Assess the Microbial load in and around Market and medical survey in order to determine the health effects

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Abstract

From our studies it revealed that in the Mysore City due to the increasing population and pollution the microbial quality of air has been deteriorated. Hence it is necessary to take remedial steps to prevent disease epidemics. We found three types of bacterial colonies (Staphyloccus, Bacillus Micrococci) and seven types of Fungal colonies (Penicillium, Fusarium, Alternaria Alternata, Aspergillus niger, Curvularia, Aspergillus Fumigatus, Mucor in the market area and was impeccably identified. The occurrence of these bioallergens in the market area is an opportunistic pathogenicity, which causes infection such as damaged skin or mucous membranes and these are parasitic on mummlian skin. These are also responsible for respiratory ailments in the human body, like Asthama, allergic rhinitis etc., Due to favourable conditions the concentration of Bacillus species, Aspergillus Niger, Aspergillus Fumigatus, Curvularia, Penicillium were found high. The results of the medical survey showed astonishing effects of bioallergens on shop keepers. These vendors mainly had symptoms of Rhinitis and Influenza which includes nasal congestion, irritation of throat, cough and shivering. Also showed symptoms respiratory disorders. Eye irritation, skin infection was commonly seen. Hence, to curtail the concentrations of these bioallergens it is utmost important to maintain good sanitations levels, proper containers should be provided to different sections in the market to maintain health and aesthetics of the environment.

Key words: Bioallergens, microbes, health effects

Introduction

Many bacteria and fungi are also responsible for certain immunotoxic diseases like Organic Dust Toxic Syndrome (ODTS). It is the most common immunotoxic disease caused by inflammation of small airway and the alveoli of the lungs due to mycotoxins and endotoxins produced by bacteria and fungi. The symptoms of this disease resembles those of influenza with high temperature, shivering, nasal congestion, irritation of throat, headache, cough etc.,

It is known that allergen sensitization plays an important role & even critical role in the chronic inflammation characteristic of asthma. Since market is a place of high commercial activity where major and minor business transactions are carried out where in maximum amounts of bioallergens are aroused from these markets due to improper sanitation, decaying of vegetables and fruits, improper dumping facilities etc. Here, we are focusing mainly on the microbial load inside and around markets.

Mysore is one of the developing cities, which has seen steady growth in all fields resulting in pollutants of all kinds affecting the health of the people in and around the city. A study has therefore been undertaken to quantify the microbial population in the air in some selected market areas of the city and identify the potent Airborne Bioallergens that cause various types of allergies

Vegetable markets are one of the many environment that produce airborne microorganisms such as bacteria, fungi, actinomycetes and other living propagules. The vegetable markets, where the concentration of airborne fungal microorganisms may be significant, as the human population of all the living status is exposed.

OBJECTIVES

The main objectives of this project is to **"To assess the Microbial load in and around Devaraj Urs Market".**

Specific objectives are

1. To determine the concentration of airborne bioallergens in and around the market.

2. Identification of Bioallergens
3. To conduct medical survey in order to determine the health effects of the workers in and aroudn Dearaj Urs Market under doctor guidance.

MATERIALS AND METHODOLOGY

This present study was conducted to assess the concentrations of Bioallergens in Devraj urs market and their ill effects on the workers.

Sampling of air-borne microorganisms aims at their removal from air on to the surface for further microscopic examination or culturing to observe post-growth development. It is conducted for various purposes and may be quantitative or qualitative.

- Quantitative sampling refers of the percentage contribution of different types of microorganisms in air spora and one can determine the specific nature of microorganisms within some related group in qualitative analysis.
- Qualitative sampling refers to different type of microorganisms in air spora and one can actually determine their concentration in sampled air by quantitative analysis. The sampling methods vary greatly depending upon individual interest.

STUDY AREA

Mysore District is a popular tourist destination, offering several attractions ranging from the royal splendour of Mysore City and its fabulous Dasara Festival to exquisite temples, pilgrimage centres and scenic spots. The district lies on the undulating table land of the southern Deccan plateau, within the watershed of the Kaveri River, which flows through the northwestern and eastern parts of the district. The Krishna Raja Sagara reservoir, which was formed by building a dam across the Kaveri, lies on the northern edge of the district. Nagarhole National Park lies partly in Mysore district and partly in adjacent Kodagu District.

Mysore city (12^{o} 18’ N, 76^{o} 42’ E) situated about 140 Kms southwest of Bangalore, in Karnataka. It is situated at an altitude of 770m above mean sea level. The population of Mysore city is about 1,038,490 (according to 2001 census). Mysore has a moderate climate. The temperature in the district varies from 15^{o}C in winters to 35^{o}C in summers. Mysore district receives an average rainfall of 785mm.

Devaraj Urs market is one of the main market where fruit, flowers, vegetables and others basic things are found,It is laid over the filled and planned shopping along the Sayyaji Rao road with styled frontages and bables with a market plaza. This market stretches along the Western side of Sayyaji Rao Road south of Dhanvanthri Road. The market comprises of 722 shops.

Devaraj Urs market is the place, where major and minor business transactions takes place. Due to the improper sanitation, improper dumping and poor maintenance in the market area leads to the generation of harmful bioallergens. Therefore we are concentrating mainly on the market area in order to protect the health of workers and other peoples from allergies and infectious diseases.

SAMPLING SITES

In the present study six sampling sites are selected randomly in and around Devaraj Urs market. Fig. 3.1 shows the sampling sites in the study area.

