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The Inhibitory Effect of some Chemical Food Preservatives on the Growth of some Isolated Dairy Products Fungi

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ABSTRACT

Fungi are an important food borne pathogen involved in a variety of invasive disease so the current study aimed to detect the frequency of contaminated dairy products by fungi. Sixty samples of milk products were collected randomly from different areas in Port-Said governorate namely dry cheese (Romi cheese), shredded cheese (mozzarella cheese), processed cheese (triangle cheese), soft cheese (tallaga cheese) and packed cheese (tetra pack cheese). Fungi were isolated by cultivation on selective media (chloramphenicol yeast extract agar). The isolated fungi included *Aspergillus flavus* (2.63%), *Fusarium oxysporum* (13.16%), *Mucor circinelloides* (2.63%), *Penicillium italicum* (10.53%), *Penicillium freii* (2.63%), *Rhodotorula mucilaginosa* (2.63%) and *Saccharomyces cerevisiae* (65.79%). It was also found that Romi cheese is the most contaminated product with fungi with a rate of 47.37%. One of the goals of this study is to determine the effect of the chemical food preservatives used in cheese manufacturing on fungi according to Egyptian standard specifications. *Saccharomyces cerevisiae* is the most sensitive one where it had the highest zone of inhibition of 25 mm for Natamycin. While *Aspergillus flavus* is the most resistant fungus.

Keywords
Dairy products, fungi, chemical food preservatives

1. INTRODUCTION

Since crude human launch to develop yields and nourishment storage, decay spoilage fungi have requested their offering. Fluffs, oozes of dark, red, white, green, orange and earthy colored which have quietly attacked – acidifying, maturing, staining and crumbling, delivering nutritious products not pleasant to taste or dangerous [1].

So far, fungi possess generally been thought to be causing only unattractive food spoilage, in spite of the truth that some related to humanitarian illness over since two hundred years, and also the acute poisoning of fungi which are

macro has known from a long time. The Western world turns into mindful that ordinary decay moulds could create huge poisons. Mould infection cause liver disease and gastrointestinal disorder in human because of its ability of fabricating (mycotoxins), so it has a dangerous effect on humans and animals health. Fungi affect the characters of the merchandise such as physical characters (flavour and smell) and chemical characters (Textures and PH) and that is will leading to reduce the quality of the merchandise [2].

The use of drying in food preservation is one of the oldest methods used over the years, and it is still used until now because it is considered one of the cheapest and most successful methods [3]. Recently, many methods have been used to preserve food, such as: Cooling, preservatives and salting (to discourage the growth of microorganisms), freezing and irradiation (to kill non spore forming microorganisms) all of this to extend the shelf life of the product.[4].

Clearly, preservatives to be used in nourishment ought to be secure for human. Beneath that restriction, preservatives which have weakly acidic feature: nitrous, sorbic, benzoic, acetic and sulphurous or their esters are utilized. During the concentrations authorized via the rules of the food, the acids mentioned are functional only at pH levels up to their pka plus one pH unit, because to be functional they have to be present because un dissociated acid. For studies of the mechanism of action of preservatives which have weakly acidic feature see[5]; [6]; [7] and [8]. The action of chemical preservatives in nourishment is registered through legislation in World Health Organization (WHO) to relatively low standard and to particular types of nourishment. Some species of fungi have resistance mechanism to preservatives which have acidic feature, *Zygosaccharomyces bailii* is the first memorable yeast. It can grow and ferment in cordials of the fruit with (2.9 to 0.3) PH, containing 800 mg/L of carboxylic acid. Another type of fungus able to develop within the existence of acetic acid with (4%) concentration and still alive with concentration of (10%) [9].

Therefore, this study was planned to examine some of dairy products to determine the total moulds and yeast count and the role of chemical preservatives in inhibition of the isolated moulds and yeast growth in cheese samples.

2. MATERIAL AND METHODS

2.1 Sample collection

Randomly Sixty samples consisting of 12 samples of each kind of these cheeses: Dry cheese (Romi cheese), shredded cheese (mozzarella cheese), processed cheese (triangle cheese), soft cheese (tallaga cheese) and packed cheese (tetra pack cheese) were collected in the year 2018 during the four seasons (winter, spring, summer and autumn) from various tradesmen in Port-Said city in Egypt for microbiological estimation.

2.2 Preparation of samples

One ml of sample was added to 9 ml of 2% saline solution in test tubes to be homogenized by vortex apparatus and be ready for cultivation in petri plates on chloramphenicol yeast extract agar media which is a selective agar media used for counting and isolation of moulds and yeasts in dairy products then incubate in 25◦c for 7 days[10].

