Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2020, 9(12): 52-58

Effect of Benzoin Resin Fumes on Indoor Environmental Microbes

Mashael W. Alruways, Intisar E. Elrayah*, Mariam A. Mansi

Department of Clinical Laboratories, College of Applied Medical Science, Dawadmi, Shagra University, Saudi Arabia

**Corresponding e-mail: elreyagh@su.edu.sa*

ABSTRACT

Objective: *This work aimed to investigate the antimicrobial effect of traditional Saudi Arabian incense (white benzoin resin, Jawi) on indoor environment microbes in Shaqra University, Dawadmi Female Student Campus, and bacterial isolates from patients in Dawadmi General Hospital.* ***Methods:*** *The effect of white benzoin resin fumes was tested directly on clinical bacterial isolates. The bacterial culture plates were incubated with benzoin resin fumes within a confined space. The microbial quality of indoor air in four laboratories, lecture rooms, library, and restroom of the students was measured, and the effect of benzoin resin fumes on these indoor microbes was determined. The settle plate method with open Petri dishes containing different culture media was used to collect samples two times daily before and after benzoin resin fumes were used.* ***Results:*** *The growth of Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans was completely inhibited after exposure to benzoin resin fumes for 90 min. The growth of Klebsiella pneumoniae, Escherichia coli, and Enterobacter aerogenes was inhibited by 79%, 68%, and 55%, respectively, after the same incubation time. Fumigation of selected university premises with the incense decreased the total bacterial count from 23.5 Colony-Forming Units (CFU) to 6.25 CFU.* ***Conclusions:*** *The application of benzoin resin fumes in some university premises showed a promising effect on decreasing the total number of microorganisms in the air of some rooms and inhibiting the growth of some pathogenic bacteria.*

Keywords: Benzoin resin fume, Bacterial isolate, Indoor environment microbe, University premise

INTRODUCTION

Indoor environments are the most important environmental factors that can influence health. The air quality of indoor environments is one of the main factors that affect the health, safety, and productivity of people. The presence of microorganisms, including bacteria, molds, and viruses, is one of the major problems that affect the quality of indoor air [1,2]. People spend 90 % of their time indoors breathing an average of 14 m^3 of air per day [3,4]. As such, people are highly exposed to indoor microorganisms. A review published by the World Health Organization on some epidemiological studies showed adequate evidence for the association between indoor dampness-related factors and a wide range of effects on respiratory diseases, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze, and dyspnoea [1].

Ceremonies were performed in the past to purify the environment by burning wood and odoriferous medicinal herbs [5]. Since the earliest times, the burning of frankincense and myrrh in places of worship for religious purposes had hygienic functions, can refine smell, and minimize pollution by purifying indoor air [5]. The fumigation of an operating theatre with fumes of mustard, butter, and salt may be an early kind of "antisepsis" of the air, although it had been additionally performed to eliminate evil spirits [6].

Nowadays, different traditional practices involve the use of fumigation smokes within confined spaces to purify or disinfect the air and to clean the environment.

The traditional use of fumes of resins in Arabic countries has many purposes: resin fumes are used for spiritual purposes, to provide a pleasant smell in homes and eliminate bad smells, improve the mood and eliminate negative energy, feelings of fatigue, lethargy and laziness and respiratory problems, such as colds and flu [7].

Recent studies have proven the efficacy of holy stick fumigation against infectious bacteria, as well as the efficacy of medicinal smoke on airborne bacteria [5,6]. Many substances, including Jawi (benzoin resin), oud, frankincense, aromatic wood, herbs, flowers, essential oils, and perfume, are used to produce incense and to eliminate undesirable odors in an indoor environment.

Jawi is the most common fume incense used in daily traditional practice in Arabic countries. Jawi is used for its aromatic scents.

