Antifungal Assessment of Selected Food Products Obtained From the Local Markets of Mardan KPK Pakistan

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ABSTRACT

Foodborne diseases are very easily transmitted from contaminated food and food handlers. Bacteria and fungi are the major factors for fruit spoilage. Food wastage causes losses to manufacturers, suppliers, and customers in the form of abridged quality and quantity and higher prices. The objective of this study was to isolate and evaluate the antifungal analysis of food-borne fungal pathogens. For the evaluation of pathogenic fungi Milk, Chutney, Apple, Spinach, Salad, Guava, Fish, and Peas were taken. All the other food samples were taken in polythene zip bags. The vegetables and fruits were cut into small pieces with a sterilized blade and then plated on Sabouraud dextrose agar (SDA) aseptically and then incubated for 5 days. To find foodborne pathogenic fungi in various food items, a total of 640 were collected for evaluation. Out of the total food samples (130), 20.31% were found positive for fungal pathogens in various foodstuffs while 510 (79.68%) food samples were found negative. Among the positive food samples, Fusarium spp was more predominant 56(43%) led by Aspergillus spp 50(38.5%). In comparison, Mucor and Cladosporium were found in low frequency with percentages of 14(10.8%), 10(7.7%). For antifungal activity, a panel of selected drugs was used to assess the susceptibility of pathogens. Among the antifungals best activity was shown by Voriconazole and intermediate activity was shown by Fluconazole. While high resistance was observed in Nystatin as compared to other antifungals.

Key Words: Antifungal, Pathogens, Foodstuffs, Contamination, Food spoilage

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INTRODUCTION

Foodborne diseases are very common and can easily transmit from contaminated food and food equipment. Among the foodborne pathogens, strains of Staphylococcus aureus, Bacillus cereus, Listeria monocytes, and Clostridium are very important because of their presence in a wide range of foodstuffs (**Khan et al 2019**).Weather conditions can selectively promote the growth of particular fungal species, which cause rotting and spoilage of grape berries before harvest. The fungi may also lead to produce mycotoxins and cause contamination (**Mikušová et al 2012).**

Food spoilage is any change in the appearance, smell, or taste of food products. Food decay causes losses to manufacturers, suppliers, and customers in the form of reduced quality and quantity and higher prices. The factors that affect microbial growth in food and constantly the association that develops also determine the nature of spoilage and any health risks. The influence of each factor on growth in a food system the factors are present together and exert effects on microbial growth in combination, either favorably or adversely (**Anwer, et al 2017**). Bacteria are the foremost and important factor for fruit degeneration. They will survive in suitable temperatures with the presence of food and water, which caused changes in the appearance, color, and smell of the fruits (**Hasan et al 2018**) Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminate pathogens. The washing of vegetable fruits and can reduce fungal levels but cannot eliminate pathogens. Eating or drinking contaminated foods or drinks can cause food-borne disease (**Yaradua, S. S. 2018)** Post-harvest fungal infection of fruits and vegetables is mainly caused by fungal pathogens that can be harmful to both humans and animals as they produce mycotoxins, post-harvest diseases in fruits and vegetables are a serious problem that results in the loss of a large percentage of crops and some fruits. Microorganisms, counting bacteria and fungi, affect significant financial losses by indulging not only reaped fruits and vegetables but also crops in their fields. The identification of food-borne fungi is a good step to control them (**Saleh, I., & Al-Thani, R. 2019**) The wide array of available dairy foods challenges the microbiologist, engineer, and technologist to find the best ways to prevent the entry of microorganisms includes spoilage include aerobic psychotropic, Gram-negative, bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria (**Samuel et al 2016).** Certain fungi such as Aspergillus, Fusarium, and Penicillium are commonly occurring filamentous fungi found in vegetables and their growth may result in the production of mycotoxins, which can cause a variety of illnesses in humans, from an allergic response to immune suppression and cancer. The transmission of this disease by hand, food, and kitchen surfaces had been reported from 5 to 100%. Several reports have been indicated that food-borne diseases are a global problem. Polluted food affects 1.5 billion cases of diarrhea in children yearly, Foremost to More Than 3 Million Deceases All over the World (**Muhammad et al 2018).**

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MATERIALS AND METHODS

This research study was conducted in the Microbiology Research Laboratory, Abasyn University Peshawar from March 2023 to May 2024. The work focused on isolation, identification and antibiogram analysis of pathogenic fungal species from various food stuffs.

