***Original Research Article***

# 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 freshners 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: *Staphylococus 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 *Staphylococus 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

# INTRODUCTION

## Background to the study

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

Despite rapid advances in the characterization of airborne microbial communities through rRNA surveys, metagenomics, proteomics, and metabolomics, limited information is available about actual concentrations of airborne microorganisms in built environments. In one of the few studies of concentrations of total bacteria and viruses in indoor air by air sampler, Prussin *et al.* (2015) found virus-like and bacteria-like particle concentrations of approximately $10^5$ and $10^6$ particles $m^3$ in various indoor and outdoor air environment, respectively(Shelton *et al.*, 2002).More over an average viable airborne fungi concentration of 80 CFU/$m^3$ were reported in samples collected from schools, hospitals, residences, and industrial buildings; However, in some instances concentrations were as high as $10^4$ CFU m$^3$.Such information should be forthcoming as methods for quantitative metagenomics analyses air samplers become more powerful (Shelton *et al.*, 2002;Frank *et al*., 2011*;* Gilbert *et al*., 2011;Duhaime *et al*., 2012).

In confined environments geared for both industrial and non-industrial activities, the presence of microbial pollutants may elicit the deterioration of indoor air quality (IAQ). Generally, in healthy indoor occupational environments, microflora concentrations are lower than outdoor concentrations (ACGIH 1989; Macher *et al*., 1995). In indoor environments, air from identifiable sources may be responsible for exposure to microbial pollutants through phenomena like diffusion, accumulation and concentration. As people spend 80–95% of their time indoors, air pollution is frequently reported to cause health problems (WHO 1983, 1984). Diverse studies have demonstrated that dust particles, macromolecular organic compounds, Gram-negative bacteria and total volatile organic compounds may cause nasal, optical and physiological changes and sensory symptoms exemplified by irritation, slugginess, sleepiness, headache and reduced ability to concentrate (Gyntelberg *et al*., 1994; Pan *et al*.,2000). The presence of any type of microorganism can be problematic to IAQ, particularly bacteria and fungi (Stetzenbach*etal*., 1998). In residential and public buildings like schools. Microbial growth is associated with adverse health effects (Husman *et al*.,1996; Haverinen *et al*., 1999). Airborne concentrations of *Cladosporium, Epicoccum and Coprinus* spores were associated with peak expiratory flow rates (PEFRs) deficiency in children (Neas *et al*., 1996). The presence of moisture damage in school buildings was a significant risk factor for respiratory symptoms in schoolchildren (Meklin *et al*., 2002). Because of their lower water activity (Aw) requirements compared with bacteria, fungi are the principal contaminant in various types of substrates. They tend to colonize a wide variety of humid building materials wetted by floods, condensation or plumbing leaks. Consequently, when fungal proliferation occurs, aerospores are abundantly distributed on and around the surfaces, and the indoor environment becomes a source of exposure to occupants. Knowledge of indoor environmental mycoflora is especially important from an allergologic view-point, which, in many cases differs from that observed in outdoor environments. Although less frequent than the possible dangers caused by exposure to pollen and acari, fungal exposure causes hypersensitive reactions which characterize allergic respiratory pathologies like bronchial asthma and rhinitis (Burge 1989). Fungi may elicit allergic symptoms similar to those caused by pollen.

With an ever-increasing population utilizing different types of aerosols as insecticides and air fresheners, in order to improve and sustain health and vitality; and consuming products in which these supplements are used as room flavors, it is essential that these products are safe for human use. A very critical indicator of safety is the microbiological quality of these products. To improve the prediction of dispersion models and the environmental health assessment on the one hand and to get an insight on the airborne micro-organisms in other relevant environments, e. g. living spaces. However, these studies give insight in the internal structure of bio-aerosols and the distribution of micro-organisms on airborne particles themselves for developing guidelines in order to achieve and maintain safe microbial levels in these products.

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

## MATERIALS AND METHODOLOGY

### Study area

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

### Collection of the samples

Eight (8) different samples of chemical aerosols were purchased from shoprite shopping mall, alagbaka, Akure, Ondo State, Nigeria. The selected aerosols were; insecticideImidacloprid, 1R-trans Phenothrin, pyrethroids, pyrethroid compound. as categorized as Insecticides, while hydroxypropyl beta-cyclodextrin,dipropylene glycol methyl ether,allyl 3-cyclohexylpropionate, allyl caproate, benzyl alcohol, butylated hydroxytoluene (BHT) and Cyclodextrin were purchased as air fresheners/fragrance,

### Experimental design

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

### Microbial isolation and determination of total viable counts

The method used for isolation and identification of microorganisms was as described by Olutiola*et al.*(1991). Twenty (20ml) of nutrient agar and acidified potato dextrose agar cooled to $45^0C$ was poured separately onto each of the plates in triplicate and the plates were gently swirled and allowed to solidify. The plates were exposed to air in the room before and after spraying with aerosols for 10 minutes. Thereafter, the nutrient agar plates were incubated in an inverted position at $37\pm2^0C$ for 24 hours for isolation of mesophilic bacteria while Potato

Dextrose Agar plates were incubated at $28\pm2^0C$ for 72 hours. Anaerobic plates were inverted in the anaerobic jar at $37\pm2^0C$ for 24 hours for isolation of anaerobic organisms present in the samples. After incubation, colonies on the plates were counted using colony counter and the number of viable cells obtained to be the total viable counts of the isolates. The viable colonies were sub cultured from mixed culture plate to obtain a pure culture. The colonies were then identified directly by their size, shape, colour of the pigment (chromogenesis), opacity, elevation, surface, edge and consistency and stored on agar slants for further biochemical tests.

## Determination of microbiology of the air samples

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

## Identification of fungal Isolates

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

**Calculation of Percentage frequency of the isolates**

The isolation frequency (Fq) of each isolate from the eight rooms was calculated according to the formula by Gonzalez *et al*. (1999). This was used to determine the distribution of the isolates in the eight sample rooms.

$$\text{Frequency of occurrence (\%)} = \frac{\text{Number of isolates of a genus x 100}}{\text{Total number of samples collected}}$$

**Data Analysis**

The experiment was conducted using a completely randomized design. Means of three replicates were computed using computer software Microsoft Excel.

