

## FUNGAL CONTAMINATION IN FRESH RAISIN JUICE IN MOSUL CITY

May Akram Krmo<sup>1</sup>

nineveh Education Directorate, Mosul-Iraq

[1mayakram9@gmail.com](mailto:mayakram9@gmail.com)

Maha Akram Mohammad Ali Al\_Rejaboo<sup>2</sup>

Department of Biology, College of Science, University of Mosul,  
Mosul- Iraq

[2mahaalrejaboo2@uomosul.edu.iq](mailto:mahaalrejaboo2@uomosul.edu.iq)

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**Abstract: Background:** Fungal contamination in food and beverages, including fruit juices, poses significant health risks. **Specific Background:** This study investigates the extent of fungal contamination in fresh raisin juice collected from Mosul City and its suburbs, including Bashiqa, Al-Hamdaniya, Bartella, Al-Shekhan, Aqra, and Dohuk, over a period from January 2023 to April 2024. **Knowledge Gap:** Despite the potential for fungal contamination in fruit juices, detailed analyses of specific contamination rates and fungal species in this region have been lacking. **Aims:** The study aimed to identify and quantify fungal contamination in fresh raisin juice samples using Potato Dextrose Agar and Sabouraud Sucrose Agar, and to characterize the fungal isolates morphologically, biochemically, and molecularly. **Results:** Of the 40 samples tested, 100% were contaminated with fungi, yielding 121 isolates of various species. The highest contamination rates were observed for *Candida* spp. and *Aspergillus* spp., each constituting 27.3% of isolates. Other notable contaminants included *Wickerhamomyces anomalus* (12.39%), *Saccharomyces cerevisiae* (11.57%), and *Rhodotorula* spp. (8.26%). Additionally, lower rates of contamination were found for *Penicillium* spp., *Exophiala dermatitidis*, and several other species. **Novelty:** The study revealed a high prevalence of fungal contamination, including the identification of a novel *Candida oleophila* strain (GenBank accession PP961930.1). **Implications:** These findings highlight the significant fungal contamination in fresh raisin juice, which could have implications for food safety and quality control. The identification of specific fungal species and the novel strain underscores the need for stringent monitoring and improved processing practices to mitigate contamination risks.

**Keywords:** Fresh Raisin Juice, Fungal contamination, Mosul City, Its Suburbs



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### Introduction

Raisins are dried grapes. Grape is a berry fruit that generally grows in a group of deciduous woody vines belonging to the Vitaceae family. (Venkitasamy et al., 2019). Grapes are an important and economically well-known fruit all over the world (Kui et al., 2020). Grape production is estimated at more than 74.5 million tons per year, based on a cultivation area of 7.12 million hectares. China, the United States, Italy, Spain, France, Turkey, Argentina, India and Iran are the largest producers of grapes (Anjum et al., 2020). It is mainly consumed as food such as raisins, juice and wine and as fresh fruit or used in various products such as vinegar. It is considered a rich source of vitamins, minerals, carbohydrates, dietary fiber and substances chemical vegetarian like polyphenol and promotes general health (2023, Jaiswal et al). It is also considered antibacterial, antioxidant and anti-inflammatory due to its content of Polyphenol (Imran et al., 2017). Black grapes have large amounts of antioxidants and

grapes are considered a seasonal fruit that grows abundantly and thus they were dried and converted into raisins for storage and use throughout the year, either by the old method of drying under the sun or in the air (2015,.Wang et al). As for modern drying using microwaves, ovens and drying under infrared rays (Wang et al.,2016 and Khiari et al., 2018a). Old and new drying techniques greatly affect changes in the volatile organic compounds that contribute to the aroma of raisins.((Torres et al., 2015; Wang et al., 2017). Raisins have benefits for blood pressure and cholesterol low-density lipoproteins, triglycerides, and fatty acids oxidizing in the blood and cytokines that cause inflammation, this indicates the possibility that raisins greatly reduce the risk of developing type 2 diabetes.) (T2DM, cardiovascular disease, cancer, and gut health, indicating that raisins consumed as part of a healthy diet are rich in nutrients (Das, 2008 and Restani et al., 2016).

