Detection of The Presence of Aflatoxin, Cyclopiazonic Acid and Other Alkaloids in Raisin Juice in Mosul City and its Suburbs

May Akram Krmo[1], Maha Akram Mohammad Ali Al_Rejaboo[2]
[1]*Nineveh Education Directorate, Mosul-Iraq. Email: 1mayakram9@gmail.com*
[2]*Department of Biology, College of Science, University of Mosul, Mosul-Iraq*

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ABSTRACT
28 samples of fresh raisin juice were collected randomly from the city of Mosul, the right and left sides, and its suburbs. The samples were collected from the areas of Bashiqa, Hamdaniya, Bartella, Aqra, and Dohuk. The presence of Aflatoxine by the ELISA method and Cyclopiazonic acid (CPA) and other alkaloids by the modified Ehrlich reagent method, where there was a variation in the extent of contamination of the samples with Aflatoxine, ranging from 0.0235 ng/L, the highest concentration in the R1 sample, the sample was from the Bashiqa region, and the source of the raisins was from Mosul herbalists, to the lowest concentration 0.002 ng/L in the two R31 samples. This sample was collected from Mosul, left side, Al-Majmoua Al-Thaqafiya neighborhood, the sample R37 is also from Mosul, left side, Al-Masarif neighborhood. As for the other samples, the concentrations ranged from (0.007, 0.005, 0.013, 0.0135, 0.009, 0.0075, 0.01, and 0.0205, 0.012, 0.003, 0.005, 0.008, 0.017, and 0.002) ng/L. As for Cyclopiazonic acid (CPA), there were samples contaminated with this poison, and there were samples with a small percentage of contamination, as the highest concentration was in samples R2, R3, R5, R7, R8, R9, R12, R13, R15, R19, R20, R34, R35, R37, and R38. The lowest concentration is in samples R1, R6, R17, R22, R23, R39, and R40. There are samples that contain other alkaloids, as in samples R16, R21, R24, and R30. All of these samples were the source of raisins. It is either from Attarin, Mosul or northern Iraq.

1. Introduction

Mycotoxin is a toxic compounds resulting from secondary metabolism by filamentous fungi found in foods (Greeff-Laubscher *et al*., 2019). It is the most widespread fungus in the world and produces toxins Aspergillus, Fusarium, Penicillum (2017, Pitt and David Miller). He observed more than 400 toxic compounds produced by more than 100 species of fungi (Jard *et al*., 2011). These toxins are transmitted to humans and animals directly through consumption. Foods contaminated with them are indirectly transmitted through eating animal products contaminated with these toxins (Gacem *et al*., 2020), which affects human and animal health, as large doses lead to poisoning, while exposure to small doses leads to the emergence of chronic symptoms such as liver damage, immune deficiency, and mutations (Amuzie *et al*., 2015). One of the most common mycotoxins in fruit juices and beverages is Aflatoxins (AFs), Ochratoxin A (OTA), Patulin (PAT), Fumonisin (FB), Trichothecenes (TCs) and Zearalenone (ZEN) are mainly produced by the genera Fuzuriam, Penicillum and Aspergillus and Alternaria toxin is produced by the genus Alternaria (WHO, 2017). It is one of the most common and most dangerous toxins in food contamination in the world Aflatoxine, which causes poisoning, mutagenicity, and carcinogenesis in humans and animals, is produced by species of the genus (Aspergillus), which includes A.flavus. A.parasiticush, and A.niger, which are found in various foodstuffs, as well as their products in juices, dried fruits, grains, and other crops (Caceres *et al*., 2020), and the contamination of juices with mycotoxins increases during the process transportation, drying, and storage until use as the period of time exposed to contamination increases. Zohri and Abdel-Gawad (1993) revealed the presence of mycotoxins{Aflatoxins (AFs), Ochratoxin A (OTA), Patulin (PAT), Fumonisin (FB), Trichothecenes (TCs), Zearalenone (ZEN) in raisins, all samples were free of these toxins. There are other toxins that contaminate foods and drinks, such as: Cyclopiazonic acid (CPA) and Indol-tetramic and other alkaloids that have a significant impact on public health, as they inhibit enzyme function ATPase located in the network Endoplasmic and Sarcoplasmic is an active crystalline compound without color and odor, it is soluble in methanol, chloroform, acetonitrol, dichloro, methane, and sodium bicarbonate with an approximate amount of 20 mg/ml. It is also soluble in dimethyl sulfoxide (DMSO) but it is insoluble in water, it may be found in juices in general and especially dried fruits and acid. It affects many foods, cheeses and food products. (Seidler *et al*.,1989 and Ostry *et al*., 2018). There are other alkaloids such as Ergot product

by species of the genus and *Clavicepes sp. and Penicillium sp* especially *P.cyclopium* which leads to poisoning and death when ingested. Symptoms appear gradually and lead to death, such as cramps, weight loss, dehydration, and loss of the ability to move (Bullerman and Bianchini, 2014).