Inside the Market

S_1 Flower section

S_2 Vegetable section

S_3 Fruit section

Outside the Market

S_4 Meat Section

S_5 Godowns

S_6 Outside dumping section

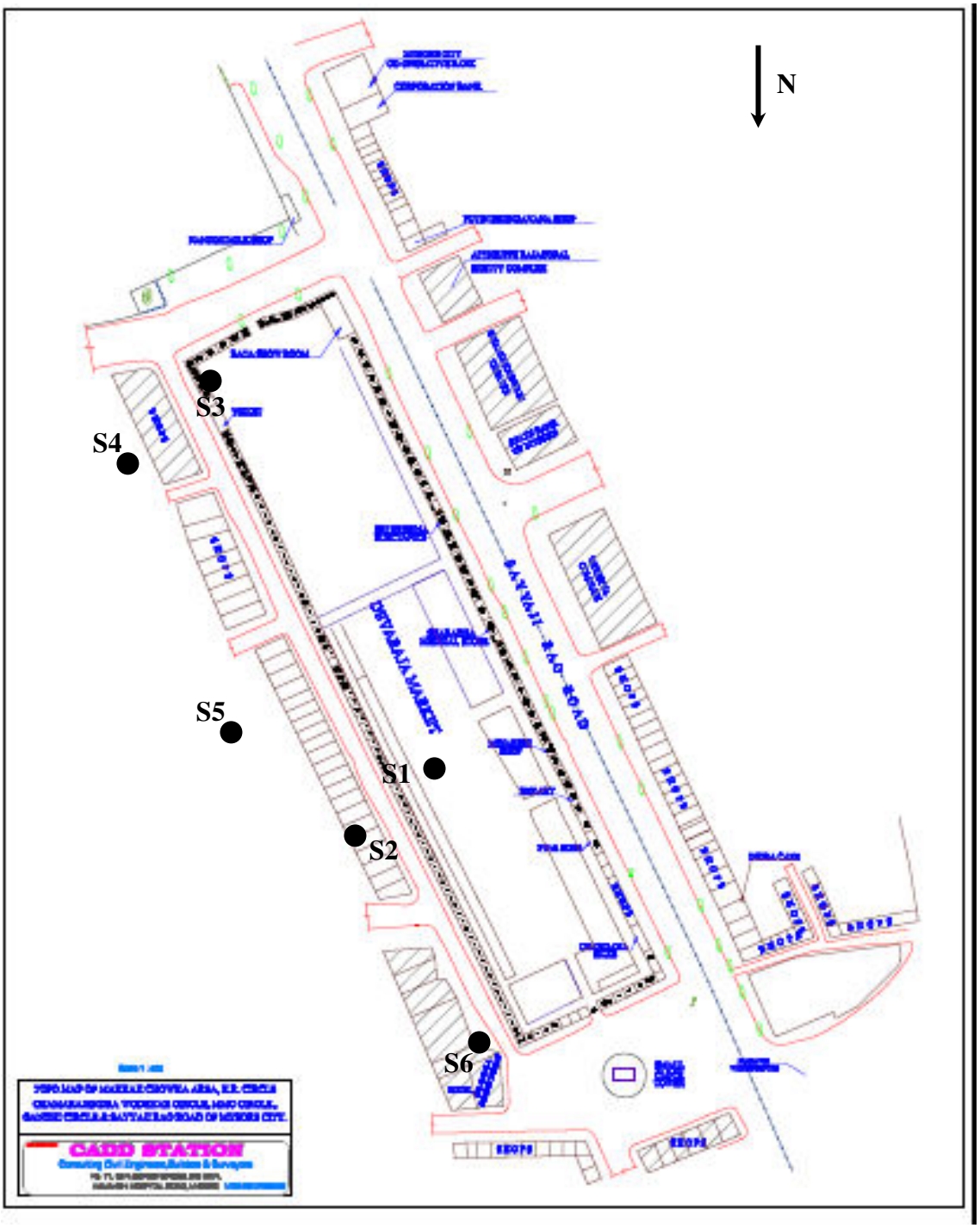


Fig 1: Map of the Study area

This experiment is based on the air sampling method which is also known as gravitation method. It involves direct isolation technique for bacterial and fungal isolation. Samples was collected from six sampling sites between morning that is from 10:00 AM to 11:00 AM and evening that is from 3:30 PM to 4:30 PM, by exposing Petri plates to air.

Sampling carried out by Gravitational Petriplate Method, petriplates were exposed in the ambient air of the sampling sites for 10 minutes, at the height measuring 1.5 meter above from the ground surface to approximate breathing height.

EXPERIMENTAL PROCEDURE

Culture Media Used and Method of Preparation

Nutrient agar – Nutrient agar is used for culturing bacteria. The composition of the agar is as follows:

- Agar, 15 g/L
- Meat Extract, 1g/L
- Peptone, 5 g/L
- Sodium chloride, 5g/L
- Yeast Extract, 2g/L

Czapek dox Agar – Czapek dox agar is used for culturing fungi

It is used for the routine cultivation of fungi, especially Aspergillus, Pencillium and non-sporulating molds.

METHOD OF AGAR PREPARATION

1. Soak the ingredients in small amount of water.
2. Add soaking ingredients to boiling water and bring to the boil again, by stirring continuously.
3. Dispense for slopes as required.

PROCEDURE FOLLOWED PRIOR TO SAMPLING

1. All surgical gloves, surgical masks are to be sterilized.
2. All glass wares used for preparation of culture media are sterilized for around 1 hour.
3. About 15 to 20 ml of Nutrient Agar and About 15 to 20 ml of Czapekdox Agar are poured into each of six petridishes.
4. The Petri dishes are kept undisturbed for 20 minutes for the Agar to solidify.
5. After solidification of culture media, the Petriplates are exposed to various sampling sites.
6. The exposed Petriplates are subjected to incubation and counting.

INCUBATION

The marked Petri plates were sealed kept in plastic covers and incubated, for a period of seven days, during which period, observation were made.

The Petri Dishes containing Nutrient Agar are incubated in the bacterial incubator at 35^{o} C for a period of 7 days.

The Petri dishes containing Czapekdox Agar are kept at room temperature for a period of 7 days.

COUNTING

Colony counter was used for Counting of the bacterial and fungi colonies growing on the medium of al 6 plates, was done on the third, fifth and seventh day of incubation, in order to obtain a more accurate results (to avoid missing out of any colony) .

The counts of the total bacterial colonies and total fungal colonies were recorded.

IDENTIFICATION

Identification was done for both bacterial and fungal colonies, based on their morphological characters. However, further identification to the genus level was done only for the fungal colonies by microscopic examination of the material. For small piece, of the material was taken from the colony with the help of a sterile needle. This was mounted on a slide with a drop of lactophenol or cotton blue in lactophenol, cover slip was placed over it and gently tapped.

IDENTIFICATION OF FUNGI

- Procure young cultures (5-7 days old) of fungi growing on culture medium.

- Put a drop of Lactophenol cotton blue is training solution for fungi
- Transfer a portion of mycelial mat from fungal colony into the drop of mounting fluid with the help of flamed and cooled needle.
- With the help of two needles gently spread the fungal propagules so that the mycelia should be mixed with stain.

Prepared slide is observed under Stereo Binocular Microscope and based on the structure and arrangement of the conidia (spores), the various fungi were identified, making use of standard manuals. The colony morphological features, were recorded using a stereobinocular microscope.

IDENTIFICATION OF BACTERIA

Bacteria produce acidic products when they ferment certain carbohydrates. The carbohydrate utilization tests are designed to detect the change in pH which would occur if fermentation of the given carbohydrate occurred. Acids lower the pH of the medium which will cause the pH indicator (phenol red) to turn yellow. If the bacteria do not ferment the carbohydrate then the media remains red. If gas is produced as a by product of fermentation, then the Durham tube will have a bubble in it.

The following are the biochemical tests that we perform are

1) Citrate utilization
2) Gelatin utilization
3) Starch hydrolysis
4) Indole Production
5) MRVP (Methyl Re-Vogues Proskauer)
6) Catalase
7) Oxidase test

CITRATE UTILIZATION

Tests for the ability of bacteria to convert citrate (an intermediate of the Kreb's cycle) into oxaloacetate (another intermediate of the Kreb's cycle). In this media, citrate is the only carbon source available to the bacteria. If it can not use citrate then it will not grow. If it can use citrate, then the bacteria will grow and the media will turn a bright blue as a result of an increase in the pH of the media. To inoculate this slant, use the transfer loop.

