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Effect of Ozonation on some Mycotoxins and Fungi Associated in Stored Grain from Qalubyia Governorate

El-Sayed T.I.[1], El-Desouky T.A.[2] and Abd El Aziz A. M*.

[1] Faculty of Science, Botany Department, Benha University, Qalubia, Egypt.
[2] Food Toxicology and contaminants Department, National Research Center, Dokki, Cairo, Egypt.

ABSTRACT

This study assessed the efficacy of the ozonation on total fungal colonies (TFC) associated in grains samples at 30 and 60 ppm with exposure time 10 and 20 min. also impact of ozonation on reduction of aflatoxins (AFs) and ochratoxin A (OTA) in spiked grain samples with 10 (µg/kg). Results showed that the infection percent of TFC decreased after ozonation at 30 ppm for 20 min to 87.5%, 66.6%,.85.7% and 100% in case of white corn ,yellow corn , wheat and rice samples, respectively. While the infection percent decreased to 100% after ozonation at 60ppm for both exposure time 10 and 20min. also the results reflected that the percentages of reduction of AFG1, AFB1, AFG2 and AFB2 were 81.7, 78.5, 83.8 and 74.8% with white corn. While in yellow corn samples were 74.4, 80.8, 80.2 and 79.5%, respectively. Whereas rice samples the percentages of reduction were 87.5, 84.2, 82.5 and 86%, respectively.in addition the results showed the percentage of reduction of OTA after ozonation at 60 for 10 and 20 min with white corn ,yellow corn , wheat and rice. after ozonation for 10min the reduction percentage of OTA into artificially contaminated white corn, yellow corn, wheat and rice were 54.8, 62, 73.4 and 75.6, respectively. After exposure time for 20 min the reduction percentage of OTA was73.8, 82, 86.5 and 87.2% with white corn, yellow corn, wheat and rice.

Keywords: Aflatoxin, ochratoxin A , grain and ozonation.

Corresponding author

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INTRODUCTION

Many food crops are susceptible to fungal attack either both in the field and storage. Some of these fungal species can produce as secondary metabolites a diverse group of chemical substances known as mycotoxins. Aflatoxins (AFs) and ochratoxin A (OTA) are a group of highly carcinogenic mycotoxins produced by three genera: AFs, produced by some *Aspergillus* species, OTA produced by both *Aspergillus* and *Penicillium* [1]. Mycotoxins have been reported to be carcinogenic, teratogenic, mutagenic, immunosuppressive, tremorgenic, hemorrhagic, hepatotoxic, nephrotoxic and neurotoxic [2].Fungal growth and mycotoxin production only occur under three categories: physical, nutritional and biological factors such as temperature, relative humidity of the atmosphere, water activity and moisture. in addition the mycotoxins production in the substrate can happen in the field and in storage conditions between 20 and 40 ºC with a 10- 20% of moisture and 70-90% of relative humidity in the air [3-5].Fungal growth on stored grain causes significant reductions in both the quantity and the quality of the grain. For example, in the United States, more than 400 million tons of grain are stored every year and the annual storage losses caused by fungi and insects are estimated at more than 500$ million **[**6]**.**The development of AFs in stored grains can be avoided by controlling the growth of toxin-producing molds. Fungal growth can be prevented by ensuring that moisture and temperature conditions favorable to growth and proliferation do not occur [7]. Once the mycotoxins contamination has occurred, control measures must be established and applied to reduce the risk of exposure to these toxins. Necessary approaches include physical, chemical or biological removal, or use of chemical or physical inactivation. One method of decontamination for AFs-affected commodities that has been a focus of attention is ozonation, a physical/chemical oxidation method [8].Ozone gas is a powerful oxidant capable of reaction with numerous chemical groups. Ozone is a powerful oxidising agent with a demonstrated ability to reduce populations of bacteria and fungi in a diversity of use situations [9]. So the objective of this work was study the effect of ozonation on total fungal colonies and degradation of AFs in some grains purchased from local markets of Qalubyia governorate.