2. Methodology

Detection of afla toxin by ELISA method

28 samples were collected from different areas of the city of Mosul, the right and left sides, and its suburbs (Bashiqa, Bartella, and Hamdaniya), they were stored at a temperature of -20 degrees celsius using the ELISA technique via Total Aflatoxin ELISA Test Kit From a company Shenzhen Lvshiyuan Biotechnology Co. Ltd

Extraction of poison from samples Fresh daily raisin juice:

The solutions were prepared in the following manner, according to the kit

Preparation of solutions:

Take 5ml of each raisin juice sample in a 50ml centrifuge tube and added to it 20ml of solution Methanol 70% which consists of methanol(V): redissolving solution V = 3:7

It was shaken for 3 minutes and then inserted into the centrifuge at 4000 revolutions per minute for 10 minutes at room temperature. take100 ul supernatant solution and add 700 ul of sample redissolving solution {wash buffer: wash buffer diluted 20x with deionized water, wash buffer 20x(v): redissolving solution (V) 1:19 then stir to mix the sample completely take 50ul of each sample for ELISA testing as shown in the kit detectionand other alkaloid Cycloiazonic acid (CPA) using the modified method of the Ehrlich reagent

Detection of Cycloiazonic acid (CPA) and other alkaloids using modified Ehrlich reagent method:

Ehrlich reagent was adopted as a principle for detection of Cyclopizonic acid (CPA) and other alkaloids by utilizing the extract of the poisons used in the previous method. 100 ml of the extract was taken and placed on Filter papers whatman No.1. A , drop of Ehrlich reagent was added to it and The results were recorded based on the time period in which the violet aura appeared, either in a short period of time, i.e. after (2-6 minutes) its clear appearance with a strong reaction, or in a long period of time, i.e. between (7-10 minutes), with a weak reaction. (Samson et al., 2004)

3. Results and Discussion

28 samples were collected from different areas of Mosul city, the right and left sides, and the areas of Baashiqa, Bartella, Hamdaniya, Aqra district, and Dohuk governorate. The samples from Baashiqa area were sourced from the herbalists of Mosul, while the samples from Hamdaniya and Bartella were from Al-Faisaliah and northern Iraq. The samples from Aqra district were also sourced from northern Iraq. As for Dohuk governorate, the source of the raisins was from Kurdish villages. As for the city of Mosul on both sides, the source of the manufactured raisins was from Aqra district and northern Iraq and from the herbalists of Mosul. They were stored at a temperature of -20 degrees Celsius using the ELISA technique, as Figure (1) adopted it in determining the level of Aflatoxin concentration by projecting the optical density of the samples into the standard curve to obtain the concentration of the toxin in ng/L.

Figure (1) Standard curve for a testAflatoxin using the ELISA device

Since the maximum internationally permissible limit found in juices is 2 ug/kg EC (European Commission, 2006) which is equivalent to 0.002 ng/L, when comparing the samples with the permissible limit, we notice (26) samples in which the concentration of afla exceeded the permissible limit, and this is not suitable for human or animal consumption. Two samples of the samples R31 and R34 were within the permissible limits.