The two main types of benzoin resins are Sumatra benzoin and Siam benzoin, which are available in markets. These two varieties grow in different geographical areas. Siam benzoin grows in Laos, whilst Sumatra benzoin grows in Indonesia [8]. The three types of Styrax benzoin resins are red, grey, and white, which contains compounds, such as benzoic acid, benzaldehyde, and benzyl benzoate, exhibiting bactericidal, germicidal, antiviral, and fungicidal properties. The areas exposed to the smoke of benzoin resin are disinfected. Benzoin oil also has anti-flatulence and carminative properties [9,10]. The antimicrobial effects of some known constituents of the different type's benzoin resin have been studied in detail by extracting essential oils from resin.

The obtained extracts were tested against two types of pathogenic bacteria, namely, *Staphylococcus aureus* and *Escherichia coli*. The results showed that the extracts have good antioxidant and antibacterial effects [11].

Regarding the effect of resin fume on microbes, several studies have investigated the effect of burning incense and its smoke on microbes by evaluating their effect on bacteria and fungi by using a smoke chamber or air samplers [12-15] inside a confined area in the laboratory.

In the present work, we selected white benzoin resin (Al-Jawi) as a subject because of its widely popular use as traditional incense fume in Saudi Arabia. We tested the direct effects of incense fume on selected clinical pathogenic bacteria isolates collected from the hospital and examined its short-term effects on cleaning and sterilizing confined university premises from environmental microbes.

METHODOLOGY

The study was carried out in the College of Applied Medical Sciences, Dawadmi, Shaqra University between March and November 2019.

Samples

White benzoin resins: Al-Jawi is a balsamic resin obtained from the bark of several species of trees in the genus *Styrax* [8]. The fresh samples used in this study were obtained in March 2019 from a local market in Dawadmi City (Figure 1) and were used in the crude form similar to the ones traditionally used in the homes on a daily bases.

Figure 1 White benzoin resin (crude sample purchased from Dawadmi City market, KSA)

Bacterial isolates: Five strains of bacteria (*E. coli*, *S. aureus Klebsiella pneumoniae*, *Enterobacter aerogenes,* and *Pseudomonas aeruginosa*) isolated from clinical cases in the General Hospital of Dawadmi City were used in the study.

In addition to these pathogenic bacteria, airborne microbial isolates were collected from different university premises using the settle plate method.

The isolated microbes were incubated aerobically at 37°C for 24 h. Based on the morphological analysis and the Gram reaction, two isolates were considered for further analysis. One desecrate colony from each type was obtained and dissolved in 1 mL of sterile physiological saline. Suspensions were adjusted to standard turbidity (0.5), which corresponds to approximately 1.0×10^8 CFU/ml. An automated device (VITEK® 2, BioMérieux) was used to identify the microbial isolates. The identified isolates were *S. aureus* and *Candida albicans*.

Experimental Setting

The experiments were conducted in two phases: the first phase aimed to test the effect of the white benzoin resin on the known clinical bacteria isolates and airborne environmental isolates under laboratory conditions. In the second phase, the effect of resin fumes was assessed in confined places in university premises to determine the effect of fumes on total air microbial count before and after burning the resin in these places.

First phase: The clinical isolates were used for the in vitro determination of the antibacterial efficiency of crude resin following the method of Fontes et al. with some modifications (nutrient agar was used instead of blood agar) [16]. Bacteria were inoculated on nutrient agar plates and incubated aerobically at (35 ± 2)°C for (18 to 24) h. The standardized inoculum was prepared using a direct colony suspension by making a saline suspension of isolated colonies selected from nutrient agar plates. Each bacterial suspension was adjusted to 10^5 CFU/mL. An aliquot of 10 µL of this suspension was inoculated by lawn culture in four nutrient agar plates with a final concentration of 10^3 CFU/mL. Then, the plates were divided into four groups. The first group was exposed to the benzoin resin fumes by burning of 1 g of benzoin resin in a sealed container with a dimension of 50 cm × 50 cm × 50 cm for 30 min. The second and third groups were exposed to incense fume for 45 min and 90 min. The resin was burned using an electric incense burner provided with a hot plate (without using charcoal). The fourth group of plates was the negative control group. Then, the plates were incubated at 37°C for 24 h, and the CFU for each plate was determined using a colony counter.

