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Evaluation of the plasma jet effects on the Citrinin and Ochratoxin A producing species of the genus *Penicillium*

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Mycotoxins are small toxic molecules produced by a variety of microorganisms. They are often made of several secondary metabolites and often have a variety of chemical functions and structures. Citrinin and Ochratoxin A produced by different species of *Penicillium* contaminate various types of foods. Today, the cold atmospheric pressure plasma method has potential for mycotoxin detoxification. In this study, cold atmospheric pressure plasma (CAPP) operating parameters were investigated and optimized to increase the effect on Citrinin and Ochratoxin A in wheat, oat, corn and rice. In this study, the output of power supply first was set to 50 kV, 100 watts and the electron frequency of 30 kHz (optimum conditions for stable and effective plasma) to perform the tests. Then, the effect of cold atmospheric plasma jet (argon) on the gas flow establishment (gas rate 6 6 l/min) for 30, 60 and 360 sec was investigated. The results showed that CAPP method effectively reduces the pure mycotoxins. Overall, there are more than 40% and 50% reduction in the mean initial concentrations of Ochratoxin A and Citrinin, when plasma-treated at 100 W powers. The degree of destruction of various mycotoxins varies according to their structure. It was also shown that the presence of a combination of mycotoxins in foods reduces the plasma's effect but does not prevent their destruction. Finally, in this study, Citrinin and Ochratoxin A were successfully detoxified on all specimens. In addition to mycotoxin detoxification, the CAPP method is neutral in terms of biologic effects and has least negative effects on the nutrients compared to the conventional methods of disinfection of food products.

Keywords: Mycotoxin, Citrinin, Ochratoxin A, *Penicillium*, Cold atmospheric pressure plasma, Detoxification

INTRODUCTION

Penicillium is one of the most commonly found fungi in nature. They belong to the class of *Ascomycota* and subdivide into subgeneas such as *Furcatum*, *Aspergilloides*, *Beverticllium* and *Penicillium*. *Penicillium* is present in nature (water, air, soil and plants). They have many benefits, including the ability to produce various enzymes, antibiotics, antitumor and antifungal drugs, as well as effective compounds against insects. The study aimed to investigate the effect of atmospheric cold plasma on the reduction of mycotoxins produced by *Penicillium* isolated from 5 different food sources (wheat, oat, corn, rice and flour).

Today, plasma science is a new way of overcoming the problems faced by food biotechnology researchers. The unique properties of plasma make it widely available in industry and medicine. Classification from a point of view of pressure, divides the plasma into two general classes: low pressure plasma and atmospheric pressure plasma (APP). Currently, more attention is paid to APP due to its simplicity and low cost compared to low pressure plasma. Atmospheric electrical discharge has been extensively investigated in recent years due to advantages such as cost-effectiveness of the test, performance without vacuum, accessibility and sample processing without limitations in size and high reactivity. The use of APP is a new technology that has been considered as a non-thermal method in recent years due to its high potential for the destruction of microorganisms and its low cost.

For the first time, Kung *et al*. [1] stated that cold plasma is a very effective bacteria killer. Cold atmospheric plasmas have the ability to generally disable microorganisms under controlled laboratory conditions [1]. Mortazavi *et al*. [2] announced that plasma would degrade proteins and cause lipid peroxidation of *E. coli* gram-negative bacteria, thereby destroying the bacterial membrane of the microorganisms [2].

Mortazavi *et al*. [2] also reported that one of the most important mechanisms for killing bacteria, in addition to destruction of DNA, proteins, etc. by cold plasma, is the destruction of bacterial membrane through membrane lipid oxidation. Since plasma can produce reactive oxygen species, therefore, by increasing treatment time, the level of membrane lipids oxidation should increase, as a result more bacteria in different times will be

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eliminated [2]. Fernandes and Thomson [3] reported that the need to meet the consumer demand for fresh products without challenging the microbial safety of food and its quality, has attracted the interest of the food industry to new low-temperature products. Compared to the thermal processes, these technologies depend on physical processes, such as high hydrostatics, ionizing radiation, ultrasonic, ultraviolet light and cold plasma, which are capable of destroying microorganisms at limited temperatures. This latter treatment (plasma jet) is one of the promising technologies for food maintenance [3].

In this study, 5 *Penicillium* samples from flour, wheat, corn, oat and rice (their storage sites) were isolated and then cultured for observing their important features in oatmeal agar. After the preparation of the culture slide, the morphological features such as colony surface and back color, colony texture and pigmentation production were determined. To increase the mycotoxin production, their fungal isolates were cultured in two broth types (malt extract + sabouraud dextrose broth) and (yeast extract + sabouraud dextrose broth) and after an incubation period, the toxins produced by superior strains were isolated by solvent extraction and their initial concentrations before the treatment were examined by ELISA test, finally the effect of mycotoxin detoxification of APP was studied at 3 different times - 60, 120 and 360 sec.

