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Medicinal smoke reduces airborne bacteria

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Abstract

This study represents a comprehensive analysis and scientific validation of our ancient knowledge about the effect of ethnopharmacological aspects of natural products' smoke for therapy and health care on airborne bacterial composition and dynamics, using the Biolog® microplate panels and Microlog® database. We have observed that 1 h treatment of medicinal smoke emanated by burning wood and a mixture of odoriferous and medicinal herbs (*havan sámagri* = material used in oblation to fire all over India), on aerial bacterial population caused over 94%

reduction of bacterial counts by 60 min and the ability of the smoke to purify or disinfect the air and to make the environment cleaner was maintained up to 24 h in the closed room. Absence of pathogenic bacteria *Corynebacterium urealyticum*, *Curtobacterium flaccumfaciens*, *Enterobacter aerogenes* (*Klebsiella mobilis*), *Kocuria rosea*, *Pseudomonas syringae* pv. *persicae*, *Staphylococcus lentus*, and *Xanthomonas campestris* pv. *tardicrescens* in the open room even after 30 days is indicative of the bactericidal potential of the medicinal smoke treatment. We have demonstrated that using medicinal smoke it is possible to completely eliminate diverse plant and human pathogenic bacteria of the air within confined space.

Keywords: Medicinal smoke; Ethnopharmacology; Antimicrobial activity; Bacteria; Airborne; Pathogen

Article Outline

1. Introduction

From time immemorial, human beings have used smoke of medicinal plants for curing disorders. Smoke produced from natural substances has been used extensively in many cultures and famous ancient physicians have described and recommended such use. Under the continuous Saraswati–Indus civilization going back to ~7500 BC [i.e., ~9500 Before Present (B.P.)] (Nigam and Hashimi,

2002) the great *rishis* (saints) used to perform *agnihotra–yagnas* to purify the environment as described in *Rigveda*—the most ancient compilation of knowledge on earth by sublimating the *havana sámagri* (mixture of wood and odoriferous and medicinal herbs) in the fire accompanied by the chanting of *Vedic mantras* described in *Rigveda*—the most ancient compilation of knowledge on earth (Kalyanraman, 2004). The records, written on clay tablets in cuneiform, from Mesopotamia dated about 2600 BC; are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation (Mohagheghzadeh et al., 2006). Smoke produced at high temperatures is a simple way of administering a drug, which exhibits rapid pharmacological activity when inhaled. In early Indian writing (Sushruta 800–600 BC), the fumigation of an operating room with fumes of mustard, butter and salt might be considered an early form of "antisepsis" of the air, although it was also used to get rid of evil spirits (Ayliffe and English, 2003). During 522–486 BC, smoke by burning esfand (*Peganum harmala*) and/or sandalwood (*Santalum album*) was used to protect the king from evil and disease (Farahvashi, 2003). Throughout the medieval period, including the terrible years of the bubonic plague caused by the causal bacterium *Yersinia pestis*, the main prophylactic measure against infectious diseases was fumigation by burning incense, herbs and aromatic essences (Ayliffe and English, 2003). Recently, mono- and multi-ingredient herbal and non-herbal remedies administered as smoke from 50 countries across the 5 continents are reviewed (Mohagheghzadeh et al., 2006).

To the best of our knowledge, no detailed scientific study has ever been conducted to elucidate what effect medicinal smoke from the *havan sámagri* may have on the bacterial composition in the atmosphere. This paper, therefore, reports on scientific validation of our ancient knowledge on antibacterial activity of the medicinal smoke during the treatment of a room with the medicinal smoke, emanated by burning wood and a mixture of odoriferous and medicinal herbs (*havan sámagri*, i.e., material for oblation to fire), by generating it within confined spaces so as to purify or disinfect the air and to make the environment cleaner.

In this study we have designed an air sampler for microbiological air sampling during the treatment of the room with medicinal smoke. In addition, elimination of the aerial pathogenic bacteria due to the smoke is reported too.