Raisin juice is considered one of the popular juices it is used as a healthy alternative to caffeinated drinks such as coffee, tea and soft drinks. It has many benefits such as antioxidants, anti-cancer and anti-diabetic properties, and vitamins and minerals play an effective role in preventing a cancer, heart disease, diabetes and high cholesterol (Madappa et al., 2018). It contains 70% of its weight sugar and 2% of its weight protein. It is also rich in elements, especially K, Na, P, Mg, and Ca<sup>+</sup> and vitamins A, B3, C. there are many contaminated according to the type of storage until consumption and thus affects the production of juice and wine (Welke, 2019) . This results in contamination of raisin juice with mycotoxins (Pena et al.,2010).Studies have shown that fungal contamination attributed to climate conditions and physiochemical and for grapes and wine and its effects on production (Freire et al., 2017).

## Methods

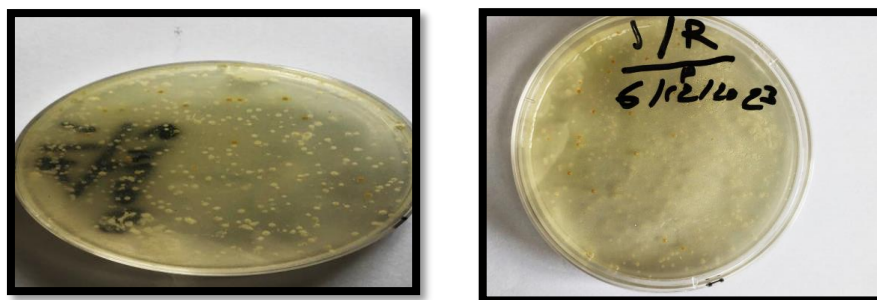
(40) samples of fresh raisin juice were collected from Mosul city to the right and left and its suburbs, including the areas of Bashiqa, Al-Hamdaniya, Bartella, Al-Shekhan, Aqra and Douw, with 50 ml of each sample, and placed in special sterile boxes to avoid contamination and take information about each sample in terms of the source of the raisins, the date of making the juice, the method of storing it and the components of the juice. The sample collection period was from January 2023 to April 2024, and the samples were cultured using the dilution method on the media (PDA) Potato Dextrose Agar and Sabouraud Sucrose Agar (SSA) (Ronald et al., 1959 and Marth, 1978). To determine the extent of fungal contamination, it was diagnosed morphologically by relying on the color of the colony in the dish from both sides, the diameter of the colony, the presence and color of secretions, and the appearance of the fungal hyphae. Microscopically, a small piece of the colony was taken with a small amount of the medium on a glass slide, then mixed with a drop of lactophenol dye or crystal violet dye, then the cover glass was placed on it and examined under a microscope at a magnification of 40x (de-hoog and Gurro,1997 ; Pitt and Hocking ,1997 ; Samson and Frisvad ,2004).Yeasts were diagnosed using differential media based on the taxonomic key (Pitt and Hocking, 1997), where 7 differential media were used, including MEA25, MEA37, MEA28, Malt Extract Agar, Czapek Agar (CA), Malt Acetice Agar (MAA), Malt Extract yeast 50% Glucose Agar (MY 50G), and Extract Yeast Malt 5% or 10% salt 12% Glucose Agar (MY10-12). The isolates were cultured after purification on SSA medium for two days until diagnostic tests were performed, and the general characteristics were recorded in terms of colony color, texture, and size. The diagnosis of the fungal isolate was confirmed using molecular methods, where DNA was isolated from two-day-old yeast, its purity was checked, PCR was performed, and DNA was isolated by electrophoresis and sequencing. The result was then sent to the fungal isolate for the purpose of conducting a nitrogenous base sequence determination test after the sample was diagnosed using ITS primers in the United

States of America.