MATERIALS AND METHODS

Materials

Sampling

Samples of grains destined for human consumption were randomly purchased from local markets of Qalubyia governorate. Collected samples were conserved in plastic bags and then stored in a dark and dry place until analysis.

Solvent and Chemical

Methanol, acetonitrile, sodium chloride, isopropanol and tri Fluor acetic acid were purchased from Sigma chemical Co.(St. Louis, MO, U.S.A.). Potato dextrose agar (PDA) was obtained from Sigma-Aldrich, France. All solvents were of HPLC grade. The water was double distilled with Millipore water purification system (Bedford, M A, USA).

Aflatoxins and ochratoxin A standards

AFs and OTA standards were purchased from Sigma, chemical Co. (St.Louis, MO, U.S.A). Stock solutions and standards were prepared and assayed according to AOAC Method 971.22, 2000).

Apparatus

Wrist-action shaker, Burrell Model 75 or require lent (Burrell corp., Pittsburgh, PA, USA); Blender; filter paper-reeve angel 802, 32cm diameter, pre-pleated (WhatmanInc, Clifton, NT). Auto sampler vial (5, 10 ml) with Teflon lined crimp top (hew lett- pakard, avondale, PA); the immune affinity column AflaTes® and OchraTest HPLC were obtained from VICAM (Watertown, MA, USA).The High Performance Liquid Chromatography (HPLC) system consisted of Waters Binary pump Model 1525, a Model Waters 1500 Rheodyne manual injector, a Watres 2475 Multi-Wavelength Fluorescence Detector,

and a data workstation with software Breeze 2.A phenomenexC_{18} (250x 4.6 mm i.d), 5 um from Waters corporation (USA

Ozone generator

Ozone gas was produced from air using ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford,Quebec Canada (http://www.ozomax.com).

Methods

The (PDA) was prepared according to the manufacturer's directions by adding 40 g of the dehydrated PDA to 1000 ml of distilled water. The culture media was then mixed and autoclaved at 121°C for 15 min.

Preparation of artificially grains samples

Three replicates of each whole (white corn, yellow corn, wheat and rice) samples were spiked with 10 µg/kg for AFs including (AFG_1, AFB_1, AFG_2 and AFB_2) and followed by shaking to 10-15 min. In addition samples control with spiked without treatment (O3) and samples control without spiked with treatment. Standard stock was prepared according to the (AOAC,2000) method.

Preparation samples treatment

The sample was transferred into a 500mL flask and the sample flask was plugged with a silicone stopper with 2 holes in it. One hole was for the ozone line and the other was for tubing connected to the ozone destruct unit. The samples were treated for 10 and 20 min at room temperature with two different ozone concentrations (30 and 60 ppm). Ozone gas was produced from air using ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (http://www. ozomax.com).

Detection and determination of mycotoxins

Aflatoxin and Ochratoxin A, were extraction in samples using the immune affinity column according to [10]. Determination of AFs and OTA by High Performance Liquid Chromatography (HPLC) was according to [11].

RESULTS AND DISCUSSION

Impact of ozonation on fungi contaminated grains

Data in Fig (1) show the total fungal colonies (TFC) associated in grains samples were ozonation at 30 ppm for 10 and 20 min. The results indicated that the total fungi colonies count on the plate agar media in control samples from (white corn, yellow corn, wheat and rice) were 8 TFC, 6 TFC, 7 TFC and 4 TFC, respectively. The infection percent (total fungal colonies) decreased after ozonation at 30 ppm for 10 min to 75%50% , 71.4%and 50% in case of white corn yellow corn , wheat and rice samples, respectively While The infection percent (total fungal colonies) decreased to 87.5%,66.6%,.85.7% and 100% when ozonation for 20 min at the same dose of ozone gas . Exactly the contrary the infection percent reached to 100% after ozonation at 60ppm both 10 and 20 min Fig (2). Ozone in gaseous or aqueous form is reported to reduce levels of the natural microflora, as well as bacterial and fungal contamination in grain. Fungal inactivation and subsequent decontamination of toxins depends upon several factors including ozone concentration, exposure time, pH and moisture content of the grain mass [12]. According to many researches ozonation destroys microorganisms by the progressive oxidation of vital cellular components. The cell surface has been suggested as the primary target of ozonation. Two major mechanisms have been identified in ozone destruction of the target organisms. First mechanism is that ozone oxidizes sulfhydryl groups and amino acids of enzymes, peptides and proteins to shorter peptides. The second mechanism is that ozone oxidizes polyunsaturated fatty acids to acid peroxides. Ozone degradation of the cell envelope unsaturated lipids results in cell disruption and subsequent leakage of cellular contents. Double bonds of unsaturated lipids are particularly vulnerable to ozone attack [13- 16]. On the other hand cellular death can also occur due to the potent destruction and damage of nucleic acids. Thymine is more sensitive to ozone than cytosine or uracil. .Since the cell wall of the