2. Materials and methods

2.1. Plant material

Impact of medicinal smoke on aerial bacteria by burning wood and a complex mixture of odoriferous and medicinal herbs (*havan sámagri* = material used in oblation to fire all over India) obtained from Gurukul Kangri Pharmacy, Haridwar, UA, India, without any rituals and mantras, was studied in an indoor environment. Mixture consisted of *Aegle marmelos* (L.) Corr. (Rutaceae) wood; *Alpinia galanga* (L.) Willd. (Zingiberaceae) rhizome; *Aquilana malaccensis* Lam. (Thymelaeaceae) wood; *Aquilana agallocha* Roxb. (Thymelaeaceae) wood; *Azadirachta indica* A. juss (Maliaceae) wood; *Butea frondosa* Koen. ex Roxb. (Fabaceae) whole plant; *Cedrus deodara* (Roxb. Ex D. Don) G. Don f. (Pinaceae) rhizome; *Cedrus libani* Loud. (Pinaceae) bark; *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae) fruit; *Cocos nucifera* L. (Palmae) husk fiber; *Commiphora mukul* Engl. (Burseraceae) gum resin, *Cyperus scariosus* R. Br. (Cyperaceae) root; *Ervatamia divaricata* (L.) Burkill (Apocynaceae) whole plant; *Euryale ferox* Salisb (Nymphocaceae) whole plant; *Ficus bengalensis* L. (Moraceae) whole plant; *Ficus glomerata* Linn. (Moraceae) bark and leaf; *Ficus religiosa* L. (Moraceae) bark and stem, *Mangifera indica* Linn. (Anacardiaceae) leaf and wood; *Peganum harmala* L. (Zygophyllaceae) fruit; *Pistacia vera* L. (Anacardiaceae) fruit; *Prosopis spicigera* L. (Mimoseae) leaf; *Prunus dulcis* (P. Mill.) D.A. Webber (Rosaceae) fruit; *Santalum album* L. (Santalaceae) wood; *Sesamum indicum* L. (Pedaliaceae) seed; *Syzygium aromaticum* (L.) Merrill and Perry (Myrtaceae) bud; *Valeriana wallichii* DC. (Valerianaceae) root; *Vitis vinifera* L. (Vitaceae) fruit and seed; *Withania somnifera* (L.) Dunal (Solanaceae) root and *Zanthoxylum armatum* DC. (Rutaceae) fruit and seed. Odoriferous substances consists of *Crocus sativus* L. (Iridaceae) stigma; *Mimulus moschatus* (Scrophulariaceae) flower, *Gelidium amansii* f. elegans (Kützing) Okamura (N.O. Algae) thallus; *Tabernaemontana divaricata* (L.) R. Br ex Roem et Schult. (Apocynaceae) root; *Santalum album* L. (Santalaceae) wood; *Elettaria cardamomum* Maton. (Zingiberaceae) fruit; *Pelargonium fragrans* Willd. (Geraniaceae) whole plant; *Myristica fragrans* Houtt. (Myristicaceae) fruit; *Cinnamomum camphora* Sieb (Lauraceae) leaf and *Cinnamomum macrocarpum* Hook. f. (Lauraceae) leaf; substances with healing properties: clarified butter (ghee), milk, fruit, *Linum perenne* L. (Linaceae) seed, and cereals like *Triticum vulgare* Vill. (Poaceae) seed; *Oryza sativa* L. (Poaceae) seed; *Hordeum vulgare* L. (Poaceae) seed and legumes like *Panicum decompositum* R. Br. (Gramineae) seed; *Vigna mungo* (L.) Hepper (Fabaceae) seed; *Pisum sativum* L. (Fabaceae) seed; Sweet substances: sugar, dried dates, resin, honey, etc; some commonly used herbs are *Tinospora cordifolia* Miers (Meninspermaceae) leaf; *Bacopa monnieri* (L.) Pennell (Scrophulariaceae) leaf; *Convolvulus pluricaulis* (L.) Choisy (Convolvulaceae) whole plant; *Mesua ferrea* L. (Guttiferae) leaf; *Glycyrrhiza glabra* L. (Leguminosae) rhizome; *Terminalia bellirica* (Gaertner) Roxb. (Combretaceae) fruit; dry *Zingiber officinale* Roscoe (Zingiberaceae) rhizome and *Terminalia chebula* Retzius (Combretaceae) fruit ([Acharya, 2001] and [Mohagheghzadeh et al., 2006]).