### Results and Discussion

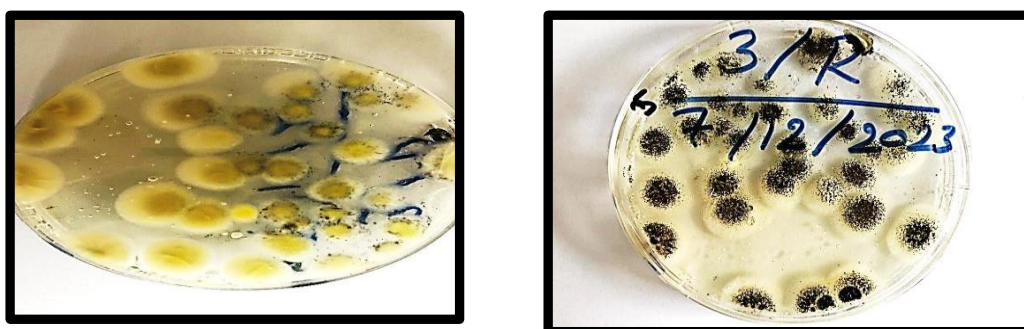
Isolated and identified the contaminating fungi for (40) sample of natural raisin juice sold in the markets of Mosul and its suburbs from December 2023 to April 2024. Elected (28) sample from shops randomly on the side in Mosul right and left it included all of the shops in Al-Muthanna district, Al-Zahour district, Al-Majmoua Al-Thaqafiya district, Mosul University, Al-Darkazliyya, Al-Masarif district, three samples from Bashiqa district, two samples from Sheikhan district, two samples from Bartella, and one sample from Al-Hamdaniya and two samples from Aqra district and four samples from Dohuk governorate, where the location of the shop and the source of the raisins were taken, as well as the date of making the raisin juice from all the selected shops. The fresh juice was composed of (dried raisins, sugar, and a dried mint leaves and a Synthetic dye). The results of the study showed pollution for large samples-Mold and Yeast, there were fungi growing on both media SSA, PDA and fungi grown on the only one medium. Sample R1, R2 and R5 isolated from Bashiqa stores contained on a mold and yeast in both media SSA, SDP which was the source of raisins from the perfumers of Mosul from it *C.oleophila* one types of *Candida* which was registered as a new genotype in NCBI named *Candida* strain MAYKAIY and we have shown *Aspergillus niger* and *Saccharomyces cerevisiae* and *P.atramentosum* was found in the plate containing SSA medium and not in SDP medium. The fungus *C.tropicalis* was one of the most contaminated fungi in these samples.

**Figure (3): *C.tropicalis* yeast**



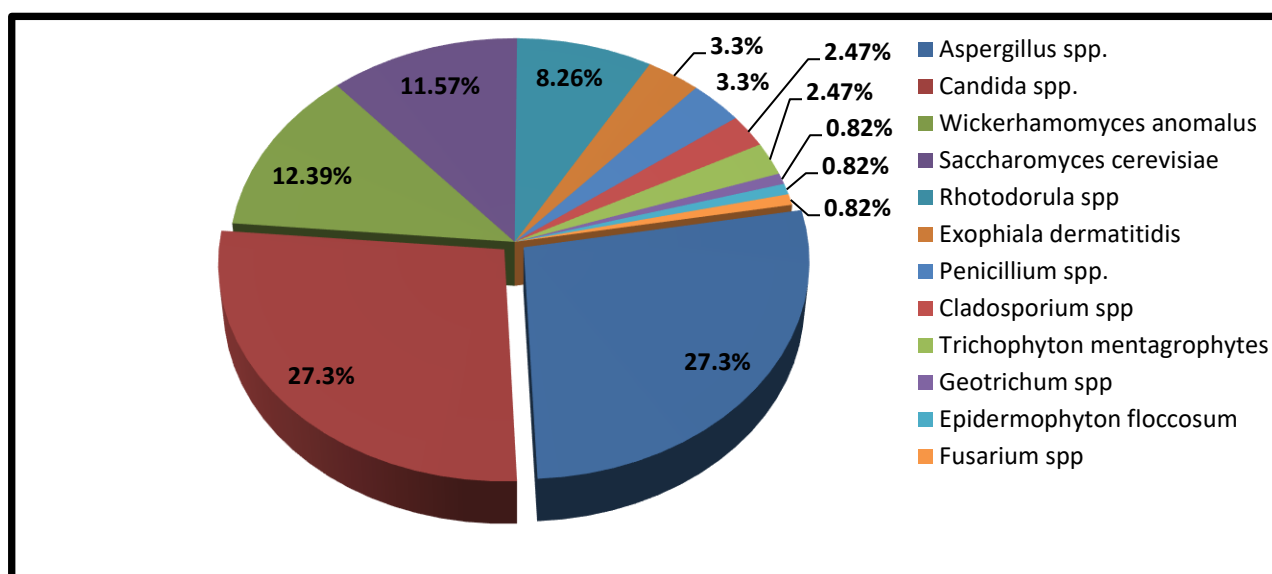
As for the samples taken from Aqrah district, it was found that: R27 contaminated only With *A.niger* and R28 contaminated with *A.niger*, *Rhodotorula* and *Wickerhamomyces anomula* and *C.parapsilosis*, where we grew up *Rhodotorula* On each of PDA did not grow on SSA, but the most common species that appeared between the two samples was *A.niger*, while the Samples R3 and R13 Taken From the judiciary the Two old men grew mushrooms *A.niger*, *C. oleophila*, *Saccharomyces cerevisiae* in both SDP The most frequent fungus in both samples *A.niger*