fungus is made up of several layers containing 80 % carbohydrate and 10% protein and Glycoprotein also contains many cells on the wall of the bilateral ties of sulfur, which makes them subject to oxidation by ozone [17].

Impact of ozonation on Aflatoxins and ochratoxin A in artificially contaminated grain

In the study we investigated the influence of ozonation at 60 ppm for exposure time 10 and 20 min on the degradation of AFG_1, AFB_1, AFG_2 and AFB_2 inartificially contamination grain .

Data presented in Fig (3) showed the percentages of reduction of AFs in artificially contaminated grain 10 µg/kg after ozonation at 60 ppm for 10 min with white corn were 62.7, 57.2, 63.4 and 60.4% for AFG_1, AFB_1, AFG_2 and AFB_2, respectively. While in case of yellow corn samples the percentages of reduction of AFG_1, AFB_1, AFG_2 and AFB_2 were 58.5, 58.2, 61.2 and 60.5%, respectively. The percentages of reduction of AFG_1, AFB_1, AFG_2 and AFB_2 were 66.6, 61.4, 65.7 and 65% with wheat sample. While the rice samples showed the highest percentages of reduction for AFG_1, AFB_1, AFG_2 and AFB_2 were 68.1, 62.6, 6.2 and 68%, respectively. On the other hand, observed increased on the percentages of reduction for of AFG_1, AFB_1, AFG_2 and AFB_2 after ozonation for 20 min as shown in Fig (4). The percentages of reduction of AFG_1, AFB_1, AFG_2 and AFB_2 were 81.7, 78.5, 83.8 and 74.8% with white corn. Whereas rice samples the percentages of reduction of AFG_1, AFB_1, AFG_2 and AFB_2 were 87.5, 84.2, 82.5 and 86%, respectively.

Data presented in Fig (5) showed the percentage of reduction of OTA after ozonation at 60 for 10and 20 min. after ozonation for 10min the reduction percentage of OTA into artificially contaminated white corn, yellow corn, wheat and rice were 54.8, 62, 73.4 and 75.6, respectively. After exposure time for 20 min the reduction percentage of OTA was73.8, 82, 86.5 and 87.2% with white corn, yellow corn, wheat and rice, respectively. Effects of ozone treatment on decontamination of toxins depends upon several factors including ozone concentration, exposure time, pH and moisture content of the grain. [18] suggested that the primary reaction site of ozone in AFB_1 is the C_8-C_9 double bond at the terminal furan. This reaction resulted in the formation of aflatoxin molozonide which is further change to aflatoxin ozonide. This compound is unstable and change to aldehydes, Ketones, acids and CO_2 as shown Fig (6).

Ozone acts by direct or indirect oxidation by ozonolisys, and by catalysis. The three major action pathways occur as follows (1) direct oxidation reactions of ozone, resulting from the action of an atom of oxygen, are typical first order, high redox potential reactions.(2) in indirect oxidation reactions of ozone, the ozone molecule decomposes to form free radicals which react quickly to oxidize organic and inorganic compounds.(3) ozone may also act by ozonolisys, by fixing the complete molecule on double linked atoms, producing two simple molecules with differing properties and molecular characteristics[19].

