exposing petri plates in a closed room before and after fumigation; results displayed a substantial decrease in concentration of fungal load [14]. The investigation performed by Abhang 2014 reported that before yagya the spore count increases and after yagya the spore count decreases. Agnihotra fumes decrease microbial load in air up to 30 feet; load in the air can be controlled by performing agnihotra [15].

Abhang 2015 conducted agnihotra and reported that the average microbial colony count after agnihotra was 52, which was 70% less than the before agnihotra count of 171 [16]. In this present study fungal colony counts after agnihotra were 48 and before agnihotra it was 152. In this present study microbial colony counts obtained after agnihotra were substantially reduced compared to the microbial count before agnihotra. This suggests that agnihotra fumes may have antimicrobial properties. It was reported that agnihotra fumes show antimicrobial properties by killing or decreasing the growth of microbes, which results in the reduction of microbial load in the surrounding environments [16]. In this present study the agnihotra fumes reduced the microbes' growth.

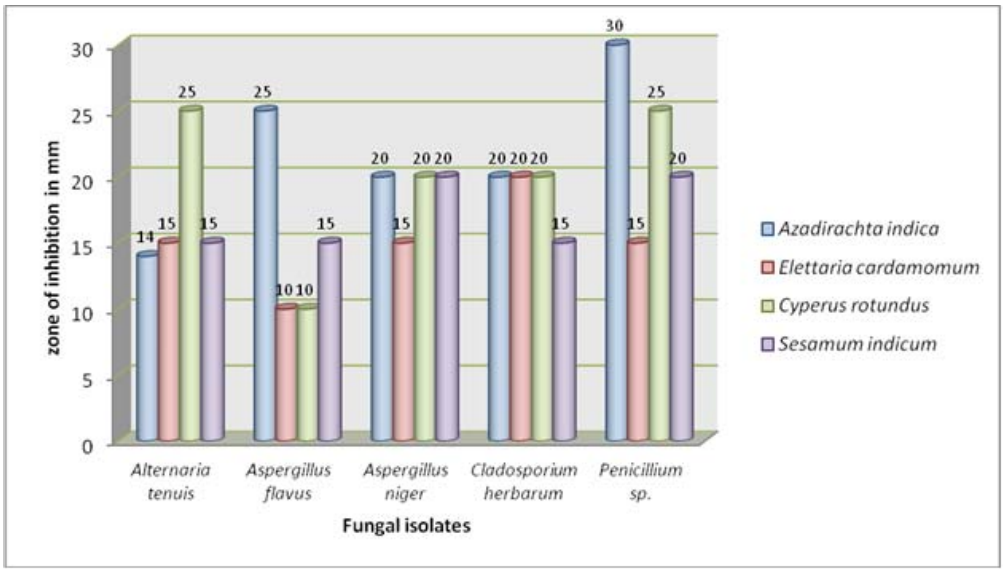


Figure 5: Sensitivity of fungal isolates against different methanol extract of Herb (Yagya samagri).

Agnihotra yagya controls the aeromicroflora. During agnihotra it purifies the surrounding environment by preventing air pollution. According to Patil et al. 2008 [17], agnihotra is an effective antimicrobial agent. The agnihotra was able to reduce the number of air spora. In this present investigation a small yagya was performed at the house. Due to the effect of yagya, the number of fungal spores was decreasing every week, which showed that yagya smoke has the potency to kill aeromycoflora, because 68.42% less spore count was reported during the last 5th week of sampling.

The indigenous plants are used as traditional medicinal plants for decades, which are useful in the treatment of numerous contagious diseases. Active components present in plants are responsible for the biological activities of the natural plants during the metabolism of plant species. To conduct the study of agnihotra fumes on effect on aeromycoflora, in this study agnihotra yagya was carried out by using Cyperus rotundus, Elettaria cardamomum, Sesame seeds and Azadirachta indica as oblations to the fire. This investigation was focused on analyzing the antimicrobial activity of plant material (yagya samagri) against five dominant fungal isolates.

