Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.24, 2015
www.iiste.org

Evaluation the Non-Thermal Plasma Application Activity in AFB_1 Detoxification

Muna T. AL-Mossawei[1], Labeeb A. AL-Zubaidi[2], Saja Y. Abduljaleel[3], Hammad R. Humud[4]
1 Department of Biology, College of Science for Women, University of Baghdad. Iraq.
2 Directorate of Environmental and Water, Ministry of Science and Technology. Iraq.
3 Department of Biology, College of Education for Women, University of Anbar, Iraq.
4 Department of Physical, College of Science, University of Baghdad, Iraqi.

Abstract
Contamination raw agricultural materials has been a food safety concern for human and animals. A non thermal plasma or cold plasma is a novel antimicrobial intervention, that can be reduce the level of aflatoxin B_1(AFB_1) in complete cow's feed samples .AFB_1 are carcinogenic compound produce primarily by two certain strain of *Aspergillus* include *A. flavus* and *A. parasiticus* , the contamination of feed is arise for animals health. Fifty samples from complete cow's feed were designated into imported 15 samples and local 35 samples , obtained randomly from different region in Baghdad from March 2014 to February 2015. Samples were tested for AFB_1 by ELISA and HPLC technique and exposure to application cold plasma protocol to treatment of AFB_1 contamination the samples in different time (5, 10 and 15) seconds at 3.5 cm between the plasma source and samples then tested by ELISA and HPLC. There are appears the best time successful in reducing levels of toxin at 10 sec. in local samples 3.12, 0.05 ng / g imported samples 1.21, 6.19 ng / g in HPLC and ELISA. In local and imported samples at 10 sec and that was 15 sec less time periods ability to reduce toxin level in local and imported samples, that indicated the length of exposure to NTP application is not necessary to reduce toxin level. According to the study we observed that the results from ELISA method were more sensitive, accuracy and simplicity when compared with results from HPLC technique.
Keyword: Cold plasma, decontamination,AFB_1, HPLC, ELISA.

1- Introduction

Mycotoxins are structurally diverse fungal products produced by fungi that, not necessary for the growth of fungi and produced periodically under fungal stress (Martins *et al*., 2008). Some strains of fungi that (*A. flavus* and *A. parasiticus*) are produce aflatoxin. (Binder *et al.,* 2007 ; Kensler, 2011). Aflatoxin B_1(AFB_1) is the most potent toxin among different types of aflatoxins , it has been considers as a group I carcinogen for humans by International Agency for Research on Cancer (IARC, 1993; Seo *et al*., 2011; Ahmad *et al*.,2014; Manso *et al*., 2014). AFB_1 found in feed of lactating animals and about (0.3–2)% of them in animal feed is transformed to 4-hydroxylated metabolite in liver and then is excreted in milk as aflatoxin M_1 (Shouman *et al*., 2012). It is known that AFB1 causes teratogenicity, immunotoxicity, hepatotoxicity, and even death in farm animals and humans (Quintana *et al*., 2012). The Food and Agriculture Organization (FAO) estimates that many basic foods could be contaminated with mycotoxin producing fungi, contributing to huge global losses of foodstuffs, about 1000 million metric tons each year (Bhat and Karim, 2010). as well as in trade barriers unwanted raw materials and consumer products (Quintana *et al*., 2012). A non-thermal plasma (NTP) is an emerging non-thermal technology with potential applications for decontamination in the food industries , it can be used for the surface decontamination of raw produce, dried nuts and the packaging materials act).(Misra *et al*., 2011).. In NTP, also called non-equilibrium plasmas ,the gas remains at low temperature, because the energy transfer from electrons is less effective than cooling of ions and uncharged molecules (Fridman *et al*., 2008; Niemira, 2012)..Although NTPs are at room temperatures, they are extremely effective in producing activated species, e.g., free radicals and excited state atoms (Rahman *et al*., 2015).. Accordingly, in the past, NTP or cold plasma is used for sterilization of sensitive materials and now it is extended to food industries as a novel technology, Overall application of cold plasma for microbial destruction on different food substrates was discussed. Besides this, It is an eco-friendly process which is used in the preservation of food and other potential applications as an alternative to common techniques. (Basaran *et al.* 2008; Selcuk *et al*. 2008;Thirumdas *et al*., 2014).