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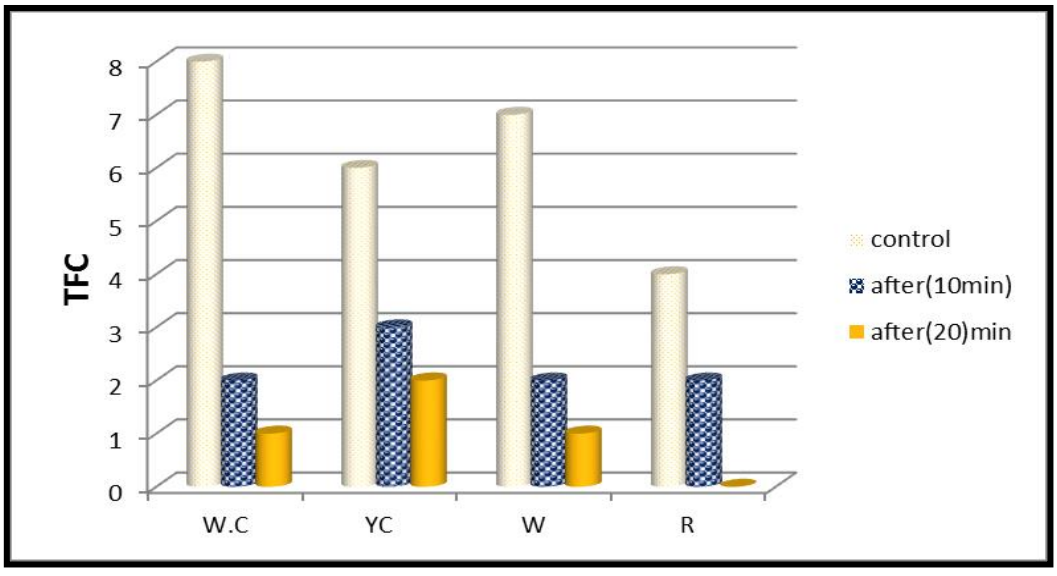

Fig.(1): Effect of ozone (30)ppm for 10 , 20 min on total fungal colonies (TFC) associated cereals samples.

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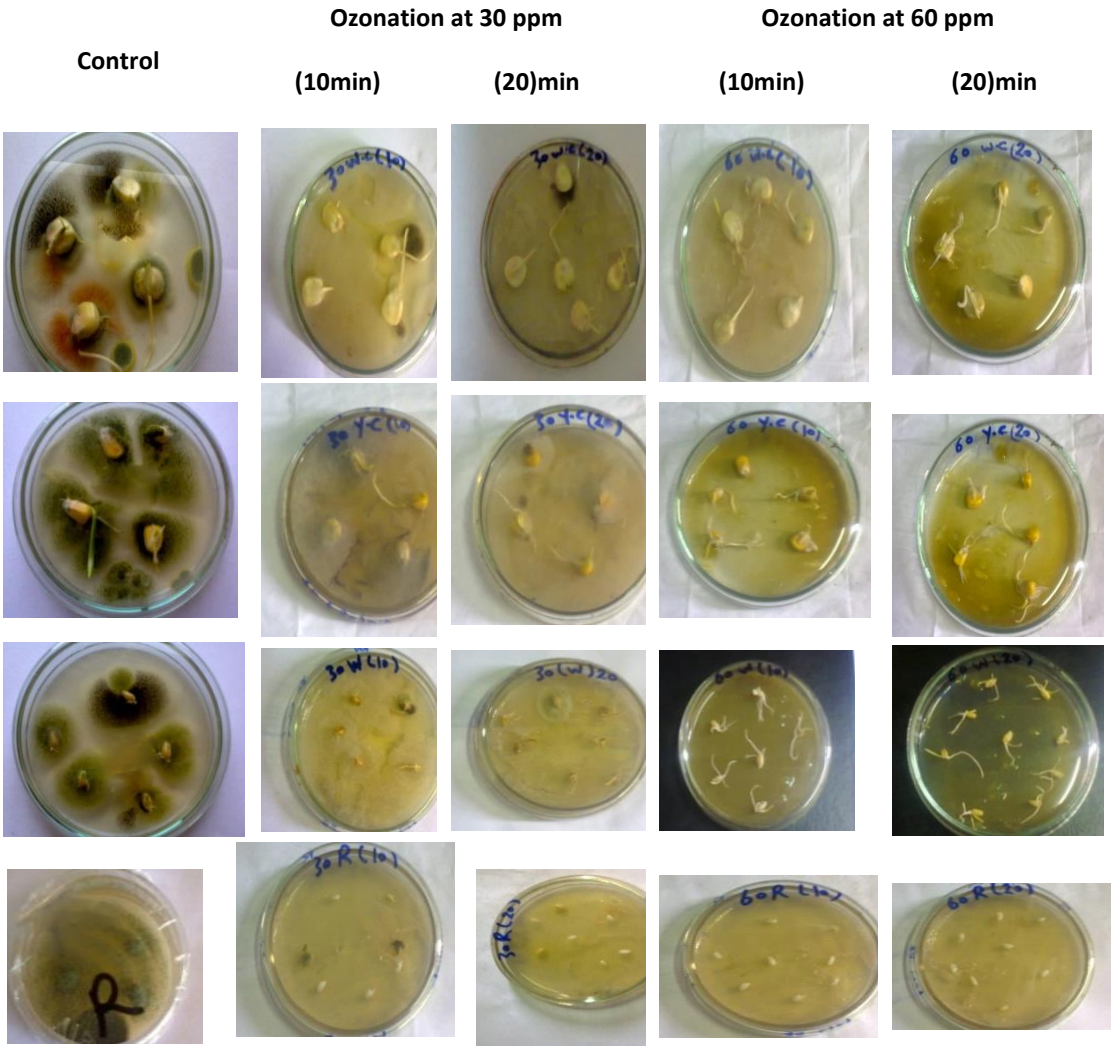