## RESULTS AND DISCUSSION

This present study was conducted to isolate and identify airborne microbes in some rooms sprayed with insecticides and air fresheners with a view to identify the microflora of the rooms and determine their sensitivity to the aerosols . A total of ten organisms were isolated from eight rooms during the course of this study. Seven bacterial genera were identified from the sampling sites as shown in table 2 comprising *Staphylococcus aureus*, *Lactobacillus jensenii*, *Bacillus coagulans*, *micrococcus* spp., *Aerococcus viridans*, *Pediococcus cerevisiae* and *Streptococcus* spp. while *Aspergillus* was the only mould generally identified *Aspergillus niger*, *A. flavus* and *A. fumigatus* are the specific species of *Aspergillus* reported. The result of eight different rooms sprayed with different aerosol as Insecticide and air fresheners are as follows:

Table 1 revealed the bacteria Isolated before and after spraying all the rooms with different aerosols are: *Staphylococusaureus, Lactobacillus jensenii, Bacillus coagulans,Micrococcus* spp, *Aerococcus viridans, Pediococcus cerevisiae*, *Streptococcus* spp. Table 2 shows the fungi isolated before and after spraying;*Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Aspergillus niger*. Before spraying the room with Mobil Insecticides, the microorganisms isolated were: *Staphylococcus aureus,Lactobacillus jensenii,Bacillus coagulans, Aspergillus flavus* and *Aspergillus niger*, after spaying the room with Imidacloprid,the Insecticide was able to inhibit the growth of *Lactobacillus jensenii, Bacillus coagulans*,However, there was an introduction of a new organisms (*Micrococcus* spp.) which was not present initially. The microorganisms isolated were 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 new organisms (*Micrococcus* spp.) which was not present initially before spraying the room with 1R-trans Phenothrin, microbes reported were: *Staphylococcus aureus*,, *Aerococcus viridans,* and *Pediococcus cerevisiae*. *Streptococcus* spp., *Aspergillus fumigatus, Aspergillus flavus,* after spraying there was inhibition of *Streptococcus* spp. only by pyrethroids thereafter before spraying pyrethroid into the rooms, microorganism isolated were:*Staphylococcus aureus, Aerococcus viridans, Pediococcus cerevisiae. Streptococcus* spp., *Aspergillus fumigatus, A. flavus, A. niger* after spraying it was discovered that pyrethroidwas able to inhibit all the organisms present initially except *Staphylococus aureus* and *Aspergillus flavus*.

Similarly, before spraying hydroxypropyl beta-cyclodextrin air fresheners, the microorganisms reported were: *Staphylococcus aureus,Streptococcus* spp., *Aspergillus fumigatus and A. niger*. Then after spraying, it was discovered that hydroxypropyl beta-cyclodextrinwas 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,* likewise before spraying with Air wick, microorganism present were: *Staphylococcus aureus,Streptococcus* spp., *Aspergillus flavus* and *A. niger*, and after spraying; 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 following microorganism were isolated:*Staphylococcus aureus*, *Streptococcus* spp., *Pediococcus cerevisiae*. *Aspergillus flavus* and *Aspergillus niger* and for Cyclodextrinspray,the isolates were:*Staphylococcus aureus, Pediococcus cerevisiae*. *Aspergillus fumigatus,* and *A. niger*. However, after spraying the room, it was discovered that there was no difference between the type of organism present before and after spraying the room with BHT. Similarly, 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.* The occurrence of *Staphylococcus aureus* (100%) was 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%) as shown on table 3 and figures:1-8. The result of the morphological, microscopic and biochemical characterization of all the organisms isolated before and after spraying are shown in table 4-6

The highest percentage occurrence (100%) is *Staphylococus 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 correlatewith several and similar findings of the studies conductedby 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. Thisstudy 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 spaying 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 *A. niger*. However, after spaying 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 insecticidewas 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 organism (*Micrococcus* spp.) which was not present initially.

Before spraying the room with 1R-trans Phenothrin, the microbes isolated were:*Staphylococusaureus, Aerococcusviridans, Pediococcuscerevisiae. Streptococcus* spp., *Aspergillus fumigatus, A. flavus, A. niger* and after spraying there was inhibition of

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

Similarly, before spraying hydroxypropyl beta-cyclodextrin, the initial microorganisms identified were:*Staphylococusaureus, 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: *Staphylococus aureus, Streptococcus* spp., *Aspergillus flavus* and *A. 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: *Staphylococus aureus, Streptococcus* spp., *Pediococcus cerevisiae. Aspergillus flavus* and *A. niger* and for Cyclodextrin, the isolates were; *Staphylococus aureus, Pediococcus cerevisiae. Aspergillus fumigatus,* and *A. 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 *Staphylococusaureus, Lactobacillus jensenii, Bacillus coagulans, Micrococcus spp., Aerococcusviridans, Pediococcuscerevisiae* 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 inconformity with the result obtained byBadri *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 (Cyrys *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.

