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FUNGAL SPOILAGE OF BAKERY PRODUCTS AND ITS CONTROL MEASURES

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ABSTRACT

Bakery products are an important part of balanced diet. It is a good source of nutrients, such as macronutrients and micronutrients that are essential for human health. Most common products are breads, bun, cupcakes, cookies, pizza base, toasts etc. These products are subject to fungal spoilage. Many filamentous fungi such as *Rhizopus*, *Mucor*, *Aspergillus* and *Fusarium* are involved in spoilage of bread due to improper handling and improper sanitation. So its economically important and commercial value is lost due to improper handling. Spoilage of these bakery products may constitute a health risk in food may cause a mild form of food illness. The chemicals are hazardous to the human health so use of natural agents is useful to maintain human health. It is not affect the food flavour. Clove, Lemongrass, Cardamom, Citrus and Edible oil are useally used in food product. These product also acts as controlling measures for these fungi. The present study is focused on the fungal spoilage of bakery products and its control measures.

KEYWORLDS: Fungal spoilage, control measures.

INTRODUCTION

Bakery products are the important staple foods in most of the country and cultures. Most common products are breads, bun, cupcakes, cookies, pizza base, toasts etc. The cereals which are used in bakery products are a valuable source of nutrients in our diet, which provide us most of our food calories and approximately half of our protein requirements. The nutrients in bakery products are carbohydrates, proteins, lipids, vitamins, calcium, iron, minerals, starch and energy.

Baked goods have been found around us for thousands of years. The bakers began to prepare bread at home in an oven, using mills to grind grain into the flour for their breads. The drastic appeal for baked goods promoted baking all throughout Europe and expanded into the eastern parts of Asia.

Bakery products once considered as sick man's diet have now become essential food items of the vast majority of population. Importance of bakery products has expanded especially the use of natural grains and other natural ingredients. Bakery industry in India is the largest of the food industries, with an annual turn over of about 3000 crores. India is the second largest producer of biscuits. Breads and biscuits from the major baked foods accounting for over 80% of total bakery products produced in the country.

Commercial bread and biscuits contain around 7.5% to 7.8% protein respectively. Bakery products are good targets for fiber enrichment. Fibers enrichment of several bakery products has recently been tested using an ingredient containing as percent short chain fructo-oligosaccharides.

Baking ingredients are wheat flour, water, sugar, salt, eggs, fats, yeast, baking soda, baking powder, cornstarch, milk, butter or margarine, honey, yogurt, essence, cocoa powder, chocolate slabs, fruit jams, sweetener etc.

Spoiled bakery products may be defined as bakery products that has been damaged or injured so as to make it undesirable for human consumption. Bakery products are subjected to spoilage problems. These include physical, chemical and microbial spoilage. So its economically important and commercial value is lost due to improper handling. The economic losses associated with bakery products, is the possibility of mycotoxins production.

Mold spores are generally killed by the baking process in fresh bread and other baked products. Therefore bakery product which become moldy, it must be contaminated either from the air, bakery surface, equipment, food handlers or raw ingredients or after baking during the cooling, slicing or wrapping operations. This means that all spoilage problems caused by molds must occur after baking. The fungal spore counts are higher in the summer months than in the winter due to airborne contamination

in the warmer weather and more humid storage conditions. Fungal spoilage caused undesirable odors and is often found on the surface of the product. The most common fungi found in bakery products are *Rhizopus, Aspergillus, Mucor, Fusarium sp.*

Many filamentous fungi such as *Rhizopus, Mucor, Aspergillus* and *Fusarium* are involved in spoilage of bread due to improper handling, improper sanitation, so losses of bakery products due to these fungi vary between 1.5% depending on seasons, type of products and methods of processing. The incidence of wheat bread spoilage caused by these fungi has increased during the last few years presumably because more bread is produced without preservative and often raw materials such as bran and seeds are added. Spoilage of these bakery products may constitute a health risk in food may cause a mild form of food illness. Consumption of these products has been association with food borne illness.

Usually bakery products are packed in plastic films after baking and cooling and they consumed within 1 or 2 months. Post process contamination is unavailable. Contamination by fungal organisms in these kind of products usually comes from the post baking cooling period as the cooking temperature is normally enough to eliminate previous contamination. This study will focus on the fungal spoilage of bakery products and its control measures.

MATERIAL AND METHOD

Collection of sample

Six different types of bakery products i.e. pizza base, bread, toast, cupcake, bun, cookies used in this study were collected from different shops such as Swadh, Anand, Paris, Kwality, King and Gujarati bakery from the local market which were decorated sampals in open stall. The collected samples were brought in sterile polythene bags to the laboratory for analysis. Open contaminated samples were brought to the laboratory environment and maintained at temperature 25-27^{0}C for the fungal growth which takes place in 7 days. Slides were prepared by scrapping the products, for identification of fungi.