In Iraq there is little or absence research in application of NTP technology in raw agricultural materials, therefore, the current research focused on the NTP application in detoxification or reducing AFB_1 in contaminated complete cow's feed .

2- Materials and Methods

2-1 Samples Collection.

Fifty complete cow's feed samples were randomly collected from feed production sites in different region in Baghdad during March 2014 to February 2015. two kilogram of each samples, there are designated into imported 15 samples and 35 local samples placed into suitable container to be avoid from any moisture that may

be cause growing fungi and increasing the amount of aflatoxin, then transferred to the laboratory until analysis (AOAC, 2005 ; Xiang *et al.*2006).

2-2 Preparation of AFB_1 Standard Curve

The standard AFB_1 solution was prepared according to AOAC (2000a) with some modification in acetonitrile at a concentration of 25 µg/ml to prepare stock solution and kept at -20 ^{0}C. The standard curve drawn with concentrations (1.25, 2.5, 5, and 10) ppm of AFB_1 apposite area by using HPLC technique Fig.1.

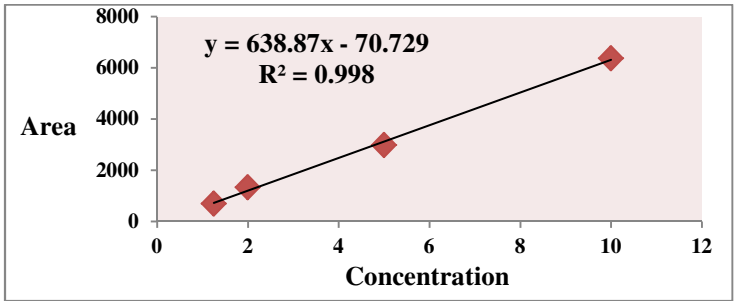

Fig.1: Standard curve of AFB_1 concentrations by using HPLC technique.

2-3 Extraction of Aflatoxin B_1

The feed and grains where extracted according to (AOAC, 2000b). with some modification, when used methanol (90%) and hexane (1:1 v/v) in separation method.

2-4 quantity detection of AFB1.

The quantity analysis of AFB1 were detect by using HPLC according AOAC (1990). The concentration of aflatoxin for each sample could be measured by application area of any peak from HPLC analysis in the standard curve equalities to gain the AFB1 concentration of the samples. For determination AFB_1 by ELISA The reagent and samples must be prepared according to the recommended Bioscientific Kit instruction. The samples 5 g were ground, add 25 ml of 70% methanol and shaker and centrifuges for 10 minutes at 4000 rpm. Dilute 1 ml of the obtained supernatant with 1 ml of 1x PBS. Vortex the samples well then use 50 µl of the diluted supernatant per well in the test. The determination of AFB_1 can be calculated by using special program with Excel functionality for Bioscientific Company.

2-5 Effect of NTP in Reduction of AFB_1 in Complete Feed Samples

To application the NTP for detoxification of AFB_1 in complete cow's feed samples were taken 50g were put in suitable containers of 100 ml capacity, divided these samples into three groups depending on the duration of exposure to the application of NTP (5, 10 and 15) sec. at 3.5 cm and used Argon gas (Nimeria, 2012a). Flow rate of air (1 liters/min) (Niemira and Sites, 2008). AC voltage variable (7.5 – 8.5 KVand 28 khz) .Was then seal samples and transported to the laboratory for analysis using HPLC, and ELISA.

3- Statistical Analysis.

The statistical analysis was conducted to extract the Mean ± Standard Error. The averages were tested using polynomial Duncan test (Duncan, 1955). Test the differences between the averages in the experiences of the effectiveness of different Numbers separately compared to the control using T-test (Steel and Torrie, 1980).