2.3 Microbiological Examination

The prepared samples were subjected to the following examination:

- Moulds and yeast total count were performed according to [11] by culturing on chloramphenicol yeast extract agar media. The total moulds and yeast counts are showed as (CFU/mL) colony forming units per millilitre [12]. The Moulds and yeast are specified by culturing on czapek yeast agar for the fundamental of aggregate microscopic and cultural characteristics represented by [13] and [14]. Each Moulds and yeast isolate from the selected dairy product sample is determined as a proportion of the total number of selected dairy product samples and registricted as the frequency [12].
- Detection of efficiency of some chemical food preservatives on cheese samples such as Sorbate (202), Nisin (234), Natamycin (235), Nitrate (251) and Sodium metabisulfite (223), according to codex with the low concentration limits of each one such as 1000 mg/kg, 12.5 mg/kg, 40 mg/kg, 50 mg/kg and 1000 mg/kg respectively according to [15] and [16].

3. RESULTS AND DISCUSSIONS

Some fungi were isolated from cheese samples and the results are shown in Figures 1-7. They were identified as *Aspergillus flavus*, *Fusarium oxysporum*, *Mucor circinelloides*, *Penicillium italicum*, *Penicillium freii*, *Rhodotorula mucilaginosa*, and *Saccharomyces cerevisiae*, respectively.

Outcome presented in Table (1) showed that moulds (20 %) and yeast (43%) were isolated from analyzed cheese samples. The acquired results indicated penurious hygiene through treatment which might lead to technological problems meanwhile processing.

Regarding to the results in Table (2), it showed that species of *Aspergillus flavus* (2.63 %), *Fusarium oxysporum* (13.16 %), *Mucor circinelloides* (2.63 %), *Penicillium italicum* (10.53 %), *Penicillium freii* (10.53 %), *Rhodotorula mucilaginosa* (2.63 %), and *Saccharomyces cerevisiae* (65.79 %) could be isolated in varying percentage from different examined cheese samples. Moulds and yeast has a dangerous effect on humans and animals health. Fungi affect the characters of the merchandise such as physical characters (flavour and smell) and chemical characters (Textures and PH) and that is will leading to reduce the quality of the merchandise. The existence of fungi in a comparatively elevation counts in analyzed Romi products may be a signal of ineffective process such as preheating through industrialization, utilizing unsterilized tools in packing as plastic cups or ineffective cooling on stockpiling [17].

The results obtained in Table (3) revealed the sensibility of several fungal cultures tested for various nourishment preservatives according to codex which was detected by inhibition zones after incubation period; the deprivation of inhibition zones around each disk indicated resistance.
The inhibitory influence of different food preservatives reported by [18] that any inhibition range lower than (4 mm) is resistant, (4 mm) to (12 mm) are intermediate while an inhibition range which bigger than (12mm) is sensitive to chemical nourishment preservatives. Sorbate had a zone of inhibition on *Rhodotorula mucilaginosa* with 22 mm and *Saccharomyces cerevisiae* with 19 mm. Natamycin had a zone of inhibition on *Fusarium oxysporum* with 13 mm, *Mucor circinelloides* with 15 mm, *Penicillium italicum* with 17 mm, *Penicillium freii* with 14 mm, *Rhodotorula mucilaginosa* with 13 mm and *Saccharomyces cerevisiae* with 25 mm.

Saccharomyces cerevisiae is the most sensitive one where it had the highest zone of inhibition (25 mm) for Natamycin as shown in Figure (8). *Aspergillus flavus* is the most resistant fungus where it had no zone of inhibition for all chemical food preservatives tested as shown in Figure (9). Nisin, Nitrate and Sodium metabisulfite have no effect on fungal cultures tested.
Descriptive statistical analytical results of yeast outcome from Table (4) showed that the bigger mean found in tallaga cheese samples (6536.94) with a maximum value (97×10^3), Romi cheese samples mean (1.91E5) with a maximum value (5×10^6) and shredded cheese samples mean (84.26) with a maximum value (17×10^2). Table (5) showed that the yeast result is highly statistically significant between groups with (.004) value which is less than .05 (level of significant).
Descriptive statistical analytical results of moulds outcome from Table (6) showed that the bigger mean found in shredded cheese samples (5.00) with a maximum value (3×10^2), Romi cheese samples mean (3.50E4) with a maximum value (5×10^5) and tallaga cheese samples mean (.56) with a maximum value (3×10). Table (7) showed that the moulds result is highly statistically significant between groups with (.000) value which is less than .05 (level of significant).
There is significant positive relation between yeast and moulds results with pearson correlation (0.144) and p value (0.009) showed in Table (8).