Preparation of media

A small fragments of these fungi were transformed aseptically to Asthana and Hawker's medium 'A'. (5 g glucose, 3.5g KNO3, 1.75g KH2 PO4, 0.75g MgSo4.7H2O and 15g agar agar). This media was autoclaved at 121^{0}C for 15 minutes. After sterilization it was allowed to cool down at about 40^{0}C. All the glass wares used in this study were sterilized in a hot air oven at 160^{0}C for 2 hours. The other materials were sterilized by autoclaving at 121^{0}C for 15 minutes. The fungal growth takes by scrapping the products preparation of media.

About 20 ml of the media was poured into each sterilized petri dish. The media in petri dish was allowed to solidify. 1g of bread sample was mixed with distilled water and a homogenate was prepared and then added on the surface of the media and spreader evenly over the surface using a sterile spreader (bend glass rod). The plates were incubated in an upright position at 30ºC for 4-5 days. The same procedure was carried out for all the samples.

The fungal count was recorded. The different types of colonies were used as inoculated to obtain pure cultures by sub culturing in Asthana and Hawker's media.

The slides were made and morphology i.e. shapes structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys available by H.L. Barnett and Barry B. Hunters "Illustrated genera of imperfect fungi and other literature.

Antifungal activity against fungal strain

Natural prevantives control measures were used against the fungal strains. There are five controlled measures are used against fungal spoilage i.e. Cardamom powder, Syzygium aromaticum powder, Citrus juice, Lemon grass juice and Edible oil. These control measures were assayed for antifungal activity against the fungal strains of *Mucor, Rhizopus,*

Aspergillus, and Fusarium. These control measures are not hazards to human being and can easily dissolve in food products.

To prepare the solvent for antifungal activity against fungal strain 100 gm of Cardamom and *Syzygium aromaticum* were taken in a grinder, for preparation of the powdered. 1 gm powdered dissolved in 10 ml of the distilled water and after 1.30 hours it was filtrated through filter paper. Collected filtrate was taken for antifungal activity against the pathogen.

Clove powder

The traditional use of clove is used as food preservative and it does not affect the food flavored. The clove consists of the many chemical compounds such as phenolic compound, phenolic acid, Gallic acid and Eugenol. These are the main bioactive compound of clove. Clove powder is reported to have strong antifungal activity against many strains. The essential ingredient responsible for its antifungal activity is Eugenol from the clove. Clove powder caused growth inhibition of fungi.

Lemongrass extract

Lemongrass is widely used in traditional medicine in many countries around the world.

The lemongrass extract consist of the many chemical compound, these are alpha pinene, cis- sabinene hydrate, 1–8 cineole, Geranly acetate, Geraniol, Terpinolene, B-caryophyllene, Linalool, Limonene, 3–myrcene, neral, Geranial. A mixture of neral and geranial is called citral.

These citral major fractions exhibited the greatest antifungal activity. Lemongrass extract was found to be highly fungicidal and highly growth inhibition of fungi. The leaf extracts effect on fungi as reduction in condition, loss of pigmentation and disrupted conidiophores structure. It was effective in inhibiting fungal viability and spore germination.

Cardamom powder

The traditional use of the cardamom in food as a flavoring agent is seen from ancient time.

Cardamom consists of many chemical compounds such as cineole, and other aromatics compound present such as tripinyl acetate, terpineole, spathulenol, borneole, terpinene etc. The other constituents of the cardamom seeds are fixed oil, starch and proteins. The cardamom contains volatile oil to the extent of 2% to 8%. The active constituent of the volatile oil is cineole. Cardamom powder was found to be performed against the fungi as the strong antifungal activity. It was effective in inhibiting the completed growth of fungi.

Citrus extract

In citrus the lime flavors are used in the beverage, confectionery, cookies and desserts. The citrus extract consist of the many chemical compound such as geranial, nerol, geranyl acetate, geraniol, beta -caryophyllene, nerol, neryl acetate. The citrus consist of major components is limonene. The citrus extract is reported to have strong antifungal activity against the many strains of fungi. It strongly inhibits the spore production and germination of the fungal pathogen.

Edible oil

In many oil seedlings soyabean oil are very useful in cooking which also acts as an antifungal agent. The soybean oil is specially included as a main component in food which is very nutriceus. Soybean oil is often used as adjuvant, in agricultural spray to facilitate spread of the active. Product derived from Soybean crops are not only global food staples, but are also used in industry and agriculture. This oil consist of 12% saturated acid and 80% unsaturated acid. The edible oil is reported to have strong antifungal activity.

Calculation-It was done by using the formula dc – dt/dc
Where, dc – control reading of Species, dt – reading of target Species.