MATERIALS AND METHODS

In this study, a non-thermal atmospheric plasma (99% argon and 1% air with a gas flow rate of 6 l/min for a single jet with high sinus voltage at a frequency of 30 KHz for treatment of samples was used). The general stages are:

- Sampling
- Initial tests (identification)
- Culture tests and detoxification testing
- Culturing and determining the amount of toxic production by ELISA method
- Execution of plasma radiation and measurement of mycotoxin detoxification
- Review of raw data and statistical data analysis

Materials and culture media

In this study, an oatmeal agar medium was used, and sabouraud dextrose broth 250 ml + malt extract 7.5 ml and sabouraud dextrose broth 250 ml + yeast extract 7.5 ml were used (to enrich and increase mycotoxin production by collected samples). All culture media and chemical materials used to isolate and produce mycotoxins were from the German Merck Co. (According to the instruction on the cans of culture media) [4-6].

Sampling

In this research, samples were from the northern regions of Iran including wheat, corn, oats, flour and rice. These samples were collected by Dr. Arash Chaichi Nosrati and were available for the study. Samples were transferred to the laboratory immediately after collection, and were kept in the refrigerator at 4 °C to determine the amount of Citrinin, Ochratoxin A, as well as mycotoxin detoxification by plasma.

Needle culture of samples in oatmeal agar media (culture in a solid medium)

To isolate the Citrinin and Ochratoxin A generic fungi, the collected samples were cultured in an oatmeal agar medium (specific culture medium) as needle culture (in 3 points at a distance of 2 cm from the edge of the plate). Each plate was transferred to the incubator for 7 days at 25 °C. Plates were examined during the incubation (every 3 days from the 7 days incubation period). Identification of isolated fungi was done according to the morphological and macroscopic characteristics of fungal colonies such as colony surface and back color, smooth or rough colony surface, powder, cotton or more, with the help of Klich, Barnett and Hunter's identification keys [7, 8].

Culture in a liquid medium (transfer of samples from solid to liquid medium)

In this step, two broths, sabouraud dextrose broth + malt extract (SB + ME) and sabouraud dextrose broth + yeast extract (SB + YE) in 10 ml falcon tubes containing 5 ml of liquid medium with sterile glass (in this study, it was used to accelerate the cell breakdown process, to extract mycotoxins from beads glass). To inoculate the samples from solid to liquid medium, the pure and isolated samples were picked by sterile loops, transferred to the liquid medium and were kept in an incubator shaker at 25 °C, 200 rpm for 7 days. To prevent drying of liquid medium during incubation (on day 3 of the 7-day incubation period) 0.5 cc of phosphate buffered saline solution (PBS) was added to each of the falcon tube by a sampler [5, 6].

Preparing culture slides from selected samples

In the preparation of culture slide in this study, the oatmeal agar medium was used. Using sterilized scalpel, parts of the medium as 1×1 cm^2 were cut and placed on the slide by observing the sterilization rules (Fig. 1). After placing the agar completely in the center of the slide, it was

horizontally placed on a "U-shaped" tube in a large glass plate (containing 10 ml of sterile distilled water). Then using a sterile anse, the pure isolation samples were inoculated on 4 points on the slide (Fig. 2). Subsequently, the sterilized slide was placed on an inoculated agar portion and stored in an incubator at 25 °C for 7 days [9]. During incubation (each 3 days from the 7-day incubation period), the plates were examined and distilled water was used to prevent the plates from drying. After the 7-day incubation period, the microscope slide was separated and two drops of lactophenol were poured on another clean slide and placed the microscope slide with a mold (purified sample) attached to it on the slide and then examined and photographed them with a (10×), (40×) and (100×) lens with an optical microscope on the next day [9].