Fig.(2) Effect of ozonation on Total fungal colonies (TFC) in grain samples.

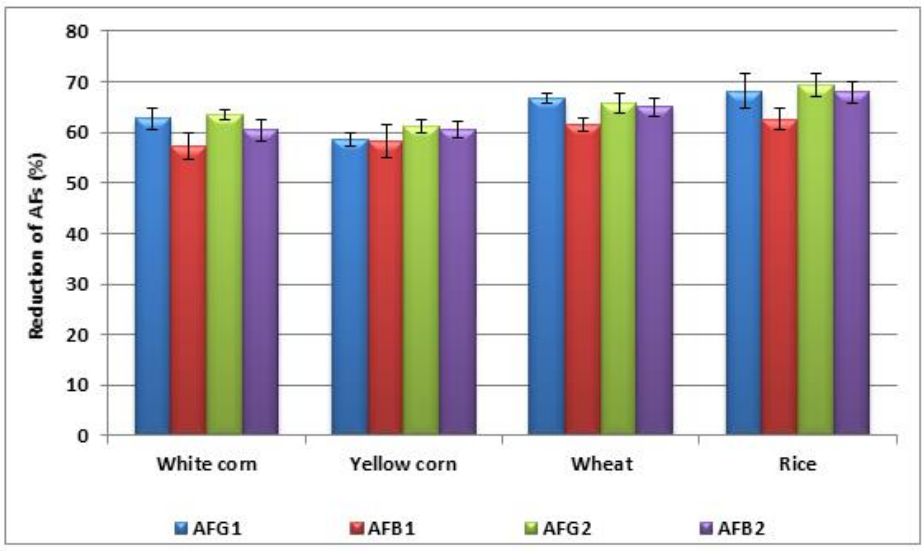

Fig (3): The percentage of reduction AFs after ozonation at 60 ppm for 10 min in spiked grain samples with 10 (µg/kg).

