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ZEARALENONE: INCIDENCE TOXIGENIC FUNGI AND CHEMICAL DECONTAMINATION IN EGYPTIAN CEREALS

(With 3 Tables)

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الزيرالينون ومدى انتشاره والفطريات المنتجة له وتكسيره كيمائيا فى بعض الحبوب المصرية

السيد عبد الله

فى هذه الدراسة تم البحث عن مدى تواجد الزيرالينون Zearalenone والفطريات المنتجه له فى عدد ١٥٠ عينة من الحبوب المصرية (٥٠ ذرة شامية ٤٥ أرز ، ٤٠ قمح) ثبت وجود الزيرالينون فى ١٥ عينة من حبوب الذرة الشاميةبمتوسط تركيز ٢٢ر٣٢ جزء / بليون وفى ٤ عينات الأرز بمتوسط ٥ر١٥ جزء /بليون ، وكذلك فى ٥ عينات من القمح بمتوسط ٨ر٨ جزء / بليون. ٧٩ عزلة من فطر الفيوزاريوم تنتمى الى ٩ أنواع تم عزلها من الحبوب المصرية وتم اختبارها لانتاج الزيرالينون فوجد منهم ٢٦ فقط منتجا له. تم دراسة تأثير H_2O_2 بنسب مختلفة (٣٪، ٥٪، ١٠٪) كمثبط للزيرالينون فى الحبوب المصرية الملوثة. أثبتت النتائج أن H_2O_2 يعتمد على تركيز درجة الحرارة كذلك مدة التعرض فوجد أن أعلى نسبة تثبط هى ٩ر٨٣ ٪ بتركيز ١٠٪ H_2O_2 عند درجة حرارة ٨٠ °م لمدة ١٦ ساعة تليها نسبة تثبط هى ٧٥٪ لنفس الظروف عند ٨ ساعات ثم أقل نسبة كانت لتركيز ٣٪ H_2O_2 عند ٥٠°م لمدة ساعتين.

SUMMARY

An investigation for occurrence of Zearalenone (ZEN) and toxigenic fungi in cereals (Corn, 50 samples; rice 45 samples; and wheat, 40 samples) collected from Egypt. ZEN was detected in 15 of 50 corn samples with an average 22.32 ppb. The incidence value of ZEN in rice samples was of 8.9% (4 samples of 45), and the average was 15.5 ppb. Out of 40 wheat samples 5 samples were cantaminated with ZEN (12.5%) with an average 8.8 ppb. Seventy-nine *Fusarium* strains belonged to 9 different species were isolated from Egyptian cereals, and tested for ZEN production, only twenty-six isolated were Zearalenone producer. Efficiency of H_2O_2 for destruction of ZEN in contaminated corn was studied at different concentrations (3%, 5% and 10). The results revealed that percent of disappearance of ZEN was found to be dependent upon the concentration of H_2O_2, temperature and period of exposure. whereas the highest percent of degradation was 83.9%, with 10% H_2O_2 at 80°C for 16 hr, followed by 75% at the same condition for 8 hr, while the lowest one obtained at 3% H_2O_2, 50°C for 2 hr.

Key words: *Zearalenone-Toxigenic fungi-Survey-Decomposition-Cereals.*

INTRODUCTION

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by mumerous species of Fusarium. Because of its relatively common occurrence in various cereal crops. ZEN has been implicated in numerous incidences of mycotoxicosis in farm animals (Bursian et al ., 1992).

ZEN has oestrogenic effects on humans (Schoental, 1983) reported precocious sexual development in puertorco associated with ZEN contaminated Food. Zearalenol, a metabolite of ZEN (Pathre and Mirocha, 1976). ZEN is found as its glucuronide adduct in the urine of cows, rats, rabbits, and swine (Mirocha et al., 1979). A recent worldwide survey (Tanaka et al., 1988) reports Zearalenone occuring in 58% n=45) of the corn samples callected in 19 different countries. In Egypt (Abd-El-Hamid, 1990) reported 56.3% of several Egyptian foods and feeds.

Samples were Positive with a contamination average of 46.3 ± 5.2 ppb and range of 2-426 ppb, 83.3% of the positive samples were contaminated with 10 ppb or more.

Some straims of *fusarium* sp. in addition to ZEN, other related metabalites such as alpha-zearalenol and zearalenone sulfate. Mirocha et al.,1979 and Plasencia & Mirocha, 1991). The objective of this research was to investigate the presence of Zearalenone in Egyptian cereals and to investigate potential toxin production of selected Fusarium isolated growing on rice, in addition the study concerning decontamination of zearalenone in cereals.

