

The Effect of aerosols on the air microflora of the indoor air

ABSTRACT

This research work assessed the microflora of rooms sprayed with different insecticides and air fresheners with the aim of investigating the effect of the aerosols on the types of microflora in the room environment. Eight (8) different samples of chemical aerosols were used they are: Mobile insecticide (Imidacloprid), Raid multipurpose insect killer (1R-trans Phenothrin), Morten Insecticide (pyrethroids), Rambo Insecticide (pyrethroid compound), as categorized as Insecticides, while Febreze (hydroxypropyl beta-cyclodextrin), Air wick (Dipropylene glycol monomethyl ether (aka dipropylene glycol methyl ether), Glade (allyl 3-cyclohexylpropionate, allyl caproate, benzyl alcohol, butylated hydroxytoluene (BHT) and Top breeze (Cyclodextrin) were purchased as air fresheners/fragrance and eight (8) different rooms were used. Microorganisms isolated from the rooms before and after spraying with aerosols were: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus flavus*, *Aspergillus niger*, *micrococcus spp.*, *Aerococcus viridans*, *Pediococcus cerevisiae*, *Streptococcus spp*, *Aspergillus fumigatus* and *Aspergillus niger*. The result of eight different rooms sprayed with different aerosol as Insecticide and air fresheners showed that, some aerosols were able to inhibit some organisms that were initially present in some rooms while there were introduction of another organisms from some aerosols into some rooms. The occurrence of *Staphylococcus aureus* (100%) was the highest in all the rooms followed by *Aspergillus niger* (87.5) and *A. flavus* (75%). *Lactobacillus jensenii*, *Bacillus coagulans* and *micrococcus spp* had the lowest frequency of occurrence (12.5%).

Keywords: Air environment; aerosols; microflora; Indoor; microbial load

28 INTRODUCTION

29 Background to the study

30
31 Each day people are exposed to millions of bio aerosols, including whole microorganisms, which
32 can have both beneficial and detrimental effects. Assessment of the indoor of the built
33 environment, the aerobiomes is important and they are bacteria, viruses, fungi and their spores
34 are examples of bio aerosols present in the air, inhaled by human beings. According to **Smith et**
35 **al. (2013)** major sources of these bioaerosols are: humans, pets, plants, plumbing systems,
36 heating, ventilation, and air-conditioning systems, dust, suspension; aesthetic pollutant and the
37 outdoor environment. Recent advances **in molecular** sequencing have generated a rush to
38 characterize the microbiome of various environments including indoor and outdoor air (Smith *et*
39 *al.*, 2012; **DeLeon-Rodriguez et al., 2013; Kelley et al., 2013; Smith et al., 2013**). This is because
40 humans spend over 90 % of their time indoors (Klepeiset *al.*, 2001) Researchers have observed
41 that there are diverse microbial communities in indoor environments such as schools, houses,
42 and **hospital** (**Rintala et al., 2008; Tringe et al., 2008; Kembel et al., 2012**) rooms within the
43 same building. For **instance**, Dunnet *al.* (2013) **and Adams et al. (2014)** revealed that microbial
44 isolates in the bedroom differs from that of the bathroom within the same building.

45 Despite rapid advances in the characterization of airborne microbial communities through rRNA
46 surveys, metagenomics, proteomics, and metabolomics, limited information is available about
47 actual concentrations of airborne microorganisms in built environments. In one of the few studies
48 of concentrations of total bacteria and viruses in indoor air by air sampler, **Prussin et al. (2015)**
49 found virus-like and bacteria-like particle concentrations of approximately 10^5 and 10^6 particles
50 m^3 in various indoor and outdoor air environment, respectively (Shelton *et al.*, 2002). **Moreover**
51 an average viable airborne fungi concentration of $80 CFU/m^3$ were reported in samples collected

52 from schools, hospitals, residences, and industrial buildings; However, in some instances
53 concentrations were as high as 10^4 CFU m³. Such information should be forthcoming as methods
54 for quantitative metagenomics analyses air samplers become more powerful (Shelton *et al.*,
55 2002; Frank *et al.*, 2011; Gilbert *et al.*, 2011; Duhaime *et al.*, 2012).

56 In confined environments geared for both industrial and non-industrial activities, the presence of
57 microbial pollutants may elicit the deterioration of indoor air quality (IAQ). Generally, in healthy
58 indoor occupational environments, microflora concentrations are lower than outdoor
59 concentrations (ACGIH 1989; Macheret *et al.*, 1995). In indoor environments, air from identifiable
60 sources may be responsible for exposure to microbial pollutants through phenomena like
61 diffusion, accumulation and concentration. As people spend 80–95% of their time indoors, air
62 pollution is frequently reported to cause health problems (WHO 1983, 1984). Diverse studies
63 have demonstrated that dust particles, macromolecular organic compounds, Gram-negative
64 bacteria and total volatile organic compounds may cause nasal, optical and physiological
65 changes and sensory symptoms exemplified by irritation, sluggishness, sleepiness, headache and
66 reduced ability to concentrate (Gyntelberget *et al.*, 1994; Pan *et al.*, 2000). The presence of any type
67 of micro-organism can be problematic to IAQ, particularly bacteria and fungi (Stetzenbach *et al.*,
68 1998). In residential and public buildings like schools. Microbial growth is associated with
69 adverse health effects (Husman *et al.*, 1996; Haverinen *et al.*, 1999). Airborne concentrations of
70 *Cladosporium*, *Epicoccum* and *Coprinus* spores were associated with peak expiratory flow rates
71 (PEFRs) deficiency in children (Neaset *et al.*, 1996). The presence of moisture damage in school
72 buildings was a significant risk factor for respiratory symptoms in schoolchildren (Meklin *et al.*,
73 2002). Because of their lower water activity (*A_w*) requirements compared with bacteria, fungi
74 are the principal contaminant in various types of substrates. They tend to colonize a wide variety

75 of humid building materials wetted by floods, condensation or plumbing leaks. Consequently,
76 when fungal proliferation occurs, aerospores are abundantly distributed on and around the
77 surfaces, and the indoor environment becomes a source of exposure to occupants. Knowledge of
78 indoor environmental mycoflora is especially important from an allergologic view-point, which,
79 in many cases differs from that observed in outdoor environments. Although less frequent than
80 the possible dangers caused by exposure to pollen and acari, fungal exposure causes
81 hypersensitive reactions which characterize allergic respiratory pathologies like bronchial asthma
82 and rhinitis (Burge 1989). Fungi may elicit allergic symptoms similar to those caused by pollen.
83
84 With an ever-increasing population utilizing different types of aerosols as insecticides and air
85 fresheners, in order to improve and sustain health and vitality; and consuming products in which
86 these supplements are used as room flavors, it is essential that these products are safe for human
87 use. A very critical indicator of safety is the microbiological quality of these products. To
88 improve the prediction of dispersion models and the environmental health assessment on the one
89 hand and to get an insight on the airborne micro-organisms in other relevant environments, e. g.
90 living spaces. However these studies give insight in the internal structure of bio-aerosols and the
91 distribution of micro-organisms on airborne particles themselves for developing guidelines in
92 order to achieve and maintain safe microbial levels in these products.

93 Therefore, the aim of the study are to, isolate microorganism in air environment of
94 rooms sprayed with selected chemical aerosols and investigate the effect of the aerosols
95 on the load of microflora in the room environment

96

97

98 **MATERIALS AND METHODOLOGY**

99 **Study area**

100 The sampling area was an inbuilt living rooms in a house at Akure and the aerosols were
101 purchased from Shoprite shopping mall located at alagbaka, Akure, Ondo State, Nigeria.