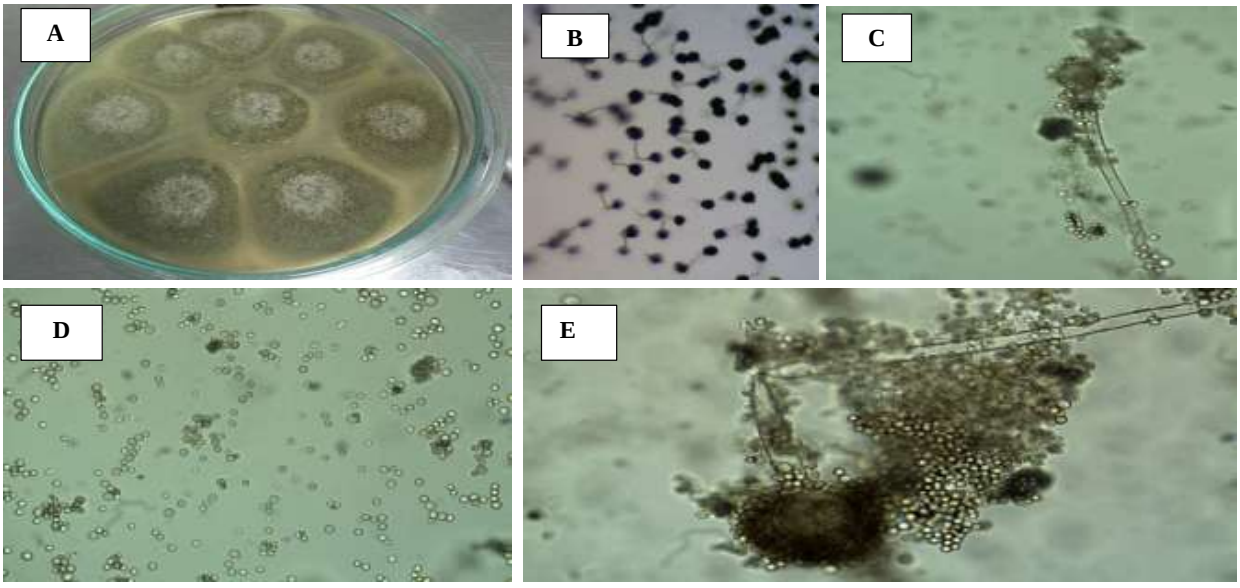

Figure (1): *Aspergillus flavus*. 7-day old colonies at (A) Czapek Yeast Agar. (B),(C) and (E) conidiophores. (D) Conidia.

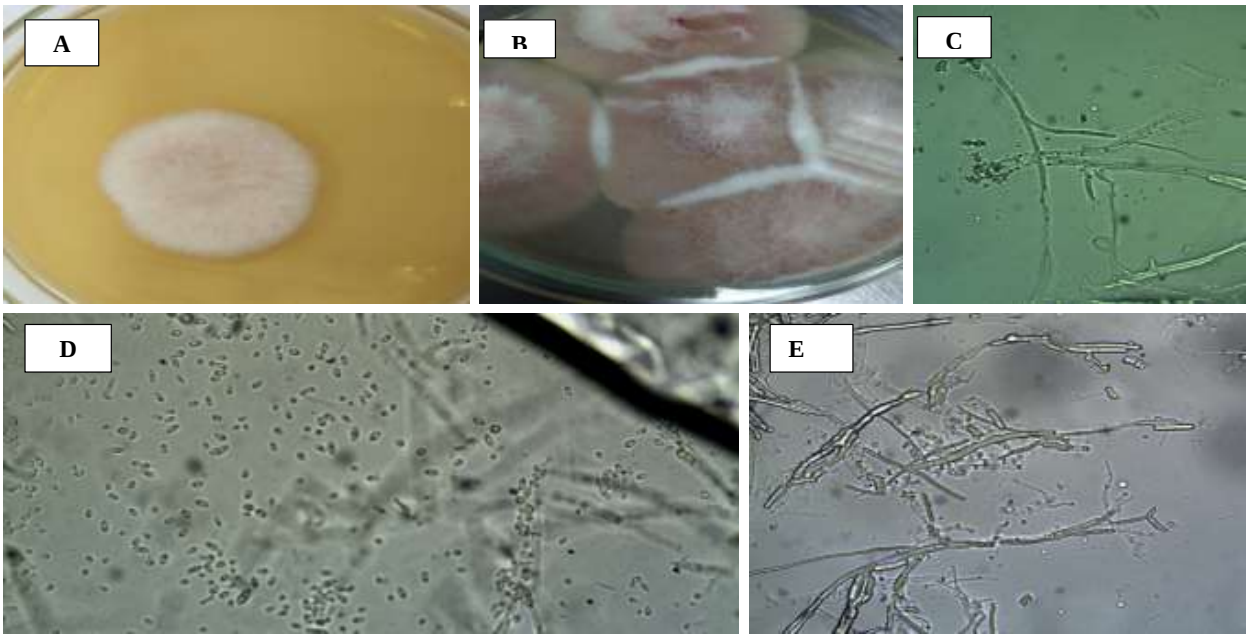

Figure (2): *Fusarium oxysporum*. 3-day old colonies at (A) Czapek Yeast Agar, 7-day old colonies at (B) Czapek Yeast Agar. (C) and (E) conidiophores. (D) Conidia.

