2013 International Conference on Agriculture and Biotechnology
IPCBEE vol.60 (2013) © (2013) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2013. V60. 15

Fungi Associated with Some Agricultural Products and Effects on Their Quality at Misurata Region (Libya)

Daghman. I. M. [1+],Sara Alshik[1] and Alhobugi A. M. [1]

[1]Microbiology Department, Faculty of Science, Misurata University, Misurata, Libya.

Abstract. This investigation aimed to isolate and identify the fungi associated with tomato, squash fruits and corn grains collected randomly from two markets in Misurata City and to evaluate the effect of some fungi on nutritional value of the tested samples. Highest fungal biodiversity was represented on squash, followed by tomato fruits. Conversely, corn grains yielded lowest fungal biodiversity. *Aspergillus* followed by *Fusarium* and *Penicillium* were the commonest genera. In contrast, *Pichia*, *Pleospora*, *Candida*, *Geotrichium*, *Ulocladium*, *Setosphaera*, and *Rhodototula* were the lowest diversity. The media DG18 and washing method contributed highest fungal population. Some nutritional value of tested fruits were variously affected depending upon the tested fungal species (*Aspergillus niger*, *Aspergillus flavus*, and *Alternaria alternata*). *A. alternata* and *A. niger* reduced carbohydrates, protein and vitamin (C) contents of tomato and squash fruits as incubation periods increased. However, vitamin (C) contents in corn grains had increased as incubation periods increased when treated by *A. flavus*. Some essential elements of the tested fruits were conspicuously affected by *A. flavus* and *A. alternata*. Phosphorus and iron clearly increased whereas the all others showed variable reduction. *A. flavus* reduced the protein and carbohydrate contents of corn grains particularly after the 10 and 13 days of incubation, respectively but showed no effect concerning the other tested elements.

Key words: Fungi, agricultural products, nutritional contents.

1. Introduction

Fungi are the most frequent contaminants of our food-stuffs and spoil our foods. Some of them cause rotting of fruits, vegetables in storage and responsible for several disorders and undesired chemical alterations for several agriculture products, particularly seeds, grains and fruits [1]. Tomato, squash fruits and corn grains are the most popular vegetable world-wide and the yield is suffered every year due to a number of pathogenic diseases. In developing countries, post-harvest losses are often more severe due to inadequate storage and transportation facilities. Fungal fruits infection may occur during the growing season, harvesting, handling, transporting and post-harvest storage and marketing conditions, or after purchasing by the consumer. Fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decayed [2]. Studies show that fungi can survive and/or grow on fresh produce and that these nutrient content (carbohydrate, protein and fat) support pathogens [3]. Numerous fungal genera such as *Fusarium*, *Penicillium*, *Alternaria* and *Aspergillus* play a crucial role in rapid deterioration and spoilage of agricultural products quality and quantity either in field or during transport and storage [4] and [5].

The high moisture, carbohydrate and other nutrient contents of the majority of fruits and vegetables encourage fungal growth and consequently lead to their deterioration and great loss quantitatively or qualitatively of yield by 5-20 % in developed and between 20–50% in no developing countries [6]. Roots and stems of tomato were the highest susceptible and sensitive to fungal damage in comparable to the leaves and fruits which were affected by 28.94%, 26,31% and 15.78% respectively. *Fusarium oxysporum* was the

[+] Corresponding author. Tel.: + 00218925503503; fax: +00218512627350.
E-mail address: (please specify).

most frequent (91.51%) during irrespective to the growth stage. In Florida, the study revealed that the nutrient contents of Apricot fruits (0.9% protein, 12% carbohydrates, 18% vitamin B_1) was conspicuously reduced as a results of fungal growth, particularly concerning proteins and carbohydrates which recorded 0.5% and 7.8%, respectively, after 10 days of incubation [7]. Some fungal species which belong to *Aspergillus* , *Alternaria* , *Penicillium* , *Rhizopus* ,and *Fusarium* cause variable retardation of some nutrient contents. However, in Libya, no comprehensive literature is available concerning the adverse effect of fungi on the nutrient contents and nutritional value of the agricultural products. Thus, the present investigation aims to study the fungi associated with tomato, squash fruits and corn grains collected randomly from two public markets (Aboshala and Almangosh) in Misurata City and to evaluate the impact of some fungi on the nutritional value of the tested samples.