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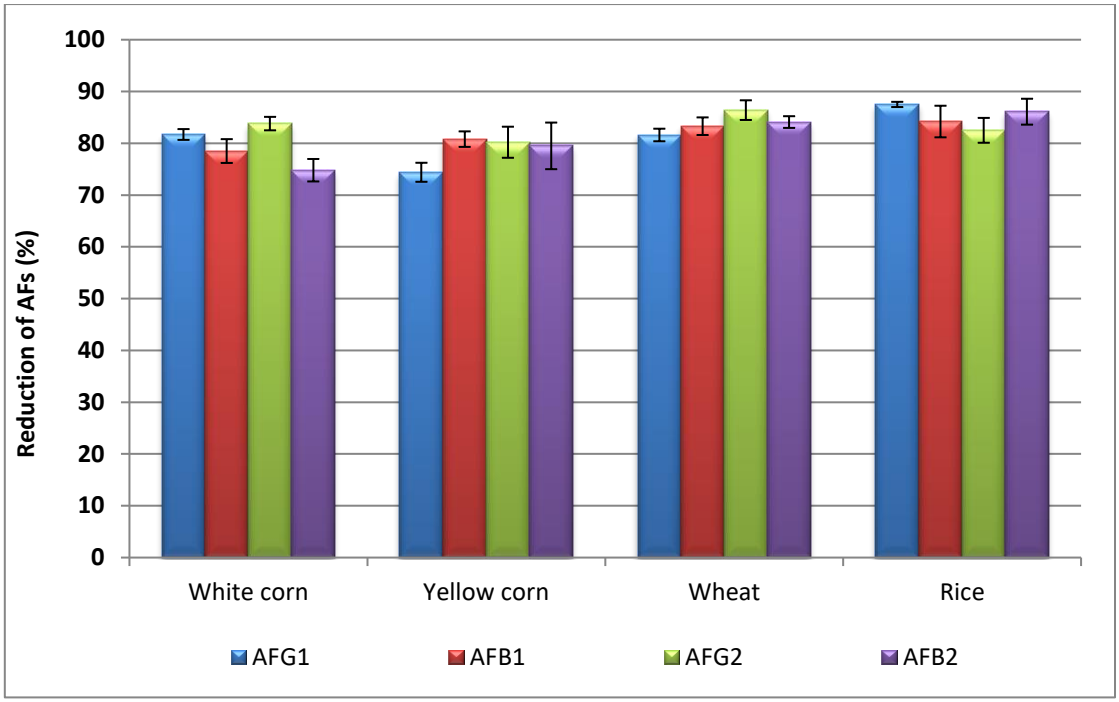

Fig (4): The percentage of reduction AFs after ozonation at 60 ppm for 20 min in spiked grain samples with 10 (µg/kg)