*Azadirachta indica*, also known as Neem, is a very common tree in India. Neem has played an important role in ayurvedic medicine. Neem's seeds, oil and roots are noted for their medicinal properties. *Azadirachta indica* leaf extract was taken to test its antifungal activity against three fungal species – *Aspergillus* sp., *Alternaria*, *Cladosporium* [6]. During this study Neem leaves were tested against fungal isolates and results showed that the neem leaves extract inhibited the growth of Penicillium sp. (30mm), Aspergillus flavus (25mm), Aspergillus niger (30mm) with the zone of inhibition. Azadirachta indica, a tree of the family Meliaceae, is known as a versatile source of components with bioactive properties. It has great medicinal importance and antiviral, antimicrobial activity. Components isolated from Azadirachta indica leaves were evaluated against fungal species viz. Alternaria tenuis, Aspergillus fumigatus, Aspergillus niger, Penicillium [18]. (Fig. 6)

Cyperus rotundus, commonly known as Nagarmotha, is widely used in traditional medicine around the world for the treatment of various diseases. The plant is also used as an anti-inflammatory. It also possesses different pharmacological actions like antifungal-antiparasitic activity [19]. Cyperus rotundus Linn extract was evaluated against Aspergillus niger. The powdered extracts were successively extracted with ethanol and water using a soxhlet apparatus. The antifungal activities were performed by the agar well diffusion method [20]. Antimicrobial activities of ethanolic extracts were examined on fungi on media containing plant extract using the agar well diffusion method. They revealed the effect of the plants by evaluating microbial growth and inhibition zone measurement [21]. In this present work antifungal activity of Cyperus rotundus was also tested on five dominant fungal isolates and it showed an inhibitory effect on Alternaria tenuis and Penicillium sp., with a zone of inhibition of 25mm, where 20mm zone of inhibition was also reported against Cladosporium herbarum. (Fig. 7)

Sesamum indicum is popularly known as sesame seeds. Sesame is a very old cultivated crop and is thought to have originated in Africa [22]. Sesame oil shows antimicrobial activity with a zone of inhibition range of 15-25mm, equal to or greater than the standard, showing the highest zone of inhibition [23]. The antimicrobial activity of sesame seeds was also evaluated against three fungal species, Aspergillus flavus and Aspergillus niger [24]. Similarly, in this investigation antifungal activity of both boiled water and methanolic extracts of Sesame seed was also reported, showing 15mm zone of inhibition against Aspergillus niger and Penicillium sp., where 15mm zone of inhibition was reported against Cladosporium herbarum. (Fig. 8)

Elettaria cardamomum, commonly known as bari ilaichi, is a well known plant used in ayurvedic and unani medicine. It has been used for various diseases and disorders. Antimicrobial activity of methanol and water extracts from leaves, root, seeds and essential oil has been tested against various microorganisms [25]. In other studies the plant extracts were screened for their inhibitory effects on fungal isolates using the agar well diffusion method. It was shown that methanol extracts were more effective compared to boiled water. Inhibition zone diameter of the methanol extract of the plants used ranged from 10 to 29mm, with inhibitory concentrations from 50-150µg/ml [26]. Similarly, during this present study the agar well diffusion method was performed with Cardamom methanolic and boiled water extract. The result obtained showed an inhibitory effect on fungal isolates with a 20mm zone of inhibition against *Cladosporium herbarum*. (Fig. 9)

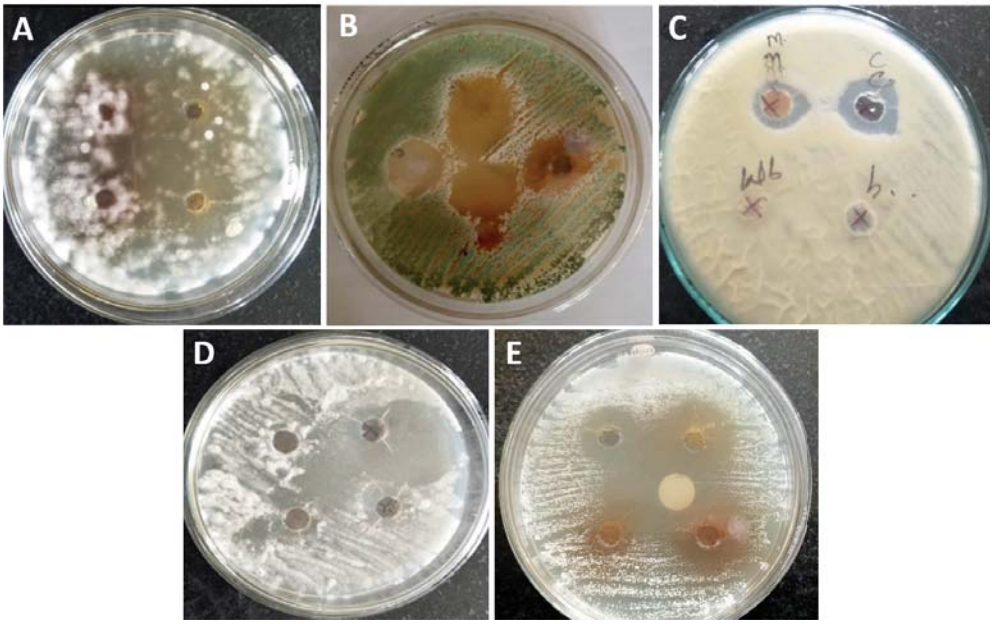


Figure 6: Sensitivity of fungal isolates against Azadirachta indica (A) Alternaria tenuis (B) Aspergillus flavus (C) Aspergillus niger (D) Cladosporium herbarum (E) Penicillium species.

