

## **Effect of Medicinal Smokes on Reduction of Fungal Indoor Air Contamination**

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### **ABSTRACT**

**Introduction:** As far as application of chemicals as disinfectants may have side effects on human health and causes drug resistance, the topic of herbal and natural products has been investigated in recent researches. Nevertheless, application of medicinal smokes in the treatment of many diseases, including bacterial and infectious diseases has long been popular in Iran. Smoke from the burning of Peganumharmala (Espand) and female donkey dung (Anbarnasa) are among these smokes which effects on ambient air fungi was investigated in the current study.

**Materials and Methods:** In this analytical research, the smoking was conducted with different masses of Espand and Anbarnasa in the room space. Before and after smoking, air fungal sampling was performed with Anderson method. After three to five days, the composed fungal colonies were counted. Data were analyzed through the SPSS Software (Vs. 18) and Microsoft Excel (2007).

**Results:** The indoor fungal removal percent by Espand smoke in 1, 3, and 5 minutes after smoking were 52.75 %, 64.72 %, and 77.28 %, respectively; and for the Anbarnasa smoke at these times were 54.6 %, 59.4 %, and 74 %. Increase in smoking time caused a significant increase ( $p < 0.05$ ) in fungi removal, but change in the mass and the kind of smoking matter (Espand or Anbarnasa) did not cause any significant change in the removal.

**Conclusion:** Smoking with Espand and Anbarnasa as well as duration of smoking can decrease the building's ambient air fungi burden significantly. Of course it is better to perform smoking in enclosed spaces especially in toilets and without the presence of the person.

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### **Introduction**

The air we breathe is not an environment completely devoid of microorganisms. Bacteria, fungi, and viruses are the dominant microorganisms found in the air. Microbes are able to spread in the environment through air follow, dust, and air droplets<sup>1</sup>. Fungi are actually part of biological aerosols that may be found anywhere and can cause problems for human health, including allergies and asthma<sup>2, 3</sup>. Closed environments such as offices, homes, and cars are places for gathering and presence of microorganisms and volatile organic

compounds of the air<sup>4</sup>. Indoor air pollution in residential and official place is very important, because the most of the people spend about 60 to 90 percent of their time in buildings<sup>5</sup>.

Today, one of the main problems in the treatment of infection is drug resistance accordingly, the use of natural alternatives is very important. Meanwhile, application of medicinal smoke in treatment of many diseases, including bacterial and infectious diseases has long been popular in Iran. The smoke resulted from Peganumharmala (Espand) and female donkey dung (Anbarnasa) is among these smokes<sup>6</sup>.

Despite the fact that a large part of consumed drugs are synthetic, it was estimated that at least one third of all pharmaceutical products either have herbal sources or were deformed after being extracted from plants<sup>7</sup>. In the past decades, herbal remedies that have been used traditionally for many years, were introduced to the market in industrial forms after passing through the clinical and research stages<sup>8</sup>.

Application of Medicinal Smoke has been popular in many countries of the world and even has had greater acceptance among the public. Smokes resulted from burning of Espand and animals' dung (manure) are among these medicinal smokes. There are ideas that such smokes have greater and more rapid therapeutic effects<sup>9</sup>.

Espand with the scientific name of *Peganumharmala* is a member of *Zygophyllaceae* family, one of the traditional herbs with various applications in traditional medicine in Iran. Espand is an herbaceous plant with divided leaves and large greenish flowers. Its fruit has a spherical capsule shape containing black seeds<sup>10</sup>. Espand contains Flavonoid and beta-carboline alkaloids antimicrobial substances (harmine, Harmalyn, harmalol, Pganyn, and kynazulyn) which are mostly found in the plant's roots, seeds, and callus<sup>11</sup>. Harmaline has toxic, fungicide, and bactericide effects. Hypothermic and hallosynogic properties were also considered for this seed<sup>10</sup>. Harmel has been used as a parasiticide and disinfection seed since ancient times. In 1980, Espand alkaloid extract was applied for treatment of dermatoses and its anti-bacterial, anti-fungal, anti-pyrogenicity, and anti-parasitic effects have been observed<sup>8, 12-15</sup>. The antimicrobial effects of Espand have been investigated in various studies. Espand callus extract, as its seed, also has anti-microbial properties against microbes such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*<sup>16</sup>.

