Investigating the Effect of Plant Resin Fumes on Sandstorm Allergenic Fungi Isolated from Al Zulfi, Saudi Arabia

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Abstract

Introduction: In Saudi Arabia, resin fumes have traditionally been used for home fragrance. Sandstorms pose health risks due to dust-borne fungal allergens; our study is to investigate the effectiveness of these fumes against such allergens. Objective: This study examined the efficacy of commonly used resin fumes, including oud, myrrh, and benzoin, against fungal allergens isolated from sandstorm dust in Zulfi, Saudi Arabia. Materials and Methods: Resin fumes (5 g) were generated using charcoal cake and introduced into a bell jar chamber containing pre-inoculated plates of sandstorm fungal isolates, including hyaline fungi (Penicillium spp., Fusarium spp., Aspergillus niger) and dematiaceous fungi (Ulocladium spp., Bipolaris spp., Cladosporium spp.). Control plates received no fume exposure. Fungal colonies were counted after 3-5 days of incubation and analyzed for log reduction. Results: Myrrh fumes demonstrated balanced efficacy against both hyaline (98.18% reduction) and dematiaceous fungi (84.3%). Oud fumes exhibited superior effectiveness against hyaline fungi (98.52%) but lesser activity against dematiaceous fungi (64.2%). Benzoin fumes showed moderate effectiveness against both fungal types. Synthetic incense cones resulted in a 100% reduction of fungal growth due to toxicity toward both types of fungi. Conclusion: This first report demonstrated the antifungal properties of traditionally used resin fumes, with myrrh fumes showing superior efficacy against both hyaline and dematiaceous fungi. The research identifies potential health risks from synthetic incense cones and highlights the lack of scientific studies on fume exposure for fungal control.

Key words: Agarwood, dematiaceous fungi, fumes, hyaline fungi, sandstorm dust

INTRODUCTION

atural plant resins such as frankincense, benzoin, myrrh, copahu balm, galbanum, labdanum, and styrax play a significant role in the perfume industries. These resins are valued for their distinctive aromatic qualities, therapeutic properties, and potent fragrances.[1] In Saudi Arabian culture, resin fumes have been traditionally used for multiple purposes, including enhancing home fragrance, eliminating unpleasant odors, and controlling airborne microorganisms.[2,3] Three resins are particularly prominent in Saudi Arabia: Oud (derived from Aquilaria trees), Myrrh (Commiphora myrrha), and Al-Jawi (Styrax benzoin). Remarkably, Oud resin is deeply embedded in Saudi cultural practices, is believed to offer various health benefits, and symbolises warm hospitality.^[3] Oud, or agarwood, is especially valued for its opulent aroma and is commonly used in the form of bakhoor, which consists of scented wood chips soaked in fragrance oils and burned in both public places and private homes.^[4] Myrrh and al-jawi (benzoin) are also popular for

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Received: 28-03-2025 **Revised:** 29-05-2025 **Accepted:** 12-06-2025 their aromatic properties and are used similarly to improve indoor air quality and create a pleasant environment.^[5-7]

Conversely, sandstorms (also called dust storms) are common in the desert regions of Saudi Arabia, especially in the Middle East. They carry a wide range of microbial particles, with high-blowing dust can cause significant health issues and disrupt outdoor activities.[8,9] These sandstorm particles, which frequently contain fungal spores, can cause several allergic reactions and respiratory problems, raising serious public health concerns.^[9,10] Recent research indicates that sandstorms can transport vast amounts of dust and microorganisms over long distances, exacerbating respiratory problems and increasing the incidence of asthma, bronchitis, and other respiratory ailments.[11-13] The World Meteorological Organization has cautioned about the considerable dangers of airborne dust, which may carry bacteria, viruses, and other harmful substances. These dust particles easily infiltrate housing and workplace areas through minute gaps in windows and doors. Children and elderly individuals are at high risk of developing respiratory ailments due to the microbial particles in dust. Recent reports from Kuwait and Saudi Arabia have documented increased hospital admissions among children and elderly people during sandstorm events.[11,14] The increasing frequency of sandstorm events in Saudi Arabia has resulted in a rise in respiratory illnesses.[15] Controlling indoor airborne allergens is crucial, despite numerous microbiological studies having analyzed them. However, research on controlling allergens using plant resins is very limited. [3,16] In particular, there are no available reports specifically addressing sandstorm fungal indoor allergens. While some studies have examined the effects of resin fumes against common indoor fungal isolates and other clinical isolates in Saudi Arabia and globally, there is a notable lack of research specifically targeting sandstorm fungal isolates.[3,16]