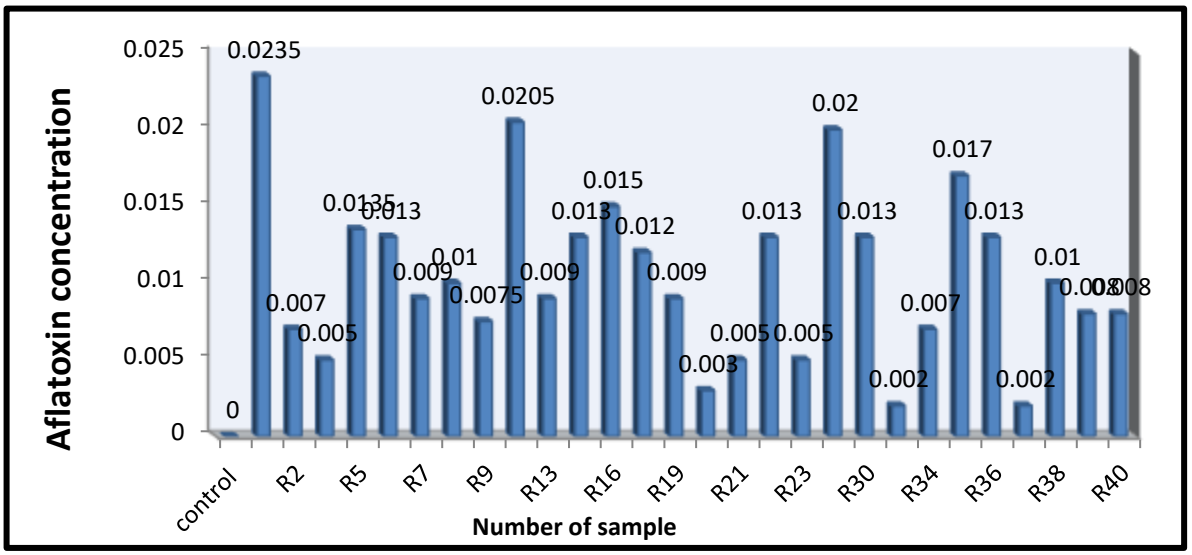

Figure (2) Detection resultsAflatoxin for raisin juice samples using ELISA technique

From Figure (2), there was a variation in the extent of contamination of samples with Aflatoxin, which ranged from 0.0235 ng/L. The highest concentration was in sample R1, this sample was collected from the Bashiqa area, and the source of raisins was from the herbalists of Mosul, where the shop was small and the juice was made daily, to the lowest concentration of 0.002 ng/L. The two barns R31, taken from the left side of the City of Mosul, from the Thaqafi group neighborhood, the source of raisins was from herbalists of Mosul and R37 from the AL-Masarif district. The source of raisins was from northern Iraq, where the two stores were larger, more expansive, and cleaner. The juice was fresh and made daily, and the rest of the samples were in the middle. The afla concentration was 0.007 ng/L for the R2 sample from Bashiqa area. The source of raisins was from Mosul soft drinks, ready-made juice was added to the juice. An amount of mint was added at a concentration of 0.005 ng/L for the R3 sample. The sample was collected from Sheikhan district and at a concentration of 0.0135 ng/L. Sample R5 was collected from Bashiqa with a concentration of 0.013 ng/L. Sample R6 was taken from the University of Mosul and the raisins were sourced from northern

Iraq and Baghdad with a concentration of 0.009 ng/L. Sample R7 was from the city of Dohuk, with a concentration of 0.01 ng/ L. Sample R8 it was collected from the Thaqafi group neighborhood with a concentration of 0.007 ng/L. Sample R9 was collected from the city of Mosul on the left side, originating from Aqra and Baghdad district, and with a concentration of 0.0205 ng/L. Sample R12 was collected from Al-Hamdaniyah, and the source of the raisins was from northern Iraq, with a concentration of 0.009 ng/L, for sample R13 was taken from Sheikhan district, with a concentration of 0.013 ng/L. R15 from the city of Mosul on the right side, and with a concentration of 0.015 ng/L. Sample R16 was collected from the right side of the city of Mosul, with a concentration of 0.009 ng/L. Sample R19 it was collected from the Thaqafi group neighborhood, and the source of raisins was from Aqrah district, with a concentration of 0.003 ng/L. Sample R20 is from the left side of the city of Mosul, Nabi Yunis neighborhood, with a concentration of 0.005 ng/L, for this R21 sample was collected from the right side of Mosul, from the Dawasa neighborhood, and the source of the raisins was from Aqra district, with a concentration of 0.013 ng/L. Sample R22 was collected from Mosul-left side of the Thaqafi group neighborhood, with a concentration of 0.005 ng/L. Sample R23 It was collected from Dohuk. The source of the raisins was from Northern Iraq, from the village of Barwari, with a concentration of 0.02 ng/L. sample R24 was collected from Dohuk, and the source of raisins was in Aqra district, with a concentration of 0.013 ng/L. For sample R30, this sample was collected from the University of Mosul, and the source of raisins was from Dohuk, with a concentration of 0.007 ng/L. R34 sample was taken from the right city of Mosul, Al-Zuhur neighborhood, also sourced from Aqra district, with a concentration of 0.017 ng/L. Sample R35 was also collected from the city of Mosul, left side, Al-Muthanna neighborhood, the source of raisins was from Aqra district, with a concentration of 0.0013 ng/L. Sample R36 taken from Mosul city, left side, the source of raisins is from Aqra district, with a concentration of 0.01 ng/L. Sample R38 was collected from the city of Mosul, the left side of Al-Zuhur neighborhood, and with a concentration of 0.008 ng/L. R39 was collected from the city of Mosul, from the left side of Darazliyya, and with a concentration of 0.008 ng/L. Sample R40, which was collected from Mosul city, North Garage. The percentage of contamination with poison in these samples was close, and there were samples with equal concentrations, and the samples contained mint, which is added to it when preparing the juice. All the mentioned percentages were not within the international permissible limits for aflatoxin present in juices. The percentage of contamination with mycotoxins was high, but there were only two samples within the permissible limits, which were R31 and R37. The percentage of contamination with aflatoxin was lower.