A total of 640 food samples were examined. For the evaluation of pathogenic fungi Milk, Chutney, Apple, Spinach, Salad, Guava, Fish and Pea were taken. All the others food samples were taken in polythene zip bag. The fruits were cut into small segments with a sterilized blade and then plated on Sabouraud dextrose agar (SDA) aseptically and then incubated at 28ºC for 5 days. Culture was obtained and sub-culturing each of the different colonies that emerged onto the SDA plates and were incubated at 28ºC for 5 days. As a control, each of the healthy food samples was sterilized with 75% ethanol.

Polythene zip bag

Sampling

Isolation of Fungi

A total of 640 randomly selected spoilt fruits and another 200 healthy looking fruits were examined. The fruits were cut into small segments (3 mm in diameter) with a sterilized blade, surface sterilized in 1% hypochlorite for 2 min, plated on Sabouraud dextrose agar (SDA)

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aseptically and then incubated at 28ºC for 5 days. A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the SDA plates and were incubated at 28ºC for 5 days. As a control, each of the healthy fruits was sterilized with 75% ethanol. The fruits were cut into small segments (3 mm in diameter) with a sterile blade, placed on SDA and then incubated at 28ºC for 5 days

Identification Method for Fungi

Agar disc diffusion method was used for screening antifungal activities of each antibiotic. Yeast inoculums in 0.85% NaCl solution was spread on the surface of yeast extract-peptone-glycerol (YPG) agar plate. Sterile filter paper discs with 50 µg of Nystatin, 25 µg of Fluconazole, 1 µg of Voriconazole were used. Sanitized water was use as negative control.

RESULTS

To find foodborne pathogenic fungi in various food items, a total of 640 were collected for evaluation. Out of the total food samples (130), 20.31% were found positive for fungal pathogens in various foodstuffs while 510 (79.68%) food samples were found negative (Tab. 1) Among the positive food samples Fusarium spp were more predominant 56(43%) leading by Aspergillus spp 50(38.5%). Mucor and Cladosporium were found in low frequency with percentages of 14(10.8%), and 10(7.7%) Fig

3.**Table.1 Overall frequency of Fungal pathogens in various food items**

S.no	Food type	Aspergillus	Fusarium	Mucor	Cladosporium
1	Milk	10	12	0	0
2	Chutney	12	10	0	0
3	Apple	0	4	6	10
4	Spinach	0	10	8	0
5	Salad	8	6	0	0
6	Guava	8	6	0	0
7	Fish	12	0	0	0
8	Pea	0	8	0	0
Total	--	50	56	14	10

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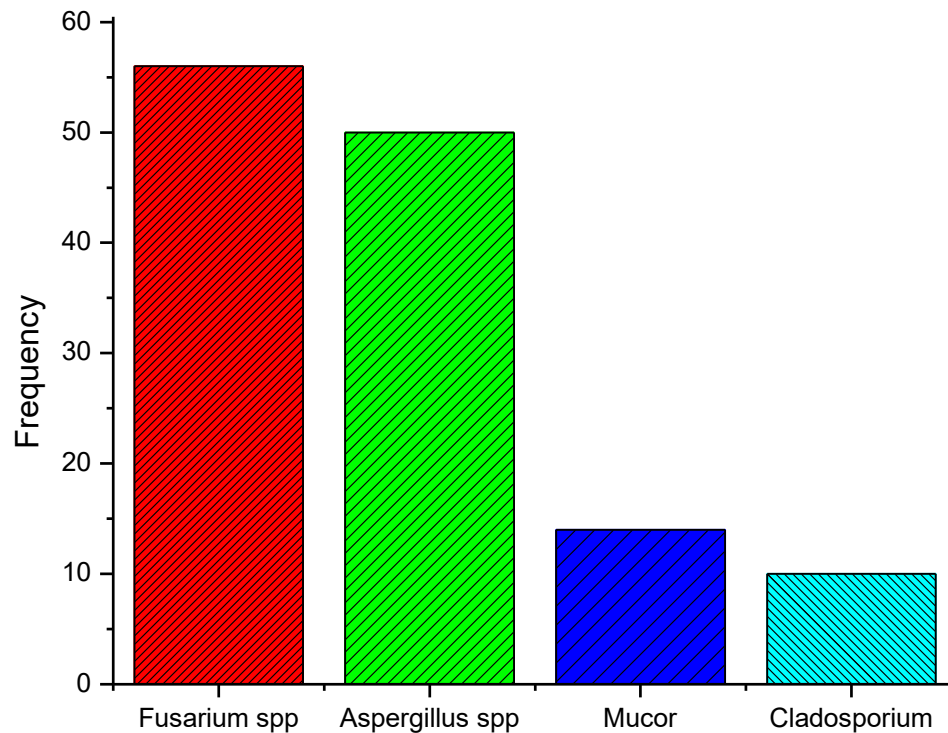
Figure.1 Frequency distribution of Fungal Pathogens