2. Materials and Methods

2.1. Fruit Samples

Tomato, Squash fruits and Corn grains (2 Kg each) were collected randomly from two public markets (Aboshala and Almangosh) at Misurata City (Libya) in sterilized polyethylene bags, and transferred separately to the laboratory for further experimentation.

2.2. Fungal Isolation

The fungi associated with the tested fruits were isolated using direct and washing isolation techniques. Dichloran Rose Bengal Chloramphenicol Agar (DRBC) and Glycerol Dichloran Agar %18(DG18) growth media were used.

2.3. Effect of Three Selected Fungal Species on Some Nutritional Values of Tested Fruits

Values of some nutritional contents (carbohydrates, proteins, vitamin C, K, Mg, Ca, Fe, Na and P) of tomato, squash fruits and corn grains as affected by *Alternaria alternata*, *Aspergillus niger* and *Aspergillus flavus* were monitored. The selected fungal species were the most predominant among the fungi associated with the tested fruits. In order to surface sterilization fruits were washed thoroughly with sterile distilled water, and then immersed in 70% ethyl alcohol for 3-4 min. Fruits were re-washed using sterile distilled water to remove the alcohol trace. The experimented fruits were bored using cork borer (5 ml) under aseptic conditions, and then inoculated with the tested fungi and incubated at 24 ± 2^0C, for 13 days, during which the nutritional contents of the experimented fruits were assayed at intervals (7, 10 and 13 days). Three replicates were used for each treatment and the nutritional contents as well as the chemical alterations were followed. High performance liquid Chromatography (HPLC) was used for assaying carbohydrate, protein and vitamin C contents. UV–VIS Spectrophotometer was used for determination the some essential element values.

3. Results and Discussion

3.1. Isolation and Identification

Fifty-four colonies forming units (CFUs) were isolated from tomato fruits using the both growth culture media (DRBC & DG18) and direct isolation technique. Of which 22 and 32 CFUs were gathered using DRBC and DG18, respectively. *A.niger* (15 CFUs) was the pre-dominant species, followed by *C.cladosporioides* (13 CFUs), and *A. alternate* (12 CFUs). *A. flavus*, *Pleospora herbarum* and *Alternaria* species were isolated exclusively on DG18. Moreover, the washing method contributed higher population (637 CFUs) using the both culture media. DG18 was the richest in fungal abundance and yielded 350 CFUs, in comparable with DRBC which recorded 287 CFUs. Highest fungal population was recorded by *Candida tropicals*. Similar results were obtained at Assiut (Egypt) where isolated 13 fungal genera from two varieties of tomato fruits using three culture media (PCA, MEA, CZ) [8], recorded that *A. alternata, A. niger, A. flavus, A. parasiticus, C.cladosporioides, F. oxysporum,* and *F. solani* were the commonest fungal species.

Forty two colonies units (CFUs) were isolated from squash fruits using both growth culture media by direct and washing techniques. Of which 16 and 26 CFUs were gathered using DRBC and DG18 respectively only by direct isolation. The *P.* chrysogenium (14 CFUs) recorded the highest CFUs followed by *C.cladosporioides* (9 CFUs).The lowest fungal population was recorded by *Ulocladium atrum.* Moreover,

the washing method contributed higher population (760 CFUs) by both culture media. Of which 433 and 327 CFUs were isolated using DRBC and DG18 culture media respectively. The highest CFUs were recorded by *Trichoderma koningii*, followed by *A. niger* and *A. flavus*. *T. harzianum* recorded 98 CFUs which isolated by DRBC culture media only. Similarly, [9] collected 8 fungal genera (*Alternaria, Rhizopus, Aspergillus, Fusarium, Geotrichum, Penicillium, Trichoderma,* and *Cladosporioides*) from ripe squash fruits using two growth media. Which recorded that *Rhizopus,* and *Aspergillus* were the pre-dominant ones. *F. oxysporum, R. stolonifer, A. flavus* and *A. niger* were the commonest species.