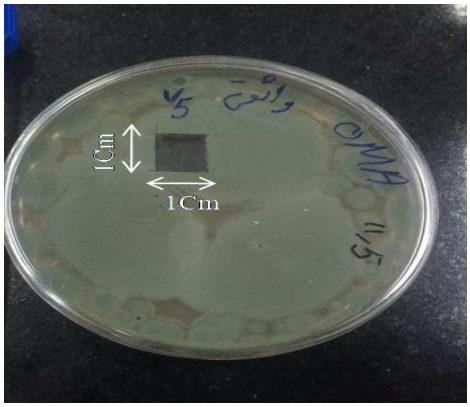


Fig.1. Slide culture from selected strains

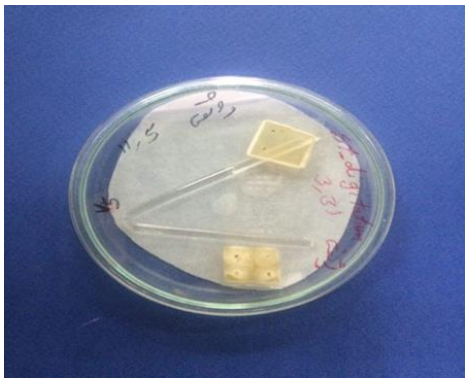


Fig.2. Preparation of culture slide on U-shaped loop

Extraction of mycotoxins from liquid

Extraction of mycotoxins from liquid media (sabouraud dextrose broth with malt and yeast extract) after incubation (seventh day) to extract and purify mycotoxins produced by pure strains requires cell-free media. For this purpose, the falcon tubes containing the liquid medium were placed in a freezer for 15 min with a 35 degree angle vortex at -70 °C. In the next step, the falcon tubes were removed from the freezer and de-frozen at the laboratory temperature. Then, 2.5 ml of extraction solvent was added to the falcon tubes and vortexed three times in three 15-minute periods at 30 to 35 degrees angle (this step was repeated to increase the extraction). A total of 5 ml of extraction solvent was used for each falcon. At the final stage of mycotoxin extraction (after two times during the vortex process and adding extraction solvent) the obtained liquid was separated by sterile Whatman filter paper (0.5 micron) and transferred to sterile microtubes (2 ml) for ELISA test and kept at a temperature of -4 °C. (Each of them was transferred to a bain-marie for 5-15 min at a temperature of 95-100 °C before the ELISA test for evaporation of the remaining alcohol from the microtubes) [10].

Extraction of mycotoxins from the solid medium (oatmeal agar)

After incubation (seventh day), cell-free media were required for extraction and purification of mycotoxins produced by the pure strains. For this purpose, using a sterile scalpel, the oatmeal agar was cut in 1×3 cm and inoculated to the sterile falcon tubes (10 cc) containing sterile beads glass and 5 cc of phosphate buffered saline (PBS) and was vortexed at 35 °C for 15 min and placed in a freezer at 70 °C.

Cold atmospheric pressure plasma (CAPP)

In this research, Citrinin and Ochratoxin A detoxification of APP of argon gas (output of the device's power supply at 50 kV, 100 watts, electron frequency 30 kHz, and gas flow rate 6 l/min) was investigated. To do the tests, first the output of the power supply was set to 50 kV, 100 watts, and the electron frequency of 30 kHz (optimal conditions for stable and effective plasma) and their exact values were measured with high-voltage probes and oscilloscopes. Then, with the gas flow (gas rate 6 l/min) and the high potential difference between the two connected electrodes, the effect of APP (argon-air) was investigated for 30, 60 and 360 sec. For treatments and mycotoxins (supernants), it was done in three replicates (Fig. 3).

RESULTS

Results of preliminary tests for the selection of mycotoxin producing microorganisms

In this research, samples were collected from northern regions of Iran including wheat, corn, oat, flour and rice. These samples were collected by Dr. Arash Chaichi Nosrati and put into the study.

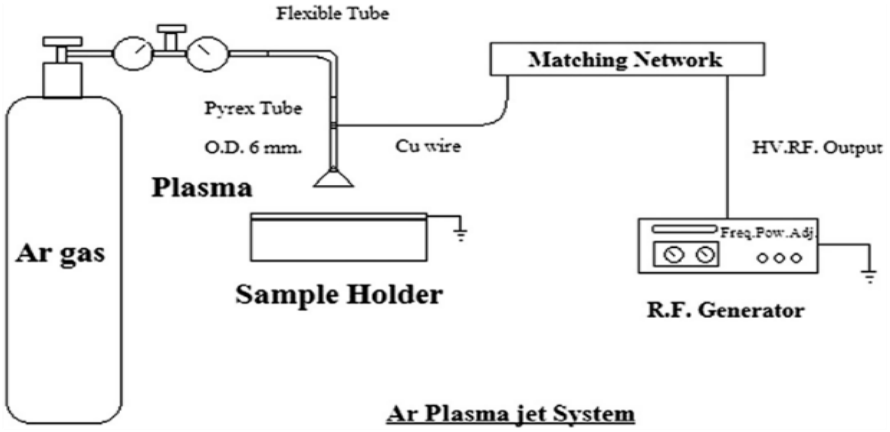


Fig.3. APPJ arrangement: Schematic image of the arrangement of plasma and plate containing the sample, as well as a view of the single-flame plasma jet produced, h of the produced plasma is 27 mm and the effective distance between the samples and the nozzle is 21 mm

In this research, 5 species of *Penicillium* genus were isolated and identified from different sources (wheat, corn, oats, flour and rice) (fungi coding as V1, V2, V3, V4 and V5).

