Innovations

Morphological Characterization and Determination of Mycotoxigenic and Non-mycotoxigenic Producing *Penicillium SP* from Bakery Food Products by UV Light and Ammonia Vapor Test

Sowmya K L
Research Scholar, Department of Microbiology, Davangere university
Shivagangothri, Davangere-577007 Karnataka.
Dr. Ramalingappa B
Professor, Department of Microbiology, Davangere university, Shivagangothri,
Davangere-577007, Karnataka

Abstract:
Products from bakeries are vital for a healthy diet. Both macronutrients and micronutrients, which are essential for preserving human health, are abundant in it and of great quality. The most popular goods are buns, bread, cupcakes, cookies, and toast. Due to inappropriate handling and poor sanitation, a variety of filamentous fungi are implicated in the spoiling of bakery food products. Mycotoxins are secondary metabolites made by filamentous fungi and found in food and feed. The purpose of this study was to identify the mycotoxigenic and non-mycotoxigenic isolates of Penicillium sp. using morphological characterization.Eighteen (18) isolates of Penicillium spwere obtained from bakery foods that were gathered from bakeries in rural locations, such as Mayakonda, Avargere, Anaberu, Alur, Attigere, Echagatta, Bathi, Kodaganur, Bada, sasalu around Davangere city. They were cultured on different media. Colony colour and texture were seen to assess macromorphological qualities, while spore colour, size, structure, conidiophore structure, conidia, and vesical form were inspected under a microscope to determine micromorphological characteristics.On coconut cream agar (CCA) medium plates, UV fluorescence of isolates was used to measure mycotoxin generation. Penicilliumsp isolates from 16 (88.88%) of the isolates were positive for mycotoxin, while 2 (11.11%) of the isolates tested negative. The prevalence of Penicillium sp., a mycotoxin-producing organism, emphasises the need for action to eradicate their presence in food. The highest incidence of Penicillium sp. was recorded in samples collected from Anaberu, followed by Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Avargere, Mayakonda, and Sasalu.

Key Words: *Bakery products, fungal spoilage, Penicillium sp, Coconut cream agar, Ammonia vapor test.*

1. Introduction

The majority of the nation and its cultures consider pastries to be vital staple foods. The most widely consumed products include breads, buns, cupcakes. Cereals account for over half of our protein demands and the majority of our dietary calories, making bakery items an important source of nutrients for our diet. Carbohydrates, proteins, lipids, vitamins, calcium, iron, minerals, starch, and energy are among the typical nutrients present in baked foods. It works well to add more fibre to bread products. Wheat flour, water, sugar, salt, yeast, baking soda, baking powder, corn starch, milk, butter or margarine, honey, yoghurt, essence, cocoa powder, chocolate slabs, fruit jams and sweetener are all materials used in baking. (Bartkiene*et al*.,2008). In fresh bread and other baked products, the baking process often destroys mould spores. Therefore, a contaminated source might be the air, a bakery surface, a component of equipment, a food handler, raw materials, or even after baking, during the chilling, slicing, or packaging processes. This implies that any issues with mold-related deterioration must arise following baking. Due to airborne contamination brought on by the warmer temperature and more humid storage conditions, the fungal spore counts are higher in the summer than they are in the winter.Unwanted odours were produced by microbial decomposition, which is frequently visible on a product's surface(Baily *et al*.,1993).

Due to the presence of several filamentous fungi including *Rhizopus*sp, *Mucor*sp, *Aspergillus*sp, and *Fusarium*spin the rotting of bakery goods brought on by inappropriate handling and sanitation, losses of bakery goods attributable to these fungi range from 1.5% to 2.5% depending on the time of year, the kind of goods, and the processing techniques used. Because more bread is being manufactured without preservatives and frequently raw ingredients like bran and seeds are included, it is likely that the incidence of wheat bread spoiling brought on by these fungi has grown over the past several years. These bakery items might be spoiled, which might be a health risk or result in a minor food disease. These goods have been linked to food-borne illnesses in previous decades. In nature, *Penicillium* sp. is extensively dispersed and mostly discovered at cereal and grains. Both before and after harvest. Temperature and relative humidity are two environmental factors that have an impact on a plant's growth. Developing qualitative cultural methods for identifying mycotoxigenic *Penicillium* species cultivated on suitable conditions using the blue fluorescence technique.(Jarvis *et al*). These techniques either use a liquid medium or a solid medium, like coconut agar medium (CAM) or potato dextrose agar (PDA). Under long-wave UV light, *Penicillium* sp., a mycotoxin generator, was discovered. Mycotoxigenic isolates are quickly distinguished from non-mycotoxigenic isolates by the emergence of blue to blue-green fluorescent, while non-mycotoxigenic is non-produce fluorescent. The quick and accurate approach of ammonium hydroxide vapor-induced colour shift is frequently used to identify toxic and non-toxic strains of *Penicillium*. Ammonium hydroxide vapor-induced colour shift is a rapid and reliable method for differentiating between hazardous and non-toxic strains of *Penicillium*. A single colony formed using this method in the centre of the Petri dish. While non-mycotoxigenic *Penicillium* strains do not produce colour, the reverse of colonies of mycotoxigenic *Penicillium* strains turned pink when their medium was exposed to ammonia vapour by dropping ammonia hydroxide on it. This study's main focus is on the rapid detection of *Penicillium* sp. mycotoxigenic generating strains that were isolated from bakery items by UV light test and ammonia vapour test, as well as its preventative measures. (Malkki*et al*.,2005).