**Figure (4): *A. niger* on the SSA medium**



the two samples R10, R11 from Bartella area were sourced in Mosul grew both *Cladosporium cladosporioides*, *A. niger*, *Saccaromyces cerevisiae*, *C. oleophila*, *C. tropicalis*, *Rhodotorula*, although both samples were taken that from two different places and also it was *A.niger* more visible in both samples and the sample R12 from area the Hamdaniya was the source of raisins from northern Iraq contaminated with- *Penicillium*, *C. parapsilosis*, *A.niger*, *Saccaromycetes services* and one sample of *Rhodotorula*, the most common fungi are: *A.niger* collect four samples of R18,R23,R24,R7 from Dohuk was the source of raisins taken from scattered areas in northern Iraq contaminated with- *C.tropicalise* and *C. oleaphila*, *Wickerhamomyces anomula*, *Rhodotorula*, and a species of *Aspergillus* spp. While *C. tropicalis* was abundant, its proportion was large. As for the samples of Mosul city on the left side, they were: R14, R4, R6, R9, R19, R20, R25, R26, R27, R30, R31, R32, R33, R34, R35, R36, R37, R38, R39, R40 most of all the raisin samples were from Aqra district and northern Iraq. All kinds of fungi grew *A. niger*, *C.oleaphila*, *Cladosporium cladosporioides*, *Rhodotorula*, *C.krusei*, *C.albican*, *C.parapsilosis*, *C.tropicalis* and species of *Penicillium*. The contamination rate was high in some samples and low in others. As for the right side of the city of Mosul, the samples were R15, R16, R17, R18 are contaminated with *Aspergillus sp* and *C.tropicalis* and *Saccaromyces cereivsiae* and *Wickerhamomyces anomalus* and *C. parapsilosis* and *Rhodotorula* and *C.krusei* and *C.albican*, AThe more contaminated the samples were *Rhotorula* it was found that all samples of raisin juice were contaminated to varying degrees with fungi, with the contamination rate being- *Aspergillus sp*. 27.3% While she was *Candida sp* Contaminated by 27.3% and 12.39% Contaminated with- *Wickerhamomyces anomalus* followed by other fungi at a rate of Say and it was kind *Aspergillus niger* and *Candida sp* From more Types Contamination and spread in samples.

**The shape (5): Shows the percentage of contamination of the study samples with fungal and yeast species.**



It was found that the most highly contaminated samples with fungi were: R1, R3, R4, R5, R7, R9, R10, R11, R12, R14, R15, R16, R17, R19, R20, R21, R22, R26, R28, R29, R30, R35, R36, R38, R39, R40 either samples are less contaminated it is: R2, R6, R8, R13, R18, R18, R23, R24, R25, R27, R31, R32, R34, R40. In general, all samples contained yeasts and mold. The results that appeared to us were consistent with what the scientist mentioned (Alomari et al., 2014) found that *Aspergillus niger* was dominant in some dried fruits during isolation and also observed different species of



*Penicillium* spp. It was agreed results the current study with (Iamanaka et al., 2005), *Aspergillus niger* is the most prevalent in raisin samples in the Brazil , (Allam et al., 2020) it was found that the genus *Aspergillus* spp is dominant by 27.3% over the rest of the other genera yeasts and other filamentous fungi, this study agreed with our study.