From the above, we note that the contamination rates in raisin juice were high compared to the internationally permissible levels 2ug/kg They found mushrooms Aspergillus niger is one of the most contaminated fungi in these samples, as they appeared in 17 samples, namely R2, R3, R5, R7, R12, R21, R20, R22, R30, R31, R35, R38, R36, R34, R37, R39, and R40. These fungi showed the ability to produce Aflatoxine, Cycloiazonic acid (CPA), and other alkaloids.

The extent of contamination of fungal growth with mycotoxins depends on internal factors such as temperature and pH. There are other factors: the shape of the grapes, the grape variety, the climate at the time of harvest and storage, as well as the period of drying the grape fruit and the manufacturing periods of the raisin juice called grape juice. All factors lead to contamination with mycotoxins. The occurrence of contamination in Foods depends on the type of product and the fungal species contaminating the foods (Milani *et al*., 2023; Butteryc,2010; Wang et al.,2017; Drusch and Ragab, 2003). The scientist (Han *et al*, 2016) reported that the average concentration of Aflatoxin in dried raisins was in small quantities of 0.8 ug/kg out of 32 raisin samples that were detected to be contaminated with the toxin. As for (Youssef *et al*., 2000), the result was that one or two samples of dried raisins were contaminated with the toxin isolated out of 100 samples. From Table (1) below it appears that most of the samples under study are contaminated with CPA, depending on the duration and intensity of the appearance of the violet aura, as well as the presence of contamination with other alkaloids. Through the appearance of a red-brown or yellow aura, the sample containing CPA in high concentration, R34, R35, R36, R37 and R38 R2, R3, R5, R7, R8, R9, R12, R13, R15, R19, R20, and

samples containing CPA in lower concentration R1, R22, R23, R17, R39, and other alkaloids. As in samples R24, R30, R16, and R21, this contamination results from the secretion of fungi contaminated with raisins and juice (Frisvard and Samson, 2004).

Table (1): Cyclopoizonic acid and related alkaloid

No. Raisin juice samples	Violet(color)	Concentration
R1	Violet	++
R2	Violet	++++
R3	Violet	++++
R5	Violet	++++
R6	Violet	++
R7	Violet	++++
R8	Violet	++++
R9	Violet	++++
R12	Violet	++++
R13	Violet	++++
R15	Violet	++++
R16	Violet(other alkaloid)	++
R17	Violet	++
R19	Violet	++++
R20	Violet	++++
R21	Red brown	++
R22	Violet	++
R23	Violet	++
R24	Yellow	++
R30	Yellow	++
R31	Violet	++
R34	Violet	++++
R35	Violet	++++
R36	Violet	++++
R37	Violet	++++
R38	Violet	++++
R39	Faintly violet	++
R40	Faintly violet	++

Violet CPA (after 7-10 min weak) +++ or ++, (after 2-6 min strong) ++++

The sample R2 was taken from the Bashiqa area, and the source of raisins, taken from the Mosul herbalists, were more contaminated than the rest of the samples, despite the shop's large size and cleanliness. The reason for the contamination may be the storage and manufacturing conditions until use. As for sample R40, it was less contaminated, coming from the left side of the city of Mosul, despite the shop's smallness and crowding. The study by (Felsociova *et al*., 2020) showed the contamination of grapes and their juice with Cyclopiazonic acid (CPA) in the city of Vrboski, Slovakia, resulting from their contamination with species of the genus Aspergillus and Penicillium. *A.niger* was one of the most contaminated fungi of grapes, as the contamination rate was 97%, and it is responsible for CPA in grape juice, and this percentage is consistent with the results we obtained were that most of the samples were contaminated with this fungus and the presence of toxins such as afla, ochre, and other alkaloids in the studied samples. Prove (Felsociova *et al*., 2020) that the percentage of *A.niger* production of CPA in raisins and their juice is 79%, and this agrees with our study that the reason for the presence of CPA in the samples is due to their contamination with *A.niger*. While it was found (Chalyy *et al*., 2021) reported that the percentage of Penicillum produced

was 19% CPA in samples of raisins or dried grapes from popular markets in Russia. The researcher (Prencipe et al., 2018) stated that the fungus *P.expansum* is responsible for the production of CPA in most foodstuffs.

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