2. Materials and Methods

2.1. Collection of samples

There are 55 various varieties of bakery items, including bread, toast, cupcakes, buns, cookies, etc. were collected from rural side (Echagatta, Bada,Bathi, Mayakonda, Avargere, Sasalu, Alur, Attigere, AnaberuandKodaganur) different bakery shops around Davangere city from which were decorated samples in open stall during July to December 2022. The collected samples were brought in sterile polythene bags to the laboratory for analysisand maintained at temperature 25-27^{0}C for further processes(Daou *et al*., 2021).

2.2. Isolation and identification of *Penicillium sp*

Using three different isolation techniques, including the spread plate approach, serial dilution method, and direct plate method, four different types of dextrose agar media, including Potato dextrose agar (PDA), Rose Bengal agar (RBA), Czapec (dox) agar (CZA), and Sabouraud's dextrose agar (SDA), were used to isolate *Penicillium sp.* (Al-kahtani, 2014). Chloramphenicol was used to produce the growth medium, which was then autoclaved at 121^{0}C for 15 minutes. The sterilised medium was placed into sterile Petri plates under aseptic conditions after cooling for 15-20 minutes. After solidification, plates were maintained at 40°C until inoculation. For the direct plate approach, 1 gm of the bakery sample was immediately poured or sprinkled over the PDA+RBA+CZA+SDA solidified agar media and incubated for 3 to 7 days at 30-37^{0}C.In the spread plate method, 1 g of sample was mixed with 9 ml of distilled water to create a homogenate, which was then evenly scattered across the surface of the media (PDA+RBA+CZA+SDA) using a sterile L-shaped spreader. Following that, the plates were maintained in an incubator for 3–7 days at 30-370C. Fungi were found and identified after incubation utilising microscopic analysis and morphological traits.(Patil &Kukade, 2020). As part of the serial dilution method, 1 gm of sample was thoroughly mixed with 9 ml of sterile water to create a 10-1 dilution, which was then serially diluted up to 10-7. Spread plate inoculation was performed using 1 ml of the solution from each dilution on PDA+RBA+CZA+SDA solidified agar medium. After that, the plates were held at 30-37°C for 3–7 days. After incubation, fungi were studied and identified using microscopic findings and morphological characteristics.(Patil &Kukade, 2020). Lactophenol cotton blue stain was used to stain the cotton, as demonstrated by Domsch et al. in 1980, Subramanian, 1983, Ellis & Ellis, 1997, Gilman, 2001, and Nagamani *et al*. in 2006; after staining; *Penicillium*sp were recognised based on morphological and microscopic observation.

2.3. Mycotoxigenic Potential Determination of *Penicillium sp*

Coconut cream agar medium (CCAM) was used to test *Penicillium* species capacity to release toxins, revealing both toxigenic and non-toxigenic isolates. Fente et al. and Davis et al. state that coconut cream agar medium was made and sterilised in Petri plates using antibiotics. A well forms in the centre of the CCAM-containing plate after solidification. Spores from a *Penicillium* species culture that had been growing for 7 days were suspended in distilled water that contained 0.025% Tween 80. The Petri plate was then aseptically loaded with a 10 microliter suspension of spores. At 28°C, the infected plates underwent a 7-day incubation period. The inoculation plates were tested for toxin fluorescence after incubation by having them checked under UV light, and the findings were recorded.

After inoculation as single colonies on CAM using a cork borer with a 5 mm diameter, the fungal isolates were grown at 28 °C in the dark for 7 days. The Petri dish was then turned upside down, and the inside of the lid was coated with one or two drops of a concentrated ammonium hydroxide solution. The Petri dish was then inverted over the control plate and lid holding the ammonium hydroxide. (Alkhersan*et al*.,2016).

3. Results

3.1. Detection of *Penicillium sp*

In bakery food items, 18 isolates of *Penicillium*sp were discovered (Figure 1). By comparing the morphological characteristics of the isolates of Penicillium sp to the main descriptions given by Domsch*etal*. 1980, Subramanian, 1983, Ellis & Ellis, 1997, Gilman, 2001, and Nagamani et al. 2006, the isolates were identified. The % detection of *Penicillium* sp in Anaberu, Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Avargere, Mayakonda, Sasalu. was 33.33% (6), 5.55% (1), 16.66% (3), 0% (0), 11.11% (2), 0% (0), 11.11% (2), 16.66% (3), 0% (0), 5.55% (1) respectively.