102 **Collection of the samples**

103
104 Eight (8) different samples of chemical aerosols were purchased from shoprite shopping mall,
105 alagbaka, Akure, Ondo State, Nigeria. The selected aerosols were; insecticide **Imidacloprid**, **IR-**
106 **trans Phenothrin**, **pyrethroids**, **pyrethroid compound**, as categorized as Insecticides, while
107 **hydroxypropyl beta-cyclodextrin**, **dipropylene glycol methyl ether**, **allyl 3-**
108 **cyclohexylpropionate**, **allyl caproate**, **benzyl alcohol**, **butylated hydroxytoluene (BHT)** and
109 **Cyclodextrin** were purchase as air fresheners/fragrance,

110 **Experimental design**

111 The experimental design is 8x3; **eight** (8) rooms were sprayed with each of the eight selected
112 chemical aerosols, Petri-dishes were prepared **a**septically in triplicates and exposed to each room
113 10 minutes after spraying with insecticides and air fresheners.

114

115 **Microbial isolation and determination of total viable counts**

116 The method used for isolation and identification of microorganisms was as described by **Olutiola**
117 **et al. (1991)**. Twenty (20ml) of nutrient agar and acidified potato dextrose agar cooled to 45⁰C
118 was poured separately onto each of the plates in triplicate and the plates were gently **swirled** and
119 allowed to **solidify**. The plates were exposed to air in the room before and after spraying with
120 aerosols for 10 minutes. Thereafter, the nutrient agar plates were incubated in an inverted
121 position at 37⁰ ± 2⁰C for 24 hours for isolation of mesophilic bacteria while Potato Dextrose

122 Agar plates were incubated at $28^{\circ} \pm 2^{\circ}\text{C}$ for 72 hours. Anaerobic plates were inverted in the
123 anaerobic jar at $37^{\circ} \pm 2^{\circ}\text{C}$ for 24 hours for isolation of anaerobic organisms present in the
124 samples. After incubation, colonies on the plates were counted using colony counter and the
125 number of viable cells obtained to be the total viable counts of the isolates. The viable colonies
126 were sub cultured from mixed culture plate to obtain a pure culture. The colonies were then
127 identified directly by their size, shape, colour of the pigment (chromogenesis), opacity, elevation,
128 surface, edge and consistency and stored on agar slants for further biochemical tests.

129

130 **Determination of microbiology of the air samples**

131 Microbiological analysis were determined according to the procedure of (Buchaman and
132 Gibbons, 1975; Gerhardt, 1981). The microbiological analysis includes isolation of
133 microorganisms from the air samples, direct and microscopic observation of the isolates,
134 biochemical identification of the isolates (Olutiola *et al* 1991). (which include gelatin hydrolysis,
135 a starch hydrolysis, casein hydrolysis, catalase test, coagulase test, indole test, urease test, nitrate
136 reduction test, sugar fermentation test, oxidative fermentation (O/F) test, methyl red Voges-
137 proskaur test, citrate test and oxidase test and motility test.

139

140 **Identification of fungal Isolates**

141 Moulds were identified based on cultural and morphological features using light microscope also
142 number of colony isolated was recorded (Barnett and Hunter, 1998; Labbe and Garcia, 2001).
143 Cultural characterization was based on the rate of growth, presence of aerial mycelium, colour of
144 aerial mycelium as well as colour on the obverse and reverse of the plates. Microscopic
145 identification was based on spore and conidiophore morphology.

169 Table 1 revealed the bacteria Isolated before and after spraying all the rooms with different
170 aerosols are: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Micrococcus spp*,
171 *Aerococcus viridans*, *Pediococcus cerevisiae*, *Streptococcus spp*. Table 2 shows the fungi
172 isolated before and after spraying; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*
173 and *Aspergillus niger*. Before spraying the room with Mobil *Insecticides*, the microorganisms
174 isolated were: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus*
175 *flavus* and *Aspergillus niger*, after spaying the room with *Imidacloprid*, the Insecticide was able
176 to inhibit the growth of *Lactobacillus jensenii*, *Bacillus coagulans*, However, there was an
177 introduction of a new organisms (*Micrococcus spp*) which was not present initially. The
178 microorganisms isolated were able to inhibit the growth of *Lactobacillus jensenii*, *Bacillus*
179 *coagulans* and *Aspergillus flavus* that were present in the room after spraying. However, *there*
180 was an introduction of new organisms (*Micrococcus spp*) which was not present initially before
181 spraying the room with *IR-trans Phenothrin*, microbes reported were: *Staphylococcus aureus*,
182 *Aerococcus viridans*, and *Pediococcus cerevisiae*. *Streptococcus spp*, *Aspergillus fumigatus*,
183 *Aspergillus flavus*, after spraying there was inhibition of *Streptococcus spp* only by *pyrethroids*
184 thereafter before spraying *pyrethroid* into the rooms, microorganism isolated were:
185 *Staphylococcus aureus*, *Aerococcus viridans*, *Pediococcus cerevisiae*. *Streptococcus spp*,
186 *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* after spraying it was discovered that
187 *pyrethroid* was able to inhibit all the organisms present initially except *Staphylococcus aureus* and
188 *Aspergillus flavus*.

189 Similarly, before spraying hydroxypropyl beta-cyclodextrin air fresheners, the microorganisms
190 reported were: *Staphylococcus aureus*, *Streptococcus spp*, *Aspergillus fumigatus* and *Aspergillus*
191 *niger*. Then after spraying, it was discovered that hydroxypropyl beta-cyclodextrin was not able

192 to inhibit all the initial organisms present. There was an introduction of three new organisms
193 which are: *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus flavus*, likewise before
194 spraying with Air wick, microorganism present were: *Staphylococcus aureus*, *Streptococcus spp*,
195 *Aspergillus flavus* and *Aspergillus niger*, and after spraying; it was discovered that There was no
196 difference between the type of organism present before and after spraying the room with
197 dipropylene glycol methyl ether. Similarly, before spraying both BHT and Cyclodextrin into the
198 rooms, the following microorganism were isolated: *Staphylococcus aureus*, *Streptococcus spp*,
199 *Pediococcus cerevisiae*, *Aspergillus flavus* and *Aspergillus niger* and for Cyclodextrin spray, the
200 isolates were: *Staphylococcus aureus*, *Pediococcus cerevisiae*, *Aspergillus fumigatus*, and
201 *Aspergillus niger*. However, after spraying the room, it was discovered that there was no
202 difference between the type of organism present before and after spraying the room with BHT.
203 Similarly, there was no difference between the type of organism present before and after
204 spraying the room with Cyclodextrin. However, there was an introduction of *A. flavus*. The
205 occurrence of *Staphylococcus aureus* (100%) was highest in all the rooms followed by
206 *Aspergillus niger* (87.5) and *A. flavus* (75%). *Lactobacillus jensenii*, *Bacillus coagulans* and
207 *Micrococcus spp* had the lowest frequency of occurrence (12.5%) as shown on table 3 and
208 figures: 1-8. The result of the morphological, microscopic and biochemical characterization of all
209 the organisms isolated before and after spraying are shown in table 4-6

The highest percentage occurrence (100%) is *Staphylococcus aureus* followed by *Aspergillus niger* (87.5) and *A. flavus* (75%). while *Lactobacillus jensenii*, *Bacillus coagulans* and *Micrococcus spp* had the lowest frequency of occurrence (12.5%). These pathogens could be linked with several infectious organisms responsible for gastroenteritis, respiratory tract infections, urinary tract infections and skin disorders. As *Staphylococcus aureus* belong to the normal flora of the human skin and nose, revealed that these organism may be originated from the nose and skin flora of the occupant of the rooms.

