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## **DEMONSTRATION OF MICROBIAL LOAD IN COFFEE POWDERS**

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Key words:

Coffee, Microbial Analysis, Fungi, Food Adulteration The study was aimed at conducting a microbial analysis of 19 samples of commercially available instant coffee powders to study the presence of various micro organisms including fungi in the samples of coffee powder. Instant coffee is a soluble product in the form of flowing powder or agglomerate derived from the aqueous extracts of freshly roasted and ground coffee, having the colour, taste and flavour characteristics of coffee. The microbial contamination of coffee powders is commonly due to mishandling and unhygienic conditions during the storage and processing of the coffee beans. Coffee is a very popular beverage amongst the Indian population. In the study that was conducted, fungal contaminants were found in 5 out of 19 samples and the bacterial contaminants were found in all the samples.

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### **INTRODUCTION**

Coffee is one of the most commonly consumed beverages in our country. There are various types of coffee POWDERS like instant COFFEE POWDERS, filter COFFEE POWDERS etc. Coffee is produced in many countries according to many different processes. Different harvesting practices can lead to discernable differences in the starting material, different processing methods may involve different 'intermediate' products with varying capacities to support the development of associated hazards, different marketing practices may lead to significant variability in the opportunity afforded for the development of related hazards.[1] The Coffee powders may be contaminated during handling packaging or storage of the product. These food borne pathogens have the potential to cause food-borne diseases which are usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food.[2] There is very limited data on food borne diseases and its impact on public health.

## **MATERIALS AND METHODS**

19 Commercially available coffee powders that were well within their expiry date, were taken as samples for the study. At the outset, four samples were used to standardise the procedure, to pick between two dilutions - 0.5mg in 1mL and 0.5 mg in 20 micro L. The concentrated sample (0.5 mg in 20 micro L) showed better growth and colony formation, so the remaining samples were plated in the dilution with higher concentration. The procedure was carried out in aseptic conditions.

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Autoclaved foil paper was used to weigh the samples, and autoclaved tongue depressors were used to carry the coffee powders from freshly opened packets. The measured samples were placed into micro-centrifuge tubes and micro-pipettes were used to pipettes out 20 micro L into each tube, with different tips for each sample. These tubes were centrifuged for 15 mins and the sediment was collected and plated onto two sets of media, BHI(Brain Heart Infusion) agar for observing bacterial growth and SDA (Sabouraud's Dextrose Agar) for observing fungal growth. The SDA was placed in a tube, and the sediment was inoculated onto the slant, whereas, the BHI was placed in a petri-plate, and the sample was inoculated in a lawn culture.

The BHI agar with the coffee sample was incubated for 24 hours at 37 degree Celsius, and the SDA with the coffee sample was incubated for 48 hrs at room temperature. After observing colonies in the agar, they were used to create smears and simple staining was done in order to identify the type of bacteria present in the colonies.

## RESULTS

According to the (fig 1), fungal colonies were observed in 5 out of the 9 commercially available coffee powders and bacterial colonies were found on all the samples, with 3 of the samples exhibiting enterococci colonies. The bacteria that were commonly observed were lactobacillus (aerobic spore bearer), staphylococcus (gram positive cocci) and enterococcus were seen in three samples.

The number of bacterial colonies ranged from 9(sample no. 8) -150(sample no.5) and the fungal colonies were from 1-2 and were most likely to be candida.

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	Nutrient A	SDA		
S.NO		Colony	Fungi	Colony
	Bacteria present	count	present	count
1	GPC, ASB	25		
2	Enterococci, ASB	103		
3	GPC, ASB	85		
4	ASB	120		
5	ASB	150	Moulds	1
6	Enterococci	35		
7	GPC, ASB	28		
8	ASB	9		
9	Enterococci	95	Moulds	2
10	GPC	15		
11	GPC, ASB	130		
12	GPC	55		
13	GPC	40		
14	ASB	65	Moulds	1
15	GPC, ASB	88	Moulds	1
16	GPC, ASB	35		
17	GPC, ASB	18		
18	GPC, ASB	15	Moulds	2
19	GPC	35		

Fig 1 colony count observed in the 19 samples



Fig 2 pie chart representing the bacterial colony numbers in the 19 samples



Fig 3 pie chart representing the type of bacterial colonies most commonly observed (ASB- Aerobic Spore Bearer, GPC- Gram Positive Cocci)



Fig 4 colonies of GPC on BHI agar



Fig 5 GPC as seen on a smear under light microscope



Fig 7 ASB as seen on a smear under a light microscope





Fig 6 colonies of lactobacilli on BHI agar



Fig 8 colonies of enterococci on BHI agar



Fig 9 enterococci as seen on a smear under a light microscope

Fig 10 colonies formed on SDA



# Fungi- colony forming units(SDA)



### DISCUSSION

The coffee beans are commonly contaminated with fungal growth, when they are stored before processing, due to moisture contamination. These coffee beans when used to make coffee powder contain mycotoxins and Ochratoxin A(OTA).OTA is a mycotoxin and a known nephrotoxin, carcinogen, teratogen and possibly genotoxic. OTA is produced by the two genera of fungi: Penicillium and Aspergillus[3] coffee (Instant coffee) powder is prepared by extraction under suitable conditions, pure, freshly roasted and ground coffee with water, The extraction is carried out in a series of percolators or extractors at suitable temperatures.

The brew thus obtained, with or without further concentration, is dried to a powder which may be agglomerated. This is packed in air-tight containers in humidity controlled rooms.[4] the contamination may even occur during the process of spray drying or freeze drying the coffee powder, where the sample may be inadequately dried, with unequal moisture distribution in the powder leading to fungal growth.

# CONCLUSION

From the study that was conducted, none of the samples were contaminant free which is quite alarming. Though these bacterial and fungal contaminants may not be fatal or life threatening, they are still contaminants and hence adulterants. Food adulteration is a topic which is not taken up very seriously in our country. People suffer from many diseases, without any known cause in our country, and these contaminants may be the reason for various complications that are not lethal but still affect the quality of life of a common Man. People should receive contaminant free sterile products for the price they pay, at least in the case of edibles, beverages and food grade materials, which enter our system on consumption of the product. Food adulteration laws must be made more stringent, and various levels of examination must be established, with further research to evaluate the levels of contamination in the currently available commercial food products and beverages.

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