**Figure(6):New mushroom pattern registration *Candida oleophila* strain MAYKAIY and give it an ID number PP961930 in NCBL.**

GenBank  
Send to: ▾

**Wickerhamomyces anomalus strain MKARWAN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**

GenBank: PP961931.1  
FASTA Graphics

Go to: ☺

LOCUS PP961931 450 bp DNA linear PLN 03-JUL-2024  
DEFINITION Wickerhamomyces anomalus strain MKARWAN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.  
ACCESSION PP961931  
VERSION PP961931.1  
KEYWORDS .  
SOURCE Wickerhamomyces anomalus  
ORGANISM Wickerhamomyces anomalus  
Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Phaffomycetaceae; Wickerhamomyces.  
REFERENCE 1 (bases 1 to 450)  
AUTHORS Karmali, M.A. and Al-Rejaboo, M.A.  
TITLE Direct Submission  
JOURNAL Submitted (28-JUN-2024) Biology, University of Mosul, Al-majmoaa street, Mosul 41002, Iraq  
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##Assembly-Data-END##  
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61 tgggtggtga gcttgcttta ctgcccaag gtctaaccac attttttta tgttaaac  
121 tttaaccaat agtcataaaa atttttaaca aaataaataa tctcaaac ttcaaac  
181 ggcctcttgg gtcttcgcaa cgaagaagaa cgcagcgaat tgcgatacgt atttgaa  
241 gcagatttgc gtgaatcgc gaactttga acgcacattg caccctcgg tattcagag  
301 ggtatgcctg ttgagcgtc atttctctc caaaccttcg ggttggat tgagtgatac  
361 tctgcaagg gtaactga atattgact tagcaagat gtactaataa gcagcttctc  
421 tgaataatg tattaggttc ttccaactcg  
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## Conclusion

The investigation of fungal contamination in fresh raisin juice from Mosul City and its suburbs revealed a high prevalence of various fungal species. The study identified 121 fungal isolates from 40 samples, with *Candida* spp. and *Aspergillus* spp. being the most frequent contaminants, each representing 27.3% of the isolates. Other notable contaminants included *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, and *Rhodotorula* spp., highlighting significant microbial diversity. The results underscore the widespread issue of fungal contamination in raisin juice, which could impact consumer health and product safety. This study's findings emphasize the need for improved quality control measures during juice production and storage. Further research should focus on assessing the health impacts of these contaminants and exploring effective methods for reducing fungal contamination in raisin juice and similar products.

## References

- [1] Mandappa, I.M.; Basavaraj, K.; Manonmani, H.K. Analysis of Mycotoxins in Fruit Juices. In Fruit Juices;
- [2] Academic Press: Wageningen, The Netherlands, 2018; pp. 763–777.
- [3] Kui, L., Tang, M., Duan, S., Wang, S., & Dong, X. (2020). Identification of selective sweeps in the domesticated table and wine grape (*Vitis vinifera* L.). *Frontiers in Plant*
- [4] *Science*, 11, 572. <https://doi.org/10.3389/fpls.2020.00572>. Do