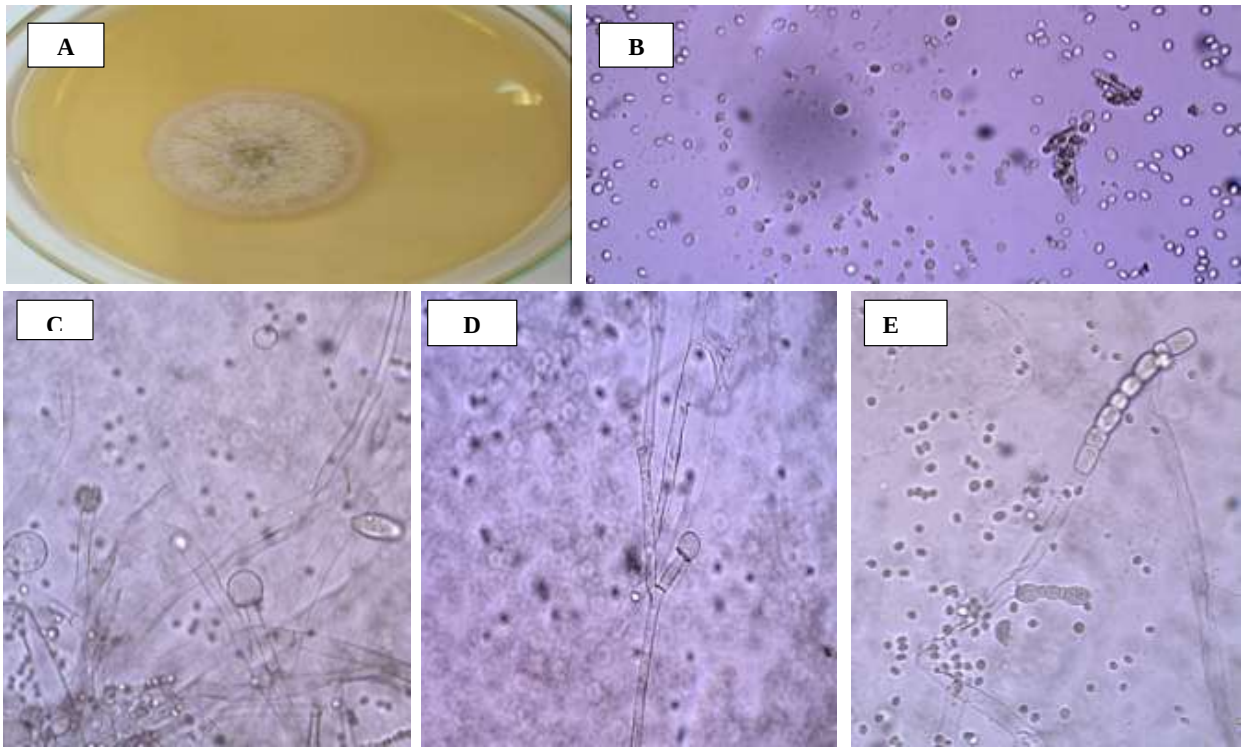

Figure (3): *Mucor circinelloides.* 7-day old colonies at (A) Czapek Yeast Agar. (B) Conidia. (C)- (E) conidiophores.