Furthermore, animal manure, or in common terms, dung is one of the substances found in the nature that has various uses<sup>17</sup>. The smoke of dung is applied to circumvent the mosquitoes in some areas. In addition, the animal dung was used to treat some human

diseases. For example, the manure of a newborn ass and ass' milk were used to treat severe cough<sup>18</sup>.

Animal manure contains large amounts of organic matters, nitrogen, and also consumed plants by animals<sup>19</sup>. It is important to note that concentration of toxic and main substances in the feces is under the influence of consumed materials and digestion of food. In fact, the feces can be a rich source of antibiotic substances with miraculous properties. Based on the experiences observed miraculous properties of feces are confirmed. On the other hand, animals have different microflora and the materials in their manure can produce different antibiotics effective on different organisms. Ancient Egyptian medicine mentioned feces smoke as a high flammable material. This feature and flammability is due to its fat and fiber contents. Lignin is one of the materials existing in dung, whose hydrolysis leads to formation of some compounds with the property to inhibit the growth of microorganisms. Based on the origin and inhibitory effects, three major groups of weak acids, furan, and phenolic compounds are formed by the effect of this hydrolysis. Antimicrobial effects of the smoke resulted from burning of dung are thus probably related to one of these three groups<sup>20</sup>.

Since the fungi, as microbial opportunistic agents of mild and severe infections in humans and animals, have received great importance in recent decades and also because treatment problems resulting from antifungal drugs have recently prevented harmless treatment of this cluster of infections, it was crucial to address the issue of anti-fungal properties of various plants in this study. Further, application of plants or plant extracts have always had better popularity and acceptance among patients than the synthetic drugs. Also, in the treatment of fungal infections of the skin and mucous, often multiple drugs are prescribed and in some cases, such as Candidiasis and LO vaginal infections, drug use problems have been observed. More, resistance to treatment in connection with a group of skin infections such as fungal nail infections and foot Dermatophytosis has been observed<sup>21, 22</sup>.

Due to the facts that most previous studies were based on chemical methods of air disinfection and thus related to the use of alcoholic extract of Espand, also because these two materials (Espand grains and Anbarnasa) are available and relatively cheap, and finally for absence of a study focusing on the effects and properties of medicinal smokes in ambient air, this study was conducted to compare the antimicrobial properties of Espand grains and Anbarnasa smokes on the amount of ambient air fungi.

### Materials and Methods

The current study is an analytical research that focused on the reduction of indoor air fungi by medical smoking. Since toilets have the highest amount of fungal burden, 4 college toilets with space range of 10-20 cubic meters, were selected as research locations, entitled as "rooms" in this study. When necessary equipment and materials of smoking were prepared, Espand in weights of 1, 2, and 3 grams and Anbarnasa in weights of 2, 3, and 5 grams were provided. Sabouraud Dextrose Agar (SDA) culture media with chloramphenicol were prepared in plates (with a diameter of 90 mm) in laboratory sterile conditions. In this study, it was necessary to measure the microbial burden (fungi) of rooms' air before and after smoking, thus, the Anderson method and Quick Take 30 (sampling device) were applied. According to NIOSH<sup>1</sup> recommendations, the sampling flow rate of 28.3 L/min and sampling duration of 2 minutes with SDA culture media selected for indoor air microbial sampling. Consequently, the sampling device needed to be placed at a height of 1.2 m from the

