Study of the effect of plasma jet on Fusarium isolates with ability to produce DON toxins

Elham Galin Abbasian (1)
Mansour Bayat (1)
Arash Chaichi Nosrati (2)
Seyed Jamal Hashemi (3)
Mahmood Ghoranneviss (4)

(1) Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
(2) AssociatePprofessor ,Division microbiology, Department of molecular & cell biology , Faculty of basic sciences , Lahijan branch, Islamic Azad university (IAU), Lahijan,Gilan, Iran
(3) Professor of Tehran University of medical Sciences, Tehran, Iran
(4) Professor of physics, Plasma physics research center, Science and Research Branch, Islamic Azad University, Tehran, Iran

Corresponding author:
Mansour Bayat
Professor of physics, Plasma physics research center,
Science and Research Branch,Islamic Azad University,
Tehran, Iran
Email: dr_mansour_bayat@yahoo.com

Abstract

Introduction: *Fusarium* species cause a variety of infections in humans, such as superficial, invasive and disseminated infections. The aim of this research is to study the effects of argon cold double atmospheric pressure plasma (DAPACP) on deactivating the cells or *Fusarium* spores which can seriously damage food products in vitro conditions.

Materials and Methods: Samples were obtained from May 2015 to September 2016 during three cultivation periods of tea in Gilan and Mazandaran province. One sample was taken from each 50 square hectares of tea plantations prepared for harvesting and also from 60 tea manufacturers. At intervals of 3, 7 and 15 days, plates were studied and the colonies on each plate were identified and isolated and then their microscopic and also macroscopic properties were documented. Finally 150 colonies were selected. We prepared Czapek and liquid environments (Czpk), then the isolated samples were cultured using stab. Plasma system is a DAPACP system (AL2O3) which used a high voltage with 25KHZ frequency and is usually applied to a high voltage electrode.

Results: The amount and type of toxins in the environment with fixed toxicity vary . In the metabolic cycle, DON (deoxynivalenol) is first produced and then is metabolized in a few steps. Plasma jet treatment causes a relative reduction in concentration of DON per solvent volume or culture media; a statistically significant negative correlation is observed here. The negative correlation between DON, which has been measured in samples previously exposed to plasma jet for 60 seconds , is affected by plasma jet more than DON. Therefore, Z = -2 / 201, Sig = 0.028 is proven, and it occurs with more intensity compared to DON.

Conclusion: This study has confirmed that argon plasma jet system has destructive effects on mycotoxins such as DON and also on the microorganisms which produce mycotoxins. Although the physicochemical properties and structural changes of mycotoxins were not explained under plasma jet treatment, this study revealed that the plasma system needs certain conditions like time and toxin concentration of food, to remove and inactivate mycotoxins.

Key words: *Fusarium*, plasma jet, DON, toxins

Please cite this article as: Please cite this article as: Abbasian E. et al. Study of the effect of plasma jet on Fusarium isolates with ability to produce DON toxins . *World Family Medicine.* 2017; 15(9):204-207
DOI: 10.5742/MEWFM.2017.93126

Introduction

Fusarium species cause a variety of infections in humans, such as superficial, invasive and disseminated infections [1]. Clinical manifestation of Fusariosis depends on the host immune system and the entrance site of the organism [2]. Superficial and localized infections occur mainly in people with healthy immune systems, while invasive and disseminated infections have been seen in immunocompromised persons [3]. *Fusarium* species are considered as pathogenic agents in plants, crops and in some animals [4]. *Fusarium* species are widely distributed in soil and water [5, 6]. More than 50 species of *Fusarium* have been identified, twelve of which are associated with disease including; *Fusariumsolani, Fusarium oxysporum* and *Fusarium* which contributes to nearly 70 % of the cases [7, 8].

Fusarium species carry several virulence factors, including Mycotoxins which are cellular and humoral immune suppressive and cause tissue problems [9]. In addition, Fusarium species have the ability to combine to prosthetic materials and could also produce protease and collagenase [10].

Deoxynivalenol (DON) is one of the several Mycotoxins produced by Fusarium species which causes contamination in maize, wheat, barley, rice and other grains during their storage life and it is considered a risk factor for humans and animals [11]. This toxin causes nausea or vomiting and was first reported in Japan in 1972 following the consumption of mouldy barley [12].

DON is chemically a member of trichothecenes mycotoxins family and structurally is apolar organic material belonging to trichothecenes type B, with three free hydroxyl groups which causes its toxicity. One of the most significant physicochemical properties of DON is high-temperature heat resistance and water solubility [13].

Plasma as a state of matter has significantly different characteristics; plasma has no fixed shape or volume and can form magnetic lines and rays under magnetic fields. In accordance to this method used, plasma can be converted into a wide range of states; from being unbalanced to thermal equilibrium. The antimicrobial effects of plasma have been known for almost 50 years [18, 16].