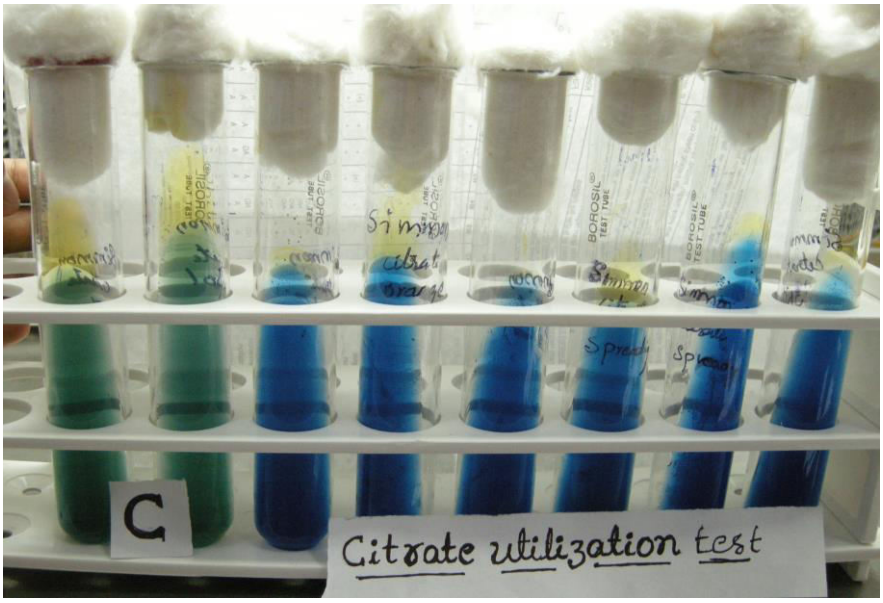


Fig 3.2: Citrate Utilisation Biochemical Test

GELATIN UTILIZATION

This media is used to test if bacteria can digest the protein gelatin. To digest gelatin, the bacteria must make an enzyme called gelatinase. To inoculate this media, use a transfer needle to stab the gelatin. After incubating the inoculated media for at least 48 hrs, transfer the tube into a refrigerator. The tube should be completely chilled prior to observation. If the media is solid after refrigeration then the test is negative (the bacteria did *not* digest gelatin). If the media is liquefied even after refrigeration, then the test result is positive…the bacteria is able to digest gelatin.

Fig 3.3: Gelatin Utilisation Biochemical Test

STARCH HYDROLYSIS

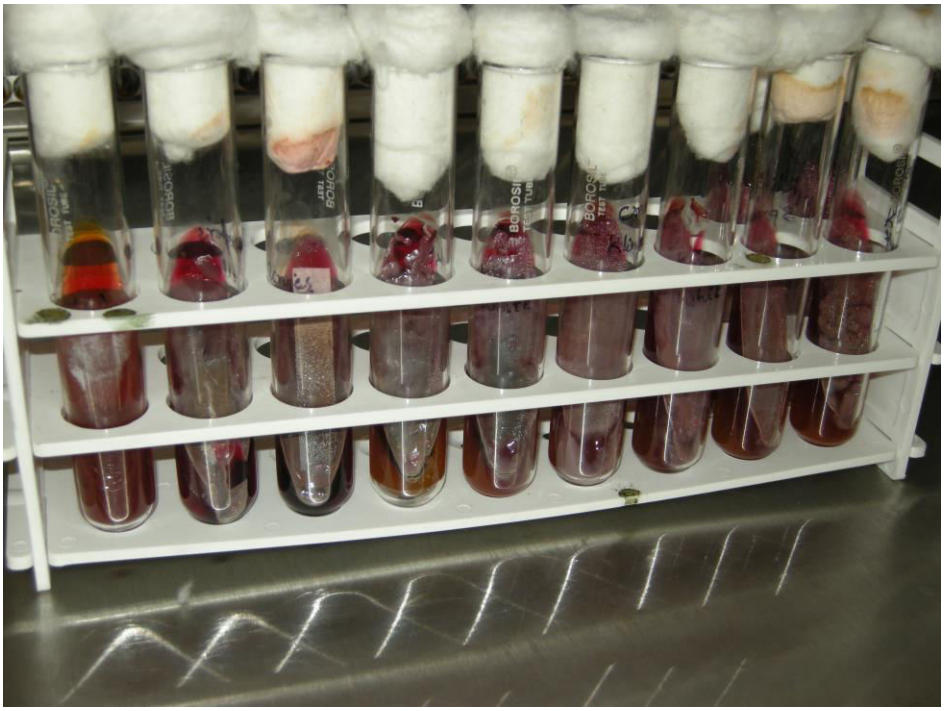


Fig 2: Starch Hydrolysis Biochemical Test

This test is used to detect the enzyme amylase, which breaks down starch. After incubation the plate is treated with Gram's iodine. If starch has been hydrolyzed (broken down) then there is a reddish color or a clear zone around the bacterial growth; if it has not been hydrolyzed then there is a black/blue area indicating the presence of starch. Simply use inoculating loop to spread bacteria onto plate surface. After the bacteria have grown, you add a few drops of Gram's iodine to the plate and look for the color immediately after adding the iodine.

MEDICAL SURVEY

In the present study, health conditions of workers are assessed by carrying out a medical survey. The QUESTIONNAIRE were prepared under the guidance of

Dr. Somashekar M.B.B.S., F.C.P,

Retired physician in K.R. Hospital

RESULTS AND DISCUSSIONS

RESULTS

This Chapter deals with the analysis and interpretation of data obtained from the experiment conducted .The results of bacterial and fungal colonies formed during the study are tabulated from table 4.1 to 4.6.