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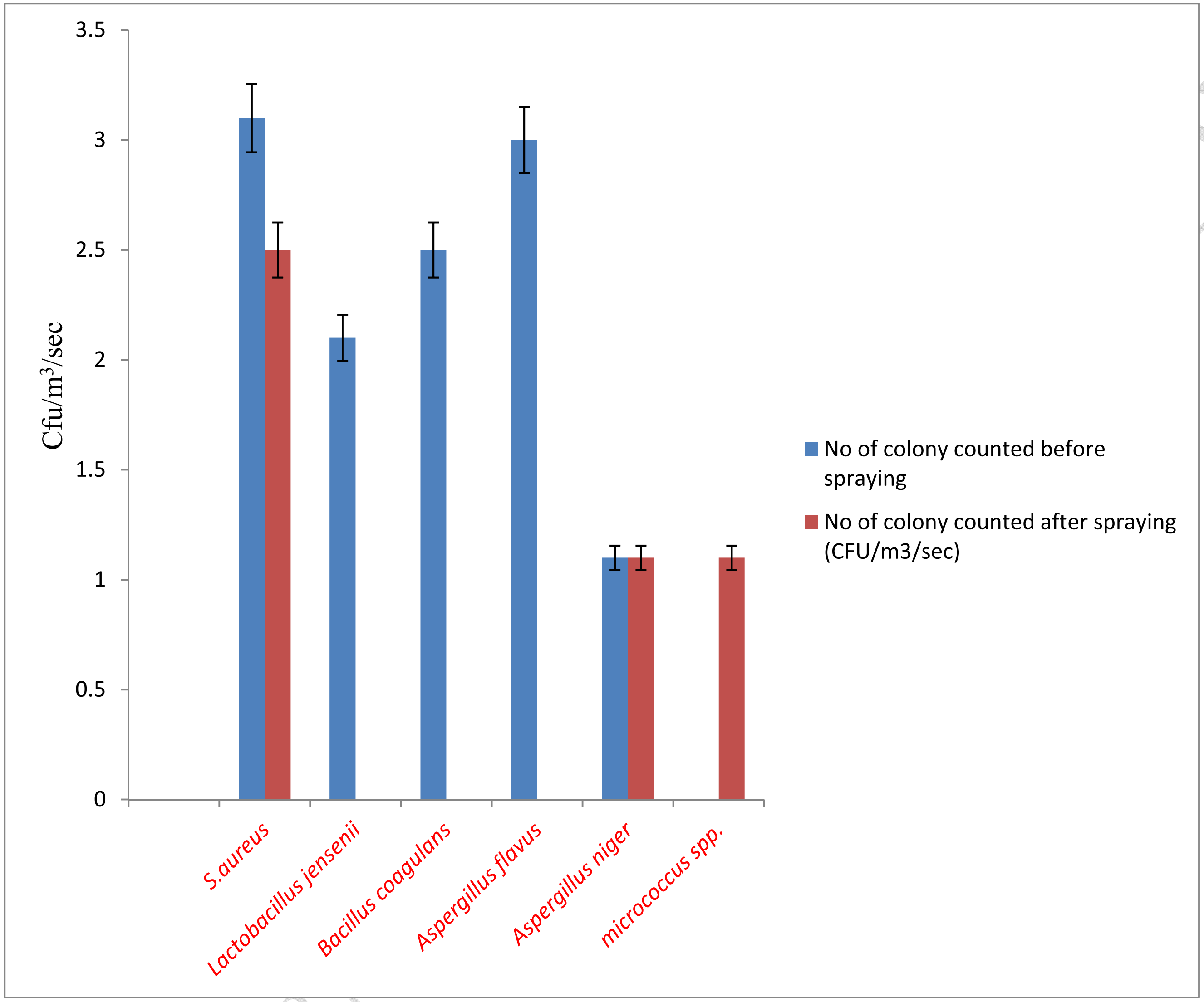

**Fig. 1**: The mean values of colony counted from each room before and after spraying with Imidacloprid aerosol *S. aureus* and *A. flavus* were recorded as ≥**300 Cfu/m$^3$/sec**

