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Evaluation of The Effects of Jet Plasma on The Production of Ochratoxin and Aflatoxin in Strains of Aspergillus Niger

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ABSTRACT

Today, phytopathogenic fungi are economically important all around the world because of their impact on a large number of crops and polluting most agricultural crops. Phytopathogen fungi often cause the reduction in the amount, yield and quality of agricultural products through producing mycotoxin. In this regard, aflatoxin and Ochratoxin produced by various species of aspergillosis of the Niger section can infect a variety of foods such as wheat, flour, barley, rice and corn. The cold atmospheric pressure plasma method has a potential for removing mycotoxidization. In this study, in order to increase the effect of plasma jet to remove mycotoxidization of human and animal food stuffs, the operating parameters of the air-pressure plasma method were optimized at low temperatures. The results indicated that the cold atmospheric pressure plasma method effectively reduces pure mycotoxins. It was also indicated in this study that the presence of a combination of mycotoxins in foods would reduce the plasma's effect, but would not prevent their degradation. Finally, the study found that removing aflatoxin B1 and Ochratoxin was conducted successfully in all samples and this indicates the high efficiency of the cold atmospheric plasma jet in this area, especially in the food industry.

Keywords: *mycotoxin, aflatoxin, Ochratoxin, aspergillus, cold atmospheric pressure plasma, mycoxidisation removal*

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INTRODUCTION

We are at the beginning of a century with new energies and new therapies. Increasing efficiency and reducing the cost of energy production and technology, including medical and non-medical, are the subjects of our time. Hence, researchers have always sought to use new methods to eliminate food microorganisms to replace existing heat treatments. Therefore, any method that can degrade microorganisms without increasing the temperature and not reducing the nutritional value is important. Today, plasma science is a new way of overcoming the problems encountered by researchers in these areas. The unique properties of plasma make it widely available in industry and medicine. Classification regarding the pressure, has divided plasma into two general classes of low pressure plasma and atmospheric pressure plasma. Currently, more attention is paid to atmospheric plasma due to its simplicity and low cost compared to low pressure plasma. Atmospheric electric discharges in recent years due to advantages such as cost-effectiveness of the test, performance without vacuum and the possibility of access and sample processing without limitations in size and high reactivity has been extensively investigated. Therefore, the use of cold atmospheric plasma jet is a new technology that has been considered in recent years due to its high potential for the destruction of microorganisms and its low cost as a non-thermal method. The cold atmospheric plasma jet contains electrons, ions, atoms and excited molecules, free radicals and radiation. The inactivation mechanism of microorganisms and the role of each inactivation factor are still under consideration. So far, efforts have been made to use different types of plasma to eliminate existing microorganisms in food. However, so far no research has been carried out in Iran on the use of atmospheric plasma jet in destroying mycotoxins. Therefore, the effect of cold atmospheric plasma on the reduction of mycotoxins produced by *Aspergillus* isolated from 5 types of different food sources (wheat, barley, corn, rice and flour) in 3 different times 30, 60 and 360 seconds was investigated. In 2016, Mortazavi et al. reported that the distribution of electrons, ions, and free radicals of oxygen in the oxygen plasma and its microbicide effect on E. coli have been studied at the level of the medical polytetrafluoroethylene, and the number of destroyed proteins and fat peroxides produced in bacterial suspension and DNA state were also investigated. They said that after sterilization in order to inactivate the bacteria, the analysis of the mechanism and its results indicated that the concentration of electrons and ions significantly decreased with the increase in distance from the induction coil center, whereas the concentration of oxygen radicals decreased much more slowly. In 2013, Hayashi et al. carried out sterilization with plasma jet. They investigated the combining of oxygen-helium and argon-

oxygen gases and found that the combination of argon and oxygen gas in 40 seconds could destroy Bacillus thorouginsis bacteria, if helium and oxygen gas in 180 seconds could reduce the number of bacteria. They also examined the effect of temperature on sterilization. They performed the previous experiment with helium and argon gas at temperatures of 55 ° C and 110 ° C, and found that the higher the degree of sterilization was the higher the temperature. Frohling et al. reported in 2012 that atmospheric pressure plasma can facilitate microbial degradation, while taking into account its low-temperature performance, it does not reduce the quality of the product, indirect treatment with plasma, new opacities Which aims to study the effects of indirect treatment with plasma on quality (color, pH, fluorescence, and reflectivity) and the health (live aerobic number) in pork. Their study showed that the potential of indirect plasma treatment for fresh pork is high, but changes in quality should also be taken into account and the advanced plasma process should be achieved to achieve a dramatic reduction in microbial load without affecting quality and guaranteeing the acceptance of the product by Consumer designed. Tian et al. (2014) reported that they used an atypical DC microjet plasma to deactivate Staphylococcus aureus and Enterococcus faecalis in the air. Their conclusion was that the destruction of bacteria is related to the abundance of active oxygen species and nitrogen and ultraviolet radiation in plasma, and the lifespan of these lethal materials and their resistance may be the codification of different inactivation curves at different treatment intervals.