Antifungal activities were screened by agar well diffusion method. The five controlled measure was tested against the fungal strains. For that 20 ml Asthana and Hawker's media was poured in to the sterile petri plates and allowed to solidify. The test fungal colonies were inoculated into the petri plates in four corners. Wells of 1cm diameter were punched in the four corners by using sterilize cork bore. In these wells 20 ml Cardamom, Syzygium aromaticum, Citrus juice, Lemongrass juice, and Edible oil extracts was poured. The plates were incubated at 27^0C for 48-72 hours. After the incubation the plates was observed for formation of clear incubation zone around the well which indicat the presence of antifungal activity. The zone of inhibition was recorded.

OBSERVATIONS AND RESULT

A) Taxonomical study of the isolates

1) *Rhizopus sp Ehren*

Family – Mucoraceae

This fungus was collected from the bakery products such as bread, bun, capcake, toasts.

These products were collected from the Quality, Swad, Paris, and Anand Bakery in month of January 2019.

Morphology of isolates

Hyphae broad, not or scarcely septate; rhizoids and stolon present; sporangiophores, brown to yellow, solitary or in tufts on the stolon, diverging from the points at which the rhizoids form; sporangia rather round; apophysis absent or scarcely apparent; sporangiophores ovold. (See Photoplate 7 fig A).

2) *Mucor sp. Fresen*

Family – Mucoraceae

The fungus was collected from the bakery products such as pizza base, bread, toasts, bun, and cup cake. Those products were collected from the Anand, Quality, Swad, Gujrati, Paris bakery in month of January 2019.

Morphology of isolates

Colonies are cottony to fluffy, fast growing, white to yellow, becoming dark grey, with the development of sporangia. Sporangiophores erect, simple or branch forming large, terminal globose, to spherical, multi spores, without apophyses and with well developed subtending columellae; sporangiopores are hyaline, grey or brownish, globose to ellipsoidal and smooth walled or finely ornamented. (See Photplate 7, fig B).

3) *Aspergillus sp*. micheli exlink Family – Trichocomaceae

This fungus was collected from the bakery products such as cap cake, toasts, cookies, pizza base. These products were collected from the Quality, Anand, Paris and Swad bakery in month of January 2019.

Morphology of isolates

Colonies white initially but soon turn in black due to the production of conidia, hyphae hyaline, branched, septate, conidiophores unbranched, hyaline, terminating in globose, vesicles, sterigmata flask shaped, producing conidia in acropetal succession in chains; conidia globose, 1 celled, venucose, dark brown to grayish black. (See Photplate 7, fig C).

4) *Fusarium sp* Link ex. Fries Family – Nectriaceae

The fungi was collected from the bakery products such as pizza base, bread, cap cake, bun.

This product was collected from the Quality, Gujarati, Swad, King Bakery in month of January 2019.

Morphology of isolates
Mycelium pale to dark brown branched, septate, conidiophores, simple, short, branched, bearing whorl Philides; conidia hyaline, variable whorl philides; conidia hyaline variable and of two kind in head, macroconidia 5-6 celled. Microconidia 1 celled, ovoid oblong borne single on conidiophores intermediate, conidia 2-3 celled oblong or slightly curved. (See Photplate 7, fig D).

5) *Alternaria Sp.*
Family – Pleosporaceae
This fungus was collected from bakery products such as toast, bread, cookies, pizza base. These products were collected from the Gujarati, Swad, Paris, Anand bakery in month of the January 2019.

Morphology of isolates
Colonies usually black. Hype dark brown, branched, wide, conidiophores arise singly or in small groups, branched flexuous, geneculated, pale to olivaceous or golden brown, smooth, thick with one or several conidial scars, conidia form in branched chain, of obcalcuate obpyriform, ovoid or ellipsoidal of often with short conical or cylindrical beak, pale to mid- gloden brown, smooth or verruculos, with upto 8 transverses and usually several longitudinal or oblique septa, black pale thick. (See Photplate 8, fig .A).

6) *Penicillium sp*. Link. Family – Trichocomaceae
This fungus was collected from bakery products such as bun, cup cake, pizza base. These products were collected from King, Anand, and Quality bakery in month of January 2019.

Morphology of isolates
Colonies broadly spreading, velvety, heavily sporulating forming condia in chains; mycelium submarch, septate; penicilia by verticulate and asymmetrical born on smooth wall conidiophores; metule forming chains, phialides born in terminal cluster; Conidia globular, elliptical, smooth. (See Photplate 8, fig .B-).

7) *Phoma sp*. Sacc.
Family – Didymallaceae
This fungus was collected from bakery products such as toast, bread, cup cake. These products were collected from Quality, Anand, and King Bakery in month of January 2019.