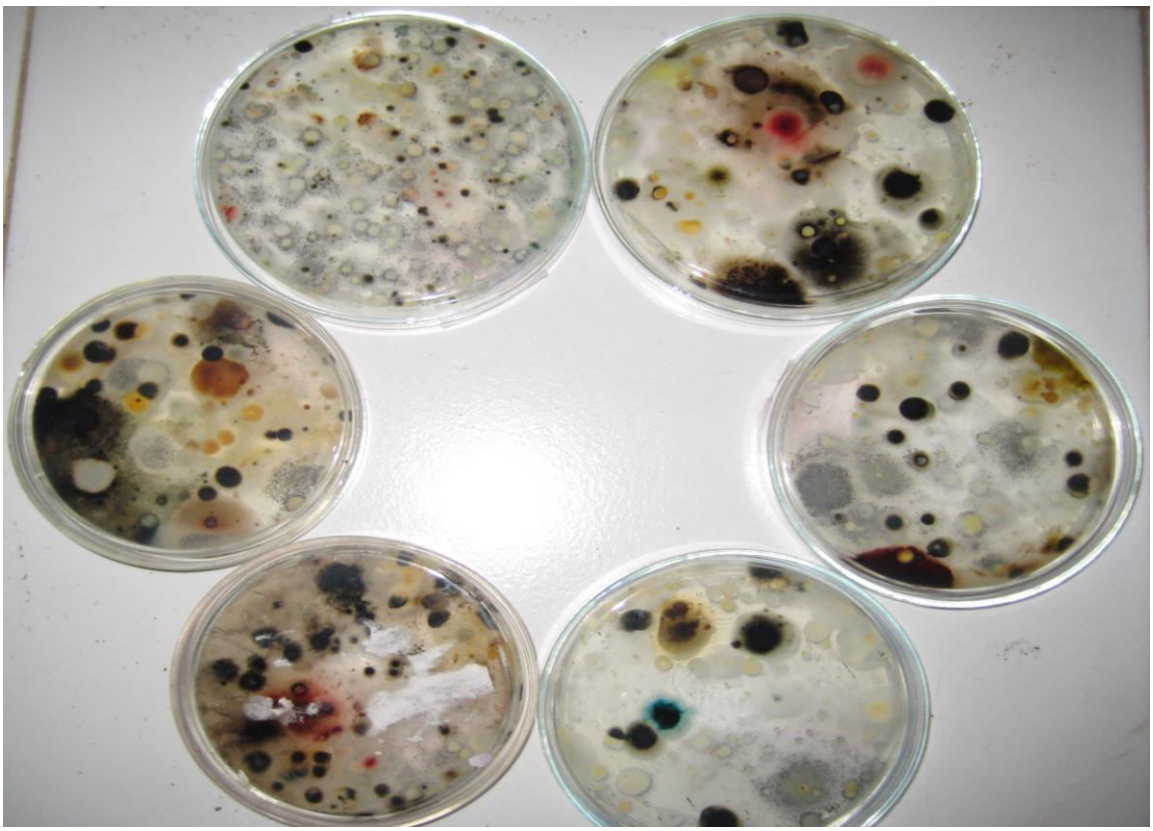


Plate – 1: Fungi Developed on Culture Media

Table 4.1: Concentration of Fungal species (cycle-1)

Species	Morning Session							Evening Session						
	Temperature	Meat	Godown	Dumping	Vegetable	Fruit	Flower	Temperature	Meat	Godown	Dumping	Vegetable	Fruit	Flower
Pencillium	35^0C	21	14	33	09	17	75	36^0C	18	32	14	16	03	06
Fusarium		14	08	12	07	16			09	11	03	10	05	03
Alternaria Alternata		Nil	13	15	16	32	19		19	22	20		05	06
Aspergillus Niger		23	10	06	32	34	20		55	13	17	26	60	52
Curvularia		Nil	13	17	13	Nil	Nil		Nil	Nil	14	05	18	Nil
Aspergillus Fumigatus		08	14	12	09	25	11		11	15	08	12	09	
Mucor		Nil	09	14	13	11	17		Nil	11	09	03	17	08

Table 4.2: Concentration of Fungal species (cycle-2)

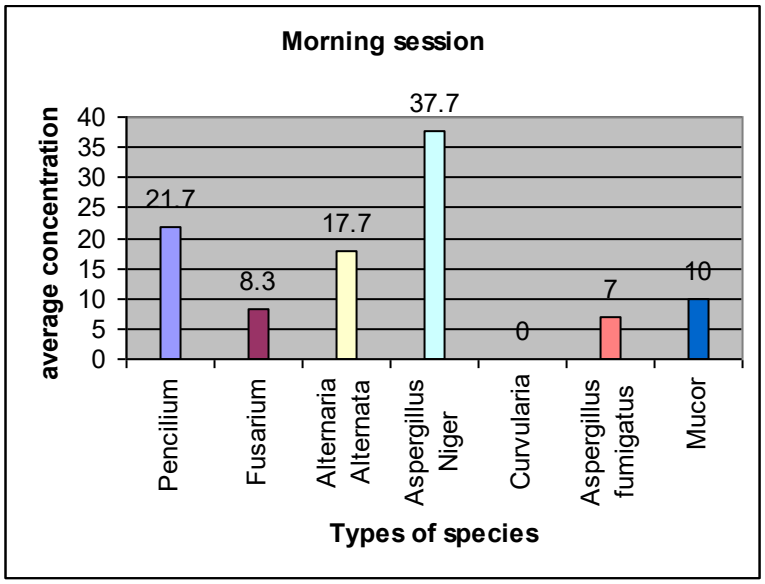
Species	Morning Session							Evening Session						
	Temperature	Meat	Godown	dumping	Vegetable	Fruit	Flower	Temperature	Meat	Godown	dumping	Vegetable	Fruit	Flower
Pencillium		23	20	14	10	18	34		19	43	13	17	08	06
Fusarium		11	07	10	03	Nil	19		05	11	10	05	36	04
Alternaria Alternata	34^0C	12	Nil	16	17	24	30	36^0C	17	24	29	06		04
Aspergillus Niger		26	06	15	43	32	20		17	14	60	52	26	17
Curvularia		Nil	12	18	11	05	Nil		08	28	15	12	15	09
Aspergillus Fumigatus		13	27	55	03	07	03		Nil	Nil	11	09	12	03
Mucor		10	09	07	13	02	11		13	12	04	Nil	03	11

Table 4.3: Concentration of Fungal species (cycle-3)

Species	Morning Session							Evening Session						
	Temperature	Meat	Godown	dumping	Vegetable	Fruit	Flower	Temperature	Meat	Godown	dumping	Vegetable	Fruti	Falower
Pencillium		Nil	08	Nil	10	02	04		17	43	13	13	Nil	06
Fusarium		06	14	12	02	03	06		08	07	04	05	05	08
Alternaria Alternata	35^0C	18	Nil	15	13	12	04	36^0C	19	20	Nil	16	14	13
Aspergillus Niger		21	09	06	29	31	25		17	15	37	45	23	14
Curvularia		14	07	Nil	Nil	08	Nil		13	Nil	Nil	Nil	14	Nil
Aspergillus Fumigatus		12	27	43	17	09	07		09	08	28	25	15	12
Mucor		10	13	07	09	04	02		Nil	11	11	12	09	03