2. MATERIALS AND METHODS

In this study, a non-thermal atmospheric plasma (99% argon and 1% air with an air flow rate of 6 liters per minute) containing single jet with high sinus voltage at a frequency of 30 KHz was used for the treatment of samples).

2.1 General stages of the project
- sampling
- Initial tests (identification)
- Disease and Disease Testing
- Cultivating and determining the amount of poison production through ELISA
- Performing plasma irradiation and measuring the amount of aflatoxin removal
- Review of raw data and analysis of statistical data

2.2. Materials and the medium of used cultures
In this research, the Czapek Dox Agar Medium was used for the enrichment, isolation and production of mycotoxin of *Aspergillus*. Also, the 250 ml Sabro Dextrose Broth media + 7.5 ml malt extract, and the 250 ml Sabro Dextrose Broth + 7.5 ml yeast extract (To enrich and increase the production of mycotoxins through the collected samples) were used. All culture and chemical medium used to isolate and produce mycotoxins are from the Mercury Corporation in Germany. (The cultivation medium was prepared according to the instruction on the cans) (Cobans et al. 2010) and (Cecilano et al. 2016) and (Dui et al. 2017).

2-3-Sampling
In this research, samples from the northern regions of Iran included wheat, corn, oats, flour and rice. These samples were collected by Dr. Arash Chaychi Nosrati and put into the study. Samples were transferred to the laboratory immediately after collection, and were stored at 4 °C in the refrigerator to determine the levels of aflatoxin B1 and Ochratoxin A, as well as removing mycotoxin using plasma.

2.4 Preparation and cultivation of microorganisms
Pinch cultivation of samples in Czapek Dox Agar Medium (cultivation in solid medium)
In order to isolate Aflatoxin B1 and Ochratoxin A productive fungus, the collected samples were cultivated in a Czapek Dox Agar medium in a pinch cultivar (at 3 points in a distance of 2 cm apart and at the edge of the plate). Then each plate was transferred to the oven at 25 ° C for 14 days. The plates were examined during the incubation (every 5 days from the 14-day incubation period). Identification of isolated fungi according to the morphological and macroscopic characteristics of fungal colonies such as surface and back color of the colonies, the view of the colony surface in terms of crimps, radial lines or concentric circles, smooth or folded colony surface, and surface state of the colonies, such as having pearl, powder, cotton, velvet, and other modes, were conducted according the identification keys of Barnett and Hunter, Click and Dougan (Vine 2008) and (Barnett & Hunter 1998) and (Click 2002).
Culture in liquid media (Transfer of samples from the solid to liquid mediums)
In order to increase the production of mycotoxins at this stage, two mediums of Sabouraud Dextrose Broth + malt extract (SB + ME) and also Sabouraud Dextrose Broth + yeast extract (SB + YE) have been used in 10cc-Falcon tubes containing 5cc liquid medium with sterile glass shatter (In this study, crushed glass was used for extracting mycotoxins in order to speed up the process of cell failure and lysed cells). For this purpose, each of the pure and isolated samples was removed by sterile loop and transferred to the broth and held in a shaker oven at 25° c, 250 rpm round for 14 days. To prevent drying of liquid media during incubation (On day 5 of a 14-day incubation period), phosphate salt solution with Phosphate buffered saline (PBS) was added to each Falcon using 0.5cc pipette. (Sisialanoo et al. 2016) and (Devi and others 2017).

Preparation of Culture slides from selected samples:
CHAPK medium was used in slides agar culture preparation in this study. Using a sterile scalpel, part of the medium was cut in a 1cm×1cm square shape and was placed on a slide in conformity with sterile standards. After placing the agar perfectly in the center of the slide, it is horizontally placed on a "U" shape tube that is inside a large glass plate (Containing 10cc distilled water). Using sterile anise, the isolated samples were inoculated on 4 points of the medium on the slide (Fig. 1). The sterilized plates were placed on a piece of inoculated agar and kept in an incubator at 25° c for 14 days (James, 1986).