floor, with a distance of more than 1 meter from the walls and obstacles<sup>23</sup>. In this investigation, the college's toilets were considered as infected places in which microbial (fungi) air sampling was conducted while all the air escaping ways were closed. Also, after smoking with Espand and Anbarnasara in different masses and in times of 1, 3, and 5 minutes after smoking, microbial sampling from the indoor air was performed. After sampling as described above, plates were removed from the device, capped, and then transferred to laboratory to be kept at 25-27 °C for 72-120 hours. After this period, the number of colonies formed in plates was counted. According to sampling rate and duration, the results was reported in colonies per cubic meter of air (CFU/m<sup>3</sup>). So, the effect of the mentioned smoking on the microbial (fungi) burden was determined. Research experiments were performed at least twice and data were analyzed through the SPSS Software (Vs. 18) and Microsoft Excel 2007.

### Ethical issues

This study was conducted with the approval of Qom University of Medical Sciences, Medical Ethics Committee. Code: IR.MUQ.REC.1393.20

### Results

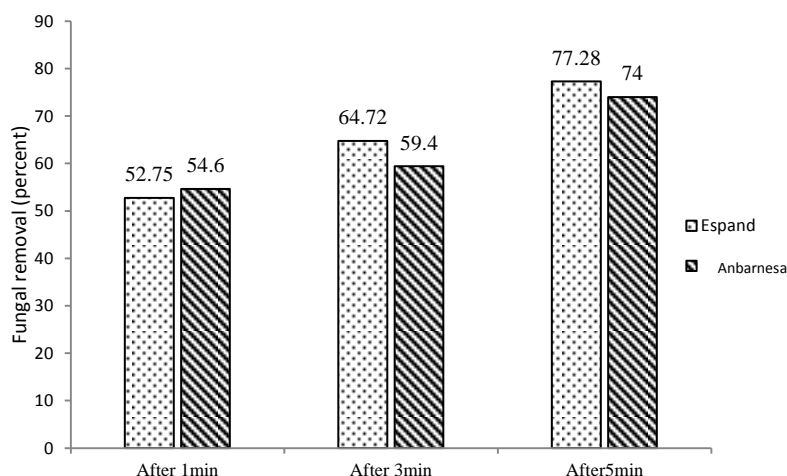
Pre-smoke samples depending on the size and location of the sampling room showed various amounts of fungal burden and ranged between 106 - 795 CFU/m<sup>3</sup> with an average of 387 CFU/m<sup>3</sup>. Table 1 represents the logarithmic rate of fungal burden removal for Espand and Anbarnasa in different times.

**Table 1:** The amount of logarithmic rate of fungi in different times

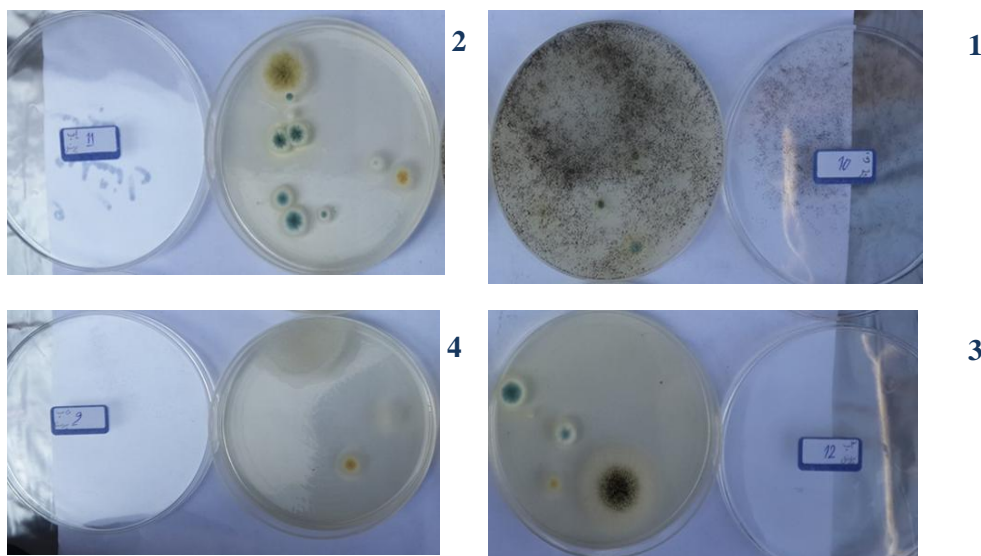
Smoking times	Logarithmic rate of fungi removal in air	
	Espand	Anbarnasa
1 minute	0.33	0.34
3 minutes	0.45	0.39
5 minutes	0.64	0.59