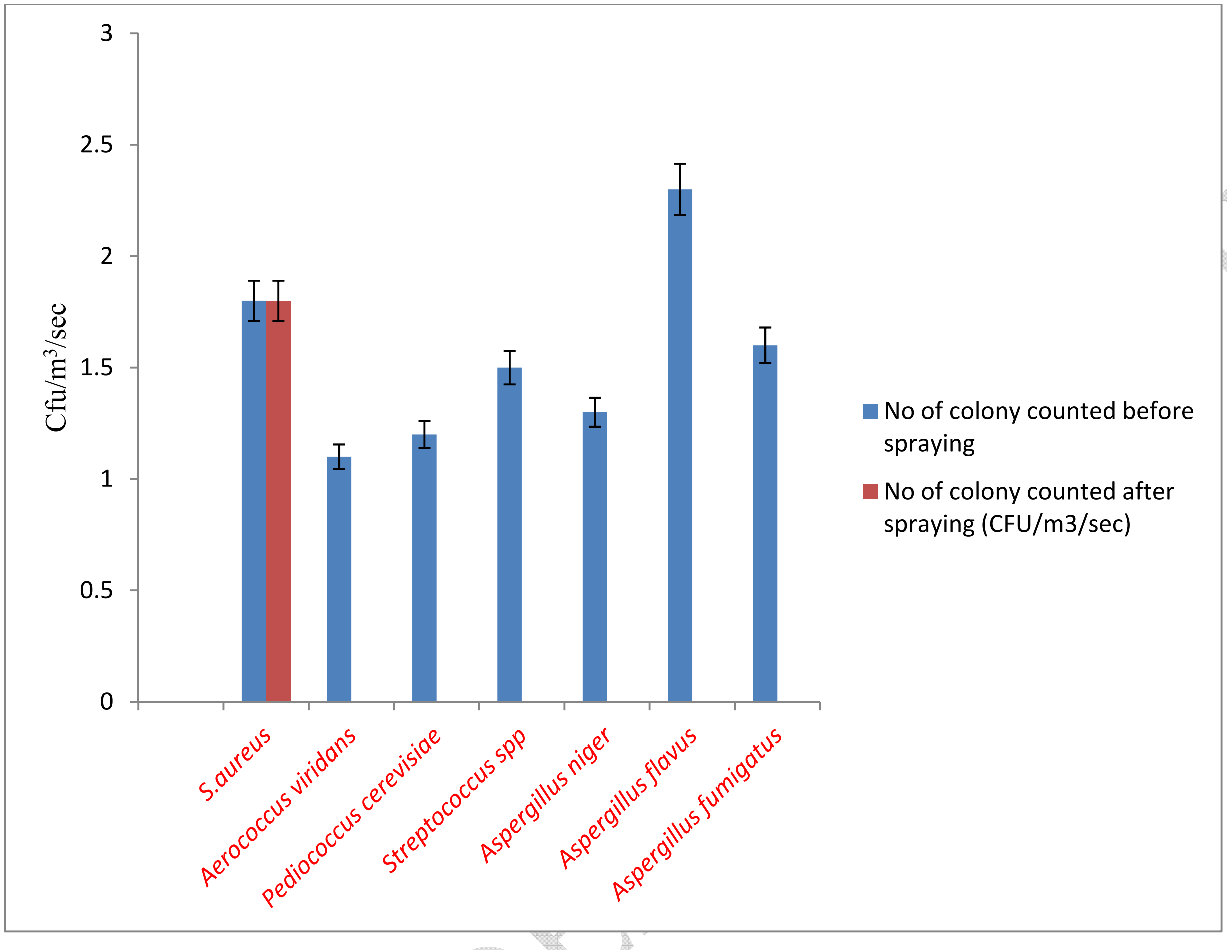

**Fig. 2**: The mean valuesof colony counted from each room before and after spraying with 1R-trans Phenothrin aerosol

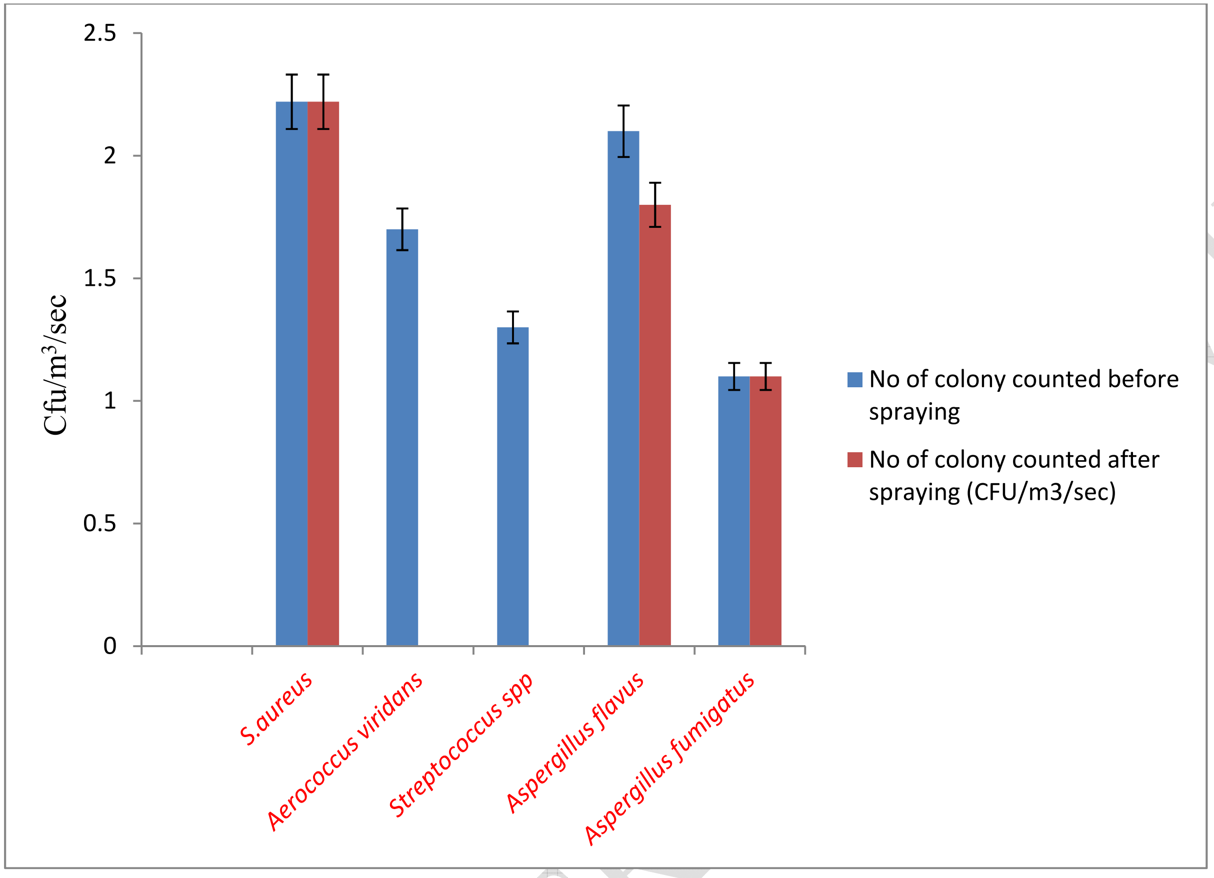

**Figure 3:** The mean values of colony counted from each room before and after spraying with pyrethroids aerosol

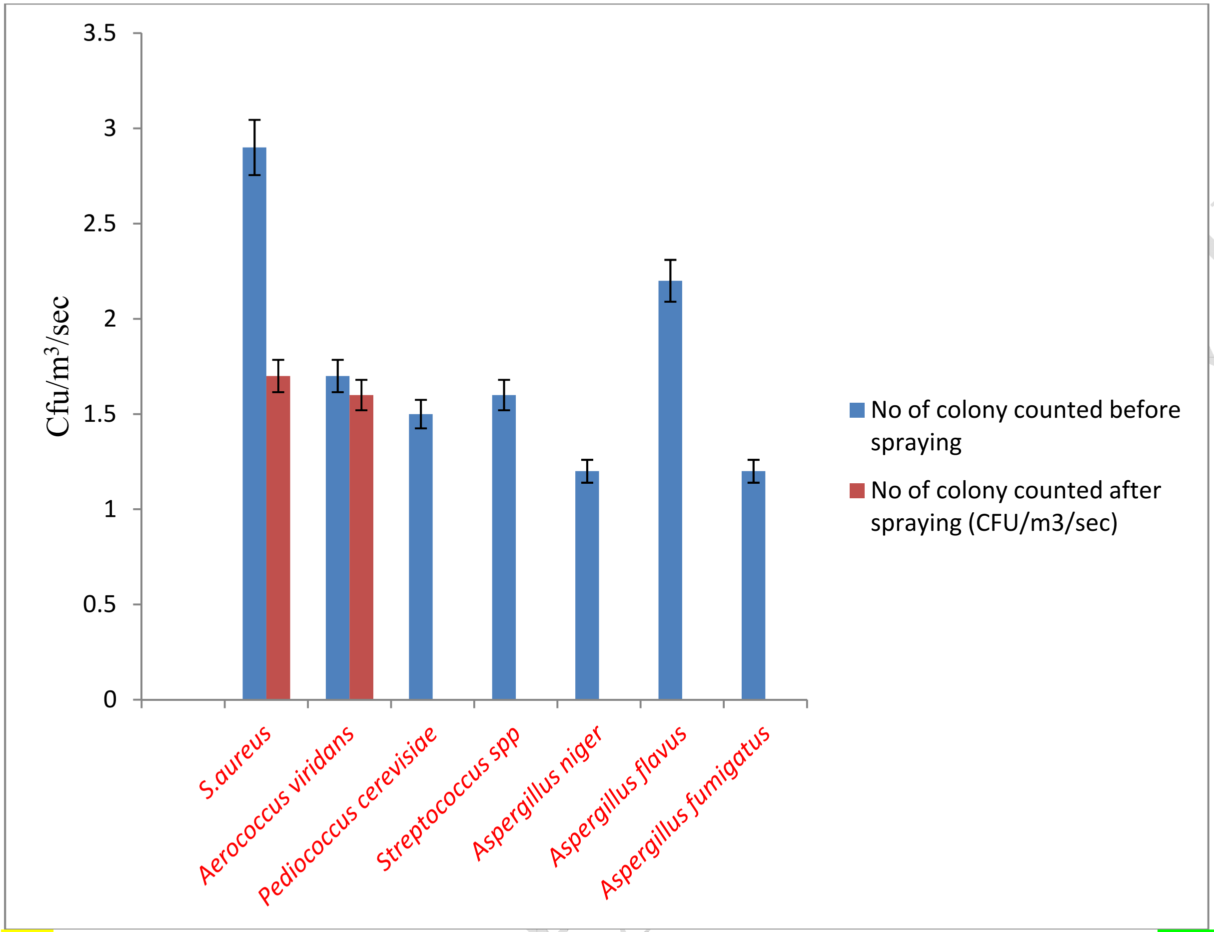