There are few reports on fume exposure effects on indoor bacterial and clinical isolates. [3] However, none of the reports are available regarding fungal isolates from sandstorm dust. People in Middle Eastern countries believe that fumes are not only used for home decoration and a pleasant odor but also for indoor microbial control. However, there is a lack of scientific studies to validate this concept. Simultaneously, some individuals use artificially produced chemical incense cones instead of natural plant resin compounds. Based on the above facts and information, this study aims to check the effectiveness of resin fumes, including oud, myrrh, benzoin, and commercially available incense cones against selected common sandstorm fungal allergens.

MATERIALS AND METHODS

Sandstorm fungal isolates

Sandstorm fungal isolates were taken from the culture storage collection from the research laboratories, at the College of Science, Majmaah University. Sandstorm fungal isolates were collected as passive air sampling methods by our previous extensive research and stored in peptone glycerol broth in microbial culture collection deep freezer (-80°C) facilities at the research laboratories, College of Science, Majmaah university (Vijayakumar *et al.*, 2017). Among the culture collections, six common sandstorm fungal allergens were selected for this study, and classified into two groups: Hyaline fungi (*Penicillium* spp., *Fusarium* spp., and *Aspergillus niger*) and dematiaceous fungi (*Ulocladium* spp., *Bipolaris* spp., and *Cladosporium* spp.). All cultures were sub-cultured and ensured their growth characteristics, viability, and integrity of the samples before proceeding with the experiments.

Resin fumes and incense materials

Three resin fumes Agarwood (oud), Myrrh gum, and Al-Jawi (also known as "Benzoin") along with one commercial incense cone, were included in this study [Figure 1]. These materials were procured from local markets in Zulfi, Saudi Arabia. To generate the fumes, 5 g of each resin was burned using charcoal, following the traditional fume-producing procedure commonly practiced in Saudi Arabia. [2,3]

Preparation of 100 colony-forming units (CFU) of fungal spores

Sandstorm fungal isolates were cultured on Sabouraud dextrose agar (SDA) and incubated at 25°C for 3 days until sufficient growth was observed. A spore suspension containing 100 CFU was prepared following standard microbiological procedures. [18] Briefly, after incubation, fungal spores were harvested by gently scraping the surface of the colonies using a sterile swab and suspending them in a sterile saline solution. The turbidity of the spore suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately

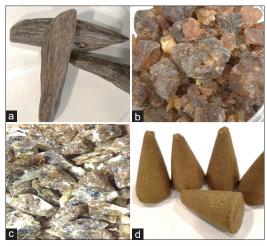


Figure 1: Selected resin fumes (a) agarwood (oud), (b) myrrh, (c) benzoin and commercial incense cone (d) for fumes exposure study

 $1-5 \times 10^6$ CFU/mL. This suspension was further diluted to achieve a final concentration of $1-5 \times 10^3$ CFU/mL, ensuring the inoculum contained approximately 100 CFU or spores per aliquot. The inoculum concentration was cross-verified by performing colony counts on agar plates. As per the dilution, a suitable aliquot of the 100 CFU spore suspension was inoculated onto the surface of sterile SDA plates and evenly spread. This step was performed immediately before conducting the fumes exposure study.

Bell jar-fumes exposure study

Fume exposure was conducted using a simple, novel method involving a sterilized bell jar. The bell jar measured 12.5 inches in height and 9 inches in diameter, providing a total exposure area of approximately 0.228 m². Five grams of each resin material (agarwood, myrrh, and benzoin) and incense were burned using a charcoal cake, following the standard procedure, and the resulting fumes were introduced into the sterile bell jar chamber. Pre-inoculated SDA plates containing 100 CFU of fungal cultures were placed inside the bell jar, ensuring no direct contact with the resin fumes. All experiments were performed on a clean, flat surface disinfected with 70% ethanol. The bell jar was tightly sealed to contain the fumes, and the fungal cultures were exposed to the fumes for a specified duration of 30 min [Figure 2]. Control plates were exposed to plain charcoal fumes without resin fumes to serve as a baseline for comparison. Environmental conditions, including temperature and humidity, were consistently maintained throughout the exposure period.