Second phase: The effect of white benzoin resin fumes on the airborne microorganisms in selected rooms in the university was determined by passive sampling. The rooms exposed to the resin fumes were the library, the students' restroom, one classroom, and the microbiology laboratory. The effects of the incense fumes on the total microbial count in the air in the selected areas were examined. Those rooms were occupied occasionally by 20-30 students. The dimensions of the rooms were 8 m × 10 m × 3.5 m. Sampling was performed early in the morning before the rooms were used by the students, and the test was performed in triplicate.

Passive sampling via the gravity method (settle plate method) was performed to determine the total microorganism count (bacterial and fungal) in the air. This number corresponds to the number of CFU counted on a petri dish with a diameter of 9 cm placed according to the 1/1/1 scheme (for 1 h, 1 m above the floor, approximately 1 m away from walls or any major obstacles). Sampling was performed two times before and after burning the incense by using nutrient agar plates [17].

Exactly 10 g of the crude benzoin resin was burned in the nave with sealed windows and doors to prevent unexpected airflow and allow the aromatic atmosphere to form. Then, passive sampling was performed as previously mentioned. Each plate was incubated at the end of the test at 37°C for 48 h. Then, the total number of CFU was determined at each plate by using a colony counter.

RESULTS AND DISCUSSION

The indoor environment is affected by many pollutants, including airborne microorganisms (bacteria and fungi). One-third of complaints regarding indoor air quality may be due to microbial contamination, and exposure to microbial contaminants can lead to allergies and respiratory and immune-toxic diseases [18,19]. Many studies have been conducted to assess the quality of microbial indoor in university premises: a high concentration of bacteria and fungi aerosols has been detected in university libraries [20,21].

The extensive use of air fresheners and disinfectants to clean the indoor environment from microbes has many side effects and may serve as allergens, irritants, or even toxins [22,23]. The effect of burning natural substances to eliminate environmental microbes has been confirmed by different studies [7,9,24].

Our major finding is confirming the ability of benzoin resin fumes to inhibit or reduce the growth of indoor airborne microbes and clinical bacterial isolates.

Figure 2 shows that the average inhibition rate of clinical bacterial isolates by burning 1 g of benzoin resin for 90 min ranged from (100-55)%. The inhibition of microbial growth (*S. aureus*, *P. aeruginosa,* and *C. albicans*) reached 100% after exposure to resin fumes for 90 min within a confined space. The benzoin resin had a weaker inhibition effect on *K. pneumoniae*, *E. coli,* and *E. aerogenes* after 90 min of exposure. Nevertheless, the inhibition effect was remarkable (79%, 68%, and 55%, respectively, Figure 2). Even after bacteria were exposed to the fumes for 30 and 45 min, bacterial growth inhibition was notable (more than 18% in all isolates except for *E. aerogenes* with almost no effect after 30 min of exposure).