The conducted experiments showed that the removed fungal burden from indoor air by Espand smoke at 1 minute after smoking was 52.75 %, 3 minutes after smoking was 64.72 %, and 5 minutes

after smoking was 77.28 %. Furthermore, the average percentages of fungi removal by Anbarnasa 1, 3, and 5 minutes after smoking were 54.6 %, 59.4 % and 64.72 %, respectively (Figures 1, 2).



**Figure 1:** Comparison the effects of Espand and Anbarnasa on reduction of air fungal burden at different smoking times.



**Figure 2:** A sample of the cultured plates before smoking (No. 1), one minute after smoking (No. 2), three minutes after smoking (No. 3), and five minutes after smoking (No. 4).

## Discussion

ANOVA Tukey test showed that there is a significant difference ( $p < 0.05$ ) between smoking retention time (1, 3, and 5 min) and the removal percentage of fungus present in the air. So that with increase of smoking retention time (Espand and Anbarnasa), the removal percentage also increased. The results of this statistical test showed that there isn't any significant difference between the reduction values of fungi by smoking with Espand or Anbarnasa in different time periods of the smoking. The results further represented that

there wasn't a significant change in removal values of fungal burdens by changing the amount of Espand or Anbarnasa (1, 2 and 3 grams). Although, it was reported that removal value of room fungi by Espand was more than that of Anbarnasa, but this difference was not statistically significant.

According to the literature, there has been no dedicated study on the effect of smoking on microbial burden of air. In the following, results of studies focusing on direct smoking of the microbial culture media are investigated. Koshakhlagh, concluded that among the alkaloid compounds of



Espand, only harmful remains unchanged in the smoke, and effects of Espand smoke probably belong to this substance. Also, increase of dung and Espand smoking time has led to an increase in antimicrobial effects of the smoke. This result shows that despite drug resistance to the dung and Espand smokes, they can be effective in removal of this important pathogenic microorganism<sup>24</sup>. Najafi et al., in their study concluded that smoke of Espand is effective against only the gram-positive bacteria, while its effectiveness against the gram-negative bacteria happens only in doses higher than 8 hours of smoking<sup>25</sup>. On the other hand, Shahverdi studied the effects of Espand smoking on *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger* and reported that this smoke is effective against all of these microorganisms<sup>10</sup>. The existing differences in the effects of the Espand smoke is probably due to the nature of surveyed microbes and the permeability of anti-microbial materials of Espand smoke in these disease-causing microbes. Mohagheghzadeh et al., in their article on medicinal smoke stated that there is a little information about composition of these medicinal smokes<sup>9</sup>.

### Conclusion

The results of this study showed that smoking with Peganumharmala, i.e., Espand (about 2 grams) and female donkey dung, i.e., Anbarnasa (about 3 grams) in a room with 10 to 20 cubic meters area for about 5 minutes can decrease the fungi growing in the ambient air. This type of medicinal smoke can also be used to disinfect the air especially in places like toilets which has pollution. Of course, it should be mentioned that the nature of smoke, as air pollution, can cause some problems for human health. Thus, smoking is better to be performed in absence of people. The results represented that Espand and Anbarnasa have different antimicrobial effects in killing indoor air fungi according to different smoking durations. By the increase of smoking duration, the antimicrobial effects of the smoke increases too. Conductance of supplementary studies especially on different species of fungus, bacteria, and their

different species can facilitate the completion of this research.

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### Conflict of interest

We have no competing interests.

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