INSIDE THE MARKET

FLOWER SECTION - FUNGI



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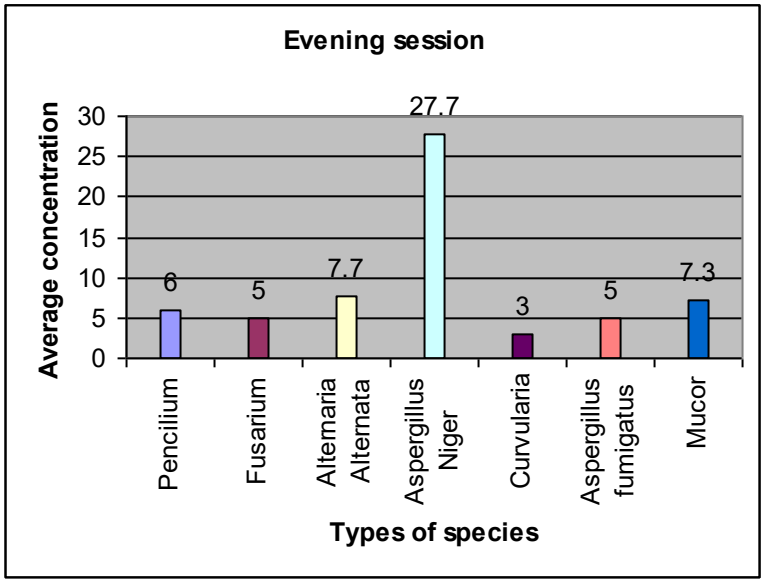
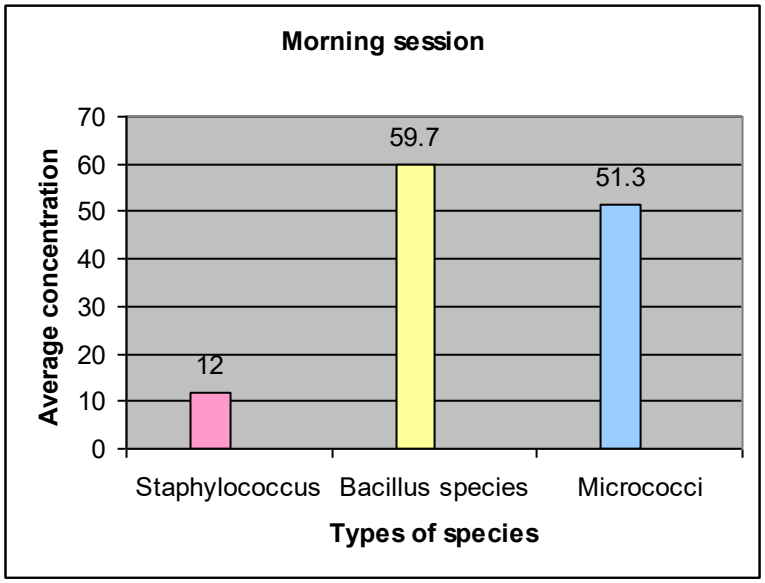


(b)

Fig 4.1: Average Concentration of Fungal Species at Flower Section

FLOWER SECTION - BACTERIA



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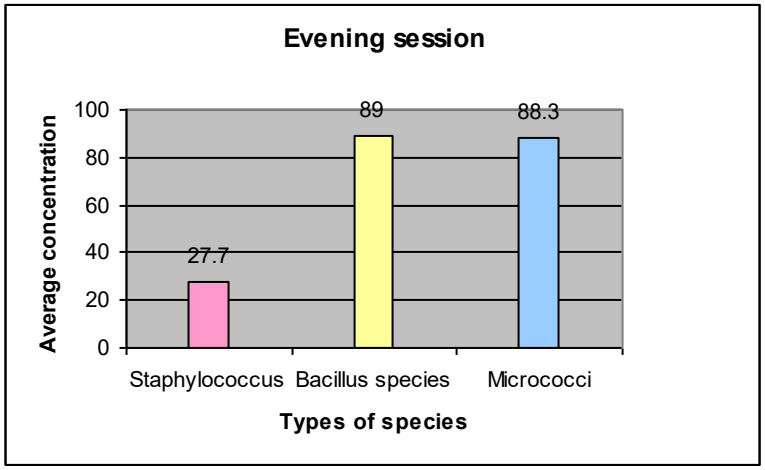
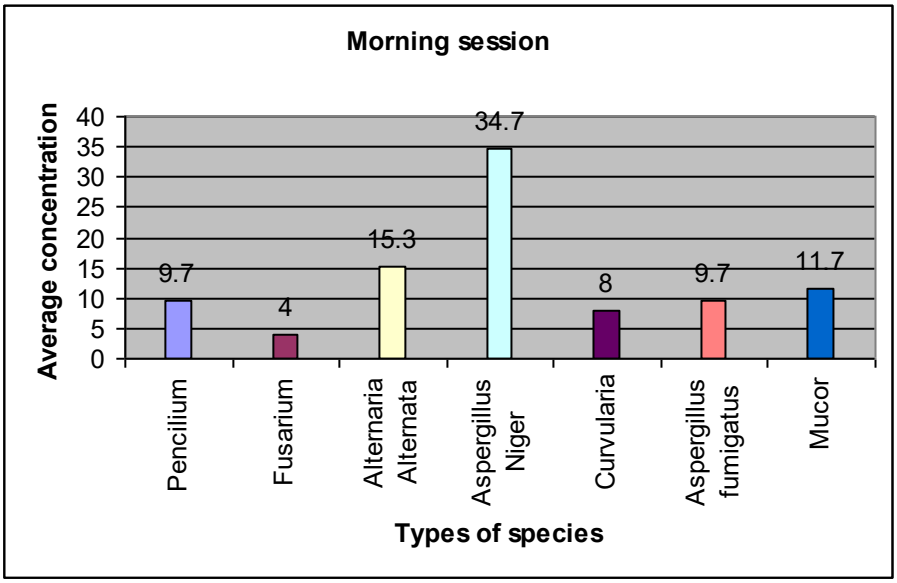


(b)

Fig 4.2: Average Concentration of Bacterial Species at Flower Section

VEGETABLE SECTION - FUNGI



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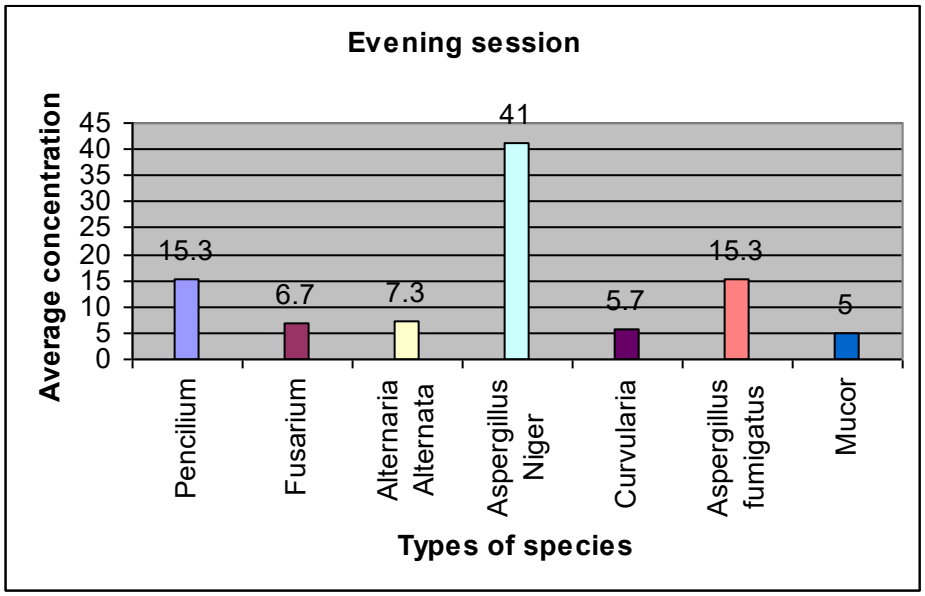