However, this higher incidence of *Staphylococcus aureus* obtained from this study correlate with several and similar findings of the studies conducted by several researchers. A study conducted by Yaghoub and Elagbash (2010) at Omdurman and El-Rhibat hospital Sudan found that *Staphylococcus aureus* was the predominant bacteria isolated from these hospitals. This study also supported the finding of Sheik *et al.* (2015), in which the occurrence was reported to be 38% in a research conducted to detect the airborne microorganism from a college in Saudi Arabia. In a review of indoor bioaerosols, Nazaroff *et al.* (2014s) suggested that the penetration efficiency of bioaerosols is close to 100 % in a naturally ventilated building, meaning that all bioaerosols flowing through leaks and openings in the building environment arrive indoors. In fact, Prussin *et al.* (2015) showed that concentrations of bacteria-like and virus-like particles were approximately two times higher in outdoor air than in indoor air, suggesting that human occupant might not be the only component shaping the microbial structure of indoor air environment.

The microbial community structure of indoor air varies geographically, depending on the external factors such as temperature, humidity, oxygen etc. However, some specific chemical air pollutants insecticides and fresheners like the samples used in the experiment, affected the distribution of some microorganisms because microorganisms were discovered before spraying and some of the microbes found before spraying might not be seen after spraying due to the fact that the chemical aerosols inhibited the growth of some of these microbes, this shows that these microbes are very sensitive to the aerosols. For those microbes that were seen after spraying, they were not inhibited by the chemical aerosols, this means they adapt or tolerate the condition, so the spray do not have effect on the microbes.

From midacloprid Insecticides the microorganisms reported were: *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus flavus* and *Aspergillus niger*. . However, after spraying the room the same insecticides, the Insecticide was able to inhibit the growth of *Lactobacillus jensenii*, *Bacillus coagulans*, from the report, there was an introduction of a new organisms (*Micrococcus spp*) which was not present initially. Furthermore the insecticide was able to inhibit the growth of *Lactobacillus jensenii*, *Bacillus coagulans* and *Aspergillus flavus* that were present in the room after spraying. However, there was an introduction of a new organisms (*Micrococcus spp*) which was not present initially.

Before spraying the room with IR-trans Phenothrin, the microbes isolated were: *Staphylococcus aureus*, *Aerococcus viridans*, *Pediococcus cerevisiae*. *Streptococcus spp*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* and after spraying there was

inhibition of *Streptococcus spp* only by **pyrethroids** Insecticide. Before spraying **pyrethroid** into the rooms, microorganism identified were: *Staphylococcus aureus*, *Aerococcus viridans*, *Pediococcus cerevisiae*. *Streptococcus spp*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger* after spraying it was discovered that the Insecticide was able to inhibit all the organisms present initially except *Staphylococcus aureus* and *Aspergillus flavus*.

Similarly, before spraying **hydroxypropyl beta-cyclodextrin**, the initial microorganisms identified were: *Staphylococcus aureus*, *Streptococcus spp*, *Aspergillus fumigatus* and *Aspergillus niger* but after spraying it was discovered that the chemical was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: *Lactobacillus jensenii*, *Bacillus coagulans*, *Aspergillus flavus*, And also before spraying with Air wick microorganism present are: *Staphylococcus aureus*, *Streptococcus spp*, *Aspergillus flavus* and *Aspergillus niger*, and after spraying the it was discovered that There was no difference between the type of organism present before and after spraying the room with **dipropylene glycol methyl ether**. Similarly before spraying both BHT and Cyclodextrin into the rooms the microorganism that were isolated were: *Staphylococcus aureus*, *Streptococcus spp*, *Pediococcus cerevisiae*. *Aspergillus flavus* and *Aspergillus niger* and for **Cyclodextrin**, the isolates were; *Staphylococcus aureus*, *Pediococcus cerevisiae*. *Aspergillus fumigatus*, and *Aspergillus niger* after spraying it was discovered that there was no difference between the type of organism present before and after spraying the room with BHT and there was no difference between the type of organism present before and after spraying the room with **Cyclodextrin**. However, there was an introduction of *A. flavus*, so a single community profile cannot be applied to all indoor settings to account for the influence of outdoor air.

Adams *et al.*, (2015) sought to determine how outdoor air and human occupancy affected bacterial microbial communities in a mechanically ventilated, office-like building. Although the authors found that human occupancy was associated with increased levels of bioaerosols associated with the human body, occupancy did not have the most profound effect on the microbiome. Rather, microbial communities observed in indoor air were closely related with those in outdoor air, and changes in microbial communities in outdoor air were mirrored by changes in indoor air. The observation recorded in this study showed an overlap in the microbial taxa in aerosol samples collected in indoor air. The observation indicated high abundances of *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *Micrococcus spp.*, *Aerococcus viridans*, *Pediococcus cerevisiae* and *Streptococcus spp.*, which are typically classified as outdoor-associated microorganism. This study led to the conclusion that outdoor air might exert a stronger influence on microbial communities than does human occupancy in the built environment that is well ventilated and has moderate occupancy.

Compared to airborne bacteria, fungi are even more strongly correlated between indoor and outdoor air Adams *et al.*, (2013). Typically most airborne fungi found indoors are presumed to originate from outdoors, except in water-damaged buildings. In residential homes, Adams *et al.*, (2013) showed that indoor and outdoor air were dominated by *Cryptococcus victoriae*, *Cladosporium spp.*, *Epicoccum spp.*, and *Penicillium spp.* and that the fungal community structure varied seasonally contrary to this finding. Lee *et al.*, (2005) found an indoor/outdoor (I/O) ratio of 0.345 for total fungal spores and 0.025 for pollen grains. Additionally, indoor fungal

and pollen concentrations followed trends in outdoor air concentrations. The low I/O ratio for pollen grains reflected the low penetration efficiency of large particles into the built environment compared to smaller spores.

This result is also in conformity with the result obtained by [Badri *et al.* \(2016\)](#), who reported *Staphylococcus aureus* as the highest bacteria isolated from their study.

In the present study *Staphylococcus aureus* was the dominant isolated organism and this bacterium is a common causative agent of various human diseases, it is responsible for many gastrointestinal tract infections, respiratory tract infections and skin disorders (Yaghoub and Elagbash, 2010). The reasons for high percentage frequency of occurrence of bacteria in this study could be due to low minimal usage of disinfection procedures against airborne pathogens,

It is well known that microorganisms is able to penetrate effectively from outdoor air into the built environment (Chen and Zhao 2011) In fact, in some cases variation in outdoor microorganisms explains the majority of variation in microorganism in the built environment (Cyrus *et. al.*, 2004)

CONCLUSION

Conclusively, it was important to determine the type of microflora present in the built environment. The outcome of this research revealed that some aerosols were able to inhibit some organisms that were initially present in experimental rooms while there were

introduction of another organisms from some aerosols into some rooms. This shows that, airborne microbiome can be emitted into any environment through the use of aerosols.