Fungal culture and observation

After the exposure period, the SDA plates were aseptically removed from the bell jar and then covered with the lid and incubated at 25° C for 3–5 days. The plates were observed daily for fungal growth or inhibition. Simultaneously, each fungal isolate's control plates were performed without exposure to fumes, and it was compared with exposed plates. After incubation, the number of colonies was counted, and their colony morphology and these fungal colonies on the treated and control plates were counted to determine the reduction in colony numbers. The effectiveness of each resin fume was evaluated based on the percentage reduction in fungal colonies and log reduction values. All results were tabulated and analyzed, and the standard statistical method one-way analysis of variance was used to compare both groups, and P < 0.05 was considered statistically significant.

RESULTS

Following the protocol established in our previous study on airborne fungal allergens in sandstorm dust, the identified fungi included *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Ulocladium* spp. *Fusarium* spp., *Curvularia* spp., and *Rhizopus* spp., all of which are recognized for their role in respiratory and allergic health issues during sandstorms. From these fungal isolates, six common airborne species were selected for the bell jar exposure study. The results of the exposure of fungal isolates to various resin fumes and incense cones are listed in Table 1.



Figure 2: Resin fumes and experimental setup of bell jar exposure study

Table 1: N	umber of colonies observed after ex	xposure of re	esin fume	es against	in bell jar ch	namber
Group of fungi	Name of the fungal isolates	Number of colonies (CFU/plate)				
		Control	Oud	Myrrh	Benzoin	Incense cone
Hyaline (HF)	Penicillium spp.	98	1	1	25	0
	Aspergillus niger	92	2	2	12	0
	Fusarium spp.	89	2	2	0	0
	Mean hyaline fungi group	93.0	1.7	1.7	12.3	0.0
Dematiaceous (DF)	Ulocladium spp.	68	2	6	16	0
	Bipolaris spp.	98	42	3	13	0
	Cladosporium spp.	91	56	32	16	0
	Mean - dematiaceous fungi group	85.7	33.3	13.7	15.0	0.0

CFU: Colony forming units. 0 represents "no growth"

The data show a significant reduction in fungal colony counts after exposure to the different resin fumes compared to the control group. For hyaline fungi, the average colony count before exposure was 93 CFU/plate. After exposure, the colony counts significantly reduced to 1.7 for both oud and myrrh fumes and 12.3 for benzoin fumes. This indicates a substantial reduction in fungal growth due to exposure to these fumes. In the case of dematiaceous fungi, the control group had an average colony count of 85.7 CFU/plate. After exposure, the colony count decreased to 33.3 for oud fumes, 13.7 for myrrh fumes, and 15 CFU/plate for benzoin fumes. These results demonstrate the varying degrees of effectiveness of the different resin fumes in controlling dematiaceous fungi.

Statistical analysis revealed that all tested aromatics significantly reduced fungal growth (P < 0.01) compared to controls, with hyaline fungi generally exhibiting greater susceptibility than dematiaceous species, particularly to oud treatment, which showed notably different inhibition patterns between the two fungal groups (98.2% vs. 61.1%, respectively). The commercial incense cones demonstrated a 100% reduction in fungal growth compared to the control, indicating their complete effectiveness in eliminating fungal colonies. Due to this conclusive result, further discussion on incense cones has been neglected from the results section.

Percentage of fungal growth reduction

The percentage of log reduction of fungal growth after exposure to various resin fumes is illustrated in Figure 3. The data indicated that oud fumes exhibited a 98.52% effectiveness in reducing hyaline fungi, slightly over the myrrh fumes, which show a 98.18% effectiveness. Both oud and myrrh fumes are significantly more effective than benzoin fumes, which have an 87.15% effectiveness in controlling hyaline fungi. In terms of dematiaceous fungi, myrrh fumes demonstrate the highest effectiveness at 84.3%, followed by benzoin fumes at 81.8%, and oud fumes at 64.2%. These data suggested that myrrh fumes are the most effective in controlling dematiaceous fungi, with benzoin fumes being moderately effective and oud fumes being the least effective in this category.