**Fig. 4:** The mean valuesof colony counted from each room before and after spraying with pyrethroid aerosol

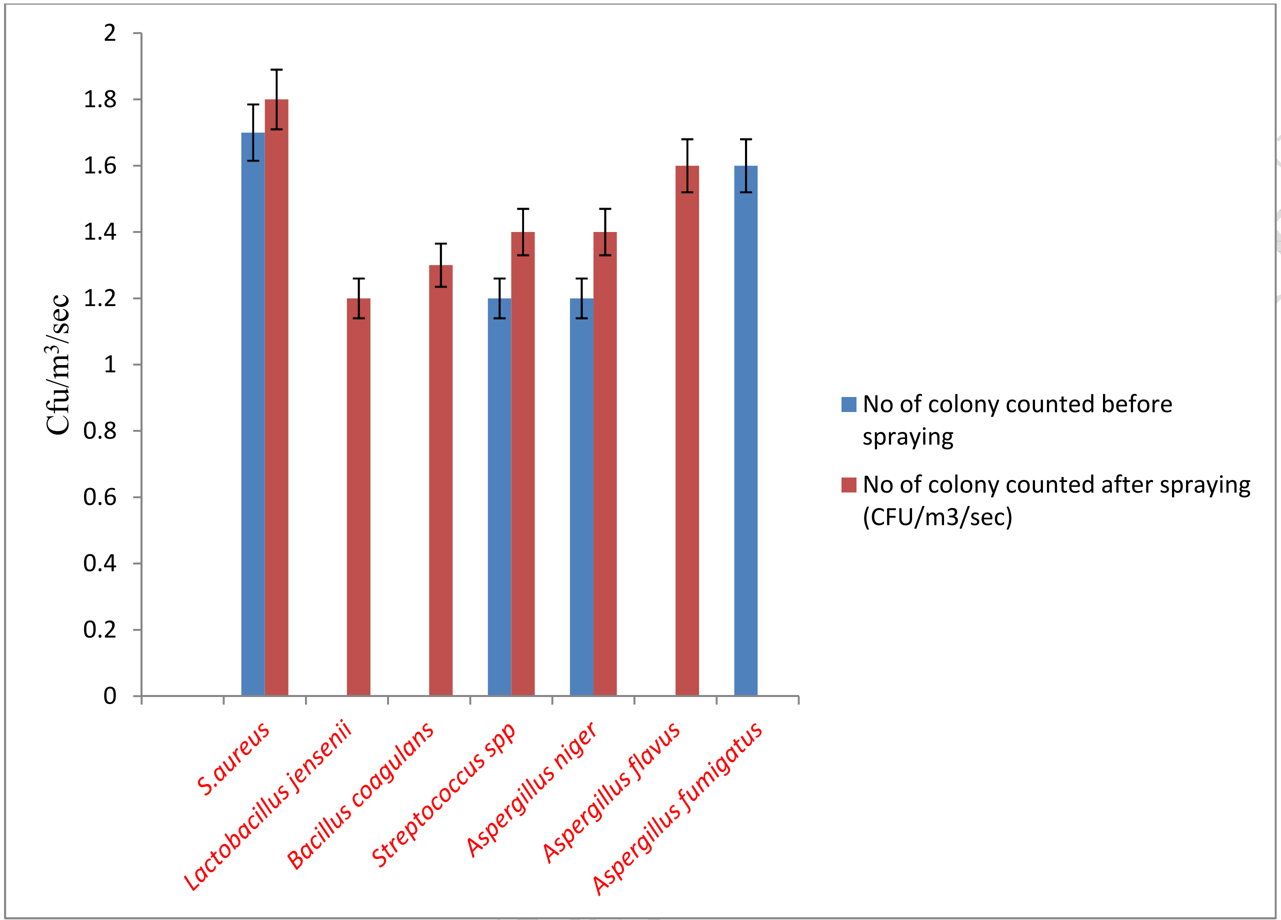

**Fig. 5:** The mean values of colony counted from each room before and after spraying with hydroxypropyl beta-cyclodextrin aerosol