Morphology of isolates
Colonies black, spreading, hyphae hyline, branched, septated, pycnidium globous to subglobulous, glabrous, conidia one celled, hyline. (See Photplate 8, fig C).

8) *Curvularia sp*. Boedijn. Family – Pleosporaceae
This fungus was collected from bakery products such as bun, toast, pizza base. These products were collected from Paris, King, and Swad bakery in month January 2019.

Morphology of isolates
The fungus appeared dark black in culture; mycelium branched, septated, subhyline, dark to light brown, conidiophores erected, slightly bent, dark brown, unbranched, septated, geniculate towards apex. Conidia produce acrogenously at the tip of conidiophores and on its successive growing point. Conidia boat shape, brown, three, septed, the tired cell from the base conspicuously large, broader and darkar than the other cells, other cells lightly curve, sub-hyline with rounded apical cell and sub-hyline somewhat of conical basal cell which bears a scar indicating point of attachment to the conidiophores. (See Photplate 8, fig .D).

Antifungal activity against fungal strain
Table 1: Antifungal activity against *Rhizopus sp.* by various solvent Control (mm) - 50.75.

Sr. No.	Solvent name	Zone of inhibition (mm)
1.	Cardamom powder	36.25
2.	Clove powder	37.75
3.	Citrus juice	35.5
4.	Lemon grass juice	41.25
5.	Edible oil	39.5

In case of Rhizopus all the five solvent show their growth of inhibition. Citrus juice, Cardamom powder and Clove powder has been observed most effective while edible oil and Lemongrass juice show less effective against the growth of Rhizopus.

Table 2: Antifungal activity against Mucor Sp. by various solvent Control (mm) – 35.

Sr. No.	Solvent name	Zone of inhibition (mm)
1.	Cardamom powder	8.5
2.	Clove powder	4.5
3.	Citrus juice	8.75
4.	Lemongrass juice	22.25
5.	Edible oil	22.75

In case of Mucor, the solvent of clove powder, Cardamom powder & Citrus juice show the most active antifungal effect while the solvent of Lemongrass juice and Edible oil show to be the less effective against the Mucor.

The solvent of Edible oil, Cardamom powder and Lemongrass juice has been observed most effective while Citrus juice and Clove powder show that less effective against the Aspergillus.

Table 3: Antifungal activity against Aspergillus Sp. by various solvent Control (mm) – 48.

Sr. No.	Solvent name	Zone of inhibition (mm)
1.	Cardamom powder	33.25
2.	Clove powder	39.75
3.	Citrus juice	37.5
4.	Lemongrass juice	36.5
5.	Edible oil	23.5

Table 4: Antifungal activity against *Fusarium Sp*. by various solvent Control (mm) - 43.25.

Sr. No.	Solvent name	Zone of inhibition (mm
1.	Cardamom powder	28.75
2.	Clove powder	8.5
3.	Citrus juice	24.5
4.	Lemongrass juice	31.25
5.	Edible oil	16.75

In case of *Fusarium* all the five solvent has been observed active in the growth of inhibition against the *Fusarium.*

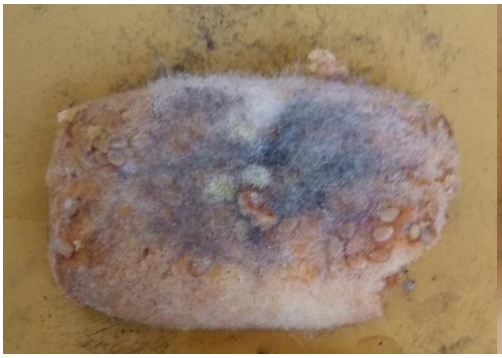
Microphotographs of bakery products infected by fungi (collected from "swad bakery"akola)
Photo plate no. 1

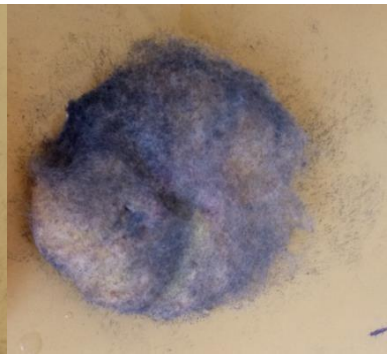
A. Microphotograph of Infected Bun **B. Microphotograph of Infected Bread**

C. Microphotograph of Infected Pizza Base.

D. Microphotograph of Infected Cup Cake E. Microphotograph of Infected Toast.

Microphotographs Of Bakery Products Infected By Fungi (Collected From "Paris Bakery"Akola) Photoplate No. 2

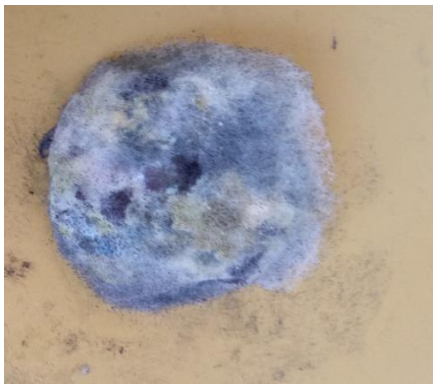




Microphotograph of Infected Cookies. Microphotograph of Infected Cup Cake.