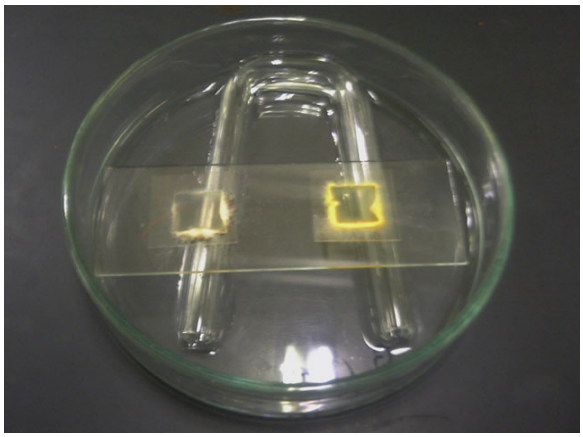

Figure 1: Slide Culture

2-5 Mycotoxins extraction from liquid and solid medium
Mycotoxins extraction from liquid mediums (SABOURAUD dextrose broth with malt and yeast extract)
After the incubation period (the fourteenth day), cell-free medium is required in order for extraction and purified mycotoxins produced by pure strains. Therefore, Falcon tubes containing liquid medium were vortexed for 15min at 35 degrees and were placed in a freezer at a temperature of -70° C. Then falcon tubes were dafrazed out of the freezer at the laboratory temperature. Then extraction solvent was added to 2.5 mL Falcon tubes and vortexed twice at an angle of 30-35 degrees for 15 minutes (the step was repeated twice to increase the extraction) that a total of 5 ml extraction solvent was used for each of the Falcon. In the final step, the resulting supernatant extraction of mycotoxins (after two vertices and adding solvent extraction) were separated by funnel and Whatman filter paper (5.0 micron) and were transferred into sterile microtubes (2 mL) for Elisa testing and kept at the temperature of -4° C. (Rahmani et al., 2009).

2-6- mycotoxins extraction from the solid medium (CHAPK agar)
Cell-free medium is required in order for extraction and purification of mycotoxins produced by net strain after the incubation period (the fourteenth day). For this purpose, CHAPK agar containing the colony was cut as 1× 3 cm using a sterile scalpel and inoculated in the sterile Falcon tubes (10 cc) containing sterilized cracked glass and 5 cc phosphate-buffered saline (PBS) and was vortexed for min15 angle of 35 degrees and placed in a freezer at a temperature of -70 ° C. Solvent extraction and filter paper were used in order to carry out the extraction procedure after removing the Falcon tubes from the freezer similar to the previous method (extraction of mycotoxins from liquid broth) (Rahmani et al., 2009).

2.7 Aflatoxin B1 and ochratoxin A analyses by ELISA
ELISA device was Biotech-ELX 800 made in America. ELISA assay kit and aflatoxin B1 and ochratoxin A standards were prepared from r-biopharm company in Germany. Extraction and test methods for mycotoxins in the sample were performed according to the manufacturer's instructions (Liszinska et al. 2001).

2-8: Clearing mycotoxins using Cold atmospheric plasma jet
A device was used to clean mycotoxins in this study to have the greatest impact of produced plasma jets on mycotoxins. The chamber within which the plasma was formed, was a helix (spiral) and the plasma jets had the maximum contact with crossing mycotoxins and performed the role of the plasma reactor. Glass tube length used in Helix manufacturing was 12 cm with an inner diameter of 2 mm and an outside diameter of 3 mm. The core diameter of spiral glass was 3 cm and step distance was 5.1 cm. The two ends of the created Helix transformed into a three-way to provide simultaneous entrance of gas and mycotoxins and can be separated in the exit. In tests, argon has been selected as the main gas to generate the plasma.

3- RESEARCH FINDINGS

3.1 The results of the aflatoxin B1 concentration obtained from the Lin / Log analysis charts in Sabouraud dextrose broth + malt extract (Aflatoxin B1 / ME + SB)
The average initial concentration of aflatoxin reaches from 16.106 micrograms per kilogram at 30, 60 and 360 seconds to 10.4, 8.32 and 7.55 micrograms per kilogram. (Chart 3)

3-2: The results of the aflatoxin B1 concentration obtained from the Lin / Log analysis charts in Sabouraud dextrose broth + intended extract (Aflatoxin B1 / YE + SB)

The average initial concentration of aflatoxin reached from 23.699 micrograms per kilogram at 30, 60 and 360 seconds to 12.82, 9.92 and 9.54 micrograms per kilogram. (Chart 3)