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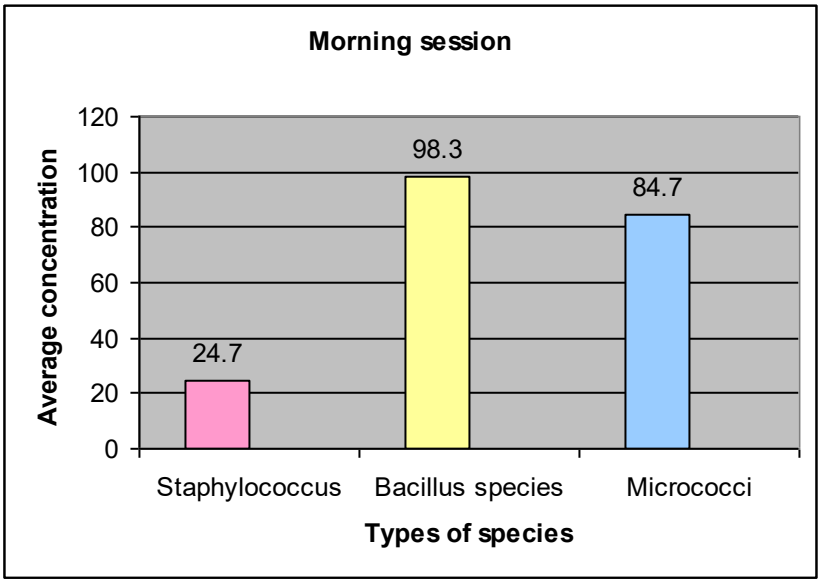
Fig 4.3: Average Concentration of Fungal Species at Vegetable Section

VEGETABLE SECTION - BACTERIA



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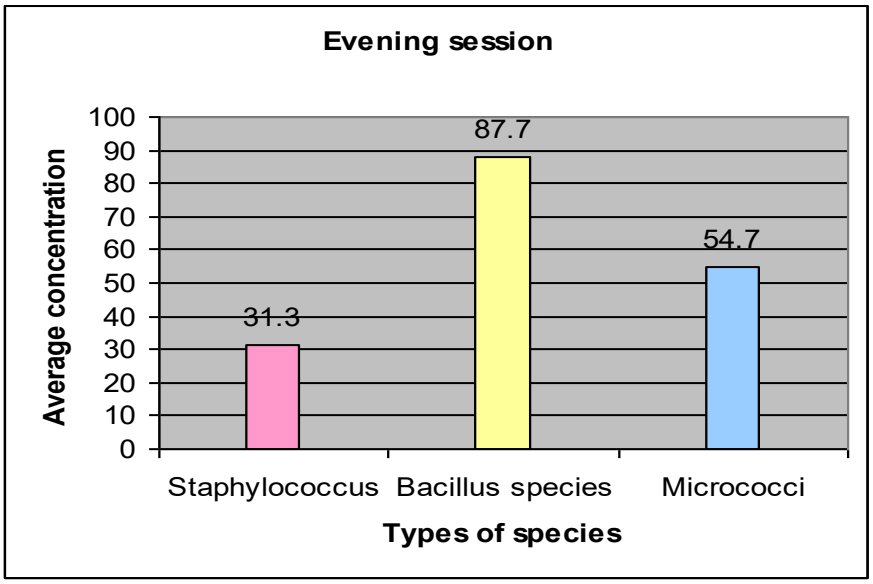
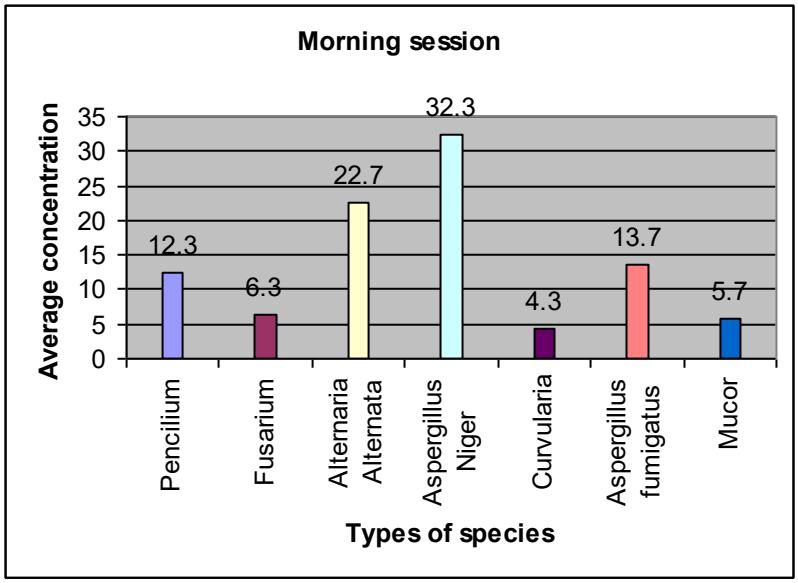


(b)

Fig 4.4: Average Concentration of Bacterial Species at Vegetable Section

FRUIT SECTION - FUNGI



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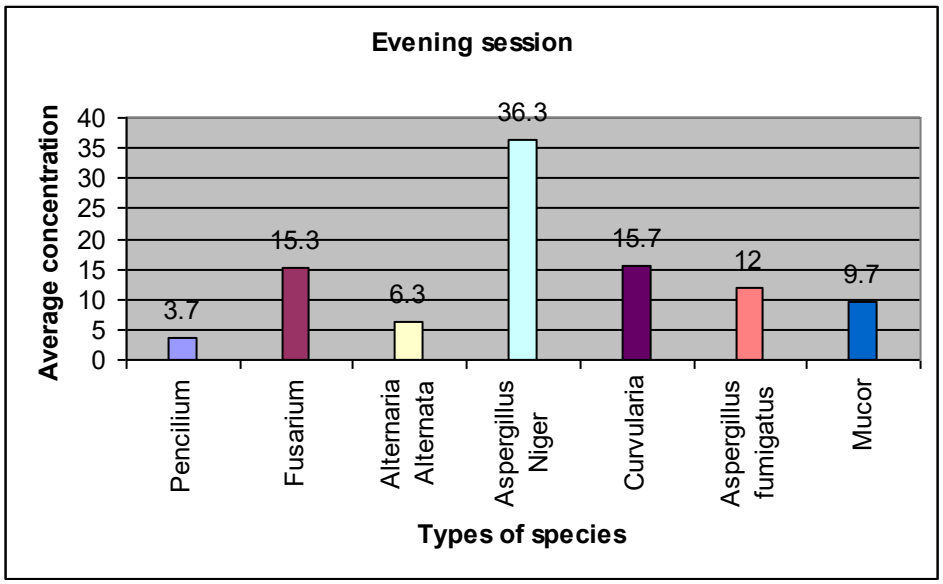