2.2. Air sampling

Our own Microbiology media preparation and washing room (space volume: 12.93 ft^3) was chosen for the experiment. An apparatus for microbiological air sampling during the treatment of the room with medicinal smoke was designed by using series of Whatman filter paper no. 1 sheet, stainless-steel grid (mesh size 1 mm), polypropylene ring (hole diameter 35 mm), membrane filter (MF; pore size 0.45 μm, diameter 47 mm; Millipore, MA, USA), polypropylene ring, stainless-steel wire mesh, stainless-steel holder connected to a vacuum pump (capacity 1.3 ft^3). The sampler developed for this study was effective to prevent clogging of MF, due to incomplete combustion of organic substances and unburnt carbon, which settle as soot (Fig. 1). Air was drawn through the MF using a vacuum pump having a suction capacity at a rate of 1.3 ft^3 for 27 min for a total of 1000 l air being filtered through MF (Fig. 1).

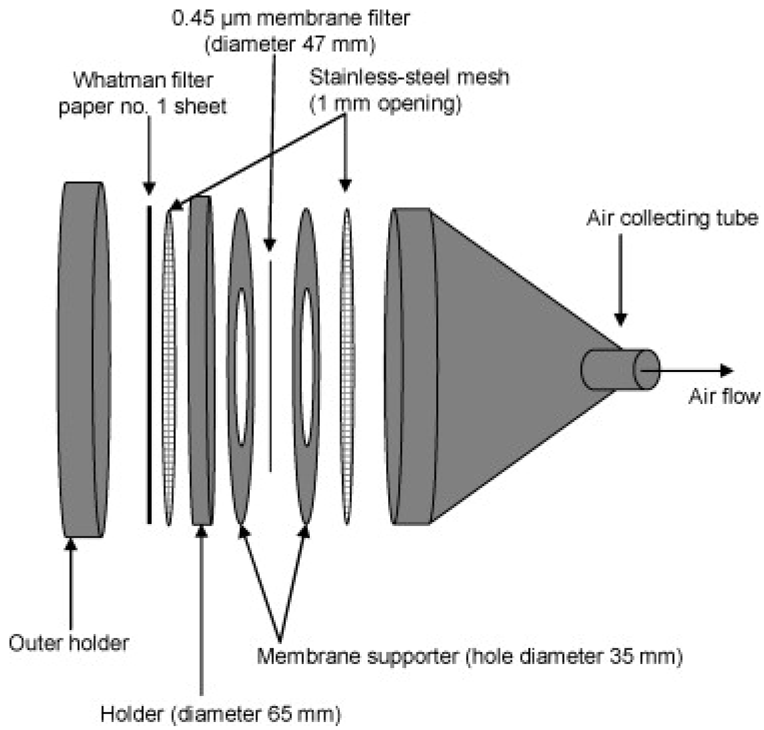

Fig. 1. Air sampling device for microbiological air sampling during the treatment of the room with medicinal smoke.

2.3. Medicinal smoke treatment

Airborne bacteria were collected in the beginning, i.e., 0–1 h after burning 1000 g *Mangifera indica* wood alone (without mixture of odoriferous and medicinal herbs), in a stainless-steel vessel served as control (Fig. 2). After 1 h, 500 g mixture of odoriferous and medicinal herbs on the burning *Mangifera indica* wood was added and air sampling was recorded up to 24 h for each round of test in triplicate to elucidate the effect of medicinal smoke on aerial bacteria (Fig. 2), in the closed room. After 24 h the windows and door of the room were opened and airborne bacteria were counted on the 7th, 15th and 30th day. Values are the mean ± S.E. of three samples.