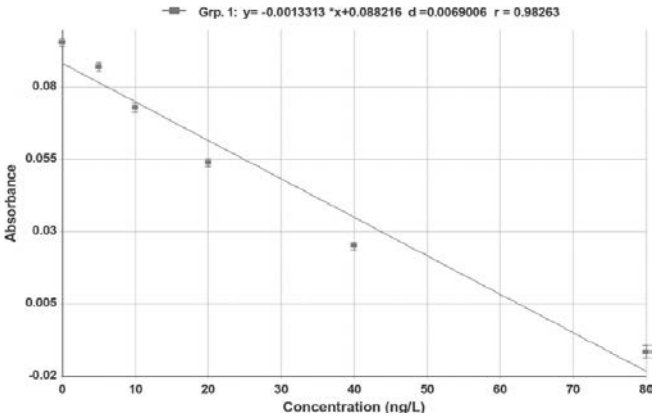

Diagram 1: Curve of Standard Aflatoxin B1

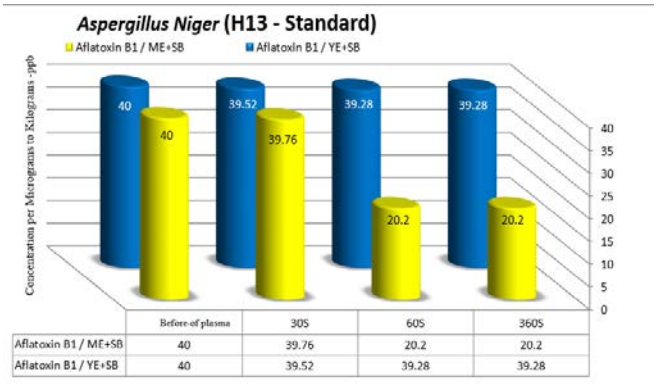

Diagram 2: Standard curve of aflatoxin in Standard *Aspergillus Niger*

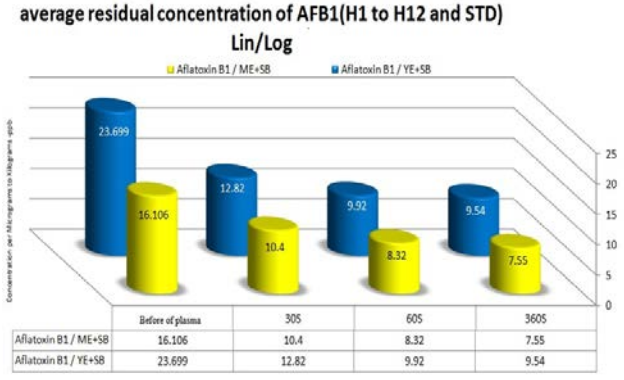

Diagram 3: Average concentration of aflatoxins before and after exposure to plasma

3.3 Determination of the concentration of aflatoxin B1 results obtained from Log / Lin table analysis in SABOURAUD dextrose broth + malt extract (Aflatoxin B1 / ME + SB)
Aflatoxin average initial concentration reduced from 20.02 micrograms per kg at 30, 60 and 360 seconds to the concentration of 6.87, 10.46 and 7.55 mg/kg, respectively (Table 1).

Table 1: Concentration of aflatoxin in Sabro Dextrose Broth media + Malt Extract

log/lig	sample Name	Before Treatment	After Treatment		
		Afla B_1 / Malt	Afla B_1/Malt/30s	Afla B_1/Malt/60s	Afla B_1/Malt/360s
H1	*Aspergillus Niger*	1.14	6.67	6.48	8.78
H2	*Aspergillus Niger*	50	6.78	7.84	7.42
H3	*Aspergillus Niger*	50	6.82	8.08	4.4
H4	*Aspergillus Niger*	0.41	6.2	5.32	6.44
H5	*Aspergillus Niger*	0.36	6.25	7.39	8.56
H6	*Aspergillus Carbonarius*	0.63	6.45	6.07	7.56
H7	*Aspergillus Carbonarius*	4.7	6.83	7.75	8.53
H8	*Aspergillus Carbonarius*	1.84	6.22	7.22	6.22
H9	*Aspergillus Foetidus*	50	6.32	7.92	6.14
H10	*Aspergillus Foetidus*	0.55	7.38	6.9	8.27
H11	*Aspergillus Foetidus*	50	7.73	7.99	7.17
H12	*Eurotium*	0.65	7.78	6.99	7.17
H13	*Aspergillus Niger* (Standard)	50	7.78	50	20.2
Mean		20.02	6.87	10.46	7.55

3.4. Results of evaluating the concentration of aflatoxin B1 obtained from the analysis of Log / Lin tables in Sabro Dextrose Broth media + Yeast Extract (Aflatoxin B1 / YE + SB) The mean initial concentration of aflatoxin in the medium has reduced from 28.35 µg / kg at 30, 60 and 360 seconds to 6.56, **8.43, and 7.02 µg / kg, respectively (Table 2).**

Table 2: Concentration of aflatoxin in Sabro Dextrose Broth media + Yeast Extract

log/lig	sample Name	Before Treatment	After Treatment		
		Afla B_1/Yeast	Afla B_1/Yeast/30s	Afla B_1/Yeast/60s	Afla B_1/Yeast/360s
H1	*Aspergillus Niger*	0.48	6.31	5.96	0.01
H2	*Aspergillus Niger*	50	6.73	6.69	0.02
H3	*Aspergillus Niger*	50	6.66	7.15	7.37
H4	*Aspergillus Niger*	1.97	5	7.53	5.38
H5	*Aspergillus Niger*	0.57	7.6	7.43	0.01
H6	*Aspergillus Carbonarius*	50	7.66	6.44	0.02
H7	*Aspergillus Carbonarius*	1.07	6.42	6.34	0
H8	*Aspergillus Carbonarius*	31.97	6.44	7.43	0.01
H9	*Aspergillus Foetidus*	0.52	5.9	7.73	0.02
H10	*Aspergillus Foetidus*	50	7.57	7.23	9.28
H11	*Aspergillus Foetidus*	50	6.99	7.96	11.58
H12	*Eurotium*	31.97	7.98	7.99	7.35
H13	*Aspergillus Niger* (Standard)	50.2	7.89	23.77	50.2
Mean		28.35	6.56	8.43	7.02

3.5. Results of evaluating the concentration of Ochratoxin A obtained from the analysis of Log / Lin tables in Sabro Dextrose Broth media + Malt Extract (Aflatoxin B1 / ME + SB) The mean initial concentration of Ochratoxin in the medium has reduced from 35.264 µg / kg at 60 and 360 seconds to 18.34 and 12.008 µg / kg, respectively (Diagram 4-30).