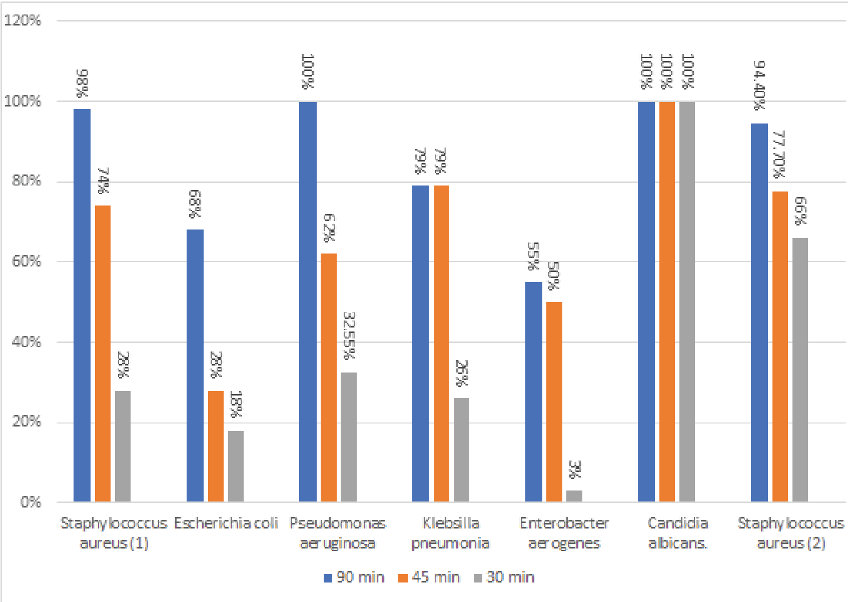

Figure 2: Percentage of inhibition of CFU formation after exposure to incense for (30, 45 and 90) min. *Staphylococcus aureus (1)*: Pathogenic strain isolated from hospital sample; *Staphylococcus aureus (2)*: airborne strain isolated from the environment

Burning 10 g of resin on the university premises for 60 min decreased the CFU of environmental microbes by 70%-75% (Figure 3).

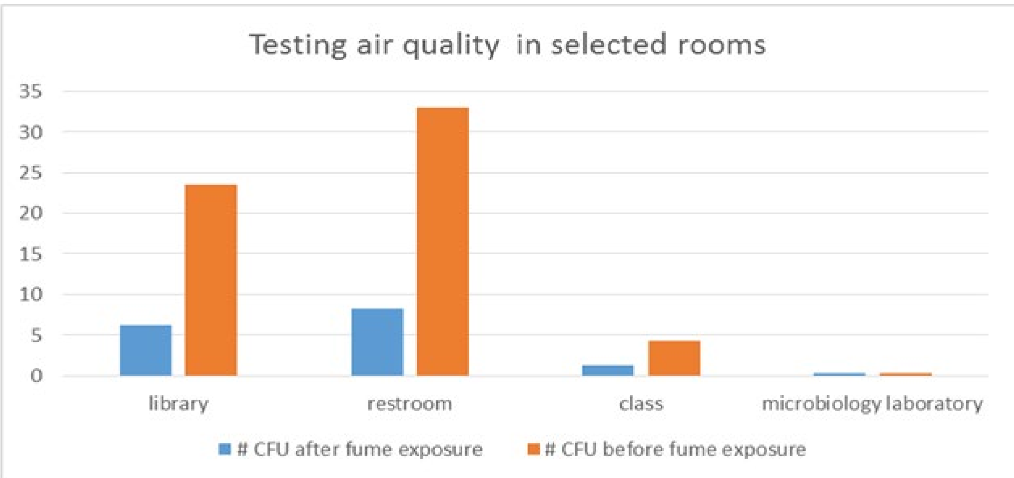

Figure 3 Difference in the number of CFU before and after applying the incense in selected rooms in the campus

The number of CFU in the library decreased from 23.5 to 6.25 (inhibition rate of 74%) (Figure 3). Also, the inhibition rate in the restroom was remarkably decreased (from 33 CFU to 8.3 CFU) after exposure to the incense fumes (inhibition rate 75%). In the classroom, the number of CFU before exposure to the incense was low (4.3 CFU) but further decreased to 1.3 CFU (70%) after exposure to incense fume. The microbiology laboratory was tested many times; no bacterial growth was detected before or after exposure to the incense fumes. This effect may be due to the regular use of disinfectants in this laboratory.

The positive effect of benzoin resin fume on microbes has been observed in other studies that used different types of incense extracts on environmental microbes [5,15,16,24].

Natulya et al., observed that burning 500 g of wood and a complex mixture of odoriferous and medicinal herbs for one hour caused over 94% reduction of aerial bacterial population counts [13]. In our study, we have used only white benzoin resin and achieved a 100% reduction in clinical isolates and a 70%-75% reduction in environmental isolates. Therefore, the present work showed the ability of the benzoin resin fumes to purify or disinfect a closed room from airborne microbes.