Thirty three CFUs also were isolated from corn grains using both DRBC and DG18 by direct technique. Of which 16 and 17 CFUs were isolated by DRBC and DG18 respectively. The *A, niger* 16 CFUs recorded the highest population followed by *A. flavus* 13CFUs. The *F. verticilliodes* only 8 CFUs. Of which washing method contributed higher population 610CFUs by both culture media. Of which 265 and 345 CFUs were isolated using DRBC and DG18 culture media respectively. The *A. flavus* 223 CFUs recorded the highest population by DG18 culture media. Followed by *T. harzianum* 184 CFUs mostly 102 CFUs isolated by DRBC culture growing media.

Our results are in accordance with those obtained by [10] who used the corn grains as culture medium for fungi and recorded that *Aspergillus, Fusarium,* and *Cladosporium* were the commonest genera. Moreover, *A. niger* and *A. flavus* were the commonest species. The study At Khartoum (Sudan) reported that 70% of the experimented corn grains were contaminated by six *Aspergillus* species (*A. flavus, A. melleus, A. niger, A. ochraceus, A. parasiticus,* and *A. versicolor*), of which *A. flavus* was the commonest one [11]. Most of similar investigation revealed that *Aspergillus* was the commonest genus on corn grains, and *A. niger* and *A, flavus* were the commonest species. This could be attributed to high enzymatic activities of these *Aspergillus* spp. Generally, the study showed that DG18 growth medium yielded more fungal population and diversity comparable to DRBC medium. This could be attributed to glycerol content of DG18 medium which fit to the osmo-tolerant fungi particularly Zygomyceteous fungi. Several authors recorded similar observation.

3.2. Effect of Three Selected Fungal Species on Some Nutritional Contents of Tested Fruits

The nutritional contents of three agricultural products (tomato, squash fruits and corn grains) as affected by three fungal species *(A. niger, A. flavus,* and *A. alternata*) were investigated. *A. alternate* exerted a significant reduction of the carbohydrate contents of tomato fruits with the increasing of incubation period into 13 days particularly after 7 days. The vitamin C content was significantly retarded with the prolongation of incubation period. Conversely, the protein content of tomato fruits was significantly induced after 7 day of incubation in comparable with the control (Table 1). The data (Table 1) revealed that the K, P, Ca and Fe contents of tomato fruits were stimulated incomparable with control. On the contrast, Mg and Na recorded a significant decrease. The quality of tomatoes mainly depends on proper handling during post harvest processes like harvest, grading, packing and transportation. The study was focused on shelf life of tomato based on the systematic survey of the distribution of tomato crop in two public markets in Misurata . Several authors reported that during the survey it was evaluated that the deterioration of the agricultural products is probable due to packing material was 25%, transportation system was 10%, means of distribution was 5%, exceeding post harvest losses up to 30% and sometimes the whole lot is lost. Time lag in transportation, bulky packing in the traditional wooden crates wrapped with papers cause high humidity making the microclimate favorable for mycoflora. The dominating fungi associated in decay process were *A. niger, A.flavus, F. oxysporum, R. stolonifer, A. fumigates*. Reduction of carbohydrate contents of tomato fruits infected with *A. alternata* is due to its consumption as a carbon source. This is in accordance with the results reported that *A. alternata* utilize the polysaccharide and organic acids of tomato as a carbon source [6].