Macroscopically, fungal colonies such as colony surface and back color, smooth or rough colony, powdery, cotton, etc., were examined (Table 1). In this study, the standard strains of *Penicillium digitatum* and *Penicillium italicum* of the microbial bank of the Iranian Institute of Plant Protection were used to compare the results and produce mycotoxins with collected samples The mean initial concentration of Citrinin reached from 47.203 ppb at 60, 120, and 360 sec to 16.38, 16.23, and 29.27 ppb, respectively (Fig. 5).

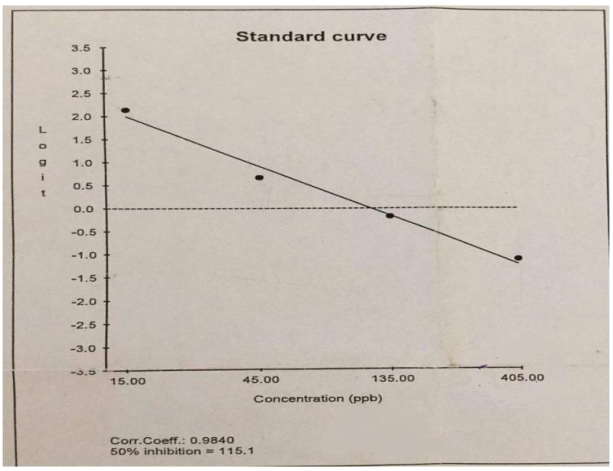


Fig.4. Standard curve of Citrinin

Results of examining the concentration of Citrinin obtained from the analysis of Lin/Log charts in sabouraud dextrose broth + yeast extract (Citrinin / YE + SB)

The mean initial concentration of Citrinin reached from 57.14 ppb at 60, 120, and 360 sec to 24.75, 30.04 and 34.78 ppb, respectively (Fig. 5).

Results of evaluation of Ochratoxin A A concentration obtained from Log/Lin tables analysis in sabouraud dextrose broth + malt extract (Ochratoxin A / ME + SB)

The mean initial concentration of Ochratoxin A in the medium reached from 25.9 ppb at 60, 120, and 360 sec to 13.66, 16.15, and 8.93 ppb, respectively (Table 4).

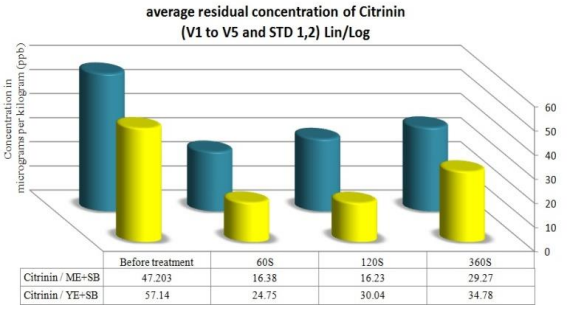


Fig.5. The average concentration of Citrinin before and after plasma radiation

Results of examining the concentration of Citrinin, obtained from the analysis of Log/Lin tables in the sabouraud dextrose broth + malt extract (Citrinin / ME + SB)

The average initial concentration of Citrinin in the medium reached from 36.05 ppb at 60, 120, and 360 sec to 41.66, 11.22 and 20.24 ppb, respectively (Table 2).

Results of examining the concentration of Citrinin obtained from the analysis of Log/Lin tables in sabouraud dextrose broth + yeast extract (Citrinin /YE + SB)

The mean initial concentration of Citrinin in the medium reached from 63.01 ppb at 60, 120, and 360 sec to 29.88, 13.49 and 31.61 ppb, respectively (Table 3).