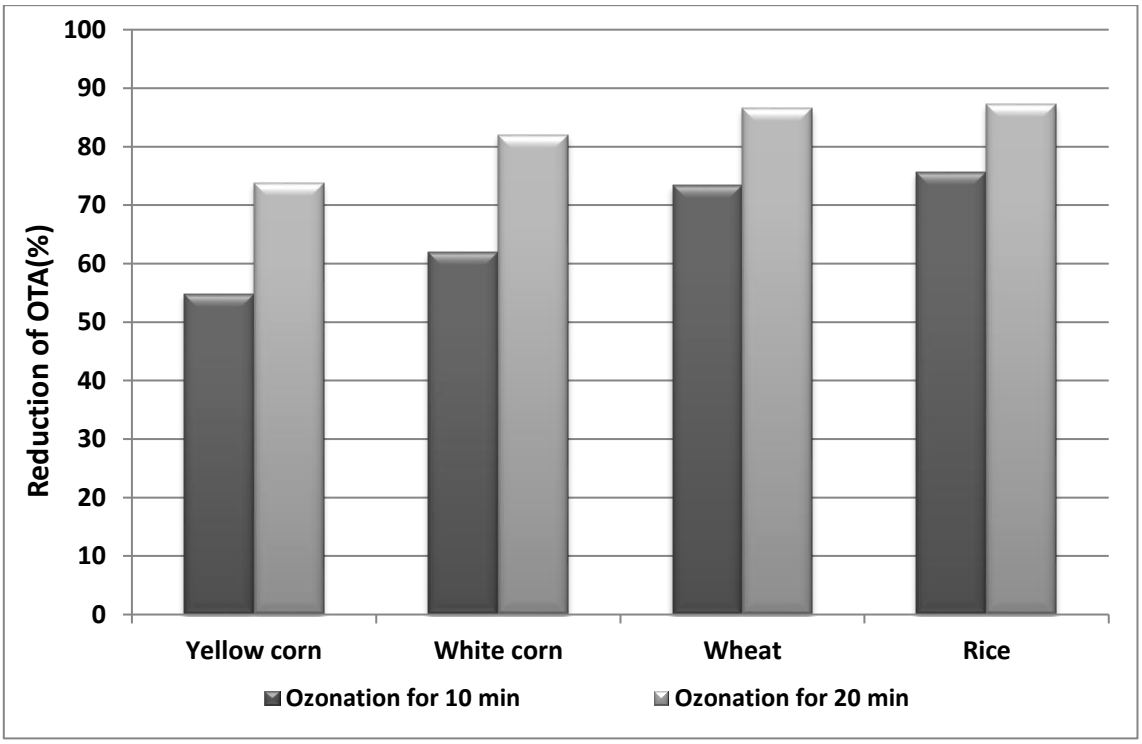

Fig (5): The percentage of reduction OTA after ozonation at 60 ppm for 10 and 20 min in spiked grain samples with 10 (µg/kg).

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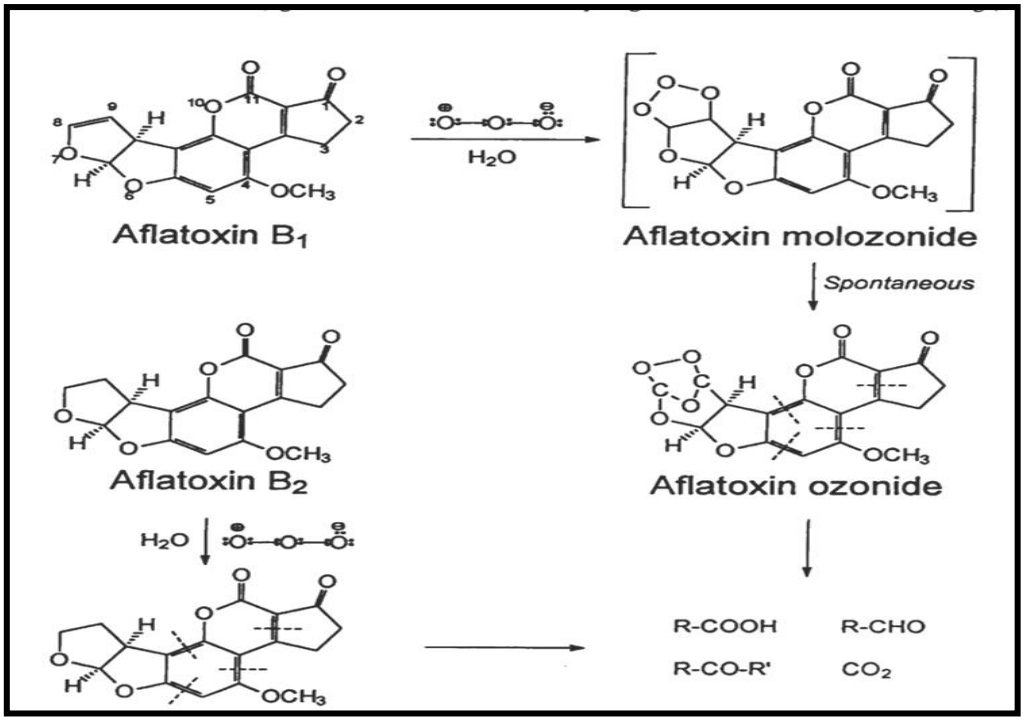

Fig (6): Mechanism for the addition of ozone to AFs accordtin g to [18].

CONCLUSION

Ozone degraded AFs and OTA in grains, and generally there was significant variation between ozonation time on reduction of AFs and OTA. Consequently, the treatment with ozone at 60 ppm for 20 min could be an effective method for protection of grain before storage from the growth of fungus and production of toxins during storage.

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