For the evaluation of pathogenic fungi Milk, Chutney, Apple, Spinach, Salad, Guava, Fish and Pea were taken (Tab. 1, Fig.2). The percentages of fungi were high in Milk leading by Chutney, Apple and Spinach. Intermediate frequency was found of Salad, Spinach, Guava and Fish while very low frequencies of fungal pathogen were found in Pea.

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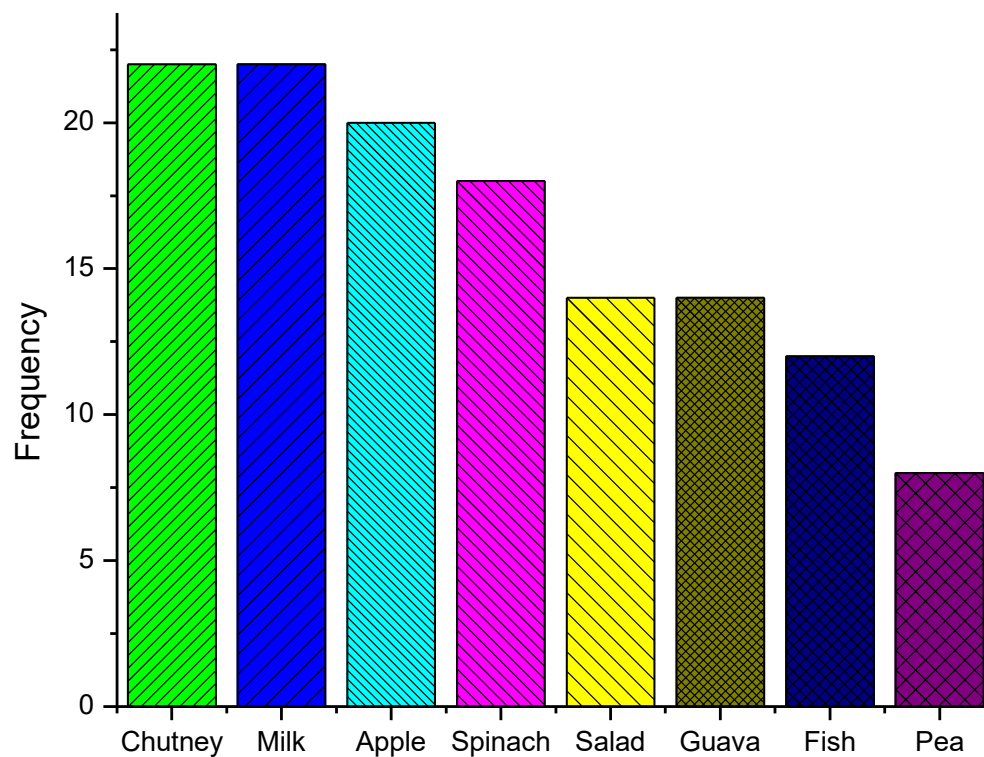
Figure.2 Food wise frequency of Fungal pathogens