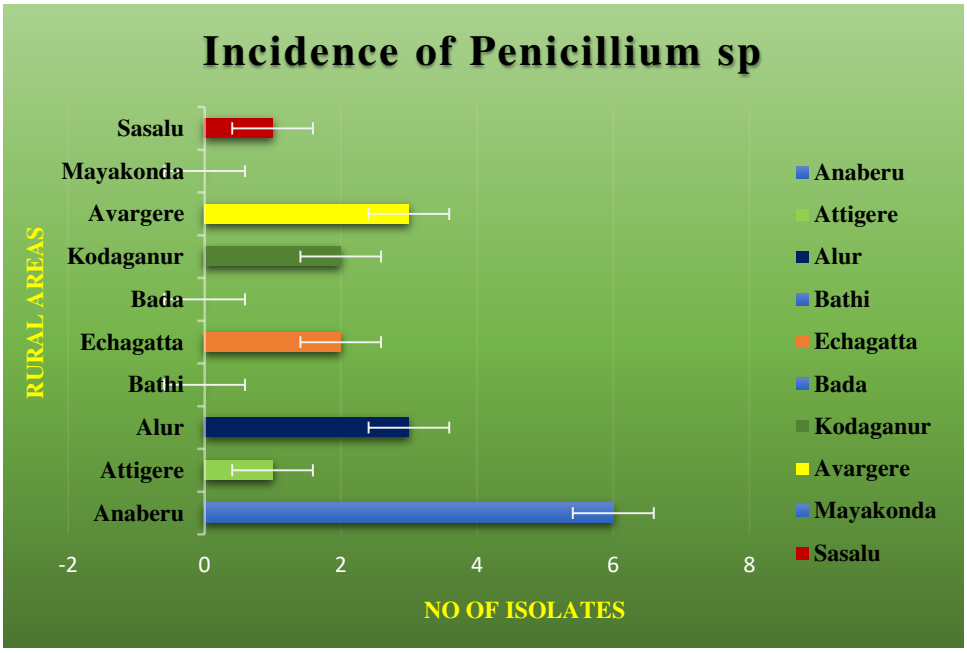
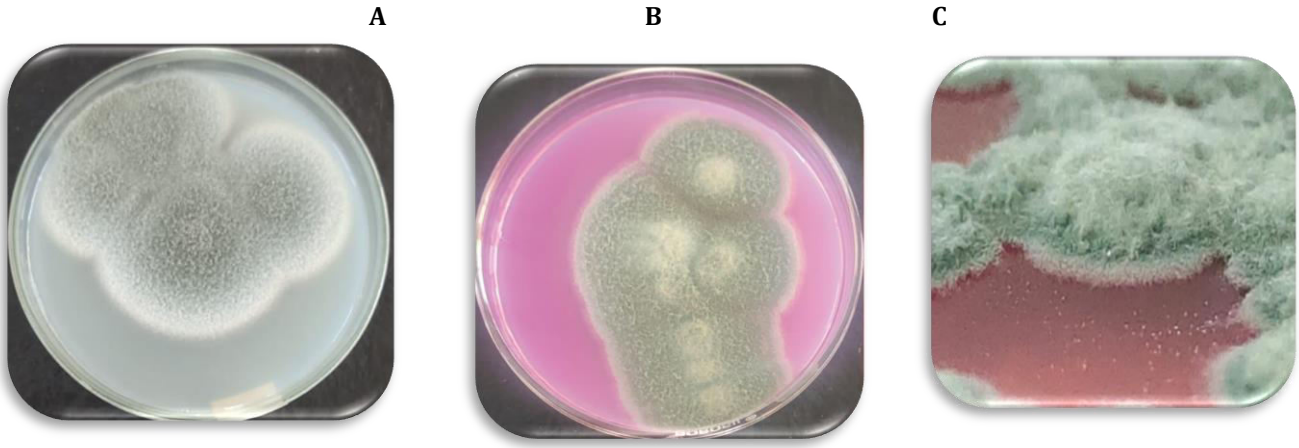


Figure.1: Prevalence of *Penicillium*isolates in Anaberu, Attigere, Alur, Bathi, Echagatta, Bada, Kodaganur, Avargere, Mayakonda, Sasalu

3.2. Macroscopic properties of *Penicillium sp* on PDA and RBA

Figure 2 displays the colony morphology of *Penicillium* sp on PDA and RBA medium plates. After two days of incubation, *Penicillium* sp.'s mycelial colour was white, and after five days of incubation, the colonies on both plates took on a blue green hue.After 14th days of incubation colonies were 80-82mm in diameter, dominated by very light green centre with white surroundings.



igure.2. Colony morphology of *Penicillium* sp. on PDA and RBA medium plates. PDA plates, RBA plates, and 1.9x magnification

3.3. Microscopic characteristic of *Penicillium sp*

The microscopic features of isolated *Penicillium* are depicted in Figure 3. Conidiophores and septate hyphae are branching. Brush like clusters were observed at the end of the conidiophores. Conidia looks like round to ovoid.