Table 1. Macroscopic and microscopic characteristics of the isolated samples

Sample	Number/ separated sources	Gender/species	Macroscopic characteristics (colony)	Microscopic characteristics
V1, V2	2 samples / rice	*Penicillium variable*	• Limited growth • Colonial diameter 10-15 mm per 7 days • Fluffy and woolly colonies • Yellow colonies	• Conidiospores are often concentrated in the center • Containing spindle-shaped soft conidia • Conidiospores with a smooth wall • Containing 5-7 units in conidiospore • Natural size of conidia is (2.5 - 2.5) × (12.5-7.0 μm)
V3	1 sample / corn	*Penicillium digitatum*	• Fast growth • Colony diameter 40-60 mm in 7 days • Low sporulation • Powder colony • Colony color (first yellow to green-brown) • Smooth colony level	• Branched and irregular conidiospores with short base and at the end 3-6 phialides • Two-row phialides around • Round conidia color (green olives) • Conidia size is (3.5 - 8) × (3 - 4) μm
V4	1 sample / from the source of wheat	*Penicillium nalgiovense*	• The colony diameter is 25-30 mm in 7 days • Colony color green to yellow • Wooly and fluffy colony • Colony back color yellow	Conidiospores are single but less than *P. Italicum* • Containing 6-2 units in Conidiospore • Conidiospores have a smooth wall • Coinidia with a diameter of 3-4 μm • Transparent Conidiospores • 2-6 high phialides at the end
V5	1 sample / cereal (storage area of wheat and oat)	*Penicillium italicum*	• Slow growth • Colony diameter 50-60 mm in 14 days • Having a fragrant smell • Colony back color is colorless or yellow-brown • Colony color (green-gray) • Smooth colony level	• Single conidiospores Conidiospores have a smooth wall having stipe with a size of (5-5.5) × (250-100) μm • Transparent conidiospores • 3-6 high phialides • Conidia color (green) • Coinidia size (5 - 4) × (2.5 - 3.5) μm
STD (1)	Bacterial Bank of Plant Protection Research Institute	*Penicillium digitatum*	• Fast growth • Colony diameter 40-60 mm in 7 days • Low sporulation • Powder colony • Colonial color (first yellow to green-brown) • Smooth colony level	• Branched and irregular conidiospores with short base and at the end of 3-6 phialides • Two-row phialides around • Round conidial color (olive green) • Coinidia size (3.5 - 8) × (3 - 4) μm
STD (2)	Bacterial Bank of Plant Protection Research Institute	*Penicillium italicum*	• Slow growth • Colony diameter 50-60 mm in 14 days • Having a fragrant smell • Colony back behind is colorless or yellow brown color • Colony color (green-gray) • Smooth colony level	• Single Conidiospores • Conidiospores have a smooth wall • Having a stipe with a size of (5-5.5) × (250-100) μm • Transparent conidiospores • 3-6 long phialides • Conidia color (green) • Conidia size (5 - 4) × (2.5 - 3.5) μm

Analysis of the concentration of Ochratoxin A in isolated samples in sabouraud dextrose broth + malt extract (SB + ME) and sabouraud dextrose broth + yeast extract (SB + YE) before and after plasma radiation

The output of the power supply was set at 50 kV, 100 watts, 30 kHz electron frequency, and the inlet gas flow rate at 6 l/min. As in the previous method, first the turbidity of Ochratoxin A of the produced samples was measured and in the next step, according to the standard curve of Ochratoxin A (Fig. 6) and standard *Penicillium* samples (STD1/STD2), the concentration of Ochratoxin A was determined before and after treatment with plasma jet as Lin/Log charts and Log/Lin tables.

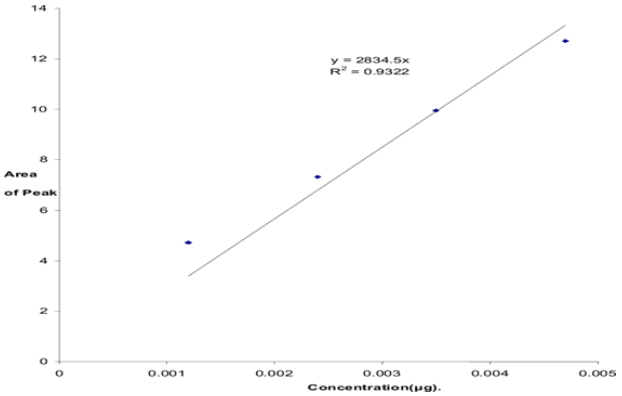


Fig.6. Standard curve of ochratoxin A

Results of examining the Ochratoxin A concentration obtained from analysis of Lin/Log charts in sabouraud dextrose broth + malt extract (Ochratoxin A / ME + SB)

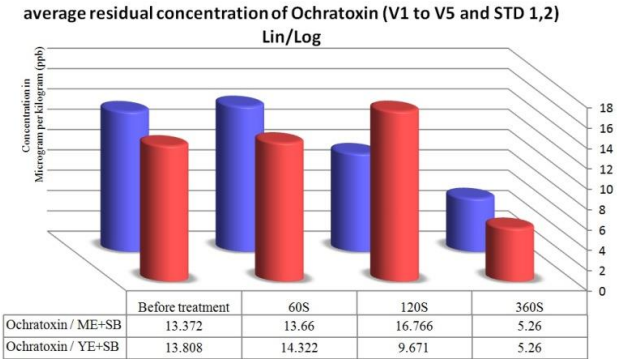
The mean initial concentration of Ochratoxin A reached from 35.264 ppb at 60 and 360 sec to 18.34 and 12.008 ppb, respectively (Fig. 7).

Results of evaluation of Ochratoxin A concentration obtained from analysis of Lin/Log charts in sabouraud dextrose broth + yeast extract (Ochratoxin A/ YE + SB)

The mean initial concentration of Ochratoxin A in the media reached from 28.625 ppb at 60 and 360 sec to 196.486 and 14.607 ppb, respectively (Fig. 7).

Results of evaluation of Ochratoxin A concentration obtained from Log/Lin tables analysis in sabouraud dextrose broth + malt extract (Ochratoxin A / ME + SB)

The mean initial concentration of Ochratoxin A in the medium reached from 25.9 ppb at 60, 120, and 360 sec to 13.66, 16.15, and 8.93 ppb, respectively (Table 4).