A. niger exhibited a significant reduction concerning the carbohydrate contents of squash fruits after 7 days of incubation, after which gradually enhanced. The protein content was significantly reduced in comparable with the control irrespective to the incubation period recording the lowest value after 13 days (Table 2). Similar significant reduction of vitamin C was recorded after 7 days of incubation, after which no alterations were recorded. The values of essential elements of squash fruits as affected by *A. niger* exhibited no regular trend regardless to the incubation. However, both Ca and K were retarded particularly after 7 days

of incubation, after which Ca recorded a significant increase. On the contrast, the values of P, Mg, Fe and Na were stimulated in comparable to the control irrespective to the incubation period (Table 2). Nearly similar results were reported by [12] concerning the chemical alterations of squash fruits infected by *A. niger*. They reported that *A. niger* exerted a pronounced ability to consume the entire carbohydrate and protein contents of squash fruits comparable to the other experimented fungal species belonging to *Aspergillus*, *Fusarium* and *Rhizopus* irrespective to the incubation conditions. It is concluded that this ability of *A. niger* is probable due to its high enzymatic activity. Inconsistence results were recorded by [13] since he reported that *A. niger* failed to utilize the carbohydrate, vitamin contents of summer squash fruits whereas it utilized some salts of P, Mg, amino acids (Methionine , Leucine ,Aspartic acid , Arginine) after 10 days of incubation.

Table 1. Effect of *Alternaria alternata* on the values of some nutritional contents of tomato fruits using different incubation periods at $24 \pm 2C^0$

		Incubation periods		
Nutritional content	**Control**	**7 days**	**10 days**	**13 days**
Carbohydrates (mg/kg)	34737.476	***8531.454	**15521.096	***108.434
Protein(mg/kg)	1981.395	**3132.540	*1865.130	*1322.100
Vitamin Cg/ 100g	13.2	**5.02	**2.64	**5.02
Potassium (mg/kg)	2273.214	*2505.878	*2715.270	*2293.235
Phosphors (mg/kg)	86.8841	*64.9942	**165.872	**157.517
Calcium (mg/kg)	45.667	*76.373	*77.768	**274.050
Magnesium(mg/kg)	872.362	**278.065	**425.018	***68.44799
Iron (mg/kg)	4.603	*10.070	*8.153	*9.145
Sodium (mg/kg)	153.514	*113.696	*124.359	*116.099

(*).simple significant difference. (**) Moderate significant difference. (***) Highly significant difference.

Table 2. Effect of *Aspergillus niger* on the values of some nutritional contents of squash fruits using different incubation periods at $24 \pm 2C^0$

		Incubation periods		
Nutritional content	**Control**	**7 days**	**10 days**	**13 days**
Carbohydrates (mg/kg)	31652.310	***11311.48	***16823.189	24728.134**
Protein (mg/kg)	3625.560	2983.740**	**1665.120	***8581.500
Vitamin Cg/ 100g	2.64	0.528*	0.528*	0.528*
Potassium (mg/kg)	3218.610	2126.168**	***432.25	*3855.136
Phosphors (mg/kg)	331.2415	*383.0661	*304.868	572.3791**
Calcium (mg/kg)	106.398	50.373*	87.806*	***1775.921
Magnesium (mg/kg)	200.00	503.8558**	*180.00	***64.26204
Iron (mg/kg)	0.686	7.526***	1.530*	3.341**
Sodium (mg/kg)	62.930	78.406*	***15.94	**95.191

(*).simple significant difference.(**)Moderate significant difference.(***) Highly significant difference.

Table 3. Effect of *Aspergillus flavus* on the values of some nutritional contents of corn grains using different incubation periods at $24 \pm 2C^0$

		Incubation periods		
Nutritional content	**Control**	**7 days**	**10 days**	**13 days**
Carbohydrates (mg/kg)	29119.047	***75130.249	***65753.123	***8759.549
Protein (mg/kg)	3227.880	**3839.520	***1709.280	***4720.800
Vitamin Cg/ 100g	3.96	***12.94	***27.21	***42.79
Potassium (mg/kg)	2003.550	***1739.191	***1789.341	**2596.145
Phosphors (mg/kg)	409.9526	**207.3549	**629.1853	***9505.8494
Calcium (mg/kg)	111.886	***1068.876	***1869.597	***1902.653
Magnesium (mg/kg)	384.40974	***1736.2805	***8543.9538	***4482,8439
Iron (mg/kg)	1.440	*5.153	*6.082	**9.341
Sodium (mg/kg)	80.992	*75.299	**50.322	80.189

(*).simple significant difference. (**) Moderate significant difference. (***) Highly significant difference.