Research has mainly focused on the use of plasma on nonliving surfaces, such as medical instruments. Plasma is a cold gas-like mixture of charged particles (free electron and proton particles) with neutral reactive particles including; gas molecules, free radicals and ultraviolet photons. When the reactor is launched in atmospheric pressure, a cool plasma jet is blown into the air [17]. Since this plasma jet can be used in sterilization of sensitive surfaces, it is a proper candidate to be applied instead/with the autoclave sterilization process for surgical and dental use as an effective agent to destroy bacteria and viruses [15].

The aim of this research is to study the effects of argon cold double atmospheric pressure plasma (DAPACP) on deactivating the cells of *Fusarium* spores which can seriously damage food products in vitro conditions. Moreover, fungi are used to investigate the efficacy of cold plasma in suppressing the produced mycotoxins.

Materials and Methods

Sampling:
Samples were obtained from May 2015 to September 2016 during three cultivation periods of tea in Gilan and Mazandaran province through following the instructions of outdoor and indoor sampling. One sample was taken from each 50 square hectares of tea plantations that were prepared for harvesting and also from 60 tea manufacturers. Sampling was carried out by placing open plates at a height of 90-110 cm, 3 to 5 days after each rainfall from 9 o'clock till 3 pm during sunshine at 25 ± 3 ° C and when their velocity was measured at 30 m / s.

Six plates with malt extract agar, yeast extract agar, Czapek yeast extract agar, Czapek agar, sabouraud dextrose agar and potato dextrose agar were all mixed with 100 ppm chloramphenicol and 50 ppm tetracycline and were examined 3 to 5 days at 25 ° C before operation. All of them were applied for one group of samples. Plates containing 15 to 25 cubic centimeter of agar after 30, 60 and 90 minutes were sampled through plantations and 15, 30 and 60 minutes through factories; 900 plates were taken and then were placed in perforated polyethylene bags and sent to the laboratory. The plates were incubated at 25 ± 2 ° C in an aerobic environment.

One plate was kept in the dark, another one in the light and finally a pair of them was kept in light-dark enviroment. At intervals of 3, 7 and 15 days, plates were studied and the colonies on each plate were detected and isolated and then their microscopic and macroscopic properties were documented. Finally150 colonies were selected.

Cultivation & Isolation:
We prepared Czapekand liquid environments (Czpk), then the isolated samples were cultured using stab (in 3 places with a distance of 2 cm from each other and from the edge of the plate). Then they were incubated for 14 days at 25 ° C. Samples were then cultured in liquid environments of sB + ME and sB + yE. They were checked regularly to avoid drying and reduction in the volume of liquid, PBS was added to these environments. The samples were collected, and the extracted solution was added and filtered.The slides of the cultures were prepared and then stained with lacto phenol.

Extracted solutions from solid and liquid environments were prepared for ELISA technique. Then the samples were exposed to cold plasma jet and were set at 30 seconds, 60 seconds and 360 seconds in certain concentrations. At this stage, sample volume was reduced.

Plasma Jet System

Plasma system is a DAPACP system (AL2O3). A high voltage with 25KHZ frequency is applied to a high voltage electrode. Voltage and the waveforms are recorded through a high voltage electrical connector with visual indicator. Argon gas is blown into the air through micro tubes to form a double plasma jet. *Fusarium* biomass and the extracted samples from culture media were collected at the bottom of the jet at a distance of 12 mm from the top of the double jet.

The emission spectrum of DAPACP jet is recorded using Spectroscopy. The spectra in Spectrograph are coupled via UV fiber.The effective gas enters a flexible tube and the plasma jet is induced by high frequency RF power supplies.

The fungus used in this study is called *Fusarium*. For inoculation, MEA is cultured on the new plates and at the end of the incubation period, spores are carefully removed and combined with 2% of Tween 80 as emulsifier and the solution is filtered.

After applying an appropriate solution, spores are released on the surface of OMMEA and they are incubated at 25 ° C for 7 days where the colonies are counted. After plasma jet exposure, the activated spores in distilled water are placed in sterilized tubes and plasma-exposed samples were used.

Colonies are regularly counted and 5 tests are used for each sample during each treatment. The experiment is repeated three times with similar conditions. Due to plasma treatment, another control sample is inoculated with the spores and is exposed to argon-oxygen gas mixture with same velocity.

This is to ensure the deactivation of plasma-exposed fungi or the removal of the spores from the surface of samples. The production of five mycotoxins which are isolated from five *Fusarium* species obtained from processing manufacturers and different foods and the spores of the investigated fungus are obtained on malt extract agar (MEA).

In preliminary tests, the isolated *Fusarium* used in this study are identified as a major source of T and DON toxin. The procedure used in this experiment is similar to what is used for spore sprouting. Inoculated fungi that had been previously sterilized without plasma treatment, are used as control in 5 culture medias and the tests are repeated three times under the same conditions.