	Before treatment	60S	120S	360S
Ochratoxin / ME+SB	13.372	13.66	16.766	5.26
Ochratoxin / YE+SB	13.808	14.322	9.671	5.26

Fig. 7. Mean concentration of Ochratoxin A before and after plasma radiation

Results of evaluation of Ochratoxin A concentration obtained from Log/Lin tables analysis in sabouraud dextrose broth + yeast extract (Ochratoxin A / YE + SB)

The mean initial concentration of Ochratoxin A in the medium reached from 27.51 ppb at 60, 120 and 360 sec to 22.45, 13.83 and 10.63 ppb, respectively (Table 5).

As can be seen from the Log/Lin tables, it can be said that detoxification of Citrinin and Ochratoxin A was investigated by radiating argon gas over 60, 120 and 360 sec, and the mean initial concentrations of samples in sabouraud dextrose broth + malt extract and sabouraud dextrose + yeast extract (before radiation) were compared with the mean residual concentrations after each plasma emission. In these studies, it was found that detoxification of Citrinin and Ochratoxin A was successful in all samples and showed a high efficiency of APP in this field, especially in the food industry.

DISCUSSION

Investigation of Pearson correlation (Lin/Log) on the concentration of pre-irradiation produced mycotoxins (Citrinin and Ochratoxin A) in two media (ME + SB) and (YE + SB)

In this section, statistical correlation between the amount of Citrinin and Ochratoxin A produced by each isolated strain (V1 to V5) was investigated in two media (ME + SB), (YE + SB) and showed that a significant incompatibility is seen between these two toxins. So, the mean concentration of Citrinin was higher than that of Ochratoxin A.

Table 2. Concentration of Citrinin in sabouraud dextrose broth + malt extract

Log/lin	Sample name	Citrinin Malt (ppb)	Citinin Malt /60s (ppb)	Citinin Malt /120s (ppb)	Citinin Malt /360s (ppb)
V1	*Penicillium variable*	44.99	42.36	9.36	22.39
V2	*Penicillium variable*	28.68	47.42	8.89	7.52
V3	*Penicillium digitatum*	40.5	88.28	0.03	0.01
V4	*Penicillium nalgiovense*	38.1	23.39	0.3	0.01
V5	*Penicillium italicum*	48.61	64.27	44.61	42.34
V STD(1)	*Penicillium digitatum*	16.81	10.43	19.82	24.38
V STD(2)	*Penicillium italicum*	34.66	15.51	5.51	24.54
Mean		36.05	41.66	11.22	20.24

Table 3. Concentration of Citrinin in the sabouraud dextrose broth + yeast extract

Log/lin	Sample name	Citrinin Yeast (ppb)	Citrinin Yeast /60s (ppb)	Citinin Yeast /120s (ppb)	Citinin Yeast /360s (ppb)
V1	*Penicillium variable*	32.43	35.04	13.54	72.2
V2	*Penicillium variable*	39.53	22.5	7.91	6.72
V3	*Penicillium digitatum*	226.68	57.8	0.03	0.2
V4	*Penicillium nalgiovense*	52.36	12.38	0.1	66.01
V5	*Penicillium italicum*	40.5	55.73	36.3	44.48
V STD(1)	*Penicillium digitatum*	15.54	12.61	17.08	8.08
V STD(2)	*Penicillium italicum*	34.04	13.11	19.51	23.58
Mean		63.01	29.88	13.49	31.61

Table 4. Concentration of Ochratoxin A in the sabouraud dextrose broth + malt extract

Log/lin	Sample name	OchraA Malt (ppb)	OchraAMalt /60s (ppb)	OchraA Malt /120s (ppb)	OchraA Malt /360s (ppb)
V1	*Penicillium variable*	29.95	13.372	20	0.1
V2	*Penicillium variable*	13.11	13.372	9.03	7.93
V3	*Penicillium digitatum*	40	13.372	0.03	0.02
V4	*Penicillium nalgiovense*	11.12	13.372	11.35	5.75
V5	*Penicillium italicum*	7.13	15.391	45.35	35.71
V STD(1)	*Penicillium digitatum*	40	13.372	15.57	5.51
V STD(2)	*Penicillium italicum*	4	13.372	11.23	7.5
Mean		25.9	13.66	16.15	8.93

Table 5. The concentration of ochratoxin A in the sabouraud dextrose broth + yeast extract