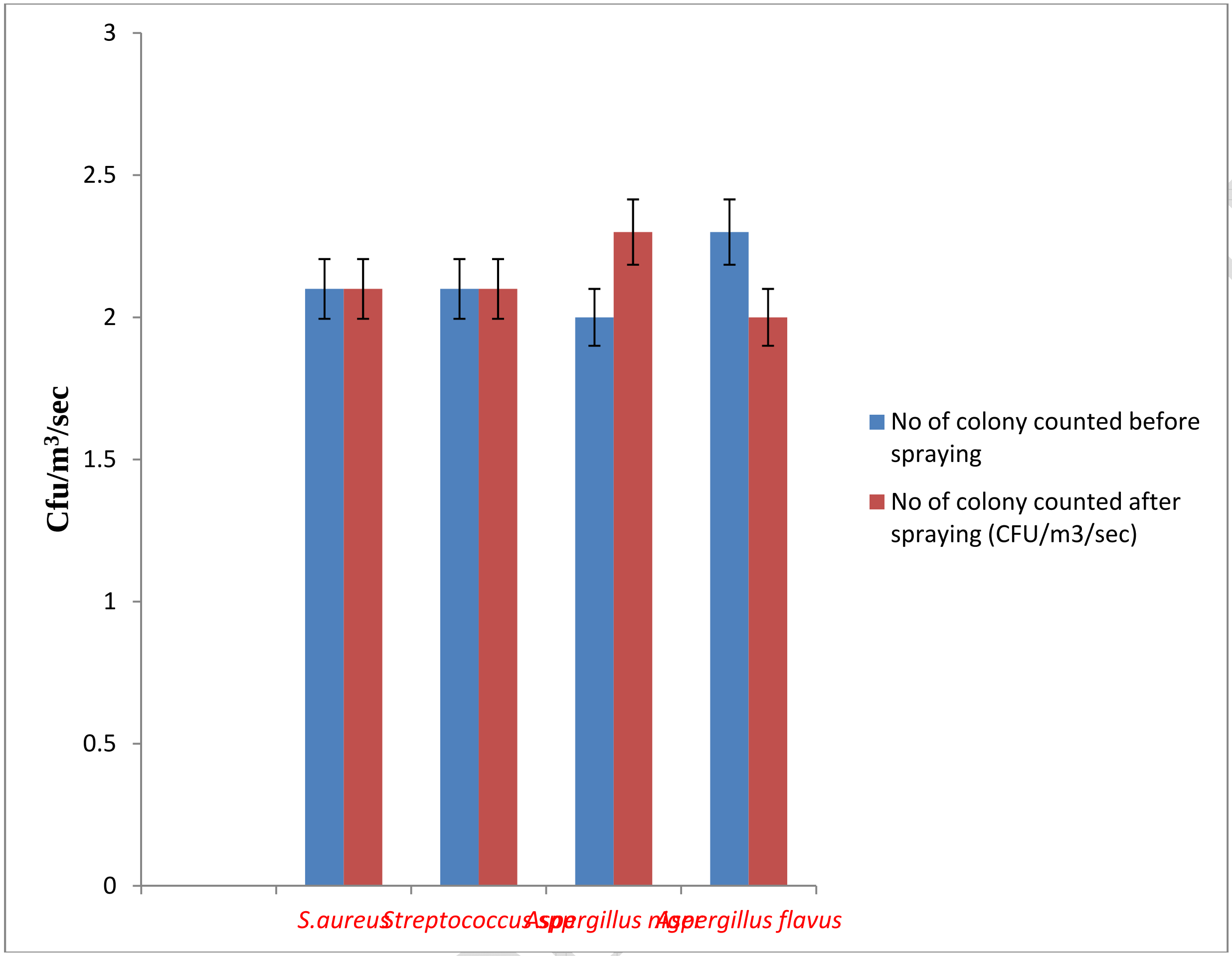

**Fig. 6:** The mean values of colony counted from each room before and after spraying withdipropylene glycol methyl etheraerosol