UNDER PEER REVIEW

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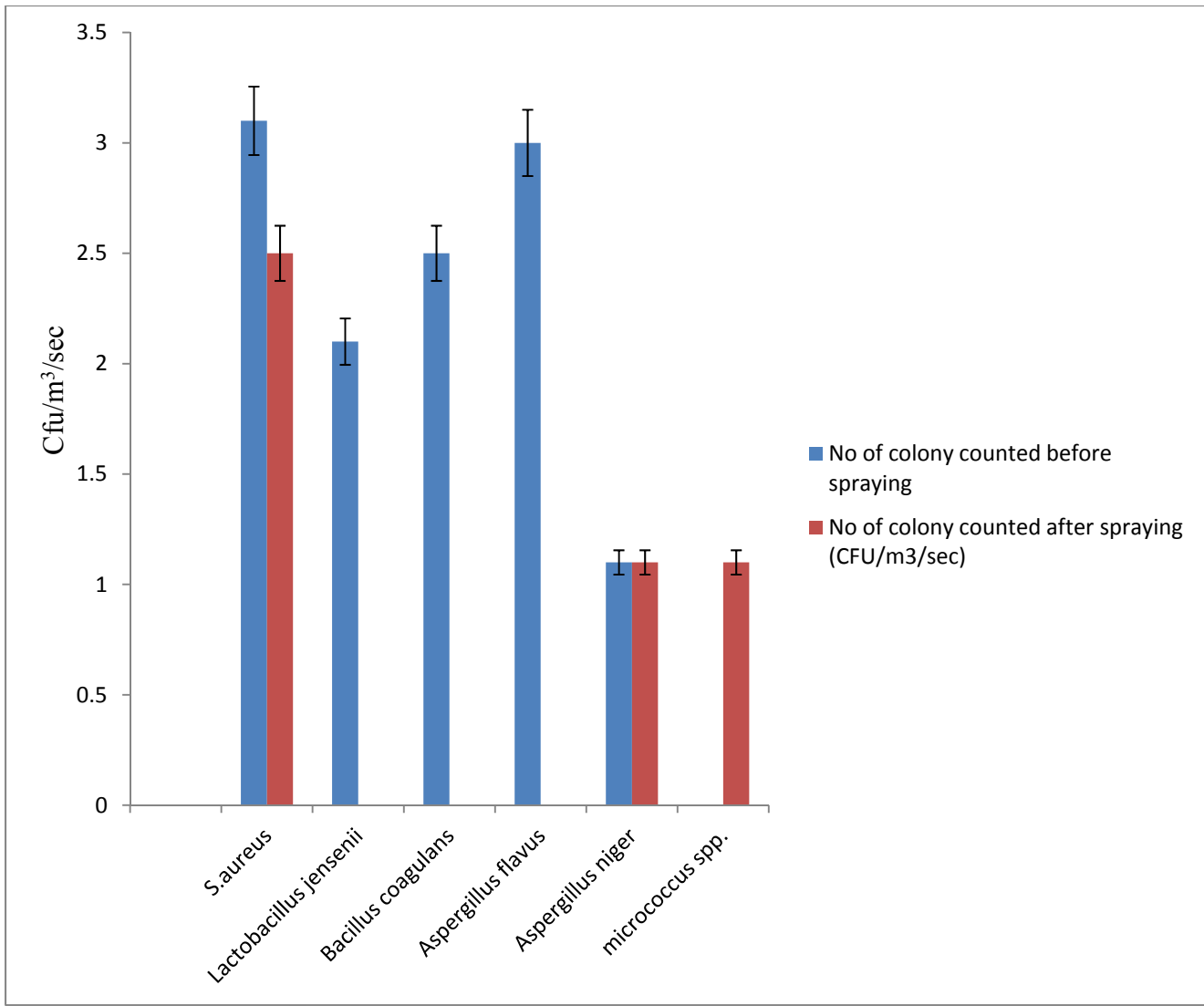


Figure 1: The mean values of colony counted from each room before and after spraying with Imidacloprid aerosol. *S. aureus* and *A. flavus* were recorded as ≥ 300 CfU/m³/sec

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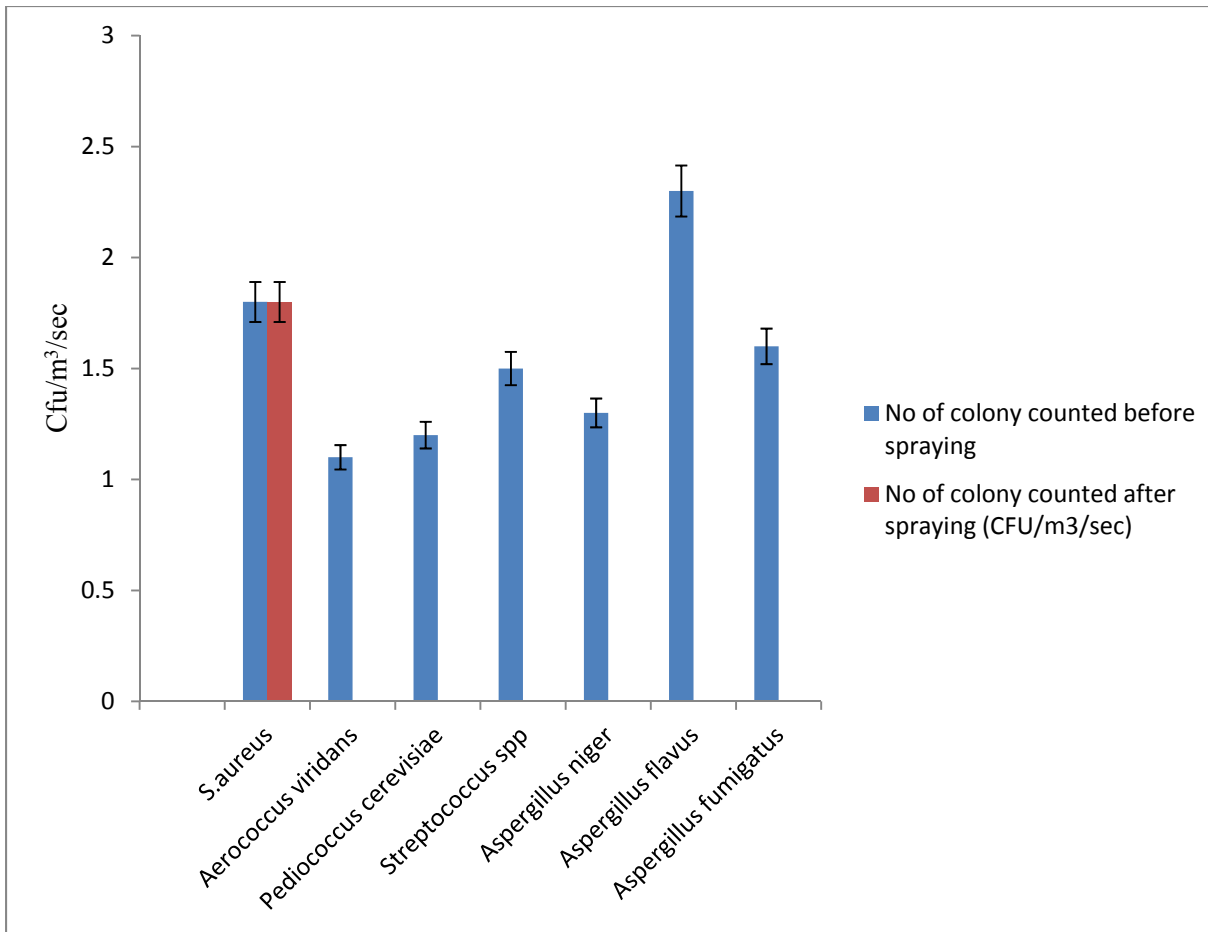


Figure 2: The mean values of colony counted from each room before and after spraying with 1R-trans Phenothrin aerosol