Log/lin	Sample name	OchraA Yeast (ppb)	OchraA Yeast /60s (ppb)	OchraA Yeast 120s (ppb)	OchraA Yeast /360s (ppb)
V1	*Penicillium variable*	23.91	62.7	11.1	0.03
V2	*Penicillium variable*	13.47	9.03	7.3	9.3
V3	*Penicillium digitatum*	40	11.6	1.51	0.01
V4	*Penicillium nalgiovense*	29.47	20.5	8.89	0.02
V5	*Penicillium italicum*	15.47	15.06	41.66	42.39
V STD(1)	*Penicillium digitatum*	40	18.8	17.3	13.79
V STD(2)	*Penicillium italicum*	40	19.5	9.03	8.89
Mean		27.51	22.45	13.83	10.63

Statistical analysis of Pearson correlation (Lin/Log) on the concentration of Ochratoxin A in two media (ME + SB) and (YE + SB)

In the study of Pearson's correlation, the correlation between the amount of Ochratoxin A produced in the two studied media showed that the fungal isolates of the two sabouraud dextrose broth + malt extract and sabouraud dextrose broth + yeast extract were able to produce toxin. In the study of the Pearson correlation, according to Table 6, the extent of Ochratoxin A reduction by plasma jet after 60 sec in both studied media showed a statistically significant correlation between them. So in both media, the toxin reduction was on average less than 10% after 60 sec.

Also, there was a significant statistical correlation after 120 sec of plasma jet treatment about comparing the amount of Ochratoxin A in the two media studied. In both media, the amount of toxin reduction after 120 sec was on average less than 5%, which is indicative of the high accuracy of ELISA test in this study.

Statistical analysis of Pearson correlation (Lin/Log) on the concentration of Citrinin in two media (ME + SB) and (YE + SB)

In the study of Pearson's correlation, the reduction of Citrinin in the medium (ME + SB) after 60, 120, and 360 sec of jet plasma treatment was more than its reduction in the medium (YE + SB) and there was a consistent and significant correlation between them.

In the study of Pearson correlation, a significant aligned correlation can be seen between the amounts of Citrinin detoxification produced after 60 sec treatment with plasma jet. The significant correlation shows that with the effect of plasma jet, Citrinin toxin levels were reduced in the ME + SB and YE + SB by 34.7% and 43.31%, respectively (Table 6).

In the study of Pearson's correlation, the statistical correlation between the amount of Citrinin toxin produced in the two studied media showed that the fungal isolates studied in both ME + SB and YE + SB are able to produce toxin. The greatest reduction and effect of plasma jet among treatments was observed for Citrinin toxin after 360 sec of treatment in YE + SB. During this time, the amount of toxin was reduced by 60.86%.

Comparison and statistical analysis of the Pearson correlation (Lin/Log) and concentration and effect of plasma jet on Citrinin and Ochratoxin A

Citrinin is known in the inter-cellular metabolism of producing fungi of the leading toxin of Ochratoxin A. In examining the correlation between Citrinin and Ochratoxin A levels in ME + SB and YE + SB, which are treated for 60 sec with plasma, there was a significant correlation between them (Table 6).

Wilcoxon test (significant numerical difference) (Lin/Log) on the concentration of pre-irradiation mycotoxins (Citrinin and ochratoxin A) in two media (ME + SB) and (YE + SB)

In evaluating the initial values of Citrinin and Ochratoxin A produced in SB + ME in statistical analysis, the result was acceptable in terms of Wilcoxon test (significant numerical difference). Therefore, the inconsistency in the study of Pearson's correlation indicates that the ratio of Citrinin to Ochratoxin A transform was relatively low. Significant statistical inconsistency between the relative amount of Ochratoxin A as reduced in SB + ME medium after 60, 120, and 360 sec treatment shows that increasing the time of treatment with plasma jet also increases the amount of toxin, although by increasing the time from 120 to 360 sec, the toxin was severely reduced.

Statistical analysis of Pearson correlation (Log/Lin) on the concentration of Ochratoxin A in two media (ME + SB) and (YE + SB)

In the study, the correlation of Ochratoxin A amount in two SB + ME and SB + YE was statistically significant. At the concentration of Ochratoxin A, after 120 and 360 sec of treatment, a correlation was observed between the reduced values. There is a statistically pair significant correlation between the amount of Ochratoxin A in the SB + ME from 120 to 360 sec, and also in the SB + YE from 120 to 360 sec.

For Ochratoxin A, the highest reduction in SB + YE occurs after 120 seconds of treatment, which decreased by about 50.27% after 120 sec. For Ochratoxin A, the highest reduction in SB + ME occurs after 360 sec of treatment, so that after 360 sec, the toxin decreased by 34.47%.