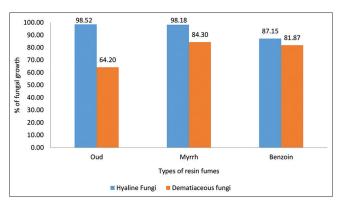


Figure 3: Percentage of fungal growth reduction after resin fumes exposure

Overall, myrrh fumes exhibited a balanced and high effectiveness in controlling both hyaline (98.18%) and dematiaceous fungi (84.3%). Oud fumes showed highly effective against hyaline fungi but less effective against dematiaceous fungi. Moderate effectiveness against both types of fungi was observed in benzoin fumes. These results highlighted the varying efficacy of different resin fumes in controlling different types of fungal growth.

Log reduction in fungal growth

The log reduction in fungal growth after exposure to various resin fumes is presented in Figure 4. Both oud and myrrh fumes were found to be equally effective against hyaline fungi, with an observed log reduction of 1.76. However, benzoin fumes demonstrated a lower log reduction of 1.15, making them less effective than oud and myrrh fumes. Regarding action against dematiaceous fungi, myrrh fumes exhibited the highest log reduction (1.0), thereby being the most effective. Benzoin fumes showed a moderate log reduction (0.75), whereas oud fumes recorded the lowest log reduction (0.7), indicating they were the least effective against dematiaceous fungi.

Overall, the log reduction in fungal growth data indicated that myrrh fumes exhibited a high level of efficacy against both hyaline and dematiaceous fungi. Oud fumes proved highly effective against hyaline fungi, although they were less effective against dematiaceous fungi. Benzoin fumes were moderately effective against both types of fungi.

DISCUSSION

For many years, the use of oud resinous fumes in homes has been a long-standing tradition in Saudi Arabia, often linked to cultural rituals and warm hospitality. Similarly, people in Asia and Africa utilize other plant resinous materials, such as myrrh and benzoin, for spiritual purposes, rituals, home fragrances, and enhancing indoor air quality. [1-3] Saudi Arabia frequently experiences sandstorms, during which dust particles carry numerous microbial particles with fungal

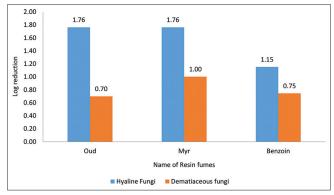


Figure 4: Fungal log reduction after the fumes exposure

allergens, posing health risks to the population. This study examined the antifungal efficacy of oud, myrrh, and benzoin fumes against selected hyaline and dematiaceous fungi isolated from sandstorm dust.

The first plant resin used in this study, "Oud," is derived from Aquilaria trees, which is formed as a natural defense mechanism when the trees are injured or infected by fungi. Its major phytochemicals include sesquiterpenes and sesquiterpenoids, which contribute to deep, woody aroma and significant antimicrobial properties.[4] Because of its unique fragrance most Asian and Middle East people used. A recent review by Rashed et al. highlighted that Aquilaria sinensis, a species of agarwood, is the most studied for its antimicrobial and anti-inflammatory properties.[19] Various studies have demonstrated its potent antifungal activity against dematiaceous (Lasiodiplodia theobromae), hyaline fungi (Fusarium oxysporum), and Candida albicans.[20] Moreover, Various authors have tested minimum inhibitory concentrations (MIC) of agarwood oil against various bacterial isolates, including species of *Bacillus*, *Enterococcus*, Salmonella, Enterobacter, Klebsiella, Proteus, Staphylococcus. [21,22] Despite extensive research on agarwood oil using methods such as Kirby-Bauer disk diffusion and MIC studies, there is a lack of data on the effects of oud fumes on bacterial and fungal isolates.[19-22] This study is the first to evaluate the antifungal efficacy of oud fumes and other plant resin fumes.