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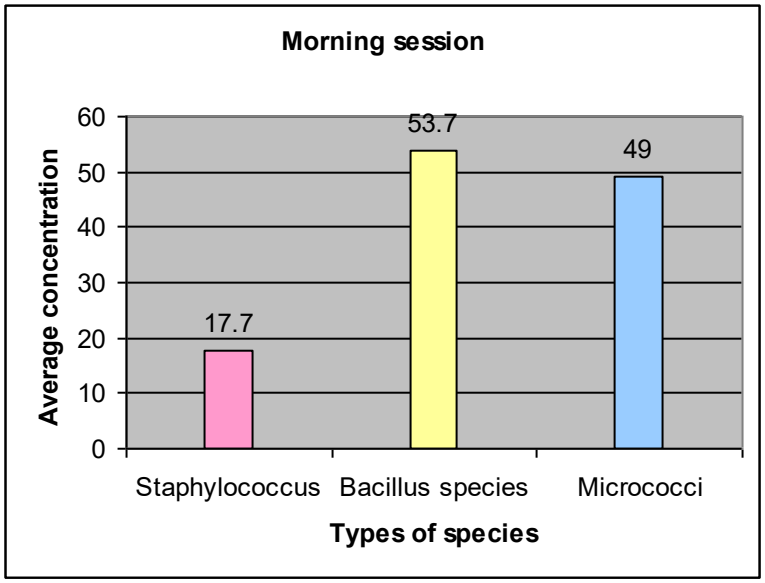
Fig 4.5: Average Concentration of Fungal Species at Fruit Section

FRUIT SECTION - BACTERIA



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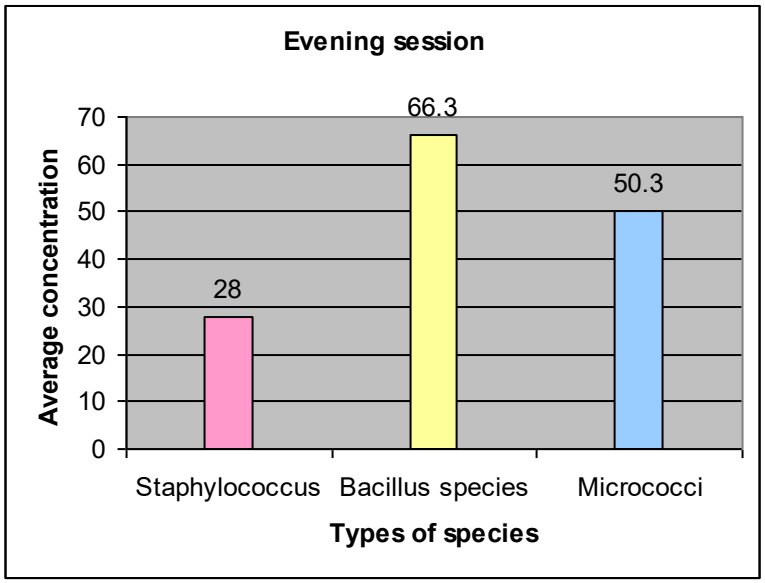

(b)

Fig 4.6: Average Concentration of Bacterial Species at Fruit Section

RESULTS OF BIOCHEMICAL TEST FOR BACTERIA

The biochemical test for bacteria is basically performed for the identification of the species and also to detmine the bioallergens which are responsible for diseases.Most of the bacteria have similar type of colonies which cannot be easily identified based on only morphological characteristics hence Biochemical Test is opted.

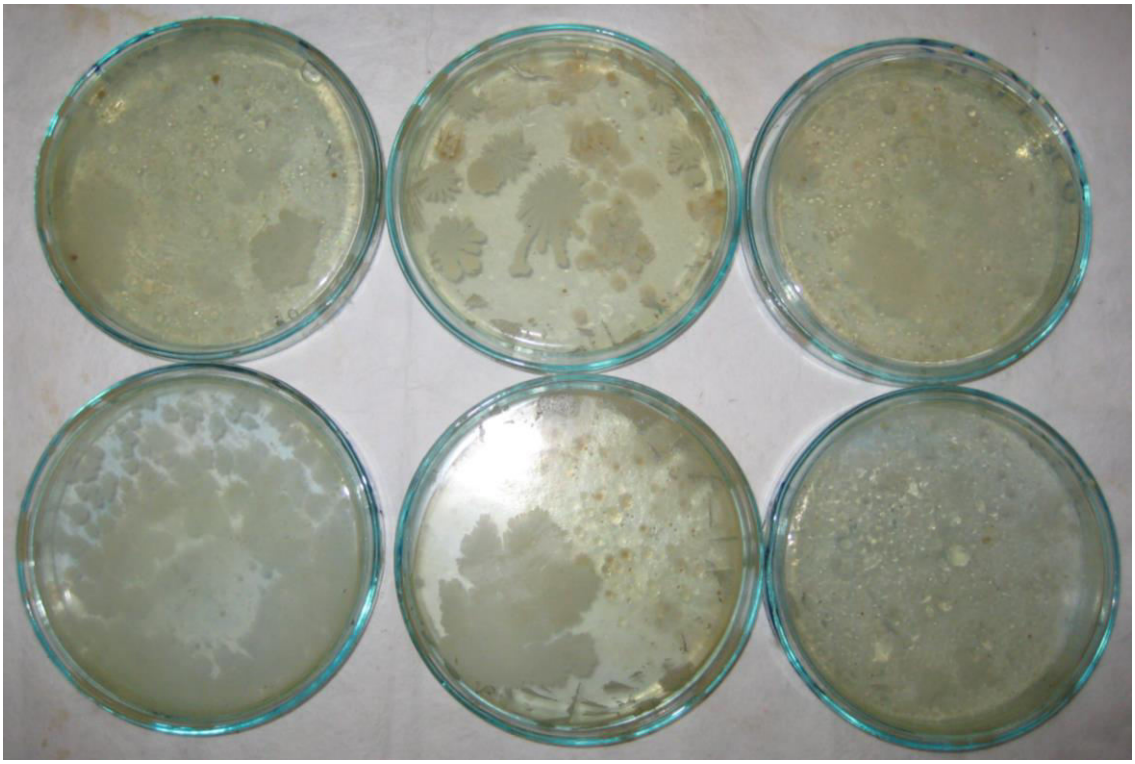

Plate 2: Bacteria Developed on the Culture Media

Table 4.7: Morphological Characteristics of Bacteria

Bioallergen	Morphological Characteristics
Staphylococcus	Non-spore forming and Gram positive cocci, circular, smooth, medium, opaque, golden yellow colony on Nutrient Agar.
Bascillus Species	Spore forming, Gram positive rods, creamy white colony on Nutrient Agar.
Micrococci	Gram positive cocci and golden yellow colony on Nutrient Agar.