Figure.2 Food wise frequency of fungal pathogens in various food items

For antifungal activity a panel of selected drugs were used for the susceptibility of pathogens. Among the antifungal best activity were shows by Voriconazole and intermidiate activity were shows by Fluconazole. While high resistance were observed in Nystatin as compare to other antifungals (Fig. 3.)
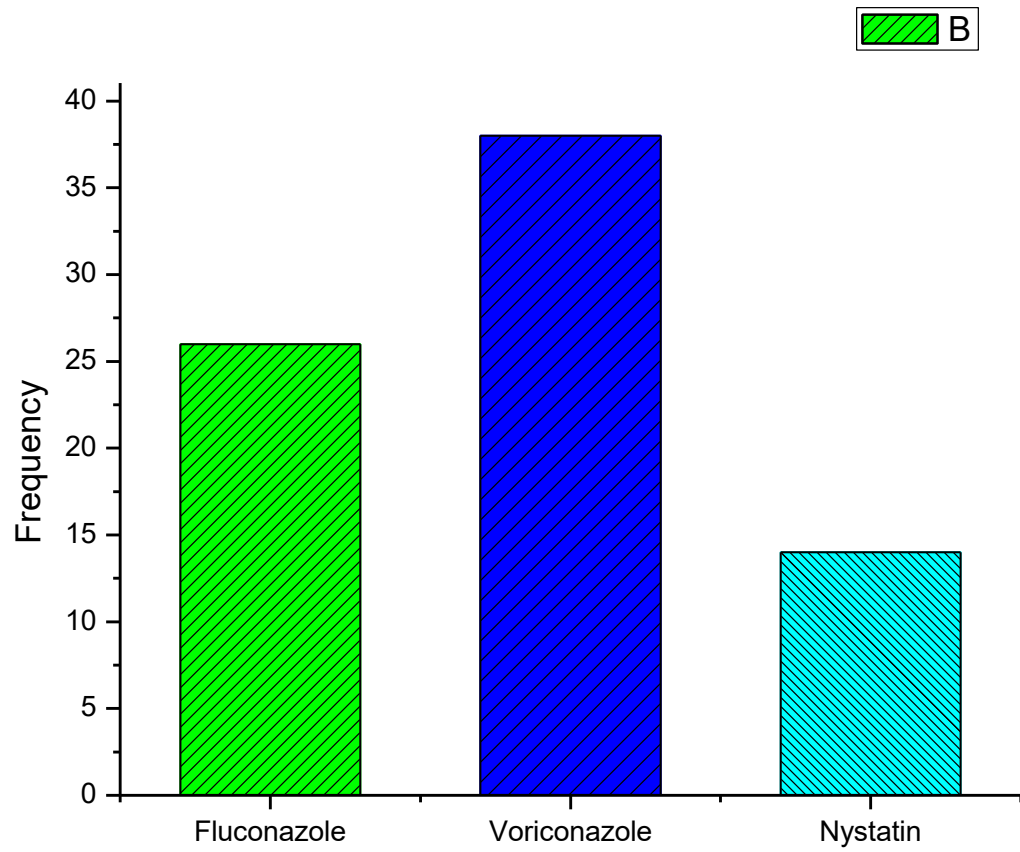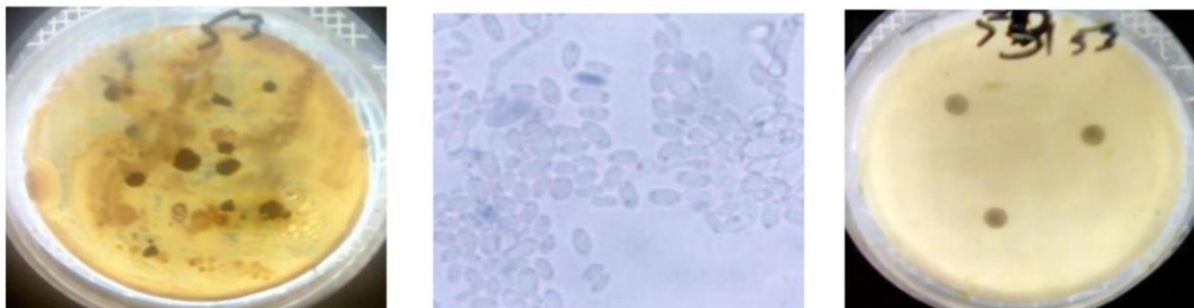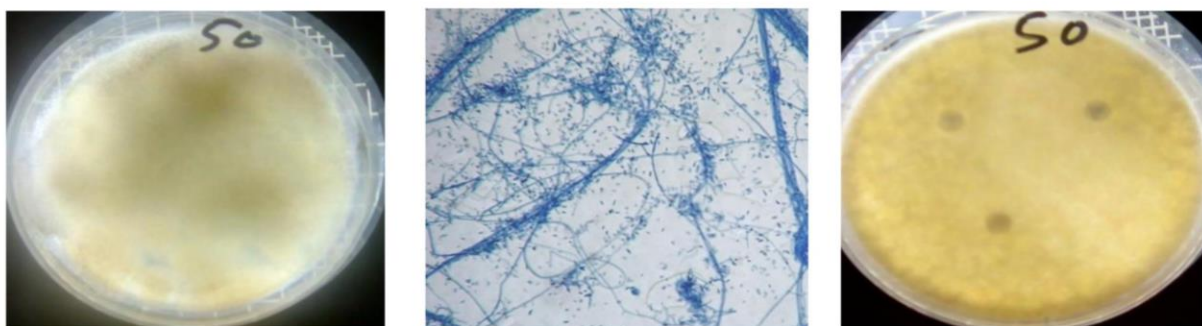
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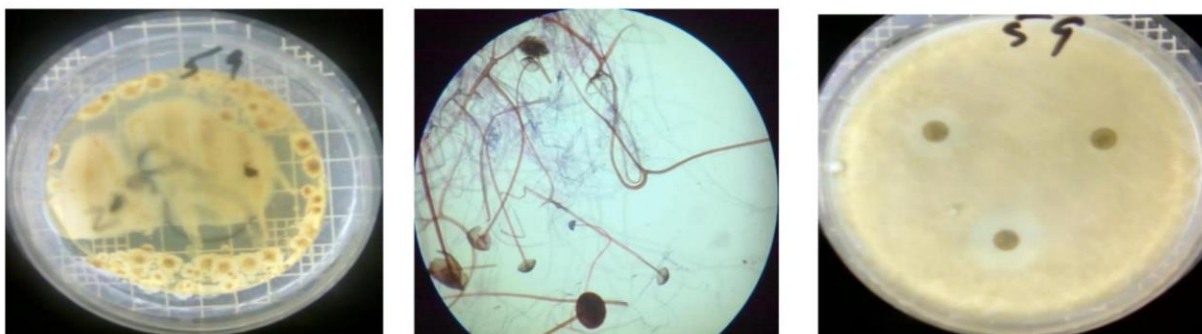
Figure.3 Sensitivity profile of selected Antifungals