Results

Correlations between V1 and V3 in DON toxin:
The amount and type of toxins vary and in the environment with fixed toxin, the purpose is to assess the correlation between V1 and V3. In other words, DON toxin production by fungal isolate has a significant positive correlation (Sig=0/PC = 0.997). If DON toxin production amount is high, T2 will be low. In the metabolic cycle, DON is first produced and then is metabolized in a few steps.

There is a significant negative correlation between the produced DON in potato culture media containing yeast extract and DON in the samples obtained from the same environments which is exposed to plasma jet for 60 seconds . Plasma jet treatment causes a relative reduction in concentration of DON per solvent volume or culture media, statistically a significant negative correlation is observed here. This feature has also a statistical significant positive correlation in the conversion of DON to non-toxicity statement after a 60-second treatment with Plasma jet (Sig=0.058 /pc=0.797)

According to the particular negative correlation which is statistically significant between DON concentration and its mean size in potato yeast extracted compared to the DON produced in the same environment but is supposed to be exposed to plasma jet pressure for 60 seconds, the results were as follows : (Sig = 0.045 / pc = -0.821). Therefore toxin reduction due to plasma jet exposure is justified. The negative correlation between DON, has been measured in samples previously exposed to plasma jet for 60 seconds. The results of Sig = 0.028 / Z = -2 / 201 indicate that in fungus strains, during their incubation period, large amounts of DON have been converted. While a negative correlation was observed in the amount of DON reduction in both environments of potato malt extract and potato yeast extract, after 60 seconds of plasma jet treatment, and we noticed a significant numerical difference.

Moreover, when Z= -1/992, Sig=0.046 (Z = numerical differentiation), the numerical differentiation of T2 in potato yeast extract with the same amount of toxin and in the same environment,where it has been exposed to plasma jet for 60 seconds, is statistically significant.

Therefore, Z = -2 / 201, Sig = 0.028 proves that the sharp decline in the value of T2 in potato yeast extract after 60 seconds treatment with Plasma jet is significant, and it occurs with more intensity compared to DON.

Kolmogorov-Smirnov test (Nonparametric):
The standards (Standard Deviation) are mostly above 2. The average deviation is between 0.5 to 2. A significant correlation was detected between the amount of DON , produced in potato malt extract in fungal samples. According to the obtained data (DON precursor in the intracellular metabolism) , there has also been a significant correlation between the produced DON in potato yeast extract and its concentration after a 60-second plasma jet treatment in the same environment.

However, there was a significant positive correlation between the amount of DON produced in potato yeast extract and the reduced concentrations after 60 seconds of plasma jet treatment. Eventually a significant negative correlation was observed between DON reduction rate after a 60-second plasma jet treatment in potato yeast extract with T2 amount in the same environment and after

of plasma jet treatment. Eventually a significant negative correlation was observed between DON reduction rate after a 60-second plasma jet treatment in potato yeast extract with T2 amount in the same environment and after the same treatment.

Discussion

Mycotoxins such as aflatoxins and zearalenones are the most common toxins in foods which can be produced in a wide range of agricultural products [17, 18]. Since mycotoxins have different toxic effects and good thermal stability, their presence in food products could potentially threaten the health of humans and animals [14]. It has been proven that they cause Mycotoxicosis in humans and animals [13,18].

Various methods have been reviewed to eliminate and destroy the toxicity of mycotoxins through physical removal of the contaminated parts of the food, such as heat, chemical and radiation treatments, in order to convert toxins to relatively harmless compounds which lead to eradication of toxin effects[12]. Some of these methods are not always effective, especially when mycotoxins are disseminated through the substance. In addition, some of these toxin elimination techniques are expensive and impractical for business consumption [11,16].

In a study by Jong-Chul Park et al. (2007. Japan) on AFB1, DON and NIV toxins, they observed that plasma jet has fine deterrence effects on toxins; they have confirmed it by using HPLC method. They have also noticed that DON and NIV toxins compared to AFB1 will be destroyed more slowly [10,14]. Our results showed that the 60-second plasma jet treatment reduces DON concentration per solvent volume or culture media,and in the case of those samples being previously exposed to plasma jet for 60 seconds, it was shown that T2 toxin is more affected with plasma jet compared to DON.

Plasma jet effect on toxin in this method is a time-dependent factor.We have set the certain concentration of plasma jet at 30 seconds, 60 seconds and 360 seconds. At this stage, the solubility volume of the samples decreases, in other words, the conversion rate of DON accumulation rate. Fungi samples tend to produce more DON toxin rather than T2 in the same environment and under the same conditions.

Conclusions

This study has confirmed that argon plasma jet system has destructive effects on mycotoxins such as DON , and also on the microorganisms which produce mycotoxins. Although the physicochemical properties and structural changes of mycotoxins were not explained under plasma jet treatment,this study revealed that the plasma system needs certain conditions like; time and toxin concentration of food in order to remove and inactivate mycotoxins.

Acknowledgment
With special thanks to The Research and Technology deputy of the Islamic Azad University, Tehran Branch.

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