Microphotograph of Infected Toast. Microphotograph of Infected Bun

Microphotographs of bakery products infected by fungi (collected from "king bakery"akola) Photoplate no. 3



Microphotograph of Infected Cup Cake.

Microphotograph of Infected Toast.

Microphotograph of Infected Bun.

Microphotographs of bakery products infected by fungi (collected from "king bakery"akola)
Photoplate no. 4

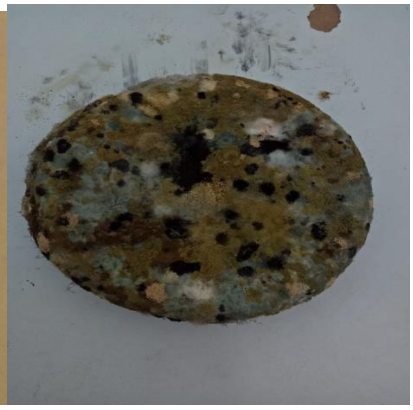
Microphotograph of Infected Toast.

Microphotograph of Infected Bread.

Microphotograph of Infected Cup Cake. **Microphotograph of Infected Pizza Base.**

Microphotographs of bakery products infected by fungi (collected from "anand bakery"akola) Photoplate no. 5

Microphotograph of Infected Brade **Microphotograph of Infected Toast**



Microphotograph of Infected Cup Cake **Microphotograph of Infected Pizza Base**

Microphotographs of bakery products infected by fungi (collected from "gujrati bakery"akola) Photoplate no. 6