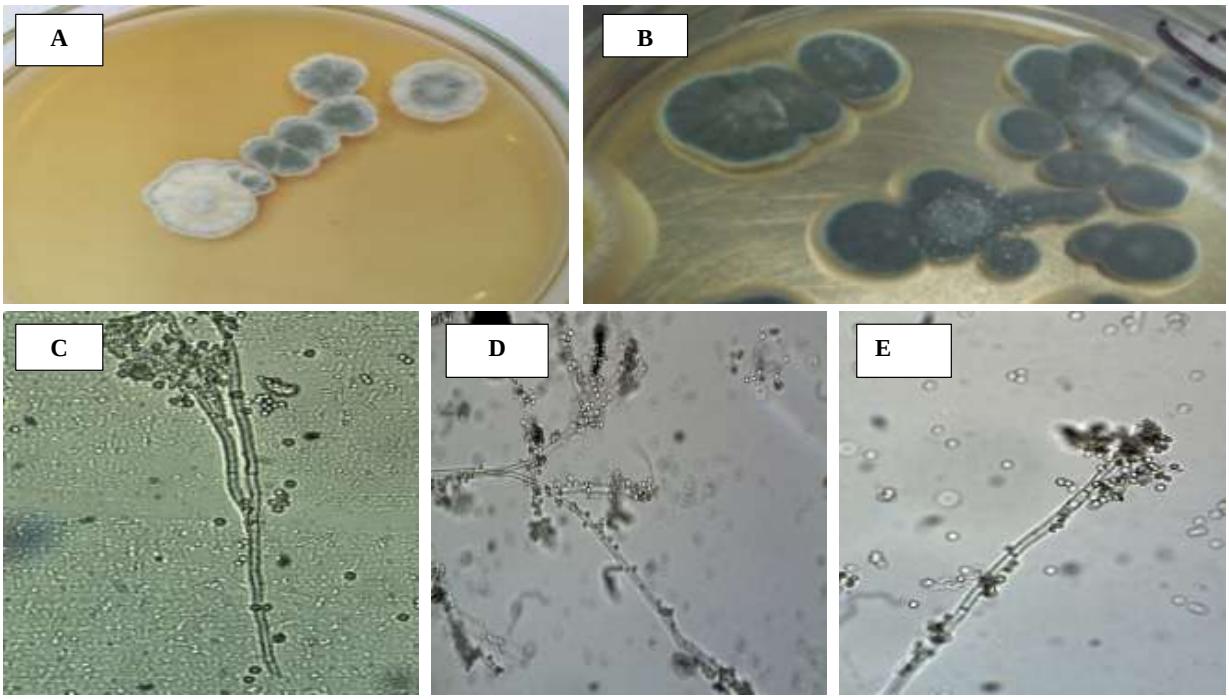

Figure (4): *Penicillium italicum*. 3-day old colonies at (A) Czapek Yeast Agar, 7-day old colonies at (B) Czapek Yeast Agar. (C)-(E) conidiophores and Conidia.

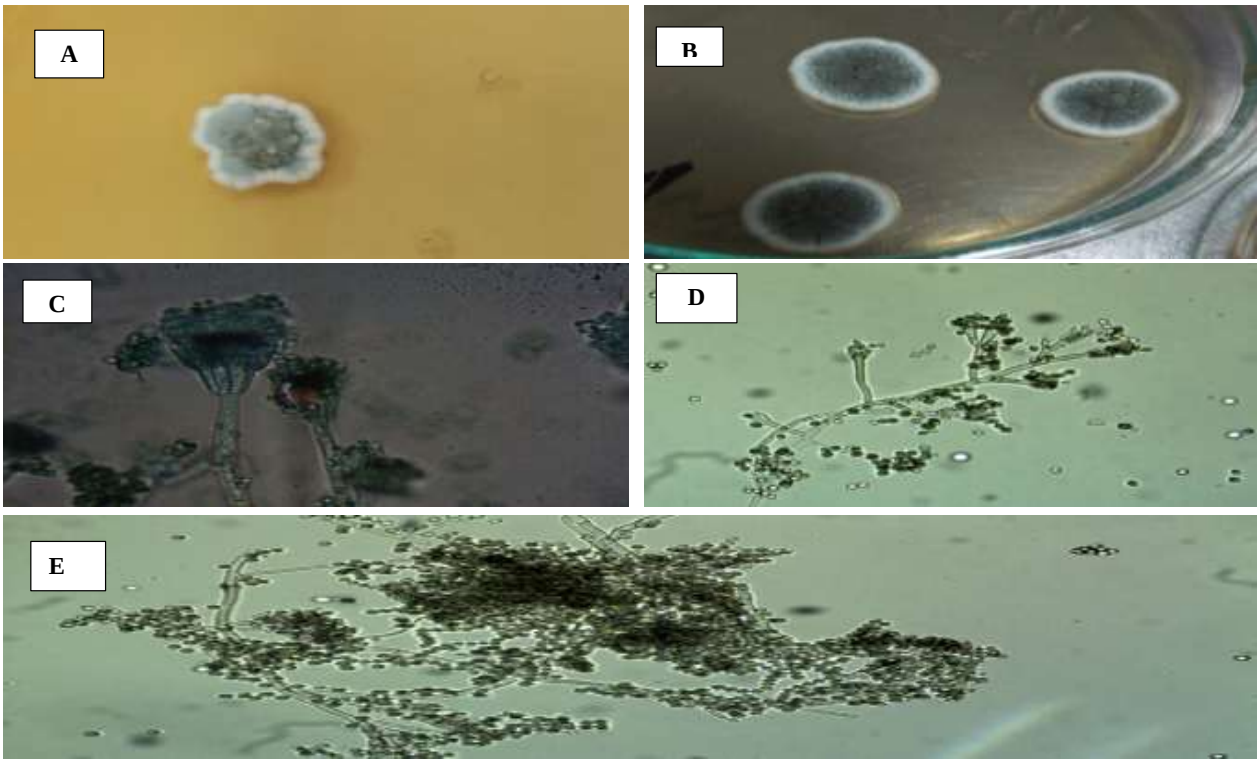

Figure (5): *Penicillium freii*. 3-day old colonies at (A) Czapek Yeast Agar, 7-day old colonies at (B) Czapek Yeast Agar. (C)-(E) conidiophores and Conidia.