3.6. Results of evaluating the concentration of Ochratoxin A obtained from the analysis of Log / Lin tables in Sabro Dextrose Broth media + Yeast Extract (Aflatoxin B1 / YE + SB) The mean initial concentration of Ochratoxin in the medium has reduced from 28.625 µg / kg at 60 and 360 seconds to 19.486 and 14.607 µg / kg, respectively (Diagram 6).

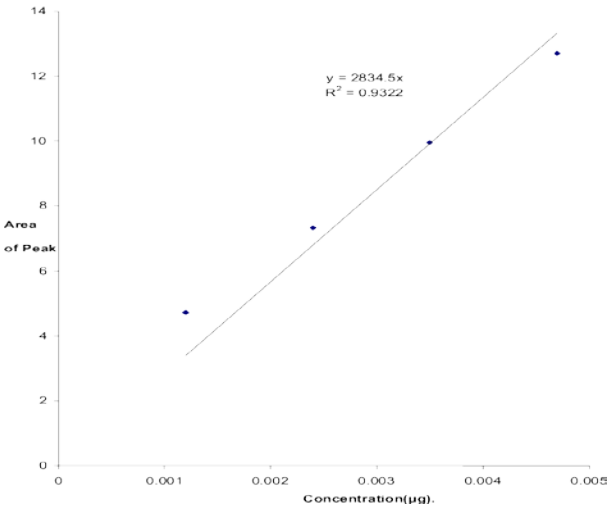

Diagram 4: Standard curve for ochratoxin A

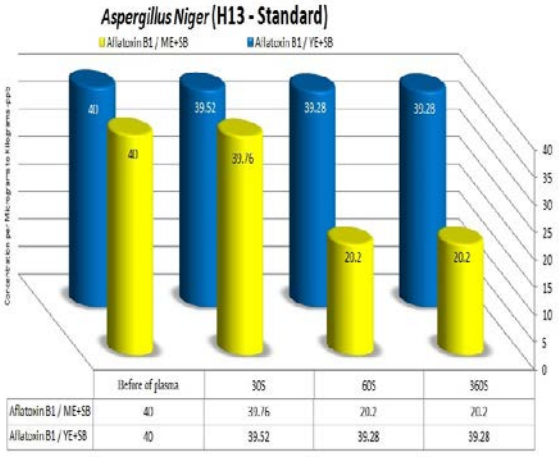

Diagram 5: Standard ochratoxin curve in standard *Aspergillus niger*

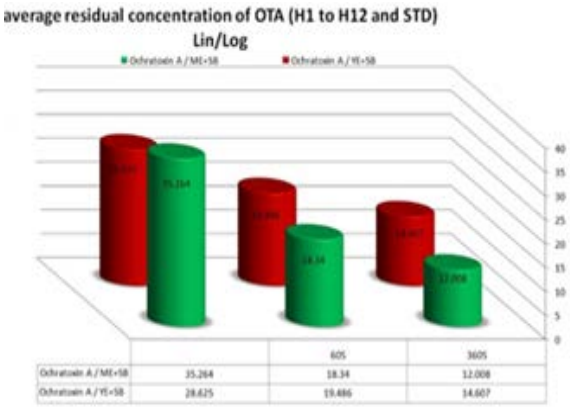

Diagram 6: Average concentration of Ochratoxin before and after plasma radiation

3.7. Results of evaluating the concentration of Ochratoxin A obtained from the analysis of Log / Lin tables in Sabro Dextrose Broth media + Malt Extract (Aflatoxin B1 / ME + SB) The mean initial concentration of Ochratoxin A in the medium has reduced from 43.22 µg / kg at 60 and 360 seconds to 26.38 and 4.82 µg / kg, respectively (Table 3).

Table 3: Concentration of Ochratoxin in Sabro Dextrose Broth media + Malt Extract

log/Lin	sample Name	Before Treatment	After Treatment	
		OTA/Malt	OTA/Malt/60s	OTA/Malt/360s
H1	*Aspergillus Niger*	7.44	50	0.1
H2	*Aspergillus Niger*	50	12.04	0.01
H3	*Aspergillus Niger*	26.93	6.62	7.68
H4	*Aspergillus Niger*	50	50	7.35
H5	*Aspergillus Niger*	50	0.02	4.64
H6	*Aspergillus Carbonarius*	27.51	23.74	17.46
H7	*Aspergillus Carbonarius*	50	0.01	0.1
H8	*Aspergillus Carbonarius*	50	25	0.02
H9	*Aspergillus Foetidus*	50	0.1	0.01
H10	*Aspergillus Foetidus*	50	50	13.51
H11	*Aspergillus Foetidus*	50	25	0.02
H12	*Eurotium*	50	50	9.69
H13	*Aspergillus Niger* (Standard)	50	50	0.01
Mean		43.22	26.38	4.82

3.8. Results of evaluating the concentration of Ochratoxin A obtained from the analysis of Log / Lin tables in Sabro Dextrose Broth media + Yeast Extract (Aflatoxin B1 / YE + SB) The mean initial concentration of Ochratoxin in the medium has reduced from 34.38 µg / kg at 60 and 360 seconds to 25.88 and 2.47µg / kg, respectively (Table 4).