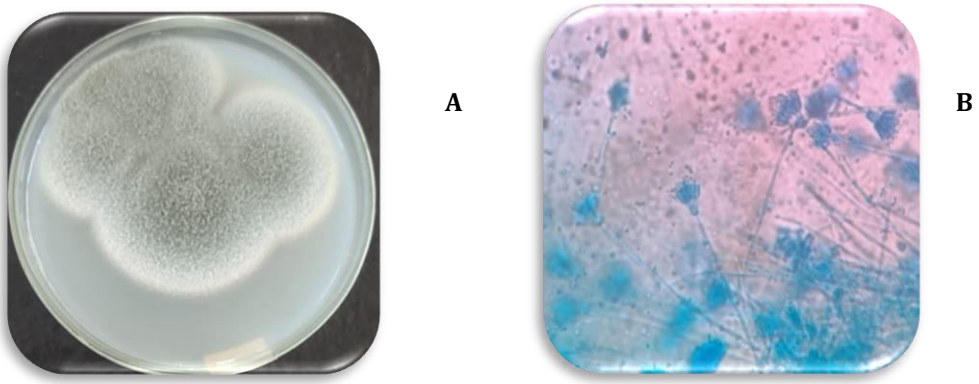
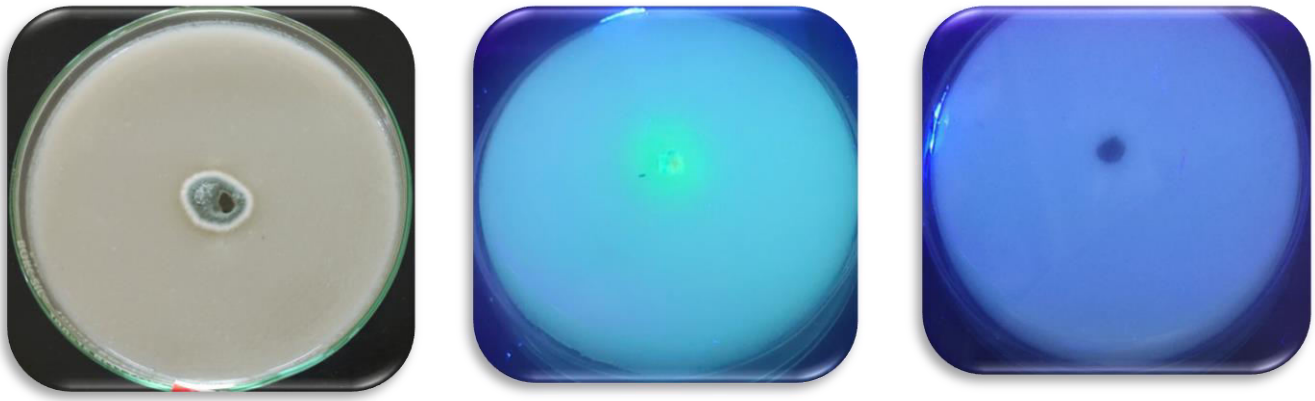


Figure.3. A. *Penicillium sp*. macroscopic features on PDA. B. *Penicillium sp* microscopic features with a 40X objective.

3.4. Screening of Mycotoxin Production

Figure 4 shows the outcomes of the *Penicillium* species UV light screening as well as fluorescent and non-fluorescent isolates of the *Penicillium* species on CCA. When exposed to UV light at 365 nm, 16 (88.88%) of the 18 isolates fluoresced blue on CCA medium plates. Table 1 reveals that of the 18 isolates, 88.88% (16) are capable of producing mycotoxin, while the remaining isolates (11.11% (2) do not exhibit blue fluorescence when exposed to UV light, indicating that they are not engaged in mycotoxin production. **Figure 5** shows the distribution of *Penicillium* isolates

that are mycotoxigenic and those that are not in ten (10) different places. Three of the six isolates from the Anaberu bakery produced mycotoxin during testing, however the other three isolates did not. One isolate from the Attigere bakery samples had mycotoxin production found, and the other isolate also had mycotoxin production.Similar to this, two of the three isolates found in the Alur bakery samples produced mycotoxin, whereas the third strain did not. Out of the two isolates found in the Echagatta bakery samples, one produces mycotoxin. Two isolates from Kodaganur bakery samples proved positive for the formation of mycotoxin, and both two isolates did so. Two of the isolates from the Avargere bakery samples proved negative for mycotoxin generation, but one strain did. Last but not least, a single isolate from a sample from a bakery in Sasalu produced mycotoxin.