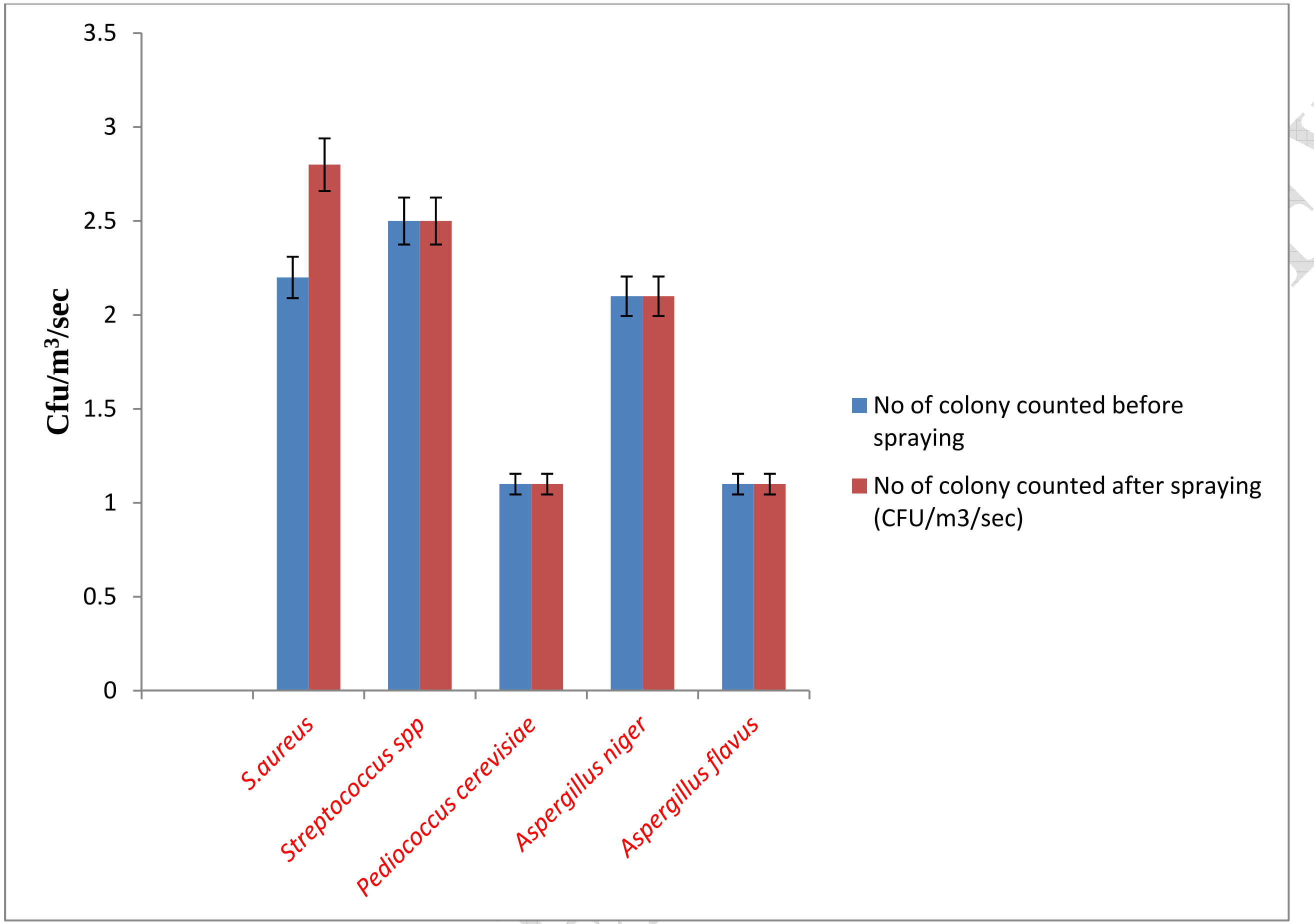

Fig. 7: The mean values of colony counted from each room before and after spraying with BHT aerosol

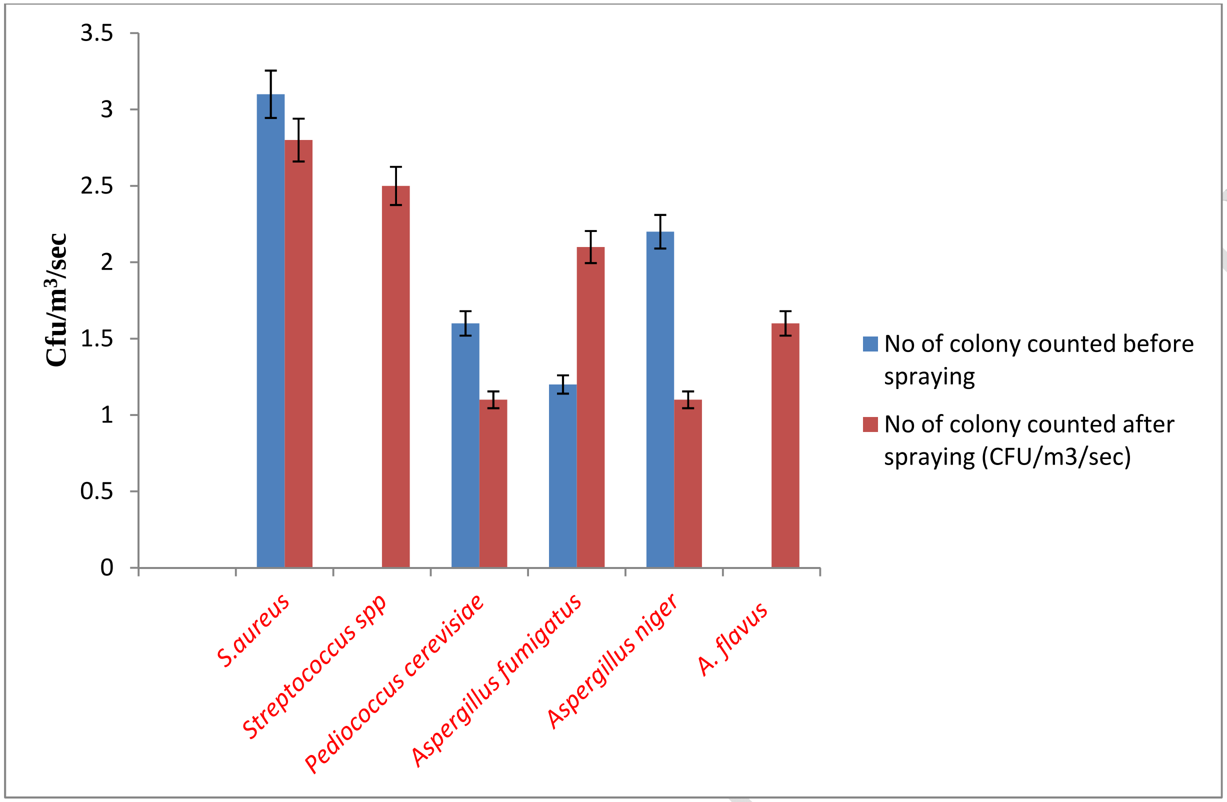

**Fig. 8:** The mean values of colony counted from each room before and after spraying withCyclodextrinaerosol *S. aureus* were recorded **as ≥300 Cfu/m$^3$/sec**

**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/ glistering | Entire | Smooth | tve | rod | Chains | -ve | -ve | -ve |
| 2 | Circular | Insoluble | Opaque | Raised | Dull | Tentate | friamble | tve | rod | singly | Oval Spore | Central | tve |
| 3 | filamentous | Insoluble | Opaque | Effuse | Smooth | Rhizoid | Friamble | tve | cocci | Pairs/ cluster | -ve | -ve | -ve |
| 4 | filamentous | Slightly soluble | translucent | raised | Dull | Rhizoid | friamble | tve | cocci | Pair/tetrad | -ve | -ve | -ve |
| 5 | Circular | Slightly soluble | Opaque | Raised | Smooth/ glistering | Entire | Smooth | tve | cocci | cluster | -ve | -ve | -ve |
| 6 | Circular | Slightly soluble | Opaque | Raised | Smooth/ glistering | Entire | smooth | tve | cocci | tetrad | -ve | -ve | -ve |
| 7 | Circular | Insoluble | Opaque | Raised | Smooth | Entire | smooth | tve | cocci | chains | -ve | -ve | -ve |