4- Result and Discussion

Table (1) were shown that all the time periods for NTP has been reduced AFB_1 level in complete cow's feed samples at the space 3.5 cm when compared with control models. The exposure complete cow's feed samples to NTP at 10 sec. was the best time period to reduce the toxin level in local and imported feed samples (3.12 and 1.21) ng/g respectively by HPLC, while (0.05 and 6.19) ng/g respectively by ELISA, while 15 sec. was less able to reduce toxin level in local and imported samples according to the result by HPLC and ELISA, also found no significant differences between treatments but it found mathematical difference, the significant differences can be shown between the treatments when compared with control ($p \leq 0.05$). In this study showed that the texture of materials affected during long exposure to NTP application that can be observed during the extracted.

Table (1): The Results of Detoxification aflatoxin B_1 by non-thermal plasma in Complete Cow's feeds by using HPLC and ELISA Technique.

Time (second)	HPLC	ELISA
	Mean ± SE	Mean ± SE
Local Control	17.34 ± 0.589[c]	45.21 ± 1.837[b]
5	4.5 ± 0.34 [a]	6.92 ± 0.33[a]
10	3.12 ± 0.288 [a]	0.05 ± 0.023[a]
15	11.1 ± 0.07 [b]	17.207 ± 0.47[ab]
Imported Control	53.37 ± 0.613 [b]	54.21 ± 1.259[b]
5	2.37 ± 0.187 [a]	7.1 ± 0.185 [a]
10	1.21 ± 0.123 [a]	6.19 ± 0.45 [a]
15	3.4 ± 0.19 [a]	18.867 ± 0.54 [a]

- Different letter into Colum refer to significant differences.
- The probability ($p \leq 0.05$).
- The mean as resulted triplicate.

In Fig. (1 and 2) can be shown that, ELISA was more sensitive to detection AFB_1 than HPLC, because AFB_1 bound to antigen protein and the ELISA technique depended on interaction between antibody and antigen, also these technique characterized by simplicity, sensitivity and adaptability (Almeida *et al*., 2011).

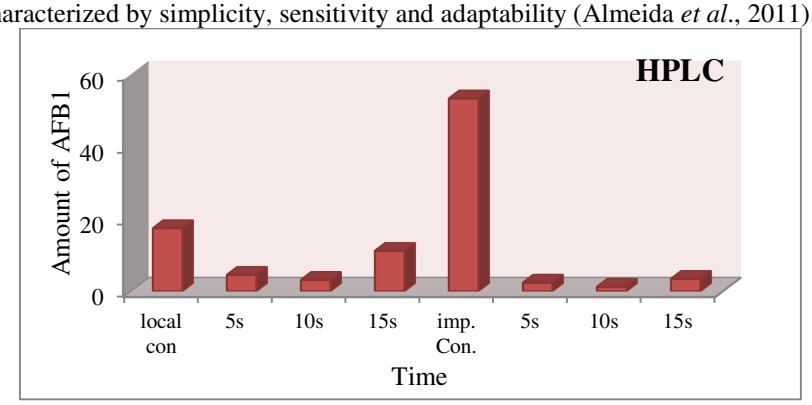

Fig.1: The compression in concentration of aflatoxin B_1(ng/ g) in complete cow feed by using HPLC.

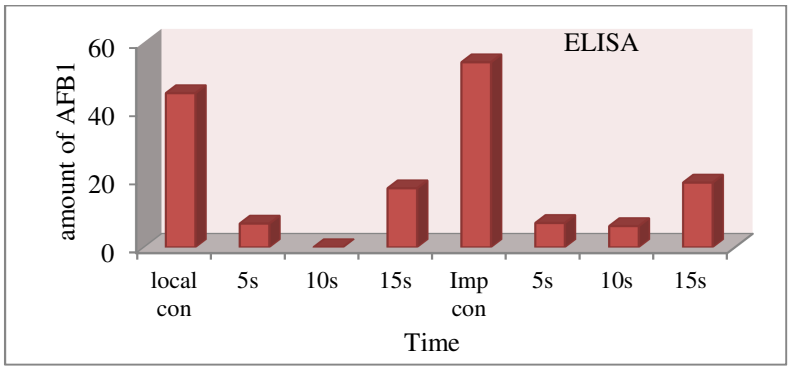

Fig. 2: The compression in concentration of aflatoxin B_1(ng/ g) in complete cow feed by using ELISA