The inhibitory effect of benzoin resin fumes may be attributed to the volatile active ingredients, which have confirmed antioxidant and antibacterial effects [11,25]; oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* have considerable amounts of volatile oils and both are applied as incense in traditional medicine aside from their other medicinal applications [26].

Therefore, the present study confirmed the microbicide effect of benzoin resin incense fumes. The fumes inhibited the growth of clinical bacterial isolates obtained from hospitalized patients and decreased the microbial load of indoor environments in the university premises. Aside from the microbiocidal effects of benzoin resin fumigation, this traditional incense promotes a pleasant smell in confined places, removes bad smells, improves the mood, eliminates negative energy, and prevents respiratory problems [7].

The use of resin fume incense may be safer than using artificial air disinfectants and fresheners. The artificial air disinfectants and fresheners used indoors have potentially harmful health effects, including sensory irritation, respiratory symptoms, and lung dysfunction [22].

The traditional daily use of benzoin resin as incense in Saudi Arabia makes the students familiar with the incense smell. The incense produces a sweet smell that improves mood and stimulates the nervous system. Besides, benzoin oil contains various bioactive components and is used as a sedative and relaxant for relieving tension, stress, anxiety, and nervousness [10].

CONCLUSION

In conclusion, our results showed that benzoin resin fume minimized microbial hazards and improved the air quality of the university premises. The fumes reduced indoor environmental microbe isolates by 70%-75% and clinical isolates from the hospital by 100%.

The use of incense fumes can prevent bacterial growth, sterilize the environmental air, and promote a healthy atmosphere by minimizing airborne transmission.

This study suggests the direct effect of crude incense on some environmental microbial isolates, such as bacteria and fungi. However, further studies are needed to determine the effects of incense on airborne viruses, such as coronaviruses, which present a major pandemic health problem at present.

DECLARATIONS

Acknowledgment

The authors would like to express their appreciation to the Laboratory Department in Dawadmi general hospital for providing the bacterial Clinical Isolates used in this study. Also, they would like to convey appreciation to Prof. Asaad Khalid MA Ahmed, Substance Abuse and Toxicology Research Center, Jazan University, Saudi Arabia for his critical reading of this manuscript.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] Heseltine, Elisabeth, and Jerome Rosen, eds. WHO guidelines for indoor air quality: dampness and mould. WHO Regional Office Europe, 2009.

[2] Wemedo, S. A., P. N. Ede, and A. Chuku. "Interaction between building design and indoor airborne microbial load in Nigeria." *Asian Journal of Biological Science,* Vol. 5, No. 4, 2012, pp. 183-91.

[3] Awad, Abdel Hameed A., and S. A. Farag. "An indoor bio-contaminants air quality." *International Journal of Environmental Health Research,* Vol. 9, No. 4, 1999, pp. 313-9.

[4] Prabhu, N., J. Rengaramanujam, and P. Anna Joice. "Efficacy of plants-based holy stick fumigation against infectious bacteria." *Indian Journal of Traditional Knowledge,* Vol. 8, No. 2, 2009, pp. 278-80.

[5] Grbić, Milica Ljaljević, et al. "Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients?." *Journal of Ethnopharmacology,* Vol. 219, 2018, pp. 1-14.

[6] Lin, Ta-Chang, Guha Krishnaswamy, and David S. Chi. "Incense smoke: clinical, structural and molecular effects on airway disease." *Clinical and Molecular Allergy,* Vol. 6, No. 1, 2008, pp. 3.

[7] Wahab, Atqah Abdul, and Ossamaa Mostafa. "Arabian incense exposure among Qatari asthmatic children. A possible risk factor." *Saudi Medical Journal,* Vol. 28, No. 3, 2007, pp. 476-8.

[8] Coppen, JJ W. "Benzoin: production, uses and international trade." *Perfumer & Flavorist,* Vol. 24, No. 3, 1999, pp. 11-22.