A B C

Figure.4. Mycotoxigenic and non-mycotoxigenic isolates of *Penicillium*observed under UV light.
A. Growth of *Penicillium sp* on CCA medium plate. B. Mycotoxigenic production. C. Non-mycotoxigenic production.

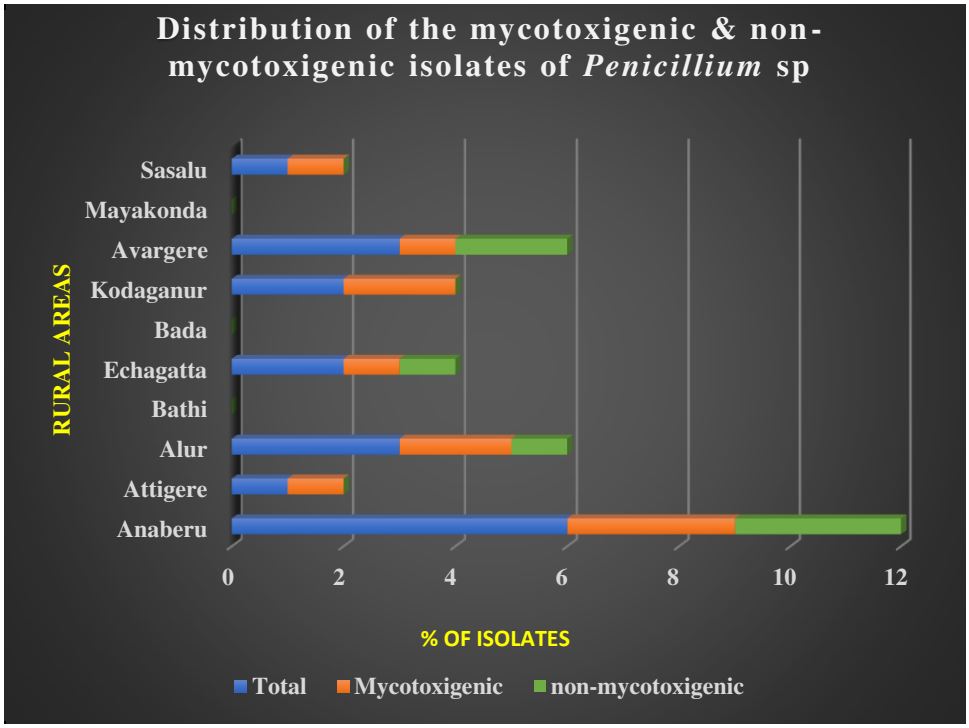


Figure.5. Distribution of *Penicillium* sp. mycotoxigenic & non-mycotoxigenic isolates in 10 bakery food samples gathered from rural locations

Table 1. Identification of production of Mycotoxin through screening by UV fluorescence

Sl.No	Isolates	Results
1	SR1	Positive
2	SR2	Positive
3	SR3	Positive
4	SR4	Negative
5	SR5	Positive
6	SR6	Positive
7	SR7	Positive
8	SR8	Positive
9	SR9	Positive
10	SR10	Positive
11	SR11	Negative
12	SR12	Positive
13	SR13	Positive
14	SR14	Positive
15	SR15	Positive
16	SR16	Positive
17	SR17	Positive
18	SR18	Positive

Discussion

Anaberu bakery samples had the highest occurrence of *Penicilliums*p (33.33%), whereas Sasalu and Attigere bakery samples had the lowest prevalence (5.55%). Environmental variations may be to blame for the variations in *Penicillium* sp. prevalence in the various bakeries. A common technique for identifying mushrooms is to observe the key macro and micromorphological characteristics of cultures of diverse fungi on different media. The morphological Characterization was conducted in this work to emphasise the necessity of such fundamental identifying procedures for the fast screening of isolates in the majority of undeveloped nations, where access to uncommon instruments is a significant challenge. *Penicillium* isolates could be consistently identified by looking at specific physical traits. The colony shape of the isolates in this investigation matched *Penicilliums*p, as seen in Figures 2 and 3, according to Klich's interpretation of taxonomic descriptions. The microscopic and macroscopic characteristics shown in Figures 2 and 3 are similar to those of A. flavus and Penicillium species that Rodrigues et al. and Diba et al. have documented.