The results of this study are corresponding to the research for Niemira (2012a) were suggested that the best period time for reduction *E. coli* O157:h7, *Salmonella, Listeria monocytogenes, Staphylococcus aureus* by cold plasma can range from 120 sec. to as little as 3 sec. Another study by) Niemira (2012b) was confirmed the best time period for the elimination of *Salmonella* and *E. coli* O157:H7 from dry almonds by cold plasma is at 20 sec. at a distance of 6 cm, also confirmed that the longer duration of treatment did not always result in enhanced reductions. many of study suggested that the food qualities of wheat and beans not affected or only marginally affected were exposure to cold plasma at 30 sec. to 30 min. and the seeds were found to be viable post plasma processing (Selcuk *et al*., 2008). The affects of cold plasma on nutritional and chemical properties of food is not known well due to few studies on the application of this technology in real food system (Afshari and Hossenini, 2014).The study of perni *et al*.,(2008). were observed the pericarps of melon and mangoes that inoculated by

Saccharomyces cerevisea, pantoea agglomerans, E. coli and *Gluconacetobacter liquefacien* after exposure to NTP ,the detection limit of these microbial corresponding to 3 log after only 2.5 sec. on both fruits whereas *E. coli* required 5 sec. to reach the same level of inactivation.

5- Conclusion

Fifty complete cow's feed samples were exposure to NTP or cold plasma as physical application to reduce AFB_1 levels in complete cow's feed samples in difference time and shown that 10 sec as best period also shown that the length of exposure to NTP application is not necessary to reduce toxin levels and observed that lead to affected on texture of these materials.