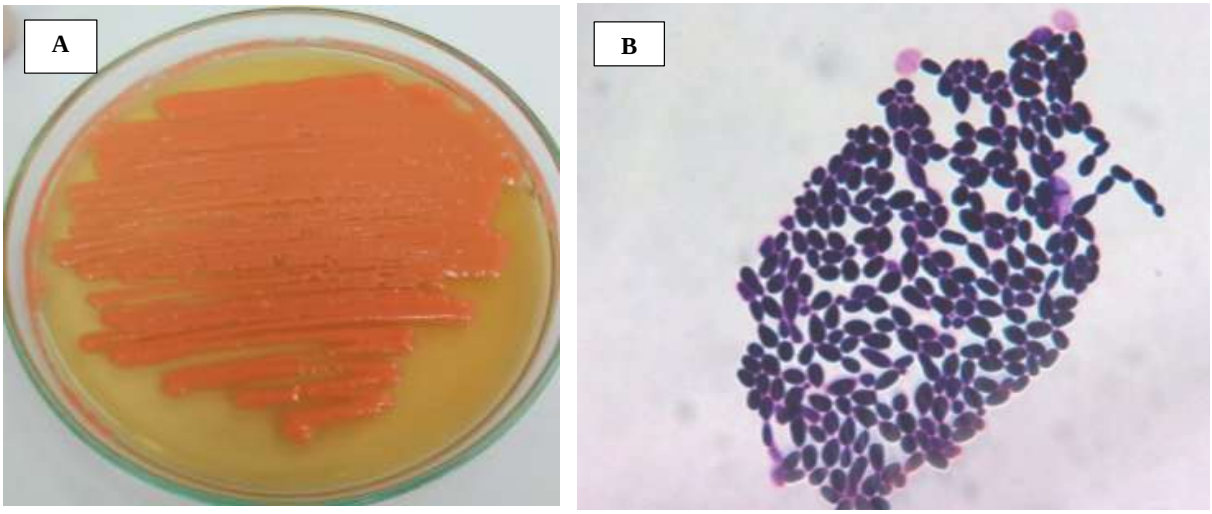

Figure (6): *Rhodotorula mucilaginous*. 7-day old colonies at (A) Czapek Yeast Agar. (B) vegetative cells.

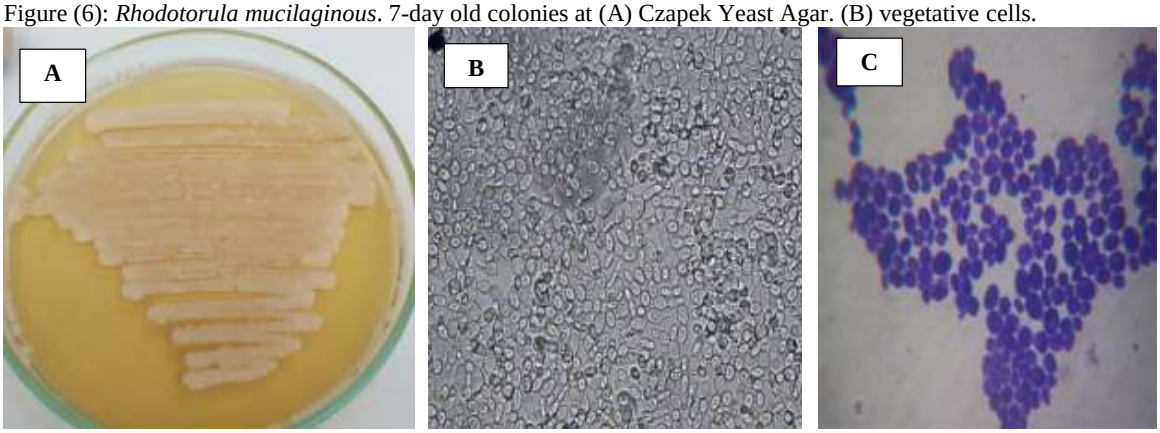

Figure (7): *Saccharomyces cerevisiae.* 7-day old colonies at (A) Czapek Yeast Agar. (B) and (C) vegetative cells.

Figure (8): *Saccharomyces cerevisiae* had zone of inhibition of 25 mm for Natamycin**.**