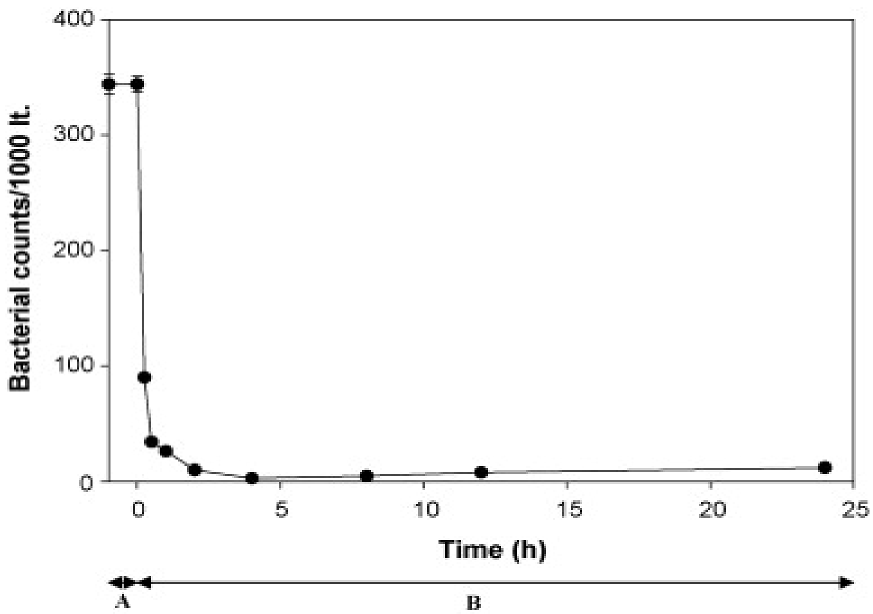

Fig. 2. Effect of the medicinal smoke emanated by burning wood (A) and a mixture of odoriferous and medicinal herbs (*havan sámagri*) (B), on the survival of aerial bacterial population (●). Values are the mean ± S.E. of three samples.

2.4. Bacterial culture media and identification

After each sample was taken, the MF was aseptically removed and placed in a 90 mm Petri dish directly on Nutrient Agar (from HI-MEDIA Laboratories Pvt. Ltd., Mumbai, India). After 72 h of incubation at 28 °C, the colonies that developed on the plate were counted and transferred to NA slants (Nautiyal et al., 2006). They were all grown on Biolog® BUG® agar for identification by the Biolog® system (Biolog Inc., Hayward, CA, USA). In preparation for analysis colonies were picked from pure cultures of bacteria and were plated as a lawn of bacteria on to a BUG® agar plate, separate plates were set up for each strain to be analyzed. The Biolog Microlog® Bacterial Identification System consists of databases combined with specialized 96-well plates (test panels). A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for "Metabolic Fingerprint" (June et al., 2006). The Gram-positive and Gram-negative panels and databases were used in this study.

3. Results and discussion

From ancient times, human beings have been studying the relationship between ill health and medicine. All cultures have strong traditions of folk medicine that include the use of plants, animals and minerals. The ancient cultures are known for their systematic collection of information on herbs and their rich and well-defined herbal pharmacopoeias. Medicinal plant therapy is based on the empirical findings of hundreds and thousands of years.

The ethnopharmacological aspects of natural products' smoke for therapy and health care have not been studied (Mohagheghzadeh et al., 2006). In this light the present report attains importance as we observed that medicinal smoke emanated from *havan sámagri* on aerial bacterial population showed very interesting inhibition effects on the aerial bacterial population (Fig. 2). There was no reduction in the number of bacteria of burning *Mangifera indica* wood alone (Fig. 2). However, medicinal smoke caused over 94% reduction of aerial bacterial counts by 60 min (Fig. 2). In the presence of medicinal smoke bacterial counts were 90, 34, 26, 10, 3, 5, 8 and 12, at 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h, respectively (Fig. 2). Bacterial counts on the 7, 15 and 30 days were 35, 86 and 128 in the room, respectively. Ability of the smoke to purify or disinfect the air and to make the environment cleaner was maintained even after removing the stainless-steel vessel used to generate the medicinal smoke, as evident form over 96% reduction of aerial bacterial counts up to 24 h in the closed room (Fig. 2). Absence of pathogenic bacteria *Corynebacterium urealyticum*, *Curtobacterium flaccumfaciens*, *Enterobacter aerogenes* (*Klebsiella mobilis*), *Kocuria rosea*, *Pseudomonas syringae* pv. *persicae*, *Staphylococcus lentus*, and *Xanthomonas campestris* pv. *tardicrescens* in the open room even after 30 days is indicative of the bactericidal potential of the medicinal smoke treatment. Work has implications to use the smoke generated by burning wood and a mixture of odoriferous and medicinal herbs, within confined spaces such as animal barns and seed/grain warehouses to disinfect the air and to make the environment cleaner.