Reference

- Afshari, R. and Hosseini, H. (2014).Non-Thermal plasma as a new food preservation method, Its present and future prospect. J. Paramedical Sciences, Vol, 5, No, (1): 116-120.
- Ahmad MM, Ahmad M, Ali A, hamid R, javad S and abdin MZ. (2014). Detection of Aspergillus flavus and Aspergillus parasiticus from aflatoxin-contaminated peanuts and their differentiation using PCR-RFLP. Ann Microbiol. 10: 0803-0805.
- Almeida, IFM.; Martins, HML.; Santos, SMO.; Feritas, MS.; de Costa, JMGN.; and Bemardo, FMD (2011). Mycotiota and aflatoxin B1 in feed from farmed sea bass (dicentrachus ladrax) . J.Toxins (3), 163-171.
- AOAC. Association of Official Analytical Chemists. (2005)."Official methods of analysis 994.08 – Derivatization of standards for aflatoxins", Chapter 49.
- AOAC. (2000). Official methods of analysis. Vol. 17. Association of Official Analytical Chemists. Washington, D.C. A
- AOAC.(1990). Official methods of Analysis of the Association Office of Analytical Chemists. 15th Ed. Helrick K.K., Ed.,AOAC. Arlington, VA. Section 970.40.
- Basaran, P.; Basaran-Akgul, N.; and Oksuz, L. (2008). Elimination of *Aspergillus Parasiticus* From nut surface with low pressure cold plasma (LPCP) treatment.. Food Microbiology, 25(4), 626-632.
- Bhat, R., Rai, R. V. and Karim, A. A.(2010). Mycotoxins in Food and Feed: Present Status and Future Concerns. Comprehensive Reviews in Food Science and Food Safety 9: 57-81.
- Binder, E.M.; Tan, L.M.; Chin, L.J.; Handl, J.; Richard, J. (2007) Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Tech., 137, 265–282.
- Duncan, D. B. 1955. Multiple range and multiple F- test biometrics 11: 1-42
- Fridman, G.; Friedman, G.; Gutsol, A.; Shekhter, A.B.; Xasilets, V.N. and Fridman, A. (2008). Applied Plasma medicine. Plasma Processes and Polymers, 5, 503-533.
- IARC,W. (1993). Some naturally occurring substance. Food and irems and constituents, heterocyclic aromatic amines and mycotoxins, IARC. Monograph on the evaluation of carcinogenic risks to humans.
- Kensler, T.W.; Roebuck, B.D.; Wogan, G.N.; Groopman, J.D.)2011). Aflatoxin 50- year odyssey of mechanistic and translational toxicology. Toxicol Sci. , 120, S28–S48.
- Manso,S. D. pezo, R. Gomez-Lus, N. Nerin, (2014).Diminution of aflatoxin B1 production caused by an active packaging containing cinnamon essential oil, Food control 45)101-108.
- Martin, HM.; Marques, M.; Almeida,L.; Guerra, MM.; and Bemardo, F. (2008) Mycotoxins in feedstuffs in Portugal: an overview. Mycotoxin research. 24(1). 19-23.
- Misra N.N. , Tiwari B.K., Raghavarao K.S.M.S and Cullen P.J.(2011). Nonthermal Plasma Inactivation of Food-Borne Pathogens. Food Engineering Reviews, Volume 3, Numbers 3-4 :159-170.
- Niemira, BA. (2012a)... Cold plasma decontamination of foods. Annual review of food science and technology,Vol 3:123-142.
- Niemira, BA. (2012b). Cold plasma reduction of *Salmonella* and pressure gases. J. Food Science, 77(3), 171-175. *Escherichia coli* O157:H7 on almonds using ambient
- Niemir B.A.;and Sites, J.(2008). Cold plasma Inactivates *Sallmonella* Stanley and Escherichia coli O157:H7 Inoculated on Golden Delicious Apples. J. Food Protection ,71(7) : 57-65.
- Perni, S.; Liu, DW.; Shama, G.; kony, MG. (2008). Cold Atomospheric plasma decontamination of the pericarps of fruit. J. Food Protection, 71(2): 302.
- Quintana, RV.; Alarcon, JS.; Tenorio, MG.; Deng, Y.; Waliszewski,SM.; and Valera, MA. (2012). Preventive Strategies alimed at reducing the health risks of aflatoxin B1 . Toxicology of inviroment and health science. 4 (2) 71-79.
- Rahman Z., Rahman H., Rahman A.(2015). Classification and Generation of Atmospheric Pressure Plasma and Its Principle Applications .International Journal of Mathematics and Physical Sciences .Vol. 2, Issue 2, pp: (127-146).

- Selcuk, M.; Oksuz, L.; Basaran,P.(2008) Decontamination of granis and legumes infected with *Aspergillus* spp. And *Penicillum* spp. By cold plasma treatment. Bioresource. Technology 99(11):5104-5109.
- Seo, J. H., Min, W. K., Kweon, D. H., Park, K. and Park, Y. C. (2011). Characterisation of monoclonal antibody against aflatoxin B(1) produced in hybridoma 2C12 and its single-chain variable fragment expressed in recombinant Escherichia coli. Food Chemistry 126: 1316-1323.
- Shouman, BO.; Doaa El Morsi, DE.; S habaan,S.; Abdel-Hamid, AH.; and Mehrim, A. (2012). Aflatoxin B1 level in relation to childs feeding and growth. India journal pediatr. 79 (1) : 56-61.
- Steel, R. G. D. and Torrie, J. H. 1980. Principle and Procedure of Statistics. 2nd Ed. McGraw Hill: New York.
- Thirumdas R., Sarangapani C. and Annapure U.S.(2014).Cold Plasma: A novel Non-Thermal Technology for Food Processing . Food Biophysics.Vol. 9(3).193-300
- Xiang, Y.; Y. Liu, and M.L. Lee. (2006). High performance liquid chromatography. J. of performance Chromatography A, 1104(1-2) : 198-202.