Building on these findings, future studies could extend this work by sampling across multiple dwellings to strengthen generalizability, incorporating statistical comparison of spore counts before and after Agnihotra, and including a standard antifungal agent as a positive control in the well-diffusion assays. Such extensions would further strengthen the evidence base for the antimicrobial and air-purifying effects of Agnihotra and Yagya samagri observed in this study.

# CONCLUSION

This study demonstrated that performing Agnihotra fumigation at home substantially reduced the airborne fungal spore load, decreasing the total spore count from 152 to 48 over the five-month sampling period. The plant material (Yagya samagri) shows notable antifungal activity against the various fungal isolates. This entire study revealed that the maximum inhibitory effect against fungal isolates was shown by extracts of *Azadirachta indica*

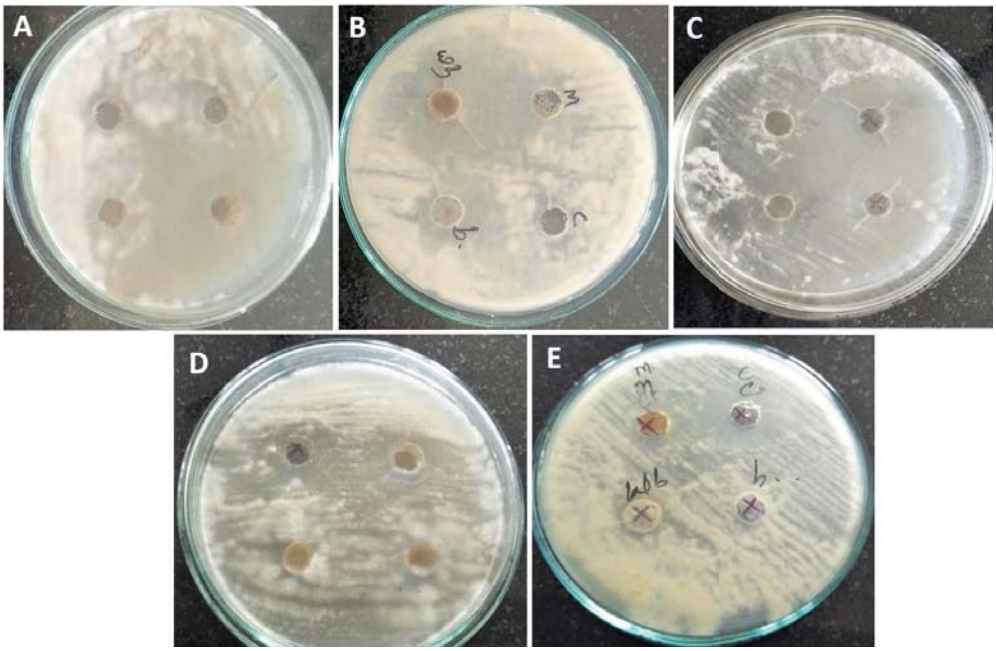


Figure 7: Sensitivity of fungal isolates against Cyperus rotundus (A) Alternaria tenuis (B) Aspergillus flavus (C) Aspergillus niger (D) Cladosporium herbarum (E) Penicillium species.