Table 4: Concentration of Ochratoxin in Sabro Dextrose Broth media + Yeast Extract

log/Lin	sample Name	Before Treatment	After Treatment	
		OTA/Yeast	OTA/ Yeast /60s	OTA/Yeast/360s
H1	*Aspergillus Niger*	50	0.01	0.02
H2	*Aspergillus Niger*	19.33	11.5	0.01
H3	*Aspergillus Niger*	11.63	50	0
H4	*Aspergillus Niger*	50	50	6.58
H5	*Aspergillus Niger*	50	0.01	0
H6	*Aspergillus Carbonarius*	13.48	27.36	0.01
H7	*Aspergillus Carbonarius*	50	0.01	0.1
H8	*Aspergillus Carbonarius*	50	23	0.02
H9	*Aspergillus Foetidus*	50	0.01	0.1
H10	*Aspergillus Foetidus*	2.23	50	7.66
H11	*Aspergillus Foetidus*	0.26	25	0.02
H12	*Eurotium*	50	50	6.44
H13	*Aspergillus Niger* (Standard)	50	50	11.13
Mean		34.38	25.88	2.47

As can be seen from the Lin / Log graphs and the Log / Lin tables, removing toxin (aflatoxin B1 and Ochratoxin A) was investigated with the help of compression of argon gas for 30, 60 and 360 seconds, and the mean initial concentration of samples in two environments Sabro Dextrose Broth media + malt extract and Sabro Dextrose Broth media + yeast extract (before radiation) were compared with the mean remaining concentration after each plasma radiation. The results of this study indicated that aflatoxin B1 and Ochratoxin A have been successfully applied to all samples, indicating the high efficiency of the cold atmospheric plasma jet plasma, especially in the food industry. According to the above and the results obtained from the study, it can be concluded that non-thermal (cold) atmospheric plasma jet can cause the removal of fungi and mycotoxin. Due to the presence of charged particles of electrons and ions, ultraviolet radiation, free radicals and reactive chemical species, the plasma can cause changes in the cell wall, morphology, or genetic characteristics of microorganisms and cause them to die. As a result, cold plasma as a new technology in various fields of food and agriculture can provide effective and effective solutions to the goals of the food industry, especially in the areas of mycotoxin dehydration.

4. CONCLUSION

Examining the Pearson correlation coefficient (Lin/Log) in concentration of produced mycotoxins before radiation (Aflatoxin B1 and Ochratoxin A) in two environments (ME+SB) and (YE+SB).

In this section, statistical correlation of produced Aflatoxin B1 and Ochratoxin A by each of the separated levels (H1 to H12) in two environments (ME+SB) and (YE+SB) was examined and meaningful discrepancy was found between these two toxins, and that the average concentration of ochratoxin A was more than average concentration of Aflatoxin B1 (the results are shown in Table 5).

Table 5: the initial concentration of mycotoxins before plasma radiation (Lin/ Log)

Initial concentration before treatment (Microgram/kilogram)	Mycotoxin production in YE+SB/ ME+SB
16.106	Aflatoxin B1 / ME+SB
23.699	Aflatoxin B1 / YE+SB
35.264	Ochratoxin A / ME+SB
28.625	Ochratoxin A / YE+SB
PC → -0.686 sig → 0.007	

Examining the Pearson correlation (Lin/Log) in Aflatoxin B1 concentration in two environments (ME+SB) and (YE+SB).
The reduction of aflatoxin B1 in environment (ME+SB) after 30, 60 and 360 seconds treatment with jet plasma was more than its reduction in environment (YE+SB) and significant statistical alignment was seen (Table 6).

Table 6. Aflatoxin concentration before and after radiation (Lin/Log)

Aflatoxin B1 concentration	Concentration after 30 seconds of radiation (Microgram/kilogram)	Concentration after 60 seconds of radiation (Microgram/kilogram)	Concentration after 360 seconds ofradiation (Microgram/kilogram)
Aflatoxin B1 / ME+SB	10.4	8.32	7.55
Aflatoxin B1 / YE+SB	12.82	9.92	9.54
Correlation amount	pc→0.656 sig→0.011	pc→0.673 sig→0.008	pc→ 0.994 sig→ 0