[9] Hanif, Muhammad Asif, et al. "Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil." *Journal of Medicinal Plants Research,* Vol. 5, No. 5, 2011, pp. 751-7.

[10] Atia Sharif, Haq Nawaz, et al. "A review on bioactive potential of Benzoin Resin." *International Journal of Chemical and Biochemical Sciences,* Vol. 10, No. 1, 2016, pp. 106-10.

[11] Hacini, Zineb, et al. "Evaluation of antibacterial and antioxidant activities of three types of benzoin resin." *European Journal of Chemistry,* Vol. 9, No. 4, 2018, pp. 408-11.

[12] Raut, Anuja B., et al. "Preparation and evaluation of antimicrobial herbal based incense sticks for fumigation against infectious bacteria." *Journal of Chemical and Pharmaceutical Research,* Vol. 3, No. 4, 2011, pp. 707-12.

[13] Nautiyal, Chandra Shekhar, Puneet Singh Chauhan, and Yeshwant Laxman Nene. "Medicinal smoke reduces airborne bacteria." *Journal of Ethnopharmacology,* Vol. 114, No. 3, 2007, pp. 446-51.

[14] Elsayed, Yehya, et al. "Chemical composition and potential health risks of raw Arabian incense (Bakhour)." *Journal of Saudi Chemical Society,* Vol. 20, No. 4, 2016, pp. 465-73.

[15] Abdallah, Emad M., and Amna E. Khalid. "A preliminary evaluation of the antibacterial effects of *Commiphora molmol* and *Boswellia papyrifera* oleo-gum resins vapor." *International Journal of Chemical and Biochemical Sciences,* Vol. 1, 2012, pp. 1-15.

[16] Fontes, Belchor, et al. "Effect of low-dose gaseous ozone on pathogenic bacteria." *BMC Infectious Diseases,* Vol. 12, No. 1, 2012, pp. 358.

[17] Napoli, Christian, Vincenzo Marcotrigiano, and Maria Teresa Montagna. "Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres." *BMC Public Health,* Vol. 12, No. 1, 2012, pp. 594.

[18] Pope, Andrew M., Roy Patterson, and Harriet Burge. "Magnitude and dimensions of sensitization and disease

caused by indoor allergens." Indoor Allergens: Assessing and Controlling Adverse Health Effects. National Academies Press (US), 1993.

[19] Douwes, J., et al. "Bioaerosol health effects and exposure assessment: progress and prospects." *The Annals of Occupational Hygiene,* Vol. 47, No. 3, 2003, pp. 187-200.

[20] Hayleeyesus, Samuel Fekadu, and Abayneh Melaku Manaye. "Microbiological quality of indoor air in university libraries." *Asian Pacific Journal of Tropical Biomedicine,* Vol. 4, 2014, pp. S312-7.

[21] Stryjakowska-Sekulska, M., et al. "Microbiological quality of indoor air in university rooms." *Polish Journal of Environmental Studies,* Vol. 16, No. 4, 2007, pp. 623-32.

[22] Kim, Sanghwa, et al. "Characterization of air freshener emission: the potential health effects." *The Journal of Toxicological Sciences,* Vol. 40, No. 5, 2015, pp. 535-50.

[23] Ibrahim ALshaer, Fatima, et al. "Qualitative Analysis of Air Freshener Spray." *Journal of Environmental and Public Health,* Vol. 2019, 2019.

[24] Alhussaini, Mohammed S., et al. "An evaluation of the Antimicrobial activity of Commiphora myrrha Nees (Engl.) oleo-gum resins from Saudi Arabia." *Journal of Medical Sciences,* Vol. 15, No. 4, 2015, pp. 198.

[25] Fernandez, Xavier, et al. "Volatile constituents of benzoin gums: Siam and Sumatra. Part 1." *Flavour and Fragrance Journal,* Vol. 18, No. 4, 2003, pp. 328-33.

[26] Hanuš, Lumír O., et al. "Myrrh-commiphora chemistry." *Biomedical Papers,* Vol. 149, No. 1, 2005, pp. 3-28.