A. flavus showed a significant induction of carbohydrate contents of corn grain after 7 days of incubation.The values of protein content showed no regular trend since they induced after 7 days of incubation, after which (10 days) recorded a conspicuous reduction, and increased after 13days of incubation.

The values of Vitamin C of corn grains as affected by *A. flavus* increased regardless to the incubation period. The values of monitored elements showed a pronounced induction with the prolongation of incubation period constituting the highest values at 13 days of incubation (Table 3).

4. References

[1] Christensen, S. D. W. and Lopez, A. U. Y. Economic Botany. The Binder third ,The World Association - Occurrence in some nutritive products and commercial products. Journal of Food Control. (New-york) : N: 187-V: 576-580 (1963).

[2] Singh D, And Sharma. R. R. Post-harvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), Sustainable Pest Management. Daya Publishing House, New Delhi, India (2007).

[3] Li-Cohen, A. E. And Bruhn, C. M. Safety of Consumer Handling of Fresh Produce. *Journal of Food Production*, 65(8): 1287-1296 (2002).

[4] Chulze, S. M. Etcheverry, A. I. Torres, M. L. Ravires, and Magan, N. D. Aflatoxin many in G. J. march and A. D marinelli Editors enfermedades del en manien argentina biblia impresores cordoba. Argantina V:103.P:103-113 (2005).

[5] Jones, M. A. Drong, A. T. and Ewana, S. A. The food problems - Article Published by Academic Journal and the Organization of Foods (London),V: (403-415) and (132-142) (2002).

[6] Perlman, A. N. Y. The Most Important Fungi The Infect With Potato And Tomato Fruits. Food Microbiology. 2nd edition. MC Grow Hill look Company - Brenistoon University «U.S.A». V:35.P:876113 (1975).

[7] Gaber, M. N. and Borane, C. M. Study Of Diseases of Apricot Fruits Caused by Fungi Species and evaluation of Apricot kernel . *lycopersici*. Phytopathology University of Florida .and Journal of Environmental Science and Technology ,N65 - pp:3465:880-886 (2002).

[8] Safwan, A. A. I. Uncovering characters of the fungi isolated from some tomato fruits by biological technique , studying spreader by Journal Science and Technological, Assiut University- the Sciences College, Num: 34 V: 465-478 (2000).

[9] Derar, S. V. Mossan, D. F. and Alveranop, M. L. History of the cultivar- groups of Cucurbita pepo, in Horticultural Reviews and different greenhouse cucumber cultivars (*Cucumis sativus* L.) in Southern Iran. African Journal of Biotechnology Vol. 8 (17), pp. 4077-4083 (1997).

[10] Joney, S. W. A. Contamination Of Maize With Fungi And Effect Aflatoxin production by fungus *Aspergillus flavus* , Journal of Environmental Science and Technology Vol. 21 pp. 567-583 (2004).

[11] Belal. Bashir, Hasan. Isolation and Identification of the fungi associated with corn grains cultivated in Khrtoum\Sudan, and study the physiological effect caused by some species of *Aspergillus*. Khartoum Agriculture Research Center. 14th edition UQ.P.90-101.(1991).

[12] Marie, A. K. Moualla, M. Y. and Boras M.G. Study of the Most Important Morphological and Productivity Characters of the Inbreed Lines of Squash *Cucurbita Pepo* L. Demashk University of Agricultural Sciences V:27 p 337-3350 - N12004 (2011).

[13] Iperng, W. F. S. Analytical Chemistry of Foods and plant (*Cucurbita Pepo*) Blackie Academic & Professional London, NY, Tokyo, Melbourne. The food problems Published by Academic Press(London) , Vol: 324-401(1983).