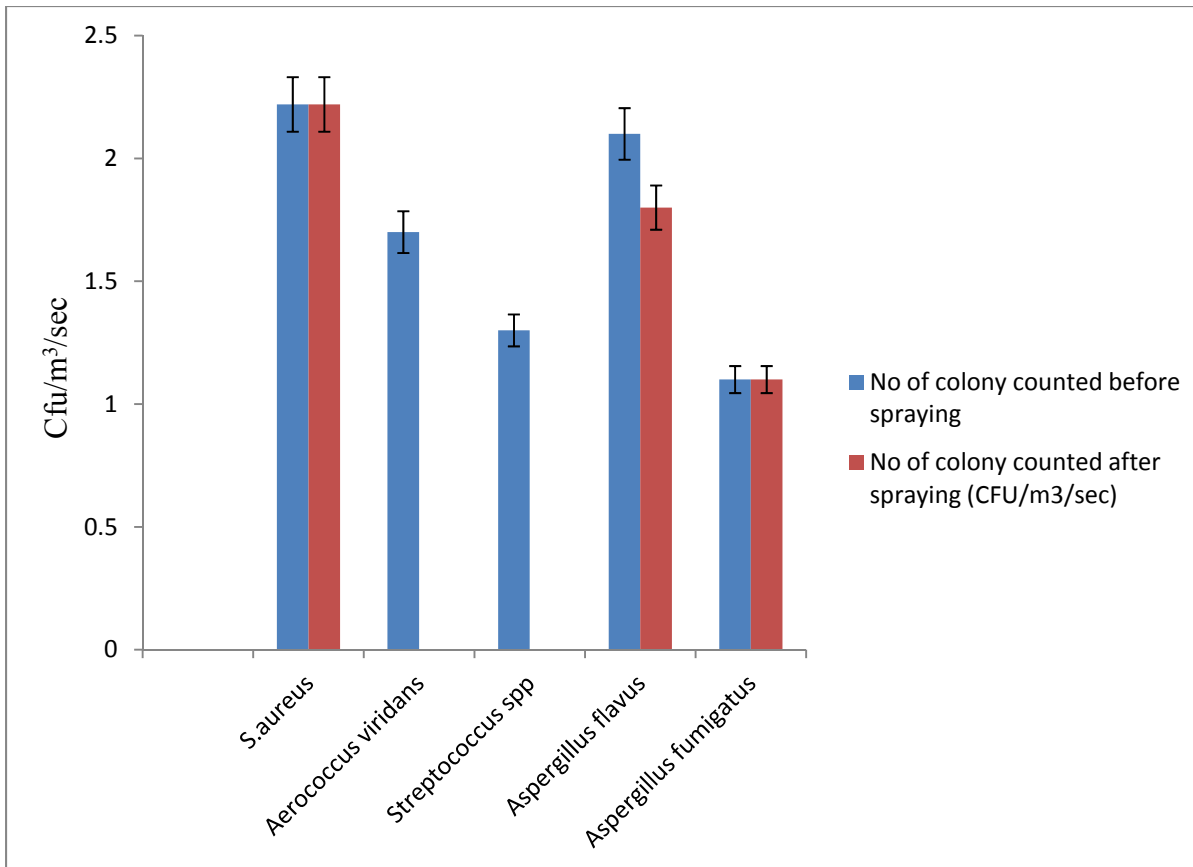


Figure 3: The mean values of colony counted from each room before and after spraying with **pyrethroids** aerosol

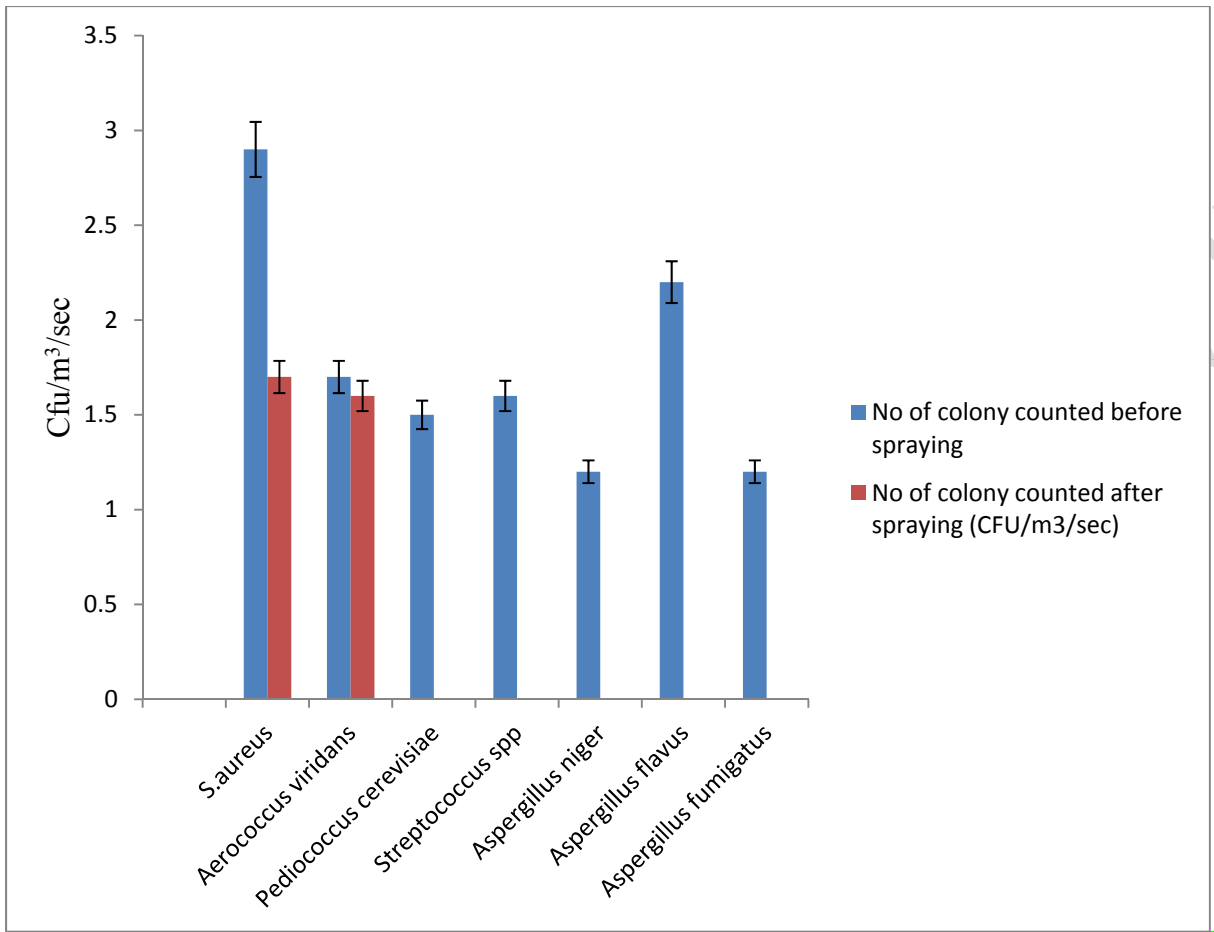


Figure 4: The mean values of colony counted from each room before and after spraying with pyrethroid aerosol