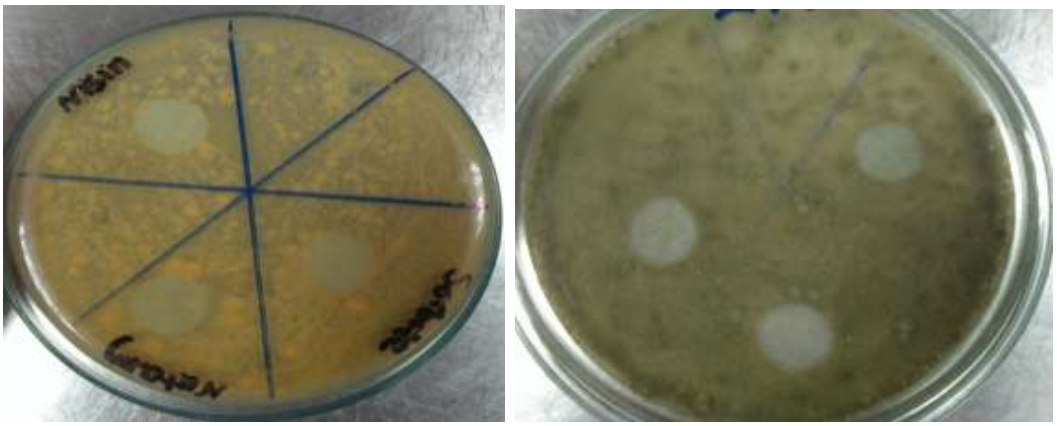

Figure (9): *Aspergillus flavus* had no zone of inhibition for chemical food preservatives tested**.**

Table (1): Results of moulds and yeasts in examined samples

Type of analyzed samples	No. of analyzed samples	Kind of detected organisms	Positive samples	
			No.	%
Cheese samples	60	Yeast	26	43%
Cheese samples	60	Mould	12	20%

Table (2): occurrence of isolated moulds and yeasts in analyzed cheese samples.

Isolates	**Romi**		**Shredded**		**Tallaga**		**Grand Total**	
	No of isolates	**%**	**No of isolates**	**%**	**No of isolates**	**%**	**No of isolates**	**%**
Moulde and yeast	**18**	**47.37 %**	**6**	**15.79 %**	**14**	**36.84 %**	**38**	**100.00 %**
Aspergillus flavus	0	0.00%	0	0.00%	1	2.63%	1	2.63%
Fusarium oxysporum	1	2.63%	2	5.26%	2	5.26%	5	13.16%
Mucor circinelloides	1	2.63%	0	0.00%	0	0.00%	1	2.63%
Penicillium italicum	3	7.89%	1	2.63%	0	0.00%	4	10.53%
Penicillium freii	1	2.63%	0	0.00%	0	0.00%	1	2.63%
Rhodotorula mucilaginosa	0	0.00%	0	0.00%	1	2.63%	1	2.63%
Saccharomyces cerevisiae	12	31.58 %	3	7.89%	10	26.32 %	25	65.79%
Grand Total	**18**	**47.37 %**	**6**	**15.79 %**	**14**	**36.84 %**	**38**	**100.00 %**

Table (3): Diameter of inhibition zone (mm) mediated by various chemical preservatives on selected fungal flora. The zone of inhibition: (4 mm) is resistant, (4 mm) to (12 mm) are intermediate while an inhibition range which bigger than (12mm) is sensitive to chemical nourishment preservatives.

Organism	Preservative material				
	Sorbate	**Nisin**	**Natamycin**	**Nitrate**	**Sodium metabisulfite**
Asperigillus flavus	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Fusarium oxysporum	**0.0**	**0.0**	**13.0**	**0.0**	**0.0**
Mucor circinelloides	**0.0**	**0.0**	**15.0**	**0.0**	**0.0**
Penicillium italicum	**0.0**	**0.0**	**17.0**	**0.0**	**0.0**
Penicillium freii	**0.0**	**0.0**	**14.0**	**0.0**	**0.0**
Rhodotorula mucilaginosa	**22.0**	**0.0**	**13.0**	**0.0**	**0.0**
Saccharomyces cerevisiae	**19.0**	**0.0**	**25.0**	**0.0**	**0.0**

Table (4): Descriptive statistical analytical results of yeasts count /ml of examined samples

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Tallaga	108	6536.94	17874.802	1720.004	3127.24	9946.65	0	97000
Shredded	108	84.26	273.760	26.343	32.04	136.48	0	1700
Romi	108	1.91E5	828090.367	7.968E4	32894.44	348819.08	0	5000000
Total	324	6.58E4	484887.531	2.694E4	12829.52	118822.46	0	5000000

Table (5): Anova results of yeasts count /ml of examined samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.535E12	2	1.267E12	5.542	.004
Within Groups	7.341E13	321	2.287E11		
Total	7.594E13	323			

Table (6): Descriptive statistical analytical results of moulds count /ml of examined samples

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Tallaga	108	.56	3.302	.318	-.07-	1.19	0	30
Shredded	108	5.00	34.546	3.324	-1.59-	11.59	0	300
Romi	108	3.50E4	114409.204	1.101E4	13127.82	56776.06	0	500000
Total	324	1.17E4	67885.283	3771.405	4232.88	19072.12	0	500000

Table (7): Anova results of moulds count /ml of examined samples

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.794E10	2	4.397E10	10.078	.000
Within Groups	1.401E12	321	4.363E9		
Total	1.489E12	323			

Table (8): Correlation results of moulds and yeast count /ml of examined samples

		yeast	mould
yeast	Pearson Correlation	1	.144"
	Sig. (2-tailed)		.009
	N	324	324
mould	Pearson Correlation	.144"	1
	Sig. (2-tailed)	.009	
	N	324	324

** Correlation is significant at the level 0.05 level (2-tailed).

4. CONCULSION

Most of fungal growth has appeared in Romi cheese and that is due to inefficient pre-heating process during manufacturing and poor hygiene on storage. Followed by tallaga cheese, since it is not exposed to heat during process and a small amount of salt is added to it, which in turn helps in fungal growth. The mozzarella cheese had the least fungal growth that is return to the storage temperature -18◦C.