In this work, appropriate growth and sporulation were enabled by the use of Potato Dextrose Agar (PDA), Rose Bengal Agar (RBA), Czapec (dox) agar (CZA), and Sabouraud's dextrose agar (SDA) medium. This enabled a successful analysis of the macroscopic and microscopic properties of *Penicillium* sp. On the intentionally infected materials, *P.chrysogenum* developed quickly and left patches of discoloration that ranged from grey to bright green, according to Nielsen et al. In this study, the quick distinction between mycotoxigenic *Penicillium* sp. and non-mycotoxigenic *Penicillium* sp. was made possible by the use of CCA media. When 16 (88.88%) of the 18 isolates were subjected to 365 nm UV light, they fluoresced blue on CCA medium plates, indicating that they had the ability to produce mycotoxins. Culture-based approaches may be helpful for quick screening of isolates in the majority of underdeveloped nations where mycotoxin determination may be delayed due to a lack of sophisticated detection tools. Culture-based approaches are generally straightforward and affordable for mycotoxin detection. However, chromatographic techniques are generally employed in conjunction with these techniques.

Conclusion

Penicillium sp. strains, both mycotoxigenic and non-mycotoxigenic, were discovered in bakery food products from various countries. Culture media and morphology are practical tools for *Penicillium* species in this study.

A rapid and reliable way for separating mycotoxigenic from non-mycotoxigenic isolates is to characterise and screen *Penicillium* isolates for mycotoxin detection under UV light (365nm).

Acknowledgment
The work was carried out in the Bioprocess and Fermentation Technology Laboratory, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka. Author is greatful to research supervisor, Department of Studies in Microbiology, Davangere University, Davangere, Karnataka, India for providing well equipped laboratory facility to carry out the present research work.

Funding: The authors declare that there is no funding.
Conflict of Interest: The authors declare that there is no Conflict of Interest.

References

- ***Abellana, M., A. J. Ramos, V. Sachis. and P. V. Nielsen. (2000).*** *Effect of modified atmosphere packaging and water activity on growth of Eurotiumamstelodami, E.Chevelieri and E. Herbariorumon a spongy cake analogue. Journal of Applied Microbiology. 88: 606- 616.*
- ***Abellana, M., sanchis, U. and Ramos, A. J. (1996).*** *Effect of water activity and temperature on growth of three Penicillium Sp. and Aspergillus flavus on a spongy cake analogue. International Journal of Food Microbiology. 71: 3151-3157.*
- ***Afzal, H., Shazad, S., Qamar, S. and Nisa, U. (2013).*** *Morphological identification of Aspergillus species from the soil of Larkana District (Sindh, Pakistan). Asian J. Agric. Biotechnol. 1:17–105.*
- ***Bailey, C. P. and Holy, A. V. (1993).*** *Bacillus spore contamination associated with commercial bread manufacture. Food Microbiology.10: 287- 294.*
- ***Bartkiene, E. G. Juobeikiene and D. Vidmantiene. (2008).*** *Evolution of deoxynivalenol in wheat by acoustic method and impact of starter on its concentration during wheat bread baking process. Food Chemistry and Technology. 42: 5-12.*
- ***Bouraoui, M., Richard, P. and Fichtali, J. (1993).*** *A review of moisture content determination in foods using microwave oven drying. Food Research International. 26(1):49–57.*
- ***Carla Lucian Gerez. and Mariaines Torino. (2009).*** *Prevention of bread mold spoilage by using Lactic acid bacteria with antifungal properties. Journal of Food Science.20: 144-148.*
- ***Cauvain, S. P. and Young, L. S. (2009).*** *Methods of determining moisture content and water activity. Bakery food manufacture and quality.228–262.*
- ***Chamberlain, N. (1993)****. Mold growth on cake. Biscuit marker and plant baker. 14: 961-964.*
- ***Chavan, J. K. and S. S. Kadam. (1993).*** *Nutritional enrichment of bakery product by supplementation with nonwheat flours. Critical Review of Food Science Nutrition.33:189- 226.*
- ***Cornea, C.P. and M. Ciuca. (2011).*** *Incidence of fungal contamination in a Romanian bakery: A molecular approach. Romanian Biotechnological Letters.16: 5863-5871.*
- ***Cotty, P.J. and Jaime-Garcia, R. (2007).*** *Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol, 119:109–115.*
- ***Cotty, P.J. and Mellon, J.E. (2006).****Ecology of aflatoxin producing fungi and biocontrol of aflatoxin contamination. Mycotoxin Res. 22:17–110.*
- ***Davis, N.D., Iyer, S.K. and Diener, U.L. (1987)****. Improved method of screening for aflatoxin with a coconut agar medium. Appl. Environ. Microbiol. 53(7):1593–1595.*
- ***Diba, K., Kordbacheh, P., Mirhendi, S.H., Rezaie, S. and Mahmoudi, M. (2007)****. Identification of Aspergillus Species Using Morphological Characteristics. Pak. J. Med. Sci. 23: 867–872.*
- ***Domsch, K.H., Gams, W. and Anderson, T.H. (1980).*** *Compendium of soil fungi. Academic press. London, New York, Toronto, Sydney, San Francisco.1:859.*
- ***Dyer, S.K. and Mccammon, S. (1994).*** *Detection of toxigenic isolates of Aspergillus flavus and related species on coconut cream agar. Journal of Applied Bacteriology. 76: 75-78.*
- ***Edward, W. P. (2007).*** *Science of Bakery Products. RSC Publication. 274.*