The biochemical characteristics of bacteria is determined by performing tests namely Catalase, Oxidase, Urease etc on the culture media obtained, the results would either be positive or negative depending upon change in the color of the culture media to pink or yellow respectively.

Table 4.8: Biochemical Tests Performed on Bacteria

Test	Staphylococcus	Bacillus species	Micrococci
Catalase	+	+	+
Oxidase	-	-	-
Motility	-	+	-
Indole	-	+	-
Methyl-red	-	-	-
Voge-Proskauer	+	+	+
Citrate utilization	-	-	-
Urease	+	-	+
Hydrogen sulphide	ND	+	ND
Strarch hydrolysis	-	-	-
Gelatin hydrolysis	-	+	-
Nitrate utilization	-	+	-

+: Positive results
-: Negative results
ND : Not Determined

Table 4.9: Results of Medical Survey Conducted in Devarj Urs Market

Name	Age	Sex	Duration of Exposure(hrs)	Work Experience(yrs)	Asthma Self reported		Allergic sensation(Rhinitis & Influenza)		Skin			Eye irritation (Yes\No)
					Breathing problem(Yes\No)	Itching in nose,throat,running nose and cough(Yes\No)	High temperature and Shivering(Yes\No)	Nasal conjestion,irritation of throat,cough(Yes\No)	Itching(observed in any part of the body)(Yes\No)	Discolouration(Yes\No)	Round warm(Yes\No)	
Mohammed	28	Male	12	14	No	No	Yes	No	No	No	No	No
Sidaraju	42	Male	10	25	Yes	Yes	No	Yes	No	No	No	No
Vijay	30	Male	8	16	No	No	Yes	Yes	Yes	No	No	Yes
Basavanna	33	Male	10	15	No	No	No	Yes	No	No	No	No
Swamy	22	Male	12	6	No	No	Yes	Yes	No	No	No	No
Rashid	59	Male	9	40	No	No	No	Yes	No	No	No	Yes
Shariff	36	Male	14	20	Yes	No	Yes	Yes	No	No	No	Yes
Kempamma	60	Female	16	40	Yes	No	Yes	Yes	No	No	No	Yes
Jabar	34	Male	14	15	No	No	No	Yes	No	No	Yes	No
Ramappa	40	Male	13	30	No	Yes	No	Yes	No	No	No	No
Nagaraj Gowda	70	Male	12	40	No	Yes	No	Yes	No	No	No	Yes
H.G.Shivalingu	54	Male	12	16	No	Yes	No	Yes	No	No	No	No
N.Satish Kumar	38	Male	14	15	No	No	No	No	No	No	No	Yes
Gopal	34	Male	14	15	No	No	No	No	No	No	No	Yes
Sayed	56	Male	12	35	No	Yes	No	Yes	No	No	No	Yes
Janardhan	54	Male	13	40	No	No	No	No	No	No	No	No
Hemavathi	18	Female	14	1	Yes	No	No	No	Yes	No	No	No
Gayathri	42	Female	8	22	No	No	Yes	No	Yes	Yes	No	No
Nataraj	52	Male	12	20	No	No	No	Yes	No	Yes	No	No
Manjunath	44	Male	13	40	No	No	Yes	Yes	No	Yes	No	Yes
Ayub	35	Male	11	15	No	No	No	Yes	No	No	No	No
Sikander	26	Male	14	10	No	No	No	No	Yes	Yes	No	No
Asif	30	Male	9	15	Yes	Yes	No	Yes	No	Yes	No	No
Subramanya	54	Male	3	40	No	No	No	Yes	No	No	No	No
Mudasir	35	Male	14	25	No	No	No	Yes	No	No	No	Yes
Syed	53	Male		36	No	Yes	No	No	No	No	No	No
Ratna	48	Male		59	Yes	No	No	No	No	No	No	Yes
Rohith	26	Male	32	No	No	No	No	No	No	No	No	Yes

DISCUSSIONS

From the present study results, it can be interpreted that,

Three types of bacterial colonies were observed namely Staphylococcus, bacillus species and Micrococci among which the most prominent was Bacillus species, found in all six sampling sites. Bacillus species are found in virtually all environments, which is a notable food spoiler.

Also there were seven types of fungal colonies such as Pencillium species, Fusarium, Alternaria Alternata, AspergillusNiger, Curvularia, Aspergillus umigatus, Mucor. Out of which Aspergillus Niger, Curvularia and Fumigatus were found in high concentrations.

Curvularia basically requires moderate to high temperatures to thrive were found mainly in dumping sites. Aspergillus fumigatus were seen in godowns where-in conditions such as dampness is come across, these species also reproduce easily in compost heaps.

In meat, vegetable, fruit, flower sections colonies of Aspergillus Niger were detected, these species are one of the most familiar moulds that germinates on contact with moist surfaces of organic matter. In fruits the enzymes penetrate well causing rapid spoilage.

Aspergillus Niger, Aspergillus Fumigatus and Penicillium species are also responsible in organic deterioration which lessens the shelf life of the edibles.

Presence of these species causes many ailments to human being, Aspergillus Fumigatus causes Aspergillosis, Aspergillus Niger as been reported to cause skin and pulmonary infections, it is a common cause of fungal related ear infections –Otomycosis.

Curvularia mainly causes Dermatitis, Phaeohyphomycosis, Sinusitis and disseminated infections. Penicillium responsible to many allergens also causes Keratitis, Peritonitis.

Bacillus causes Anthrax; Humans acquire the disease directly from contact with infected herbivores or indirectly via their products. Bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract. Bacillus

species causes two distinct food poisoning syndromes: a rapid-onset emetic syndrome characterized by nausea and vomiting, and a slower-onset diarrheal syndrome.

Medical survey was carried out in Devaraj URS market showed high levels of headaches and ringworms affected to the vendors.

The shopkeepers who directly in constant contact with these bioallergens suffer from various diseases such as respiratory problems leading to Asthma, Allergic sensations were observed to a large extent whose symptoms were shivering, nasal congestion ,cough which ultimately leads to Rhinitis and Influenza, cases involving eye irritation ringworm, discoloration of the skin were also seen.

RECOMMENDATIONS

- To control the infectious bioallergens ,the shopkeepers should keep the shops clean, the floor should be washed with disinfections ,dispose unwanted products and trash from their shops to containers in a periodic manner and the container used should preferably closed ones .The customers must wash the products thoroughly before using it.

- The market authority should make provision for the vendors to go in for a routine check up.

- The levels of bioallergens should be determined and proper legislation to be made to ameliorate the hazards of organic dust pollution.

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