MATERIALS and METHODS

Cereal Samples:

Altogether 132 samples from corn, rice and wheat were Randomly collected from some Egyptian districts between January, 1993 and May, 1995.

Zearalenone Standard:

ZEN was obtained from Sigma Camp. USA.

Isolation of *Fusarium* isolates:

Fifty grains from each samples were surface sterilized for min in 2.5% of sodium hypochlorite. They were then washed several times with sterilized water dried between sterile paper according to the method of Lichtwardth et al., (1958). The disinfected grains planted on specific medium containing PCNB, for *Fusarium* Count (Tsao, 1970). Five were placed in each of 10 plates and incubated at 28 ± 2°C. Fungi growing out from the seeds of those plated during two weeks were picked and subcultured on PDA slants. Single spore or hyphal tip techniques were used for purification of different isolates which were identified to the species level according to Gilman (1957), Barnett and Hunter (1972) and Nelson et al., 1983).

Production of Zearalenone by *Fusarium spp*:

Fusarium spp. originally isolated from collected samples (corn, rice and wheat) in Egypt were tested for their ability to produce ZEN. The isolates were grown on Commercial (oxide) potato dextrose agar (PDA) for seven days at 25 ± 2°C. Cultures were then inoculated (1 Cm diameter of agar from a PDA plates of the *Fusarium spp.*) on autoclaved rice (100g) with 30% moisture content was placed in 1L Erlenmeyer flasks. The cultures were incubated for 4 weeks at 20°C.

Preparation of Contaminated Corn:

Each 200 g of corn of 30% moisture content was placed in 1 L. Erlenmeyer flasks. Then autoclaved for 20 min at 121°C. Each flask was inoculated with 1 cm diameter disks of agar from a PDA of . equiseti (NRRL, 6470). All the flasks were kept at a temperature of 25 ± 2°C for 30 days, During the first three days, corn culture were shaken periodically to disperse inoculum uniformaly. After the incubation the cultures were dried at 60°C. Then the corn were stored at -15°C unitle treatment and ZEN analysis.

Treatment of Contaminated Corn:

Each 500 g portions of contaminated corn were soaked in 750 ml of either 3%, 5%. or 10% solution of H_2O_2 in 2-Liter flasks for 2, 4, 8 or 16 hr. at either 50°C or 80°C. Solutions were removed by passing through a coarse filter. The soaked corn, which contained about 40% moisture was then dried in a forced oven at 80°C. The corn was ground and analyzed.

The ground cereal samples and the ground mouldy rice (50g) were placed into 500ml Erlenmeyer flasks. Then add 25ml H_2O_2, 25g. diat earth and 250ml $CHCl_3$ The flasks were shaked for 30 min on wrist action shaker. The extract was filtered through What. no. 4 filter paper, and evaported to near dryness under stream of N2. The residue was washed four times with 10ml hexane. Finally rinse with 10ml CH_3CN. The mixture was transfered to separatory funel. Separate CH_3CN layer (lower). The hexane layer was reextracted with 5ml CH_3CN. Fractions of CH_3CN were combined, and evaporated to dryness under gentle stream of N_2. The residue was transfered to vial with $CHCl_3$ and evaporated under gentle stream of N_2. The quantiative and confirmation were performed according to the method described by (AOAC, 1990).

RESULTS and DISCUSSION

Natural occurrence of Zearalenone in Corn, Rice and wheat in Egypt:

The results of survey for Zearalenone (ZEN) in corn, rice and wheat in Egypt are summarized in table (1). ZEN Contamination was detected in 15 of the 50 corn samples analysed. The level of contamination ranging from 10.4 to 45.2 ppb. 4 out of 45 samples of rice were contaminated (8.9%) The main level of contamination was 15.5 ppb, with a range 5.1 to 21.9 ppb. And also detectable ZEN was found in wheat sample (12.5%) whereas 5 out of 40 samples were contaminated. The level of contamination ranging from 4.9 to 12.7 ppb, with an average 8.8 ppb. Lee et al. (1991) reported that 10.2% rice and 9.3% of soybean samples were cantaminated with ZEN. The average levels of ZEN of rice and soybean samples were 11.78 μg/kg and 7.70 μg/kg, respectively. In Egypt, Abd El-Hamid (1990) reported that all of the maize samples tested were positive, but the hydrid maize was higher contaminated (up to 79 ppb)than the native (white) maize (up to 30 ppb). He also found that the wheat samples were negative, half of the wheat bran was positive with an average of contamination 55 ppb. Chulze et al. (1989) reported that 6% of corn from Argentina were contaminated with ZEN. Lee et al. (1987) reported that 21 of 28 samples of barley harvested in 1983. contained ZEN at concentration ranging from 1 to 202 ng/g. They also found that 29 of 31 samples harvested in 1984 contained the toxins from 3 to 83 ng/g.Wheat collected from chung-buk district of Korea in 1985 were contaminated by nivalenol. deoxynivalenol, and ZEN. Tanaka et al., (1985) reported that ZEN was detected in all 18 scabby wheat grains harvested in 1984 from eighteen farms in the Tokachi district of Hokkaido, a northern island Japan. The content averaged 189 ng/g. Lee et al. (1986) found that 56% of wheat samples were contaminated with ZEN ranging from 3 to 1254 ng/g. Whereas the average of positive samples was 141 ng/g. Zearalenone was coexist in 4 (13%) out of 31 wheat samples, and the average levels in positive samples was 1 µg/kg Tanaka et al. (1986).

Egyptian government established a new standard of aflatoxins for food in 1990. But there are no action levels for ZEN in foods and feeds in Egypt. Corn, rice and wheat are very important agricultural products in diets for Egyptians. They are the main source of carbohydrate in Egypt. The significane of this finding to health of Egyptians might be further debated by the government. On the other side good information could only be obtained by collecting big number of samples from a big number of regional distribution points over the period of several years.

Formation of Zearalenone by Fusarium species isolated from Egyptian Cereals:

Toxigenic strains of *Fusarium* isolated from cereals (corn, rice and wheat) were presented in table (2). Show that sum of 79 isolates of *Fusarium* belonging to 9 species. *F. culmorum* was the highest in number of isolates followed by *F.graminearum, F.oxysporum, F.moniliforme, F.rosum, F. poae, F. solani and F.nivale* in a descending order. Many investegators indicated that *F.culmorum, F.equiseti, F.graminearum, F.sambcinum, F.solani, F.poae, F.acuminatum, F.moniliforme, F.oxysporum and F.roseum* are the most common species associated with corn, rice and wheat (Abbas et al. 1984 and 1989; Chelkowski et al. 1984; Naguib et al. 1989 Sahab et al 1989 and Logrieco et al., 1990).

Regarding to ZEN production the data showed that 32.9% (26 out of 79)were ZEN producers. It can be also noticed from Table (2) that 5204% of *F.culmorum* (11 out of 21 isolates) and 6105% of *F.graminearum* (8 out of 13 isolates) were found to be ZEN-producers. These were the most commonly isolated strains followed by *F.oxysporum* but only 803% was ZEN producing. While *F.moniliforme* and *F.nivole* were not able to produce ZEN. The highest level of ZEN was obtained by *F.culmorum; F.graminearun and F.equiseti* with average 471,319.8 and 117.5 ppm respectively. But the lowest level was produced by *F.solani (15 ppm as a mean).* F.culmorum *and* F.graminearum were the most species of *Fusarium* most frequently occurring on cereals, as well as the strongest pathogen for them (Chelkowski et al., 1984). On the other side, the strong phytopathogenic isolates of *F.culmorum* were also able to produce a high concentration of ZEN and Trichothecences of group "B" (DON and 3 AcDON) (Ueno, 1977). *F.moniliforme* (1/6, isolates) and *F.oxysporum* (2/2, isolates) were able to produce ZEN on rice medium (Jimenez et al., 1991). ZEN and Zearalenone sulfate were isolated from a cultures of *F.graminearum*-30,*F.graminearum*. 1, *F.equiseti*. 2, *F.sambucinum* N45B and *F.roseum* (Plasencia and Mirocha, 1991). Bosch et al., (1992) reported that *F.geaminearum* and *F.equiseti* originally obatained from corn and Corn-based feedstuff were highly ZEN and Trichothecence-Producing. ZEN production on ric reached 729-1943 μg/g.

Although mycotoxins were not generally detected at the time of analysis, the fungal flora might develop toxic metabalites if storage conditions favour fungal growth. Knowledge of contaminating food mycoflora is important because undetectability of a mycotoxin at the time of analysis does not mean that this metabolite could not be found later if the toxigenic species is present in the food, and if favourable conditions allow for fungal development and mycotoxin formation. Control of moisture and temperature levels of these commodities is necessary to prevent mould growth and mycotoxin production (Jimenez et al., 1991).