The results of the present study demonstrated that oud fumes achieved a control rate of 98.52% against hyaline fungi, corresponding to a 1.76 log reduction in growth. In contrast, the control rate for dematiaceous fungi was lower at 64.2% (0.7 log reduction). This difference in efficacy can be attributed to the melanin pigment present in the cell walls of dematiaceous fungi, which serves as a protective barrier against environmental stressors, including heat, ultraviolet radiation, and enzymatic lysis. [23,24] Melanin pigment also delays the penetration of antifungal agents, including resin fumes, into the fungal cells. In contrast, hyaline fungi lack melanin in their cell walls, and results were showed more susceptible to the effects of oud fumes. These differences highlight the role of melanin pigment and their pathogenicity in dematiaceous fungi.

Regarding a second resin material, myrrh, derived from the sap of *Commiphora* trees (primarily *C. myrrha*), is a resin obtained from approximately 150 species native to Africa, Saudi Arabia, and India. Historically, myrrh has been valued for its therapeutic properties, including its use as an embalming ointment and pain reliever, attributed to its bioactive compounds such as terpenes, steroids, and sterols.^[5] The resin's major phytochemicals, including furanosesquiterpenes and triterpenoids, are responsible for its distinctive warm, earthy aroma and its potent antimicrobial activity against bacterial and fungal pathogens.^[5,25] In the present study, myrrh fumes demonstrated a 98.18% effective

control of the growth of airborne hyaline fungi. These findings are comparable with previous research by Al-Sabri *et al.* and they highlighted the effective antifungal activity of *C. myrrha* L. against airborne fungal allergens. [16] Similarly, Abdel Zaher *et al.* reported myrrh's antifungal activity against *Candida* species, further validating its antifungal potential. [26] Additionally, Alqahtani *et al.* analyzed the furano-sesquiterpenoids in *C. myrrha* extract and found significant antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as antifungal activity against *Candida*. [27]

When tested against dematiaceous fungi, myrrh fumes achieved an 84.3% control rate and a 1.0 log reduction, demonstrating notable efficacy. However, research on the effects of plant resins, particularly against dematiaceous fungi, remains very limited. This highlights the need for further studies to explore the potential of plant resins in controlling dematiaceous fungal isolates. On the other hand, numerous studies have documented the antibacterial efficacy of C. myrrha resin against a range of pathogens, including Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, and Fusobacterium nucleatum.[28-30] In addition, Kim et al. highlighted the antiviral potential of myrrh, with ethanolic extracts of C. myrrha resin showing activity against the H1N1 influenza virus. [25] Collectively, these findings underscore the diverse antimicrobial capabilities of myrrh resin, spanning antibacterial, antifungal, and antiviral activities. Our results further support this, revealing a higher control rate (98.18%) against hyaline fungi compared to dematiaceous fungi. These outcomes were similar to existing research, reinforcing the potential of myrrh as a versatile antifungal agent.

The third resinous fumes evaluated in this study were derived from benzoin, commonly referred to as Al-Jawi. The primary phytochemicals identified in S. benzoin resin include benzoic acid, cinnamic acid, and esters, which are known for their potent antiseptic and anti-inflammatory properties, as documented in the literature. [6,7] In our study, exposure to benzoin fumes demonstrated significant efficacy, controlling over 80% of both hyaline and dematiaceous fungi. These findings are consistent with a similar exposure study conducted in Saudi Arabia, which reported that benzoin resin fumes reduced the microbial load by 70-75% among indoor bacterial isolates collected from university premises.^[3] While the experimental setups and test isolates differed between the studies, the results are comparable, highlighting the antimicrobial potential of benzoin fumes. Furthermore, several studies have evaluated the antibacterial activity of crude extracts of S. benzoin resin. These extracts have shown promising effects against pathogens such as S. aureus, Bacillus thuringiensis, and even Mycobacterium tuberculosis.[31-33] These findings further support the broadspectrum antimicrobial properties of benzoin resin, aligning with the results observed in our study.