The occurrences of wild fires during dry seasons and agriculture burning in many parts of the world results in large volumes of volatile compounds being released into the atmosphere ([van Staden et al., 2004] and [Wu et al., 2006]). These smoke clouds contain particulate matter, water vapour, gases and pyrolysis products which can drift over vast distances and may potentially impact on plants outside the immediate vicinity of the fire, either directly as aerosol smoke, or via aqueous media, be it moist soil or water runoff into streams, lakes and impoundments. The fascinating discovery that stimulates seed germination in nature (De Lange and Boucher, 1990) has captured the imagination of plant scientists from a wide range of interdisciplinary fields. The slow combustion of different types of vegetation and organic products at temperatures between 180 and 200 °C produces a highly active, heat stable, long lasting compound, 3-methyl-2*H*-furo [2,3-*c*]pyran-2-one (1), that stimulates seed germination was isolated from plant-derived smoke water using bioactivity-guided fractionation ([Flematti et al., 2004] and [van Staden et al., 2004]). Recent study has revealed that the effects of smoke extend beyond germination stimulation and can also act to enhance seedling vigour (Sparg et al., 2005). The physiological mechanism resulting in improved vigour is unknown. However, smoke may protect the seed and seedlings against microbial attack (Roche et al., 1997), which can result in higher seedling survival ([Sparg et al., 2005] and [Kulkarni et al., 2007]). Although it is currently unknown how smoke acts to promote germination and improve seedling vigour, aerosol smoke and smoke solutions can potentially be used for a variety of applications related to seed technology. Examples of their potential use include applications in horticulture, agriculture, seed pre-treatment, weed control, ecological management and habitat restoration (Verschaeve et al., 2006).

In this study we have demonstrated that 1 h treatment of medicinal smoke produced by burning wood and a mixture of odoriferous and medicinal herbs resulted into complete elimination of plant pathogenic bacteria *Burkholderia glumae* causing grain and seedling rot of rice (Li et al., 2004), *Curtobacterium flaccumfaciens* causing wilting in beans (Yin et al., 2005), *Pseudomonas syringae* pv. *persicae* causing necrosis of peach tree tissues (Barzic and Guittet, 1996) and *Xanthomonas campestris* pv. *tardicrescens* causing black rot in crucifers (Soengas et al., 2007) (Table 1). Work indicates that certain known medicinal constituents from the *havan sámagri* can thus be added to the burning farm material while disposing unwanted agriculture organic material, in order to reduce plant pathogenic organisms.

Table 1.

Antibacterial activity of the medicinal smoke emanated by burning wood and a mixture of odoriferous and medicinal herbs (*havan sámagri*) against the aerial bacteria strains, up to 24 h

Bacterial species	**Time (h) of sampling**[a]								
	0	**1/4**	**1/2**	**1**	**2**	**4**	**8**	**12**	**24**
Gram-positive									
Bacillus cereus	+	+	+	+	+	+	+	+	+
Bacillus megaterium	+	+	+	+	+	+	+	+	−
Cellulomonas turbata	+	+	+	+	+	+	+	+	+
Clavibacter agropyri	+	+	+	+	+	−	−	−	−
Corynebacterium urealyticum	+	+	+	+	+	+	+	+	−

Bacterial species	Time (h) of sampling[a]								
	0	1/4	1/2	1	2	4	8	12	24
Curtobacterium flaccumfaciens	+	+	+	+	+	+	+	+	−
Kocuria rosea	+	+	+	+	−	−	−	−	−
Leifsonia aquatica	+	+	+	+	+	+	+	+	+
Sanguibacter suarezii	+	+	+	+	+	+	+	+	+
Sphingomonas yanoikuyae	+	+	+	−	−	−	−	−	−
Staphylococcus lentus	+	+	+	+	+	+	−	−	−
Staphylococcus xylosus	+	+	+	−	−	−	−	−	−
Tsukamurella inchonensis	+	+	+	+	+	+	−	−	−
Gram-negative									
Burkholderia glumae	+	+	+	+	+	+	+	−	−
Burkholderia plantarii	+	+	+	+	+	+	+	−	−
Curtobacterium citreum	+	+	+	+	−	−	−	−	−
Enterobacter aerogenes (*Klebsiella mobilis*)	+	+	+	+	+	+	+	+	−
Flavobacterium johnsoniae	+	+	+	+	+	+	+	+	+
Pantoea agglomerans	+	+	+	−	−	−	−	−	−
Pseudomonas bathycetes	+	−	−	−	−	−	−	−	−
Pseudomonas corrugate	+	+	+	+	+	+	+	−	−
Pseudomonas stutzeri	+	−	−	−	−	−	−	−	−
Pseudomonas syringae pv. *persicae*	+	+	+	+	+	+	+	+	−
Sphingobacterium multivorum	+	+	+	+	+	+	+	−	−
Sphingobacterium spiritovorum	+	+	+	+	+	+	+	+	−
Sphingomonas parapaucimobilis	+	−	−	−	−	−	−	−	−
Sphingomonas sanguinis	+	−	−	−	−	−	−	−	−