Table 6. Concentration of Citrinin and Ochratoxin A before and after plasma radiation (Lin/Log)

Citrinin and Ochratoxin A	Concentration (Before treatment) (ppb)	Concentration after 60 sec of radiation (ppb)	Concentration after 120 sec of radiation (ppb)	Concentration after 360 sec of radiation (ppb)
Citrinin / ME + SB	47/203	16/38	16/23	29/27
Ochratoxin A / ME + SB	13/372	13/66	16/766	5/26
Citrinin / YE + SB	57/14	24/57	30/04	34/78
Ochratoxin A / YE + SB	13/808	14/322	9/671	5/26

Statistical analysis of Pearson correlation (Log/Lin) with the concentration of Citrinin in two media (ME + SB) and (YE + SB)

However, Pearson's correlation (significant and consistent correlation) between Citrinin and Ochratoxin A can be achieved, but the decreasing amounts of Citrinin and Ochratoxin A do not occur after these treatments, which can be detected by ELISA method and we cannot prove the extent of treatment with plasma jet, so it should be considered in another research.

Examining Wilcoxon test (Log/Lin) on the concentration of mycotoxins produced before radiation (Citrinin and Ochratoxin A) in two media (ME + SB) and (YE + SB)

Considering the significance of the numerical difference (Wilcoxon), the mean Ochratoxin A produced from the fungi studied in SB + ME medium after 360 sec treatment with plasma jet, reached from 25.9 to 8.93, indicating a non-uniform significant correlation between the data. This correlation was statistically significant in comparison with the amount of Ochratoxin A reduction in SB + ME after 120 and 360 sec of plasma jet treatment, and there was a significant inconsistent correlation in increasing the time and the requirement for toxin reduction.

The results of this study are consistent with other studies carried out in recent years, including that of Siciliano *et al*., on the effect of APP that uses argon gas to produce plasma, to study the effect of plasma on the elimination of mycotoxins produced by *Aspergillus*. In this study, they were able to use a plasma with the characteristics of a power supply output of 50 kV, 400 watts, an electron frequency of 30 kHz, aflatoxin, and ochratoxin A produced by isolates at a concentration of 25 ng/g to remove 95% after one min of radiation [5]. Also, Ouf *et al*. used the effect of APP, which uses argon gas to make plasma, to study the effect of plasma on the elimination of mycotoxins produced by *Aspergillus*. In this study, they were able to remove more than 97% of aflatoxin B1 produced by *Aspergillus parasiticus* with a power of 60 watts over a period of 15 min. In this study, they removed more than 95% of aflatoxins produced by *Aspergillus flavus* by the radiation of plasma of 60 watts in 12 min. [11].

CONCLUSIONS

In this research, detoxification of Citrinin and Ochratoxin A by APP of argon gas (output of the power supply 50 kV, 100 watts, electron frequency 30 kHz, and gas flow rate 6 l/min) was investigated. In summary, it can be said that many disinfection methods, including chemical, physical, dry or wet heat methods, are used to disinfect solid surfaces or liquids and biological surfaces such as skin with functional limitations. The use of non-thermal plasma technology in cleaning, sterilization and detoxification has advantages over existing ones, including high detoxification properties during short flow, lack of remaining toxicity, and the cost-effectiveness of argon gas. In this study, Lin/Log and Log/Lin charts and tables were statistically evaluated (mean, standard deviation, variance, co-variance, correlation coefficient, etc.).

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ВЛИЯНИЕ НА ПЛАЗМЕНИТЕ РЕАКТИВНИ ЕФЕКТИ ВЪРХУ ЦИТРИНИН- И ОХРАТОКСИН-ПРОИЗВЕЖДАЩИТЕ ФОРМИ НА *PENICILLIUM*

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(Резюме)

Микотоксините са малки токсични молекули, произвеждани от различни микроорганизми. Те често се състоят от вторични метаболити и имат различни функции и структури. Цитринин и охратоксин А, произвеждани от различни форми на *Penicillium*, замърсяват храните. Методът на студената плазма при атмосферно налягане има потенциал за детоксикация на микотоксини. В тази статия са изследвани и оптимизирани работните параметри на студената плазма при атмосферно налягане (CAPP) за засилване на ефекта върху цитринин и охратоксин А в пшеница, овес, царевица и ориз. Първоначалните опити са проведени при мощност на захранване 50 kV, 100 вата и електронна честота от 30 kHz (оптимални условия за стабилна и ефективна плазма). След това е изследвано влиянието на студената плазмена струя (аргон) върху създаването на газовия поток (скорост на газа 6 l/min) за 30, 60 и 360 sec. Установено е, че CAPP методът ефективно намалява количеството на микотоксини. Намалението с използване на плазма с мощност от 100 W е над 40% и 50% от първоначалната концентрация съответно на цитринин и охратоксин А. Степента на разрушаване на различните микотоксини варира в зависимост от тяхната структура. Показано е, че в присъствие на комбинация от микотоксини разрушаването им от плазмата е по-малко ефективно. В настоящото изследване цитринин и охратоксин А са успешно детоксикирани във всички образци. В допълнение, CAPP методът няма биологични ефекти и се отразява минимално на хранителните продукти в сравнение с конвенционалните методи на дезинфекция на хранителни продукти.