- ***Elena Guynot, M. and Sonia Marin. (2005).*** *Low intermediate moisture bakery product by MudellingEurotium Sp. Aspergillus Sp. and Penicillium corylophilum growth. International Journal of Food Microbiology. 1-5.*
- ***Ellis, M.B. and Ellis, J.P. (1997).*** *Microfungi on Land plants: An Identification Handbook. Richmond Publishers, London: Croom Helm.1-868.*
- ***Erba, S., Daniotti, B., Rosina, E., Sansonetti, A. and Paolini, R.(2016).*** *Evaluation of moisture transfer to improve the conservation of tiles finishing facades. In J. M. P. Q. Delgado (Ed.). Recent developments in building diagnosis techniques .171–194.*
- ***Fente, C.A., Ordaz, J.J., Vazquez, B.I., Franco, C.M. and Cepeda, A. (2001)****. New additive for culture media for rapid identification of aflatoxin-producing Aspergillus strains. Applied and environmental microbiology, 67(10):4858-4862.*
- ***Francesca Valerio. and Mara Favilla. (2009).*** *Antifungal activity of strains of Lactic acid bacteria isolated from Semolina ecosystem against Penicillium roqueforti, Aspergillus niger and Endomycesfipuliger contaminating bakery products. Systematics and Applied Microbiology. 32: 438-448.*
- ***Fustier, P. Lafond, A. and Champagne. (1998).*** *Effect of inoculation techniques and relative humidity or the growth of mouldes on the surface of yellow cakes. Applied and Environmental Microbiology. 64: 192-196. © 2022 IJRAR November 2022, Volume 9, Issue 4 www.ijrar.org (E-ISSN 2348-1269, P- ISSN 2349-5138) IJRAR22D1661 International Journal of Research and Analytical Reviews (IJRAR) www.ijrar.org 679*
- ***Gao, J., Liu, Z. and Yu, J. (2007).*** *Identification of Aspergillus Section Flavi in Maize in Northeastern China. Mycopathologia.164:91–95.*
- ***Gilman, J.C. (2001).*** *A manual of soil fungi. 2nd edition. Biotech books. New Delhi. 1-392.*
- ***Hailu, G. and Derbew, B. (2015).*** *Extent, causes and reduction strategies of postharvest losses of fresh fruits and vegetables – A review. Journal of Biology, Agriculture and Health care. 5:49-64.*
- ***Hamza, I. S., S. H. Ahmed and Aoda, H. (2009).*** *Study on the antimicrobial activity of Lemongrass Leaf extract. Iraq. J. Mark. Res. Consum. Protec. 1(2): 198-212.*
- ***Hara, S., Fennell, D.I. and Hesseltine, C.W. (1974).*** *Aflatoxin-producing strains of Aspergillus flavus detected by fluorescence of agar medium under ultraviolet light. Appl. Microbiol. 27(6):1118-1123.*
- ***Hicaey, C. S. (1998).*** *Sorbate spray application for protecting yeast raised bakery product. Baker's Digest. 54: 4-7.*
- ***Jarvis, B. (2001).*** *Mould spoilage of food. Process biochemistry. 7: 11-14.*
- ***Jayaraman, K. S. and Das Gupta, D. K. (1992).*** *Dehydration of fruits and vegetables – recent developments in principles and techniques. Drying Technology. 10(1):1–50.*
- ***Kim, K. B., Park, S. G., Kim, J. Y., Kim, J. H., Lee, C. J. and Kim, M. S. (2006).*** *Measurement of moisture content in powdered food using microwave free-space transmission technique. Key Engineering Materials. 321:1196–1200.*
- ***Knight, R. A. and Menlove, E.M. (2006).*** *Effect of the bread baking process of destruction of certain mould spores. Journal of the Science of Food and Agriculture.10: 653-660.*
- ***Leuschner, R.G.K., O'Callaghan, M.J.A. and Arendt, E.K. (1997).*** *Optimization of baking parameters of part baked and rebaked Irish brown soda bread by evaluation of some quality characteristics. International Journal of Food Science. 32:487-493.*
- ***Lin, M.T. and Dianese, J.C. (1976).*** *A coconut-agar medium for rapid detection of aflatoxin production by Aspergillus spp. Phytopathology. 66: 1466-1469.*
- ***Magan, N. and D. Aldred. (2006).*** *Managing microbial spoilage of cereal and bakery products. 194- 212.*
- ***Malkki, Y. and O. Rauha. (2000).*** *Mould inhibition by aerosols. Bakers Digest, 2000; 52: 47-50.*
- ***Marin, S. Guynot, M. E. and Sanchis. (2003).****Aspergillus flavus, Aspergillus niger and Penecilliumcoryophilumspoilage prevention of bakery product by means of weak acid preservatives. Journal of Food.*
- ***Mathlouthi, M. (2001).*** *Water content, water activity, water structure and the stability of foodstuffs. Food Control.12(7):409–417.*
- ***Ming-Tzaichen., Yuan-Hsin Hsu., Tzu-Sui Wang. and Shi-Wern Chien. (2016).*** *Mycotoxin monitoring for commercial foodstuffs in Taiwan. Journal of Food and Drug Analysis. 24:147-156.*
- ***Mohamed Hashem. (2011).*** *Isolation of Mycotoxin producing Fungi from fishes growing in Aquacultures. Research Journal of Microbiology. 6(12):862-872.*