Significant statistical correlation between the decrease in Aflatoxin B1 and increase of treatment time with jet plasma from 30 seconds to 60 seconds and also from 60 seconds to 360 seconds was seen in culture medium. In addition, the amount of changes of Aflatoxin B1 in culture medium (YE+SB) had significant statistical correlation with increase of plasma jet treatment time from 30 seconds to 60 seconds and from 60 seconds to 360 seconds. The reduction changes of aflatoxin B1 after 360 seconds of treatment with plasma jet has statistical alignment correlation. The analysis of results indicate that aflatoxin B1 concentration in two environments (ME+SB) and (YE+SB) reduces by the increase in plasma jet treatment time. Finally, according to table 2-5, in comparing the reduction rate and correlation changes of B1 toxin in two environments of (ME+SB) and (YE+SB) after 60 seconds of treatment with plasma jet, it was approved that the reduction was always greater in (ME+SB) than in (YE+SB). The variation domain (drop down domain) of Aflatoxin B1 in (ME+SB) and (YE+SB) continued till 360 seconds of treatment with plasma jet and had significant changes.
Examining Pearson correlation relation (Lin/Log) and Ochratoxin A concentration in two environments (ME+SB) and (YE+SB).
The reduction amount of Ochratoxin A in (ME+SB) after 60 and 360 seconds of treatment with plasma jet was more than its reduction in (YE+SB) and significant statistical alignment was also found (Table 7).

Table 7. Ochratoxin concentration before and after radiation (Lin/Log)

Ochratoxin A	Initial concentration (Microgram/ kilogram)	Concentration after 60 seconds of radiation (Microgram/k ilogram)	Concentration after 360 seconds of radiation (Microgram/kil ogram)
Ochratoxin A / ME+SB	35.264	18.34	12.008
Ochratoxin A / YE+SB	28.625	19.484	14.607

According to table 7, in examining Ochratoxin variation after treatment with plasma jet for 60 seconds and 360 seconds, despite the decrease in mean value of toxin, significant statistical alignment was not found in (ME+SB). In addition, significant correlation was not found in mean changes of ochratoxin, despite its reduction, after 60 seconds and 360 seconds treatment with plasma jet in (YE+SB). With 60 seconds of treatment in (ME+SB), ochratoxin amount was reduced meaningfully. With 360 seconds treatment, the Ochratoxin amount in c/y decreases meaningfully. The variation domain (drop down domain) of Ochratoxin A after 360 seconds of treatment with plasma jet in (ME+SB) and (YE+SB) had meaningful changes.
Comparing and examining the Pearson correlation relation (Lin/Log) in concentration rate and effect of plasma jet on Aflatoxin B1 and Ochratoxin A

Table 8: the concentration of Aflatoxin and Ochratoxin before and after plasma radiation (Lin/ Log)

Aflatoxin B1 and Ochratoxin A	Initial Concentration (Microgram/kilogram)	Concentration after 60 seconds of radiation (Microgram/kilogram)	Concentration after 360 seconds of radiation (Microgram/kilogram)
Aflatoxin B1 / ME+SB	16.106	8.32	7.55
Ochratoxin A / ME+SB	35.264	18.34	12.008

Significant and aligned correlation was seen in Ochratoxin A amount reduction and Aflatoxin B1 reduction in (ME+SB) after 360 seconds of treatment with plasma jet.
Examining Wilcoxon test (significance of numerical difference) (Lin/ Log) in concentration of produced mycotoxin before radiation (Aflatoxin and Ochratoxin A) in (ME+SB) and (YE+SB).
Examination of Wilcoxon test (significance of numerical difference), the variations in amount of produced toxin of Aflatoxin B1 and Ochratoxin A in (ME+SB) reveals that the reduction amount of Ochratoxin has been always greater than that of Aflatoxin. Therefore, it can be concluded that the mean numerical difference of Ochratoxin A is greater than Aflatoxin B and has been always under the reducing influence of plasma jet.
Examining Pearson correlation relation (Lin/ Log) to concentration of produced mycotoxin before radiation (Aflatoxin B1 and Ochratoxin A) in (ME+SB) and (YE+SB).

The statistical correlation of produced amount of Aflatoxin B1 and Ochratoxin A by each of the separated levels (H1 to H12) in (ME+SB) and (YE+SB) were investigated and it was found that there is a significant discrepancy between the two toxins. In a way that concentration amount of Ochratoxin A was more than mean level of Aflatoxin B1 concentration (according to the results shown in Table 9).

Table 9. Initial concentration of mycotoxins before plasma radiation (Log/Lin)

ME+SB / mycotoxin production in YE+SB	Initial concentration before treatment (Microgram/kilogram)
Aflatoxin B1 / ME+SB	20.02
Aflatoxin B1 / YE+SB	28.35
Ochratoxin A / ME+SB	43.22
Ochratoxin A / YE+SB	34.38

Examining Pearson correlation relation (Log/Lin) and Aflatoxin B1 concentration in (ME+SB) and (YE+SB).

Table 10. Aflatoxin concentration before and after radiation (Log/Lin)

Aflatoxin B1 concentration	Concentration after 30 seconds of radiation (Microgram/kilogram)	Concentration after 60 seconds of radiation (Microgram/kilogram)	Concentration after 360 seconds of radiation (Microgram/kilogram)
Aflatoxin B1 / ME+SB	6.87	10.46	7.55
Aflatoxin B1 / YE+SB	6.56	8.43	7.02

In examining Pearson correlation relation (Log/Lin), reduction amount variation of Aflatoxin B1 in (YE+SB) was more than (ME+SB) and after 30 seconds of treatment, significant statistical alignment was seen. This property is also seen in 60 seconds treatment and reduction amount of Aflatoxin B1 in (YE+SB) was more than (ME+SB). In examining Pearson correlation relation (Log/Lin), the variable domain (Drop down domain), in comparison to reduction amount of Aflatoxin B1 after 60 and 360 seconds of treatment with plasma jet in (YE+SB) and (ME+SB) showed statistical alignment correlation. In addition, according to the statistical findings, meaningful variations in Aflatoxin B1 amount was seen after 60 seconds of treatment with plasma jet in (YE+SB) and (ME+SB).