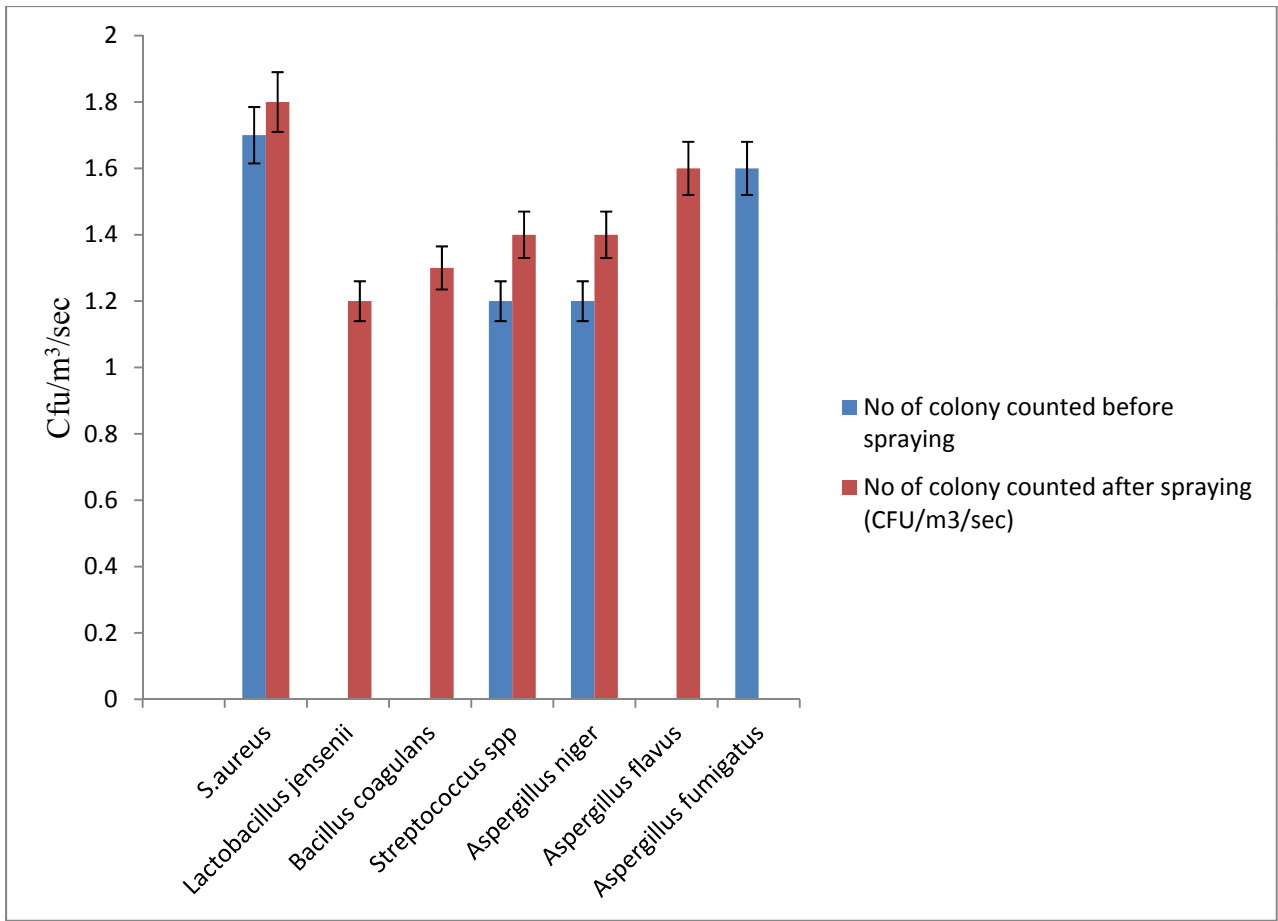


Figure 5: The mean values of colony counted from each room before and after spraying with hydroxypropyl beta-cyclodextrin aerosol

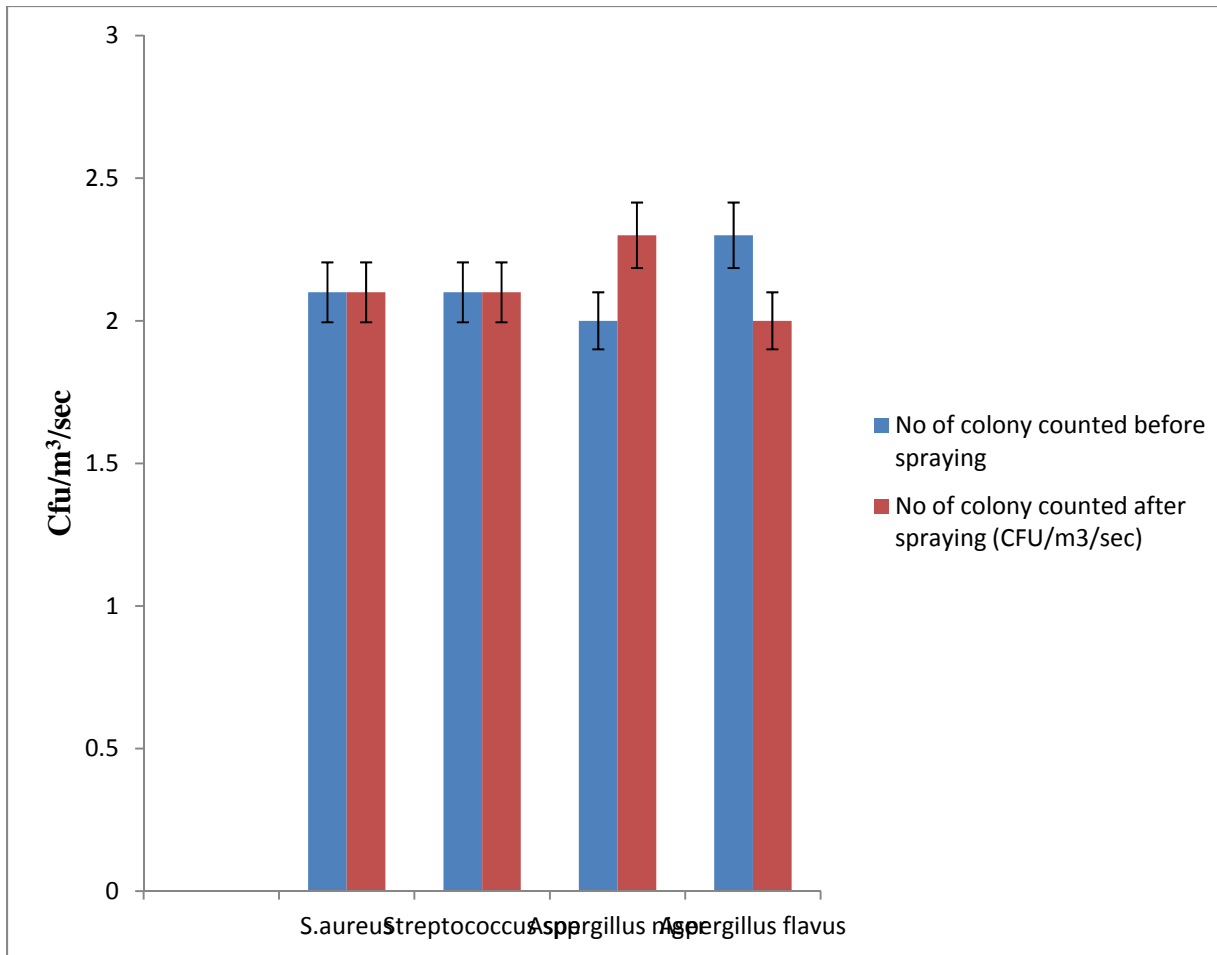


Figure 6: The mean values of colony counted from each room before and after spraying with dipropylene glycol methyl ether aerosol