Nourishment preservation suggests laying fungi in antagonistic climate so as prohibit their development or reduce their existence or be the reason of their death. Sorbate, Nisin, Natamycin, Nitrate and Sodium metabisulfite are effective in the control of most selected fungal cultures (i.e., *Fusarium oxysporum, Mucor circinelloides, Penicillium italicum, Penicillium freii, Rhodotorula mucilaginosa, and Saccharomyces cerevisiae*) causing food poisoning and down grading of the product. *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* are highly sensible to most of chemical preservatives applied in this study.

This study suggested that the concentration of chemical nourishment preservatives increased or mixed together in the cheese which not exposed to heat and can be easily contaminated by fungal flora in order to improve efficiency of chemical food preservatives to control the fungal growth beside good storage and the speed of its consumption.

REFERENCES

[1] Adebayo, C., Aderiye, B. & Akpor, O. Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented foods. African Journal of Food Science 8, 140-147 (2014).

[2] Alcano, M. d. J. et al. Susceptibility of Aspergillus spp. to acetic and sorbic acids based on pH and effect of sub-inhibitory doses of sorbic acid on ochratoxin A production. Food Research International 81, 25-30 (2016).

[3] Alimentarius, C. General standard for food additives. Codex Stan 192-1995. Pectin; Food and Agriculture Organization of the United Nations: Rome, Italy (2015).

[4] Coton, M. et al. Production and migration of ochratoxin A and citrinin in Comté cheese by an isolate of Penicillium verrucosum selected among Penicillium spp. mycotoxin producers in YES medium. Food microbiology 82, 551-559 (2019).

[5] Hu, Y., Zhang, J., Kong, W., Zhao, G. & Yang, M. Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (Curcuma longa L.) on Aspergillus flavus. Food chemistry 220, 1-8 (2017).

[6] Kumar, A., Kathuria, D. & Kumar, J. Bioactive and functional ingredients from dairy products. Functional Foods: Sources and Health Benefits. D. Mudgil and S. Barak (eds.) Scientific Publishers, India, 239-277 (2017).

[7] Little, C. L., Pires, S. M., Gillespie, I. A., Grant, K. & Nichols, G. L. Attribution of human Listeria monocytogenes infections in England and Wales to ready-to-eat food sources placed on the market: adaptation of the Hald Salmonella source attribution model. Foodborne pathogens and disease 7, 749-756 (2010).

[8] Nanda, M. Determination of zone of inhibition In: Clinical Microbiology1st Ed. Jaype Brothers Medical Publishers, New Delhi (2005).

[9] Norian, R. et al. Determination of aflatoxin M1 levels in raw milk samples using ELISA and high-performance liquid chromatography in Qazvin, Iran. Journal of Mycology Research 2, 41-48 (2015).

[10] Oyet, G., Achinewhu, S., Kiin-Kabari, D. & Akusu, M. Microbiological quality of selected street vended foods during wet and dry season in parts of Port Harcourt metropolis, Rivers State, Nigeria. Research Journal of Food Science and Nutrition 5, 35-45 (2020).

[11] Pal, M. Veterinary and medical mycology. (Directorate of Information and Publications of Agriculture, Indian Council …, 2007).

[12] Pitt, J. & Hocking, A. D. Mycotoxins in Australia: biocontrol of aflatoxin in peanuts. Mycopathologia 162, 233-243 (2006).
[13] Rawat, S. Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research 5, 47-56 (2015).
[14] Rico-Munoz, E., Samson, R. A. & Houbraken, J. Mould spoilage of foods and beverages: Using the right methodology. Food microbiology 81, 51-62 (2019).
[15] Rojo, M. C. et al. Evaluation of different chemical preservatives to control Zygosaccharomyces rouxii growth in high sugar culture media. Food Control 50, 349-355 (2015).
[16] Stoev, S. D. Food security and foodborne mycotoxicoses, risk assessment, preventive measures, and underestimated hazard of masked mycotoxins or joint mycotoxin interaction. Food Toxicology 9, 169-199 (2016).
[17] Stratford, M. & LAMBERT, R. W. Weak-acid preservatives: mechanisms of adaptation and resistance by yeasts. Food Australia 51, 26-29 (1999).
[18] Zaccheo, A., Palmaccio, E., Venable, M., Locarnini-Sciaroni, I. & Parisi, S. Food hygiene and applied food microbiology in an anthropological cross cultural perspective. (Springer, 2016).