- [5] Anjum, N., Feroze, M. A., Rafique, R., & Shah, M. H. (2020). Effect of gibberellic acid on berry yield and quality attributes of grapes cv. sultanina. *Pure and Applied Biology PAB*, 9(2), 1319–1324. <https://doi.org/10.19045/bspab.2020.90137>
- [6] Jaiswal, P. K., Kesharwani, R., Patel, D. K., Verma, P., & Kumar, V. (2023). Nutraceuticals in agriculture. In *Nutraceuticals* (pp. 223-239). Academic Press.
- [7] Khiari, R., Zemni, H., & Mihoubi, D. (2018a). Raisin processing : Physicochemical, nutritional and microbiological quality characteristics as affected by drying process. *Food Reviews International*, 1–53. <https://doi.org/10.1080/87559129.2018.1517264>
- [8] Torres, C. De, Schumacher, R., Alañón, M. E., Pérez-coello, M. S., & Díaz-maroto, M. C. (2015) Freeze-dried grape skins by-products to enhance the quality of white wines from neutral grape varieties. *Food Research International*, 69, 97–105. <https://doi.org/10.1016/j.foodres.2014.12.016>
- [9] Wang, D., Cai, J., Zhu, B. Q., Wu, G. F., Duan, C.-Q., Chen, G., & Shi, Y. (2015). Study of free and glycosidically bound volatile compounds in air-dried raisins from three seedless grape varieties using HS-SPME with GC-MS. *Food Chemistry*, 177, 346–353. <https://doi.org/10.1016/j.foodchem.2015.01.018>
- [10] Venkitasamy, C., Zhao, L., Zhang, R., & Pan, Z. (2019). Grapes. In Z. Pan, R. Zhang, & S. Zicari (Eds.), *Integrated Processing Technologies for Food and Agricultural By-Products* (pp. 133–163). Academic Press.
- [11] Imran, M., Arshad, M. S., Butt, M. S., Kwon, J. H., Arshad, M. U., & Sultan, M. T. (2017). Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. *Lipids in health and disease*, 16, 1-17
- [12] Das, U. N. (2008). Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. *Lipids in health and disease*, 7, 1-18..
- [13] Restani, P., Frigerio, G., Colombo, F., de Sousa, L. P., Altindişli, A., Pastor, R. F., & Di Lorenzo, C. (2016). Raisins in human health: A review. In *BIO Web of Conferences* (Vol. 7, p. 04005). EDP sciences.
- [14] Welke, J. E. (2019). Fungal and mycotoxin problems in grape juice and wine industries. *Current Opinion in Food Science*, 29, 7-13.
- [15] Pena, A.; Cerejo, F.; Silva, L.J.G.; Lino, C.M. Ochratoxin A survey in Portuguese wine by LC-FD with direct injection. *Talanta* 2010, 82, 1556–1561
- [16] Freire, L.; Passamani, F.R.F.; Thomas, A.B.; Silva, L.M.; Paschoal, F.N.; Pereira, G.E.; Prado, G.; Batista, L.R.
- [17] Influence of physicochemical and chemical characteristics of wine grapes on the incidence of *Penicillium* and *Aspergillus* fungi in grapes and ochratoxin A in wines. *Int. J. Food Microbiol.* 2017, 241, 181–190
- [18] Ronald, M.A.; Aifard, E.B. and Lawrence C.P. (1995). *Laboratory Manual of Experimental Microbiology*, Mosby Year book, Inc. U.S.A, pp212-220
- [19] Marth, E.H. (1978). *Standards Methods for the Examination of Dairy products*. 14 Edition, American public Health Association 1015 Eighteenth street, N.M. Washington, DC 20036, p. 416
- [20] de-Hoog, G. S. and Guarro, J. (1995). *Atlas of clinical fungi*. Universitat Rovirai Virgili, Spain, p720

- [21] Pitt, J.I., Hocking, A.D. (1997). Methods for isolation, enumeration and-3 identification. In: Fungi and Food Spoilage. Springer, Boston, MA. [https://doi.org/10.1007/978-1-4615-63914\\_4](https://doi.org/10.1007/978-1-4615-63914_4).
- [22] Samson, R. A., Hoekstra, E. S., & Frisvad, J. C. (2004). Introduction to food-and airborne fungi (No. Ed. 7). Centraalbureau voor Schimmelcultures .
- [23] Alomari, A., Bahkali, A., Asran, A., & Elgorban, A. (2014). Toxigenic fungi isolated from dried fruits: Detection of Aspergillus toxins in Saudi Arabia. Journal of Pure and Applied Microbiology, 8(1.)
- [24] Iamanaka, B. T., Taniwaki, M. H., Menezes, H. C., Vicente, E., & Fungaro, M. H. P. (2005). Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. Food additives and contaminants, 22(12), 1258-1263.
- [25] Allam, A. A. E., Tantawy, A. E. E., Mohamed, K. A. E., Morad, E. A., & El Shafei, M. A. E. (2020). otitis externa in a tertiary care hospital in zagazig, Egypt: isolated pathogens and their antibiotic sensitivity patterns. African Journal of Clinical and Experimental Microbiology, 21(1), 60-65.