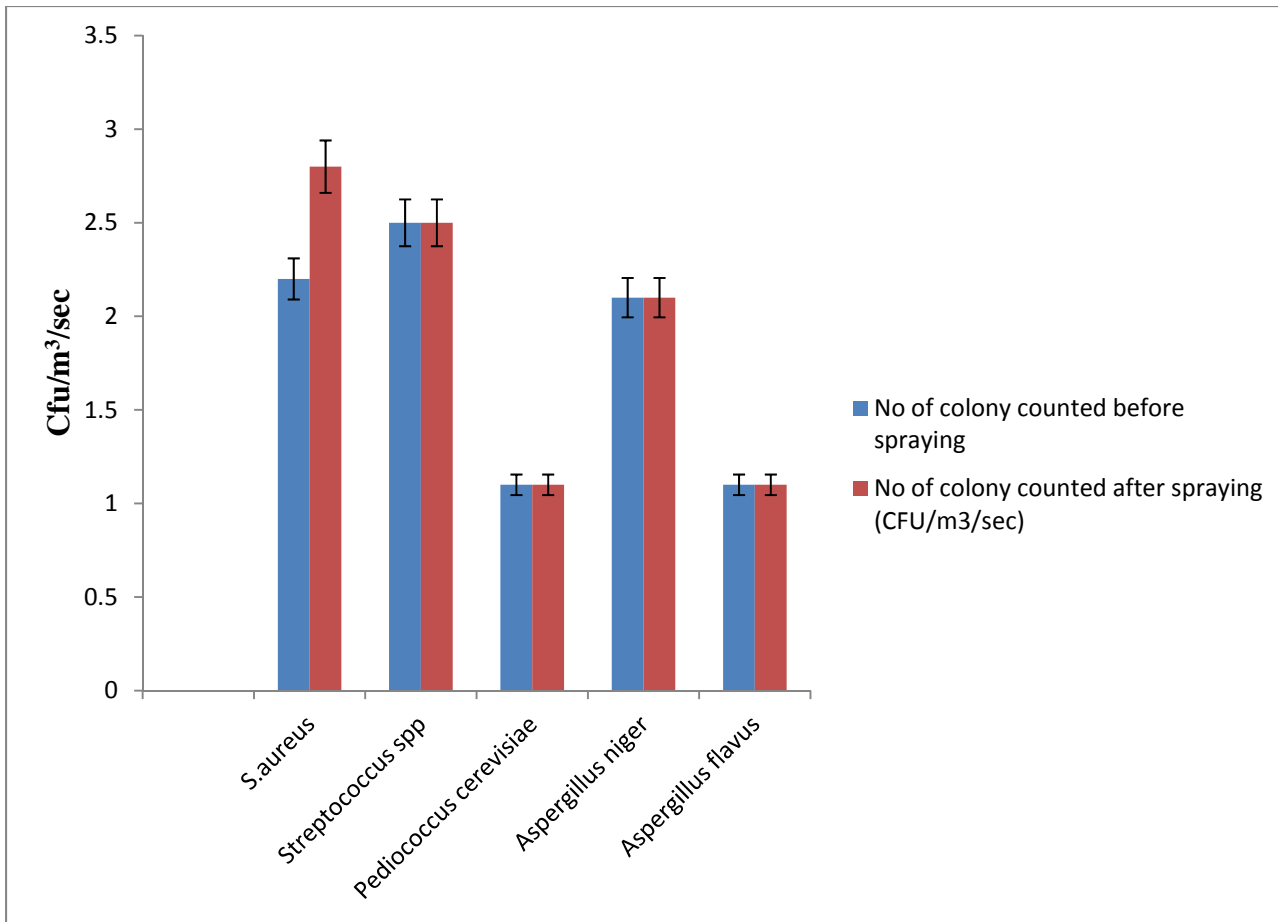


Figure 7: The mean values of colony counted from each room before and after spraying with **BHT** aerosol

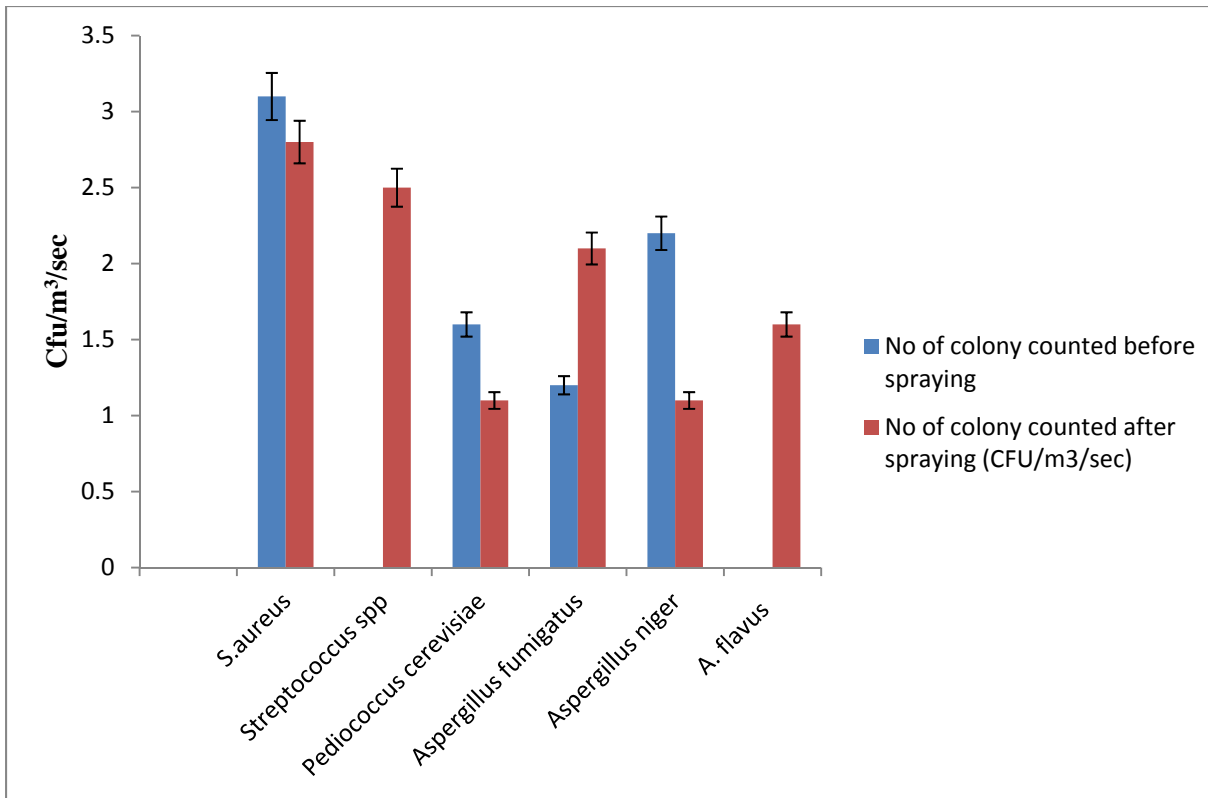


Figure 8: The mean values of colony counted from each room before and after spraying with **Cyclodextrin** aerosol *S. aureus* were recorded as ≥ 300 Cfu/m³/sec

Table 1: Morphology and microscopic characteristic of the bacterial isolates

Code	Shape on Plates	Chromogenesis	Opacity	Elevation	Surface	Edge	Consistency	Gram reaction	shapes	Arrangement of cells	Spore	Spore position	Motility
1	Circular	Insoluble	Opaque	Low Convex	Smooth/glistening	Entire	Smooth	+ve	rod	Chains	-ve	-ve	-ve
2	Circular	Insoluble	Opaque	Raised	Dull	Tentate	friamble	+ve	rod	singly	Oval Spore	Central	+ve
3	filamentous	Insoluble	Opaque	Effuse	Smooth	Rhizoid	Friamble	+ve	cocci	Pairs/cluster	-ve	-ve	-ve
4	filamentous	Slightly soluble	translucent	raised	Dull	Rhizoid	friamble	+ve	cocci	Pair/tetrad	-ve	-ve	-ve
5	Circular	Slightly soluble	Opaque	Raised	Smooth/glistening	Entire	Smooth	+ve	cocci	cluster	-ve	-ve	-ve
6	Circular	Slightly soluble	Opaque	Raised	Smooth/glistening	Entire	smooth	+ve	cocci	tetrad	-ve	-ve	-ve
7	Circular	Insoluble	Opaque	Raised	Smooth	Entire	smooth	+ve	cocci	chains	-ve	-ve	-ve