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Toxic Effects to In low doses, it is safe, but high doses safe, but high doses may may cause mild gastrointestinal irritation or sedation in animals, with limited toxicity data available. In moderate doses, it is safe, but high doses may moderate doses; can cause respiratory irritation or gastrointestinal distress in animals, and Myrrh oil can be toxic if ingested in large quantities. Safe in low to moderate doses; can cause respiratory irritation or toxicity in animals, and derivatives may cause toxicity, including metabolic acidosis or neurological effects in animals.
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References [4,19,20,22,37-39] [5,16,25-30] [6,7,32,33] [34-36]

A significant observation from this study is that commercial incense cones demonstrated a 100% control rate in fungal growth. However, this does not imply that synthetic incense cones are more effective than natural resins. On the contrary, their high efficacy may be attributed to the presence of toxic commercial agents that inhibit fungal growth. Generally, commercial incense cones are typically composed of wood waste, charcoal, synthetic perfumes, and harmful chemicals such as formaldehyde and polycyclic aromatic hydrocarbons, which are known to be toxic and carcinogenic. [34,35] Research by Elsayed *et al.* highlighted that Arabian bakhoor contains hazardous metals, chemicals, and organic compounds, including eight suspected carcinogens, posing significant risks to human health and indoor air quality. [35]

Numerous studies have analyzed synthetic incense cones and related materials, emphasizing the health and environmental risks associated with their combustion. Major health concerns include respiratory and cardiovascular complications, as well as allergic and dermatological issues, particularly affecting vulnerable populations such as the elderly and children.[34-36] Therefore, the selection of incense brands and their natural composition is a critical factor. Users are advised to investigate the composition of incense products before using them for indoor fume exposure. [34,35] In contrast, the present study highlights the importance of using natural resin fumes, such as myrrh, oud, and benzoin, which have demonstrated significant antifungal properties without adverse health effects. The traditional use of natural plant resins in homes is now supported by scientific evidence highlighting their potential health benefits, particularly in controlling fungal allergens from sandstorm dust.

To further validate, we conducted a brief literature survey comparing natural resins and synthetic incense materials. The results, summarized in Table 2, highlight the antimicrobial efficacy, chemical composition, aromatic profiles, therapeutic properties, and toxicity of these materials. While natural resins exhibit antimicrobial properties with minimal toxicity, synthetic incense cones are often highly toxic and, in some cases, mutagenic due to their synthetic chemical mixtures.

Limitations of the study

This study has certain limitations. The exposure period was limited to 30 min in a controlled bell jar chamber, which does not fully replicate the real environmental setup at homes where fumes are distributed across larger spaces with variable ventilation. In addition, temperature and humidity levels in domestic settings fluctuate, whereas the present study maintained controlled conditions; while the primary focus was on fume effects. These environmental factors may influence the efficacy and distribution of resin fumes in practical settings.

Furthermore, the chemical composition of natural resins can differ depending on their geographic origin, harvesting methods, and storage conditions. This variability may affect their antifungal effect, which was not accounted for in this study. Finally, the efficacy of synthetic incense cones depends on their chemical composition, which differs among manufacturers and is influenced by their specific formulations of active compounds.

CONCLUSION

Myrrh fumes exhibit the highest overall efficacy in controlling both hyaline and dematiaceous fungi from sandstorm dust. Oud fumes, while highly effective against hyaline fungi, show reduced effectiveness against dematiaceous fungi. In contrast, benzoin fumes demonstrated moderate efficacy against both fungal types. Although commercial incense cones achieved a 100% control rate in fungal growth, their use is not recommended due to the presence of toxic commercial agents, which pose significant health risks to humans. To the best of our knowledge, this is the first study to evaluate the antifungal efficacy of resin fumes against sandstorm fungal isolates in Saudi Arabia and globally. In addition, this study addresses the notable gap in research regarding the antifungal activity of resin fumes against dematiaceous fungi.

Future research should focus on large-scale studies to confirm these findings, investigate the detailed molecular mechanisms of action, perform microscopic analysis of fungal structures post-exposure to elucidate the specific damage mechanisms, study potential combination treatments, assess safety and toxicity, and expand testing to include more pathogenic and resistant fungal strains. Such comprehensive research will help optimize the use of resin fumes for antifungal applications and ensure their safe and effective implementation in various settings. The findings supported that the traditional use of resin fumes, particularly myrrh, may serve as a natural and effective method to control fungal allergens in indoor environments during sandstorm events, potentially reducing the incidence of respiratory and allergic reactions among the affected population. However, further research is necessary to fully understand the mechanisms of action and to refine the use of resin fumes for health-related applications.

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