Key: 1= *Lactobacillus jensenii*, 2= *Bacillus coagulans*, 3= *Aerococcusviridans*, 4= *Pediococcuscerevisiae*, 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** |
|---|---|---|
| *Aspergillus flavus* | Obverse: yellow-green becoming green with age.<br>Reverse: creamish-yellow | Conidial head showing verrucose stipe, domed-shaped vesicle and philades borne directly on vesicle |
| *Aspergillus fumigatus* | Obverse: bluish-green<br>Reverse: creamish-green. | Conidia head with philiades, metulae is absent. |
| *Aspergillus niger* | Obverse: blackish-brown often with yellow mycelium<br>Reverse: creamish-yellow to yellow. | conidial head with metulae and philades, brownishcolour 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 Jensen* |
| -ve | -ve | +ve | -ve | -ve | -ve | -ve | -ve | +ve | -ve | -ve | -ve | -ve | +ve | +ve | -ve | -ve | -ve | -ve | *Bacillus coagulans* |
| -ve | -ve | +ve | +ve | +ve | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | +ve | -ve | -ve | -ve | -ve | *Aerococcus viridans* |
| -ve | -ve | +ve | -ve | +ve | +ve | -ve | +ve | -ve | +ve | +ve | +ve | -ve | +ve | +ve | -ve | -ve | -ve | -ve | *Pediococcusce revisiae* |
| -ve | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve<br>A | +ve | +ve | +ve | +ve | +ve | +ve | -ve | +ve | *Staphylococus aureus* |
| Tve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | ND | ND | ND | ND | ND | ND | ND | ND | *Streptococcus spp.* |
| -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | -ve | +ve | +ve | -ve | -ve | -ve | *Micrococcus spp.* |

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 | *Staphylococus aureus, Lactobacillus jensenii, Bacillus coagulans* | *Staphylococus aureus*, and *Micrococcus* spp.. | The Insecticide was able to inhibit the growth of *Lactobacillus jensenii, Bacillus coagulans*,However, there was an introduction of a new organisms (*Micrococcus* spp.*)* which was not present initially |
| B | 1R-trans Phenothrin | *Staphylococusaureus, Aerococcusviridans, Pediococcuscerevisiae. Streptococcus*spp. | *Staphylococus aureus* | 1R-trans Phenothrin was able to inhibit all organisms presents initially except *Staphylococcus aureus* |
| C | pyrethroids | *Staphylococusaureus, , Aerococcusviridans, Streptococcus* spp. | *Staphylococus aureus, Aerococcus viridans* | There was inhibition of *Streptococcus* spp. only by pyrethroids Insecticide |
| D | permethrin | *Staphylococusaureus, Aerococcusviridans, Pediococcuscerevisiae. Streptococcus* spp. | *Staphylococus aureus* | permethrin Insecticide was able to inhibit all the organisms present initially except *Staphylococcus aureus* |
| E | hydroxypropyl beta-cyclodextrin | *Staphylococus aureus, Streptococcus* spp. | *Staphylococus aureus, Streptococcus* spp., *Lactobacillus jensenii, Bacillus coagulans,* | 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: *Lactobacillus jensenii, Bacillus coagulans* |
| F | dipropylene glycol methyl ether | *Staphylococus aureus, Streptococcus* spp. | *Staphylococcus aureus, Streptococcus* spp. | There was no difference between the type of organism present before and after spraying the room with dipropylene glycol methyl ether |

| | | | | |
|---|---|---|---|---|
| G | BHT | *Staphylococcus aureus, Streptococcus* spp., *Pediococcus cerevisiae.* | *Staphylococus aureus, Streptococcus* spp., *Pediococcus cerevisiae.* | There was no difference between the type of organism present before and after spraying the room with BHT |
| H | Cyclodextrin | *Staphylococus aureus, Pediococcus cerevisiae.* | *Staphylococus aureus, Pediococcus cerevisiae.* | There was no difference between the type of organism present before and after spraying the room with Cyclodextrin |

**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 | *Aspergillus flavus,*<br>*Aspergillus niger* | *Aspergillus niger* | The Insecticide was able to inhibit the growth of *Aspergillus flavus.* |
| B | 1R-trans Phenothrin | *Aspergillus fumigatus,*<br>*Aspergillus flavus,*<br>*Aspergillus niger* | | The Insecticide was able to inhibit all organisms presents |
| C | pyrethroids | *Aerococcus viridan,*<br>*Aspergillus fumigatus,*<br>*Aspergillus flavus* | *Aerococcus viridans*<br>*Aspergillus fumigatus* and<br>*Aspergillus flavus* | There was no inhibition of any microorganism |
| D | permethrin | *Aspergillus fumigatus,*<br>*Aspergillus flavus,*<br>*Aspergillus niger* | *Aspergillus flavus* | The Insecticide was able to inhibit all the organisms present initially except *Aspergillus flavus* |
| E | hydroxypropyl beta-cyclodextrin | *Aspergillus fumigatus and*<br>*Aspergillus niger* | *Aspergillus fumigatus,*<br>*Aspergillus flavus,*<br>*Aspergillus niger* | The freshener was not able to inhibit all the initial organisms present. There was an introduction of a new organisms as *Aspergillus flavus,* |
| F | dipropylene glycol methyl ether | *Aspergillus flavus* and<br>*Aspergillus niger* | *Aspergillus flavus* and<br>*Aspergillus niger* | There was no difference between the type of organism present before and after spraying the room |
| G | BHT | *Aspergillus flavus* and<br>*Aspergillus niger* | *Aspergillus flavus* and<br>*Aspergillus niger* | There was no difference between the type of organism present before and after spraying the room |
| H | Cyclodextrin | *Aspergillus fumigatus,* and<br>*Aspergillus niger* | *Aspergillus fumigatus, A. flavus* and *Aspergillus niger* | 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 *A. flavus* after spraying |

**Table 6: percentage (%) occurrence of bacteria isolates**

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

**Table 7: Percentage occurrence (%) of fungi isolates**

| **Isolates** | **No of rooms** | **No of occurrence** | **% Occurrence** |
|---|---|---|---|
| *Aspergillus flavus* | 8 | 6 | 75 |
| *Aspergillus niger* | 8 | 7 | 87.5 |
| *Aspergillus fumigatus* | 8 | 5 | 62.5 |