Decontamination of Zearalenone from contaminated corn:

The effect of hydrogen peroxide (H_2O_2) on Zearalenone (ZEN) in contaminated corn was presented in Table (3). It was observed that the decomposition of ZEN was increased by increasing the following conditions time of exposure, concentration of H_2O_2 and temperature of the treatment.

Treating contaminated corn with 10% H_2O_2 for 2, 4, 8, 16 hr at 50°C and 80°C destroyed ZEN from 67.5 up to 83.9% for 2 and 16 hr at 80°C respectively. While it was from 30.1 up to 49.2% at 50°C with the same conditions. At the lowest concentration of H_2O_2 (3%) the decomposition percent was decreased up to 28.4% and 45.6% at 50°C respectively.

The previous results indicated that the oxidizing agent can breakdown ZEN. Matsuura et al. (1979) confirm that it is possible to breakdown ZEN by oxidation, whereas the half life of the break down in an 0.5% aqueous solution of ammonium persulphate at 80-100°C amounted to only 5 min and at 60°C to about 30 min. at room temperature the breakdown rate in two solutions was 75 and 45% respectively after one day and after 7-10 days no more ZEN could be found. They also found that if the ammonium persulphate concentration was lower to 0.03% and H_2O_2 concentration to 0.01% about half of ZEN was still present after 9 days at room temperature. Lasztity et al. (1977) found that reduction in the ZEN content of maize grain and its toxicity to pigs was obtained by treatment with an aqueous solution of H_2O_2 and subsequent heating.

CONCLUSION

Food law in Egypt must be imposed to the tolerence level of ZEN in cereals. H_2O_2 treatment can be used for breakdown and removal of ZEN from contaminated corn before corn manufacture or animal feeding.

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Table 1:
Level of Zearalenone (ppb) in corn, rice and wheat collected from Egypt

Sample	Number of Samples analyzed	Number of Positive samples	Content of positive samples (ppb)		
			Minimun	Maximum	Mean ± SD
Corn	50	15 (30)[a]	10.4	45.2	22.3 ± 11.31
Rice	45	4 (8.9)[a]	5.1	21.9	15.5 ± 7.44
Wheat	40	5 (12.5)[a]	4.9	12.7	8.8 ± 3.62

Table 2:
Production of Zearalenone by Toxigenic *Fusarium* species isolated from (corn, rice and wheat) grown in corn at 25 ± 2°C for 21 days

Fusarium species	Commodity and isalate number	No.of strains tested	No.of isolates producing ZEN	Percentage of toxic strains	ZEN yieds (ppm)	
					Average*	Range*
F.culmorum	Corn Rice wheat (9) (5) (7)	21	11	52.4	471.0	325- 1300
F.equiseti	wheat (6)	6	2	33.3	117.5	35-200
F.graminearum	Corn, Rice, wheat (6) (4) (3)	13	8	61.5	319.8	24.1 - 822.0
F.moniliforme	corn, Rice (5) (3)	8	--	-----	--	--
F.nivale	Rice (4)	4	--	-----	--	--
F.poae	corn, Rice (2) (3)	5	2	40	49.0	3 -95
F.oxysporum	Corn, Rice, wheat (4) (2) (6)	12	1	8.3	35.0	0 -35
F.rosum	Corn (6)	6	1	16.7	21.6	0 -21.6
F.solani	Corn, wheat (2) (2)	4	1	25.5	15	0 -15
Total	corn rice wheat (34) (21) (24)	79	26	32.9	146.98	55.3 -355.5

* Average and Range for producer isolates

Table 3:
Destruction (%) of Zearalenone in contaminated corn treated with H_2O_2

Treatment	Temp.(C°)	Zearalenone (ppb)							
		2 hr		4 hr		8 hr		16 hr	
		level	Dest.%	level	Dest.%	level	Dest.%	level	Dest.%
Control	--	365.9	--	365.9	--	365.9	--	365.9	--
3%	50	307.7	15.9	303.7	17.0	292.4	20.1	262.0	28.4
	80	263.5	28.0	255.4	30.2	218.8	40.2	199.1	45.6
5%	50	285.0	222.1	278.1	24.0	268.2	26.7	227.2	37.9
	80	248.8	32.0	222.8	39.1	195.4	46.6	146.4	60.0
10%	50	255.8	30.1	219.9	39.9	218.8	40.2	185.9	49.2
	80	118.9	67.5	106.5	70.9	91.5	75.0	58.9	83.9