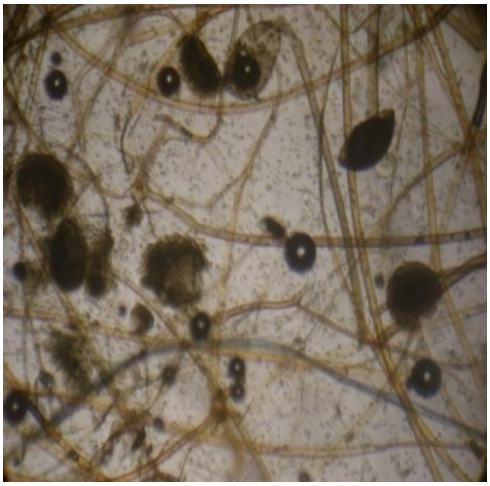
Microphotograph of Infected Toast

Microphotograph of Infected Bread

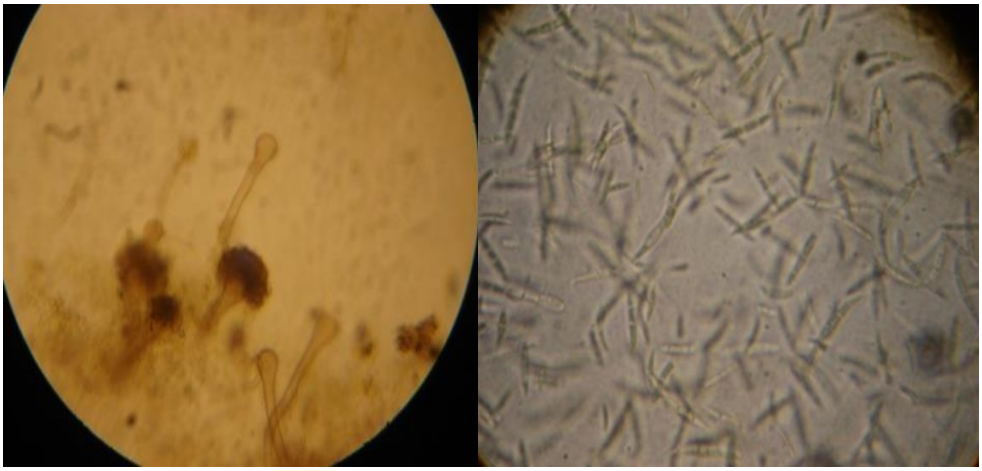
Microphotograph of Infected Bun

Fungi Identified From Infected Bakery Products Photoplate No.7

A. Microphotograph of *Rhizopus Sp.*



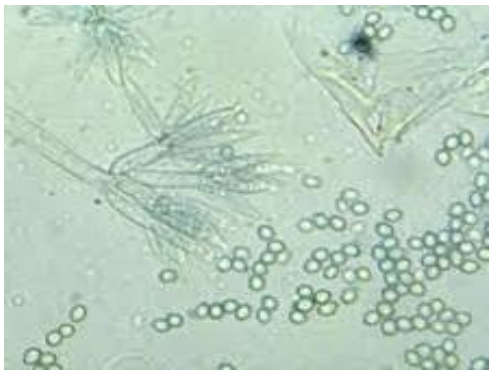
B. Microphotograph of *Mucor Sp.*



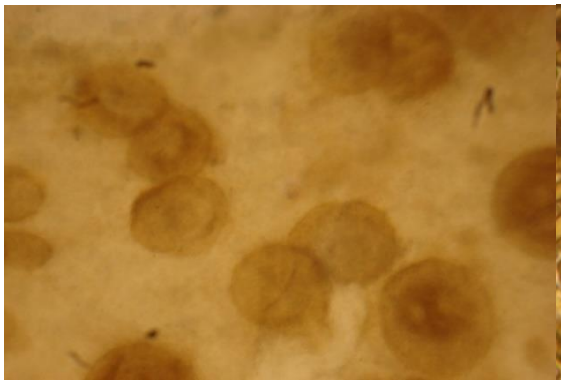
C. Microphotograph of *Aspergillus* Sp. **D.Microphotograph of *Fusarium Sp.***

Fungi Identified From Infected Bakery Products Photoplate No.8

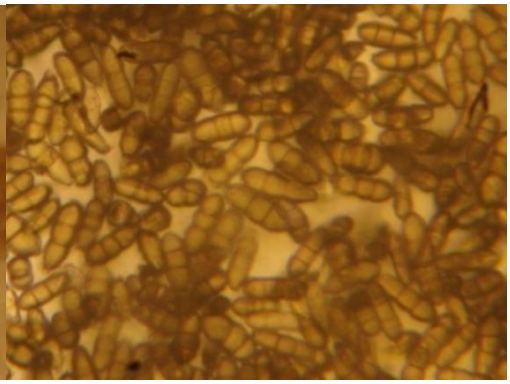
A.Microphotograph of *Alterneria Sp.*



B.Microphotograph of *Penicillium Sp.*



C.Microphotograph of *Phoma sp*.



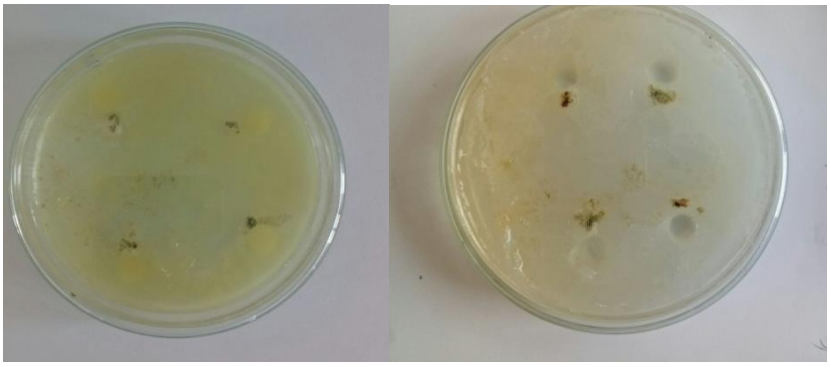
D. Microphotograph of CurvulariaSp.

Photoplate No. 09:
Antifungal Activity against *Rhizopus* by different extract of solvents.

Antifungal Activity by *Cardamomum* powder

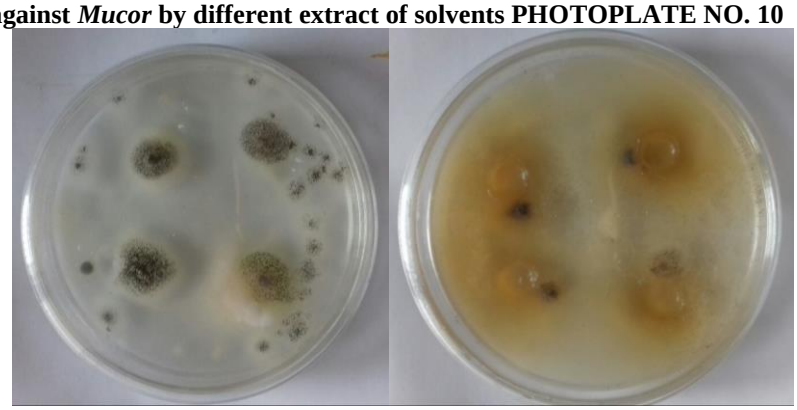
Antifungal Activity by Clove Oil

Antifungal Activity by *Citrus* Juice

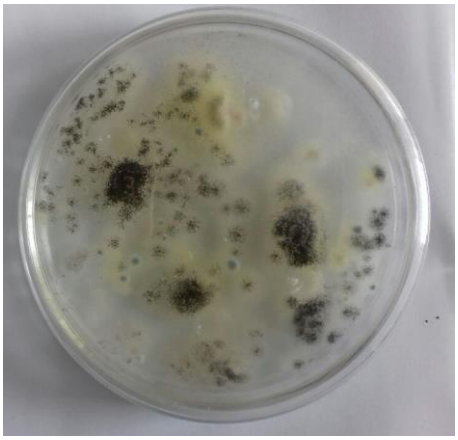


Antifungal Activity by Lemongrass Juice. Antifungal Activity by Edible Oil.