- ***Mohammadi, A.H., Banihashemi, Z. and Haghdel, M. (2009).****Identification and prevalence of Aspergillus species in soils of Fars and Kerman provinces of Iran and evaluation of their aflatoxin production. Rostanilha.23:49–50.*
- ***Montville, T. and Matthews, K. (2008).*** *Food Microbiology: An Introduction. 2nd edition. Blackwell publishers. P 432.*
- ***Nagamani, A., Kunwar, I.K. and Manoharachary, C. (2006)****. Hand book of soil fungi. I K International Pvt.Ltd. New Delhi.*
- ***Nida, M. Salem and Rafat Ahmad. (2010).*** *Mycotoxins in Food from Jordan: Preliminary survey. Food control. 21:1099-1103.*
- ***Ogawa, T. and Adachi, S. (2014).*** *Measurement of moisture profiles in pasta during rehydration based on image processing. Food and Bioprocess Technology.7 (5):1465-1471.*
- ***Okoth, S.A., Nyongesa, B., Joutsjoki, V., Korhonen, H., Ayugi, V. and Kang'ethe, E.K. (2016).****Sclerotia formation and toxin production in large sclerotialAspergillus flavus isolates from Kenya. Adv. Microbiol. 6:1–10.*
- ***Patil, V.S. and Kukade, P.D. (2020).*** *Fungal spoilage of Bakery products and its control measures. World Journal of Pharamaceutical. 1:167-181.*
- ***Pundir, R.K. and Jain, P. (2011).*** *Qualitative and Quantitative analysis of microflora of Indian bakery products. Journal of Agricultural Technology:7(3):751-762.*
- ***Raed Najeeb Alkhersan., Mohammed H.Khudor. and Basil A.Abas. (2019).*** *Rapid detection of aflatoxigenic producing strains of Aspergillus flavus from poultry feed by UV light and ammonia vapor. 1-12.*
- ***Rahim Khan., Farinazleen Mohamad Ghazali., Nor Ainy Mahyudin. and Nik Iskandar Samsudin. (2020).*** *Morphological Characterization and determination of Aflatoxigenic and non-aflatoxigenic Aspergillus flavus isolated from sweet corn kernels and soil in Malaysia. agriculture. 10:1-13.*
- ***Rodrigues, P., Soares, C., Kozakiewicz, Z., Paterson, R., Lima, N. and Venancio, A. (2007).*** *Identification and characterization of Aspergillus flavus and aflatoxins. In Microbiology Book Series–Communicating, Current Research and Educational Topics and Trends in Applied, Microbiology; Méndez-Vilas, A., Ed.; Formatex: Badajoz, Spain. 2:527–534.*
- ***Rouaa Daou., KarineJoubrane., Richard G.Maroun., Lydia Rabbaa Khabbaz., Ali Ismail and Andre El Khoury. (2021).*** *Mycotoxins: Factors influencing production and control strategies. AIMS Agriculture and Food. 6(1):416-447.*
- ***Subramanian, C.V. (1983).****Hyphomycetes, taxonomy and biology. London. New York. Academic press.410-461.*
- ***Suhr, K. I. and Nielsen, P. V. (2004)****. Effect of weak acid preservatives on growth of bakery products spoilage fungi at different water activity and pH values. International of food microbiology. 95: 67-78.*
- ***Zambrano, M. V., Dutta, B., Mercer, D. G., MacLean, H. L. and Touchie, M. F. (2019).*** *Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. Trends in Food Science & Technology, 88, 484-496.*
- ***Zhang, L., Sun, D.W. and Zhang, Z. (2017).*** *Methods for measuring water activity (a_w) of foods and its applications to moisture sorption isotherm studies. Critical Reviews in Food Science and Nutrition. 57 (5):1052-1058.*