Bacterial species	**Time (h) of sampling[a]**								
	0	**1/4**	**1/2**	**1**	**2**	**4**	**8**	**12**	**24**
Xanthomonas campestris pv. *tardicrescens*	+	−	−	−	−	−	−	−	−

[a] Results of air sampling in triplicate: (+), survived; (−), not survived.

Airborne transmission is known to be a route of infection for diseases. About 15 million (>25%) of 57 million annual deaths worldwide are estimated to be related directly to infectious diseases (WHO, 2004). One most interesting aspect of the work of great applied significance is the ability of the medicinal smoke to completely eliminate human pathogenic bacteria *Corynebacterium urealyticum* causing urinary tract infection (Nebreda-Mayoral et al., 1994), *Kocuria rosea* causing catheter-related bacteremia (Altuntas et al., 2004), *Staphylococcus lentus* causing splenic abscess (Karachalios et al., 2006), *Staphylococcus xylosus* causing acute polynephritis (Angelina et al., 1982), *Tsukamurella inchonensis* causing acute myelogenous leukemia (Yassin et al., 1995), *Enterobacter aerogenes* (*Klebsiella mobilis*) nosocomial infections (Peres-Bota et al., 2003), *Sphingobacterium spiritovorum* causing extrinsic allergic alveolitis (Sato and Jiang, 1996) and *Sphingomonas sanguinis* causing nosocomial non-life-threatening infections (Li et al., 2004) (Table 1). Smoking has some important benefits compared to other drug routes of administration. It is an inexpensive and simple way of extraction and can be a method of drug use that is acceptable to many people. More importantly, generating smoke reduces the particle size to a microscopic scale thereby increasing the absorption of its active principles. Most of the ancient drugs are still being used in the same dosage and form although smoking today is more often a form of drug abuse than as a therapeutic because of its highly effective and rapid pharmacological action. Whereas inhalation is typically used in the treatment of pulmonary and neurological disorders and directed smoke in localized situations, such as dermatological and genito-urinary disorders, ambient smoke is not directed at the body at all but used as an air purifier. The advantages of smoke-based remedies are rapid delivery to the brain, more efficient absorption by the body and lower costs of production (Mohagheghzadeh et al., 2006).

Many of human and plant pathogens reported in Table 1 can easily be considered to be of importance of its potential implications on human health, agricultural productivity, and ecosystem stability; surprisingly little is known regarding the composition or dynamics of the aerial microbial inhabitants. Using a custom high-density DNA microarray, Brodie et al. (2006) have detected in urban aerosols at least 1800 diverse bacterial types, a richness approaching that of some soil bacterial communities. Their work has also revealed the consistent presence of bacterial families with pathogenic members. Thus it is very much plausible that further research will enable more accurate scope of the utility of the use of medicinal smoke against indoor airborne infections.

This study represents a comprehensive analysis and scientific validation of the effect of ethnopharmacological aspects of natural products' smoke on airborne bacterial composition and dynamics. In particular, it highlights the fact that we must think well beyond the physical aspects of smoke on plants in natural habitats and impacts heavily on our understanding of fire as a driving force in evolution. We have demonstrated that using medicinal smoke it is possible to contain diverse pathogenic bacteria of the air we breathe. The work also highlights the fact about medicinal smoke and that a lot of natural products have potential for use as medicine in the smoke form as a form of drug delivery and as a promising source of new active natural ingredients for containing indoor airborne infections within confined spaces used for storage of agriculture commodities. The dynamic chemical and biological interactions occurring in the atmosphere are much more complex than has been previously realized. The findings warrant a need for further evaluation of various ingredients present in the complex mixture of odoriferous and medicinal herbs, individually and in various combinations to identify the active principles involved in the bactericidal property of the medicinal smoke, applied in the above-discussed fashion.

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