13: Aspergillus nigar culture, Microscopic Examination and culture sensitivity.

15: Candida albican culture, Microscopic Examination and culture sensitivity

17: Fusarium oxysporium culture, Microscopic Examination and culture sensitivity.

19: Mucor culture, Microscopic Examination and culture sensitivity.

DISCUSSION

Foodborne diseases are very easily transmitted from contaminated food and food handlers. Bacteria and fungi are the major factors for fruit spoilage. Food spoilage causes losses to producers, distributors, and consumers in the form of reduced quality and quantity and higher prices **(Khan et al 2018).** Therefore the objective of this study was to isolate and evaluate the antifungal analysis of food-borne fungal pathogens. For the evaluation of pathogenic fungi Milk, Chutney, Apple,

Spinach, Salad, Guava, Fish, and Pea were taken. The same work was conducted by (**Thiyam et al 2013**) in Nigeria A total of 200 samples were taken results showing that. The frequency of Aspergillus was found 38% high in pineapple, oranges, pawpaw, and tomatoes leading by Fusarium with the frequency of occurrence of 31% in fruits while Penicillium and Rhizopus found in low-frequency rate of 4% each food item respectively.

Among the positive food samples, Fusarium spp were more predominant 56(43%) leading by Aspergillus spp 50(38.5%). While Mucor and Cladosporium were found in the low frequency with the percentage of 14(10.8%), 10(7.7%). The same research was also performed by (**Tafinta et al 2013**) the most common fungal species were Aspergillus found in all fruits during storage of fruits. Other fungal pathogens Alternaria, Cladosporium, Fusarium, Mucor, Penicillium, and Rhizopus were common in fruits.

For antifungal activity, a panel of selected drugs was used for the susceptibility of pathogens. Among the antifungal best activity was shown by Voriconazole and intermediate activity was shown by Fluconazole. While high resistance was observed in Nystatin as compared to other antifungals. The same work was also conducted by (**Khan et al 2019**) the isolates of the fungal pathogen were Aspergillus nigar in a high-frequency rate of (50%) led by Candida krusei the frequency rate of (19%) the low frequency of Fusarium exosporium (6%) and Mucor (25%). Antifungal analysis of fungal isolates had shown differential sensitivities toward Voriconazole. While Fluconazole and Nastatin have a high resistant rate.

Conclusion

Four Fungal species were successfully isolated in eight types of food samples and identified as Fusarium spp were more predominant 56(43%) led by Aspergillus spp 50(38.5%). While Mucor and Cladosporium were found in the low frequency with the percentages of 14(10.8%), and 10(7.7%). Further research work is required to explore more about the effect of these fungal species associated with food spoilage. Improved preservation methods, and handling, and recommended to enhance the quality of food for eating.

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Competing Interests
The authors declare that they have no competing interests.

Authors' Contributions
All authrs equally contributed in the designing, experiments and wrote the manuscript. All authors read and ap proved the final manuscript.

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