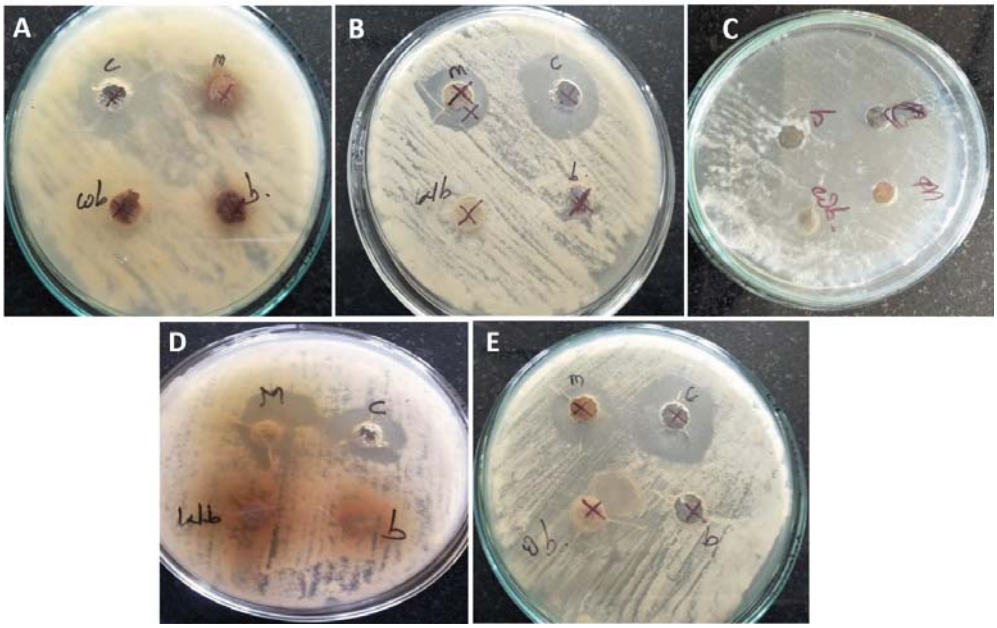


Figure 8: Sensitivity of fungal isolates against Sesamum indicum (A) Alternaria tenuis (B) Aspergillus flavus (C) Aspergillus niger (D) Cladosporium herbarum (E) Penicillium species.

plant material (Yagya samagri), with a 30mm zone of inhibition against Penicillium sp. The analysis also showed that the boiled extract of plant material has average antimicrobial activity towards various fungal isolates. It was reported that water extracts of plant material showed the lowest activity against fungal isolates. This suggests that the antifungal activity of the plant extracts was concentration-dependent, warranting further study using standardized assays (e.g., MIC/MFC determination) to characterize fungistatic versus fungicidal effects. The study indicates that these plant herb extracts may be explored in the

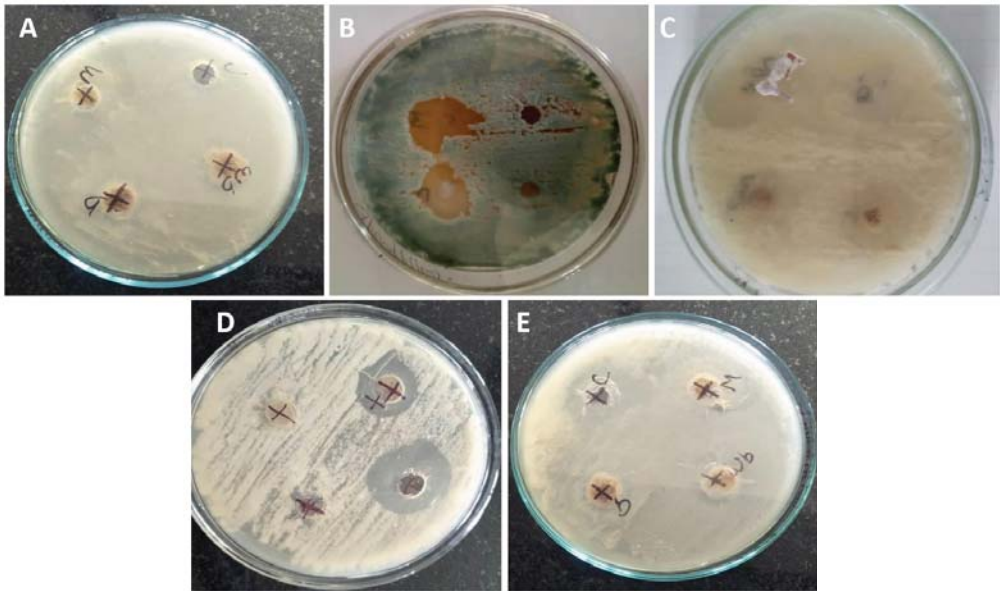


Figure 9: Sensitivity of fungal isolates against Elettaria cardamomum (A) Alternaria tenuis (B) Aspergillus flavus (C) Aspergillus niger (D) Cladosporium herbarum (E) Penicillium species.

future as effective antimicrobial agents. Separately, Agnihotra fumigation using these herbs as oblations also reduced airborne fungal spore load in this study, suggesting a complementary volatile/fumigant effect worth further investigation.

**COMPLIANCE** Not required.
**CONFLICT OF INTEREST** None.

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