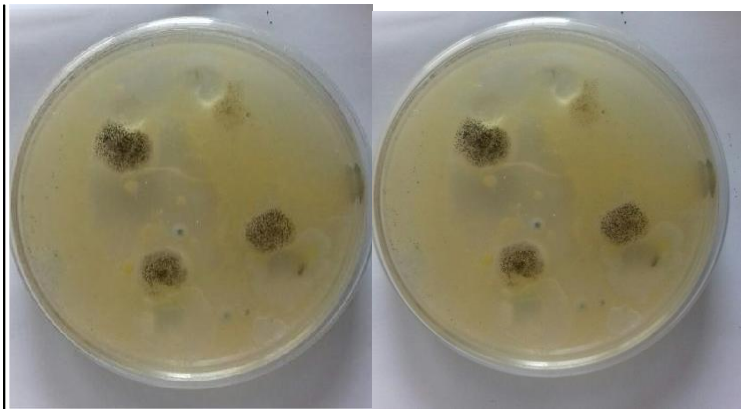
Antifungal Activity against *Mucor* by different extract of solvents PHOTOPLATE NO. 10



Antifungal Activity by *Cardamomum* powder Antifungal Activity by Clove Oil

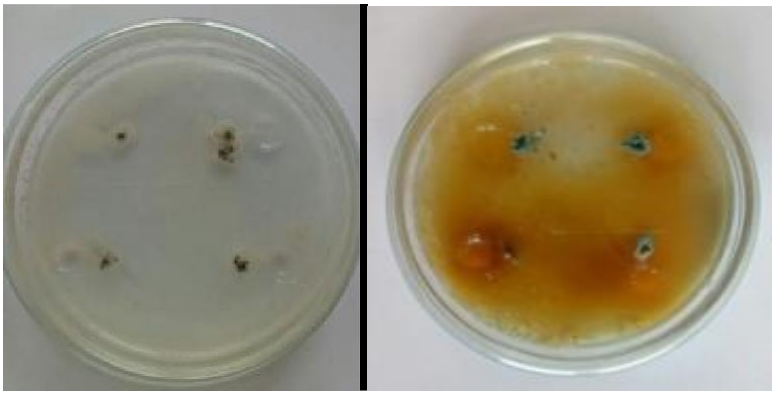


Antifungal Activity by *Citrus* Juice

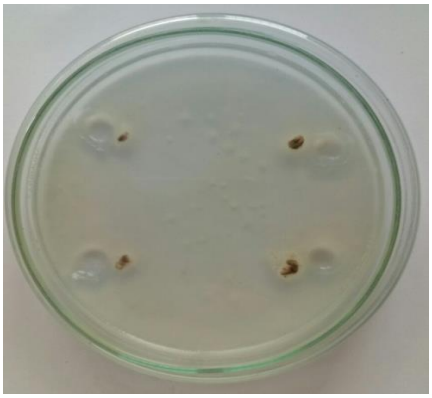


Antifungal Activity by Lemongrass Juice Antifungal Activity by Edible Oil

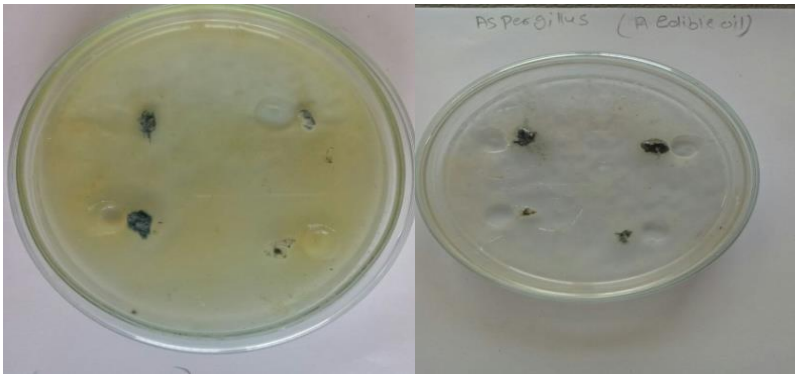
Antifungal Activity against *Aspergillus* by different extract of solvents
Photoplate No.11



Antifungal Activity by *Cardamomum* powder Antifungal Activity by Clove Oil.