Examining Pearson correlation relation (Lin/Log) in Ochratoxin A concentration in (YE+SB) and (ME+SB)

According to table 11, in examining Pearson relation, Ochratoxin A reduction amount in (ME+SB) after 60 and 360 seconds of treatment with plasma jet was more than its reduction in (YE+SB) and significant statistical alignment was found.

Table 11. Ochratoxin concentration before and after radiation (Log/ Lin)

Ochratoxin A	Concentration after 60 seconds of radiation (Microgram/kilogram)	Concentration after 360 seconds of radiation (Microgram/kilogram)
Ochratoxin A / ME+SB	26.38	4.82
Ochratoxin A / YE+SB	25.88	2.47

Ochratoxin amount reduction in (YE+SB) after 60 seconds of treatment with plasma jet had also a significant alignment. In increase of treatment time with plasma jet to 60 seconds to reduce Ochratoxin A, toxin reduction in (ME+SB) was more than its reduction in (YE+SB) and this is statistically meaningful. This property was constantly seen in treatment with plasma jet from 60 seconds to 360 seconds and is statistically meaningful. The increase of treatment time of Ochratoxin A to 360 seconds in both environments also indicated discrepant statistically meaningful correlation in Ochratoxin A amount. This property in increase of treatment time from 60 seconds to 360 seconds in (ME+SB) and reduction of Ochratoxin A amount was also statistically meaningful and discrepant. Also these conditions are also repeated in (YE+SB) and statistically discrepant correlation between Ochratoxin A and its reduction after 60 seconds and after 360 seconds was found. Therefore, treatment with plasma jet reveals that regardless of the culture medium and its condition, the increase of treatment time with plasma jet will constantly follow the decrease in Ochratoxin A amount. Non-parametric examination of the obtained data from ELIZA test according to Lin/Log function manifests that the decrease in amount of produced Ochratoxin was observed significantly from the fungal isolates of the experiment. This event was also meaningful till the increase of plasma jet time to 360 seconds in (YE+SB).

Examining and comparing the Pearson correlation relation (Log/Lin) in amount of concentration and plasma effect on Aflatoxin B1 and Ochratoxin A

Table 12. Aflatoxin and Ochratoxin concentration before and after plasma radiation (Log/Lin)

Aflatoxin B1 and Ochratoxin A	Initial concentration (Microgram/kilogram)	Concentration after 60 seconds of radiation (Microgram/kilogram)	Concentration after 360 seconds of radiation (Microgram/kilogram)
Aflatoxin B1 / ME+SB	20.02	10.46	7.55
Aflatoxin B1 / YE+SB	28.35	8.43	7.02
Ochratoxin A / ME+SB	43.22	26.38	4.82
Ochratoxin A / YE+SB	34.38	25.88	2.47

In statistical examination of Aflatoxin B1 in (YE+SB) with ochratoxin amount, discrepant and meaningful correlation was found and ochratoxin A amount was more than Aflatoxin B1, and comparing the maximum reduction amount of Ochratoxin in (YE+SB) after 360 seconds, Aflatoxin B1 in the same environment after 360 seconds demonstrated an statistically

aligned and significant correlation. Statistical meaningful discrepancy in Aflatoxin B1 mean value and Ochratoxin A in (ME+SB) indicates statistically meaningful and discrepant correlation and also proves that ochratoxin A is more than Aflatoxin B1.

Considering the mentioned reasons, it can be concluded that Non-thermal (cold) atmospheric jet plasma can cause fungus and mycotoxin dehydration. Due to the presence of charged particles of electrons and ions, ultraviolet radiation, free radicals and reactive chemical species, the plasma can cause changes in the cell wall, morphology, or genetic characteristics of microorganisms and cause them to die. As a result of this, cold plasma as a new technology in various fields of food and agriculture can provide effective solutions to the goals of the food industry, especially in the areas of mycotoxin dehydration.

SUGGESTIONS

1. Mycotoxins should be considered as the main goal of programs that are used to enhance food safety.
2. The effects of cold atmospheric plasma jet should be used to study the removal of toxin of other food contaminating fungi, including penicilliums, fusarium and alternaria.
3. Optimization of the power and conditions of the device set-up (power of the machine, the time of plasma irradiation, frequency and potential difference, the rate of gas output in different times) as well as the medium of microorganisms in terms of (temperature, pH, speed of the shaker and use From other carbon substrates) to increase the efficiency of atmospheric plasma jet radiation in the shortest possible time as well as in the future research of other low-cost gases instead of argon to be economical in the industry, especially in the food industry.

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