Key: 1= *Lactobacillus jensenii*, 2= *Bacillus coagulans*, 3= *Aerococcus Viridans*, 4= *Pediococcus cerevisiae*, 5= *Staphylococcus aureus*, 6= *micrococcus spp*, 7= *Streptococcus spp*

+ve positive
-ve negative

Table 2: Morphological identification of the fungi isolates

Isolate	Morphological Characteristics	Microscopic Identification
<i>Aspergillus flavus</i>	Obverse: yellow-green becoming green with age. Reverse: creamish-yellow	Conidial head showing verrucose stipe, domed-shaped vesicle and phialades borne directly on vesicle
<i>Aspergillus fumigatus</i>	Obverse: bluish-green Reverse: creamish-green.	Conidia head with phialades, metulae is absent.
<i>Aspergillus niger</i>	Obverse: blackish-brown often with yellow mycelium Reverse: creamish-yellow to yellow.	conidial head with metulae and phialades, brownish colour of stipe.

Table 3: Biochemical characteristic of the bacterial isolates.

ASP	GA	GL	MN	SC	LA	MA	AR	XY	RA	SO	LM	GH	SH	CA	CO	UR	IN	CI	PROBABLE ORG
-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	Lactobacillus <i>Jensen</i>
-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Bacillus coagulans</i>
-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Aerococcus Viridans</i>
-ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	<i>Pediococcus cerevisiae</i>
-ve	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve A	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	<i>Staphylococcus aureus</i>
Tve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	ND	ND	ND	ND	ND	ND	ND	ND	<i>Streptococcus spp</i>
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	<i>micrococcus spp</i>

Keys:

ND- not determined, +ve - positive, -ve -negative, ASP- ascospore, GA-galactose

GL- Glucose, MN-manitol, SC-Sucrose, LA- Lactose, MA -Maltose ,AR- Arabinose , XY- Xylose ,

RA- Raffinose, SO- Sorbitol, LM- Litmus Milk, GH-Gelatin, SH -Starch Hydrolysis,

CA- Catalase, CO-Coagulase, UR -Urease, IN -Indole, CI- Citrate.

Table 4: List of bacteria isolates from rooms before and after spraying with aerosol

Room code	Type of aerosol used	Type of microorganisms isolated from the room before spraying with aerosol (control rooms)	Type of microorganisms isolated from the room after spraying with aerosol for 10 minutes	Remarks
A	Imidacloprid	<i>Staphylococcus aureus</i> , <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i>	<i>Staphylococcus aureus</i> , and <i>Micrococcus</i> spp.	The Insecticide was able to inhibit the growth of <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i> , However, there was an introduction of a new organisms (<i>Micrococcus spp</i>) which was not present initially
B	1R-trans Phenothrin	<i>Staphylococcus aureus</i> , <i>Aerococcus viridans</i> , <i>Pediococcus cerevisiae</i> , <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i>	1R-trans Phenothrin was able to inhibit all organisms presents initially except <i>Staphylococcus aureus</i>
C	pyrethroids	<i>Staphylococcus aureus</i> , , <i>Aerococcus viridans</i> , <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i> , <i>Aerococcus viridans</i>	There was inhibition of <i>Streptococcus spp</i> only by pyrethroids Insecticide
D	permethrin	<i>Staphylococcus aureus</i> , <i>Aerococcus viridans</i> , <i>Pediococcus cerevisiae</i> , <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i>	permethrin Insecticide was able to inhibit all the organisms present initially except <i>Staphylococcus aureus</i>
E	hydroxypropyl beta-cyclodextrin	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp, <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i> ,	hydroxypropyl beta-cyclodextrin air freshener was not able to inhibit all the initial organisms present. There was an introduction of three new organisms which are: <i>Lactobacillus jensenii</i> , <i>Bacillus coagulans</i>
F	dipropylene glycol methyl ether	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp	<i>Staphylococcus aureus</i> , <i>Streptococcus</i> spp	There was no difference between the type of organism present before and after spraying the room with dipropylene glycol methyl ether

G	BHT	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i> , <i>Pediococcus cerevisiae</i> .	<i>Staphylococcus aureus</i> , <i>Streptococcus spp</i> , <i>Pediococcus cerevisiae</i> .	There was no difference between the type of organism present before and after spraying the room with BHT
H	Cyclodextrin	<i>Staphylococcus aureus</i> , <i>Pediococcus cerevisiae</i> .	<i>Staphylococcus aureus</i> , <i>Pediococcus cerevisiae</i> .	There was no difference between the type of organism present before and after spraying the room with Cyclodextrin

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Table 5: fungi isolates from rooms before and after spraying with aerosol

Room code	Type of aerosol used	Type of microorganisms isolated from the room before spraying with aerosol	Type of microorganisms isolated from the room after spraying with aerosol for 10 minutes	Remarks
A	Imidacloprid	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	<i>Aspergillus niger</i>	The Insecticide was able to inhibit the growth of <i>Aspergillus flavus</i> .
B	IR-trans Phenothrin	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>		The Insecticide was able to inhibit all organisms presents
C	pyrethroids	<i>Aerococcus viridan</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i>	<i>Aerococcus viridans</i> <i>Aspergillus fumigatus</i> and <i>Aspergillus flavus</i>	There was no inhibition of any microorganism
D	permethrin	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	The Insecticide was able to inhibit all the organisms present initially except <i>Aspergillus flavus</i>
E	hydroxypropyl beta- cyclodextrin	<i>Aspergillus fumigatus</i> and <i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i>	The freshener was not able to inhibit all the initial organisms present. There was an introduction of a new organisms as <i>Aspergillus flavus</i> ,
F	dipropylene glycol methyl ether	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	There was no difference between the type of organism present before and after spraying the room
G	BHT	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	<i>Aspergillus flavus</i> and <i>Aspergillus niger</i>	There was no difference between the type of organism present before and after spraying the room
H	Cyclodextrin	<i>Aspergillus fumigatus</i> , and <i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i> , <i>A. flavus</i> and <i>Aspergillus niger</i>	There was no difference between the type of organism present before and after spraying the room with the air freshener . However, there was an introduction of <i>A. flavus</i> after spraying

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Table 6: percentage (%) occurrence of bacteria isolates

Isolates	No of rooms	No of occurrence	% Occurrence
<i>Staphylococcus aureus</i>	8	8	100
<i>Lactobacillus jensenii</i>	8	1	12.5
<i>Bacillus coagulans</i>	8	1	12.5
<i>Micrococcus spp.</i>	8	1	12.5
<i>Aerococcus viridans</i>	8	3	37.5
<i>Pediococcus cerevisiae</i>	8	5	62.5
<i>Streptococcus spp</i>	8	6	75

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6
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8

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Table 7: Percentage occurrence (%) of fungi isolates

Isolates	No of rooms	No of occurrence	% Occurrence
<i>Aspergillus flavus</i>	8	6	75
<i>Aspergillus niger</i>	8	7	87.5
<i>Aspergillus fumigatus</i>	8	5	62.5

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