Antifungal Activity by Citrus Juice



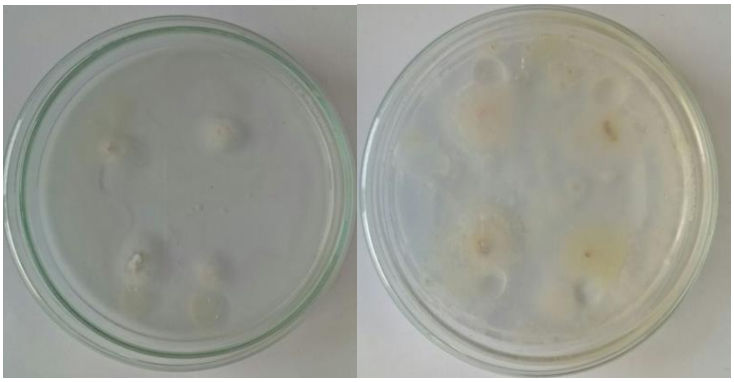
Antifungal Activity by Lemongrass Juice Antifungal Activity by Edible Oil

Antifungal Activity against *Aspergillus* by different extract of solvents Photoplate No.12

Antifungal Activity by *Cardamomum* powder

Antifungal Activity by Clove Oil

Antifungal Activity by *Citrus* Juice



Antifungal Activity by Lemongrass Juice Antifungal Activity by Edible Oil

DISCUSSION AND CONCLUSION

Bakery products are to be infected or spoilage due to the infection of fungi on products. They affect the quality of the product. The product shows discoloration, decrease the stored or packaging capacity of the product and some chemical and physical changes occurs in it. During this investigation 23 infected bakery products were collected, on these bakery products 8 fungi are commonly found. On pizza base there were several fungi grown. These fungi are *Aspergillus, Alterneria, Fusarium* and *Mucor*.

On the bread, molds like *Mucor* and *Rhizopus* are found to grow first which causes bread spoilage. This is followed by other fungi such as Aspergillus, Fusarium, and Penecilium.

On bun, growth of Mucor and Fusarium were dominant and complete spoilage takes place within seven days while on toast Aspergillus, Rhizopus and Mucor show their dominant effect.

Penecilum, Fusarium and Rhizopus are widely grows on cup cake which spoiled the cup cake completely. On cookies Alterneria, Aspergillus is hugely spread and shows their dominant effect.

By storing these products in favorable condition the quantity of these fungi is increased day by day. Microbial spoilage in bakery products causes large economic losses for both bakery industry and consumer. Fungal spoilage is the main cause of substantial economic losses in packaged bakery products and might also be regarded as sources of mycotoxins involving public health problems. Therefore control of fungal spoilage in the bakery products is extremely important and deleterious effects can be alleviated through integrated approaches.

Antifungal activity against four fungal strains by five solvent such as Cardamompowder, Clove powder, Citrus juice, Lemon grass juice and Edible oil in which the clove powder show the excellent effect on fungi similarly Edible oil and Lemongrass juice shows the good effect while Citrus juice to act as a moderate effect on the fungi and Cardamom powder show the poor effect on the growth of inhibition of fungi.

CONCLUSION

Nevertheless fungi can spoil most types of bakery products. The most notable exception is properly stored biscuits. The spoilage can appear in a number of forms, these shows visible growth on product surfaces, gas production leading to product damage or pack expansion odor and flavor changes caused by fungal metabolism. Therefore, it is a pity that fungi spoilage of bakery products has received more attention. Sources of fungi in bakeries have been identified and the most important factors governing the fungal spoilage of bakery products established. Mold spoilage is still a major problem limiting the shelf life of many high and intermediate

moisture bakery products. Losses due to mold spoilage have been resulting in lost revenue to the baking industries.

Therefore, methods to control mold growth and to extend the shelf life of bakery products is of great economic importance to the baking industry where an increased demand in global consumption exists. Other measures as good hygiene in the bakeries and if necessary complementary post packaging heat treatments or modified atmosphere packaging is the best alternatives. More investigation is needed in natural preservatives and Map for preservation of these products.

Use of antifungal reagent such as a natural agent, ie clove powder consists of many chemical compounds but main bioactive compound is Eugenol. Lemongrass extract is also consist of many chemical compound but the Citral is major fraction exhibited the greatest antifungal activity. Cardamom powder also shows the significant activity against the fungi. Citrus extract is also consisting of large amount of chemical substances but Limonene is a major component found in these extract. Edible oil it also show the antifungal activity. All these natural reagents show the inhibiting against fungal spore germination.

The chemicals are hazardous to the human health so use of natural agents is useful to maintain human health. It is not affect the food flavour. Thus by using above natural agents we can control the fungal spoilage of bakery products.

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