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EFFECT OF GAMMA IRRADIATION ON THE GROWTH OF ASPERGILLUS VERSICOLOR AND ACTIVITY OF STERIGMATOCYSTIN IN DAIRY CATTLEFEED.

BY

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INTRODUCTION

Aspergilli are ubiquitous fungi and many species occur regularly in grains stored at 13.50 to 18% moisture (Kume et al., 1983). Included among these aspergilli are members of the *Aspergillus glaucus*, *A.restrictus*, *A.candidus*, *A.ochraceus* and *A.versicolor*. groups. Of particular interest is *A.versicolor* because of its frequent occurrence in stored grains and food commodities (Aziz, 1982; Vesonder and Horn, (1985)and its ability to elaborate the carcinogen sterigmatocystin (Schroeder and Kelton, 1975). Some members of the *A. flavus*, *A. nidulans*, *A. ustus* and *A. glaucus* groups are also known to produce sterigmatocystin (Rabie et al., 1977). Reports documenting the occurrence of sterigmatocystin in foodstuff, feedstuffs and cereal grain reveal a low level incidence (Scott et al., 1972; Devi and Polasa, 1982; Manabe and Tsuruta, 1975).

The use of ionizing radiation in the preservation of feed products has proved to be suitable method for the elimination of pathogenic microorganisms (Ley et al., 1969; Aziz, 1982). The radio-sterilization

of cereals with gamma rays and radio-sensitivity of grain spoilage fungi were determined by Poisson et al. (1971); Mohyuddin et al. (1969) found that species of penicilli and aspergilli differed significantly in their resistance towards gamma irradiation.

In the present paper, studies have been carried out for the determination of the effect of gamma irradiation on the elimination of toxinogenic isolates of *A. versicolor* recovered from dairy cattle feed and the inactivation of sterigmatocystin produced in the feeds.

MATERIALS AND METHODS

Samples: Ten dairy feed samples consisted of cracked corn (60%), decorticated cotton seeds (15%) and protein mixture (25%) were collected in 1988 from different farms in Egypt, where cattle eating these feed exhibited diarrhoea with subsequent loss of milk production and death in some cases.

Mycological Examination: The mycoflora of the feed samples was determined by separately blending 20 g subsamples with 180 ml of sterile water for 15 min. A 10^{-2} to 10^{-4} dilutions were made from the resultant slurry and 0.2 ml were spread on plates of Czapek-Dox agar containing tetracycline (1-25 m/L). Plates were incubated for 5 days at 25°C. The total number of fungal colonies was determined and representative colonies were subcultured for identification.

Sterigmatocystin Production: Twelve isolates of *A. versicolor* were tested for sterigmatocystin production on autoclaved Cracked Corn and in 2% yeast-extract- 4% sucrose liquid medium (Vesonder and Horn, 1985). Triplicate solide state fermentations were carried out in 500 ml Erlenmeyer flasks

containing 50 g of sterigmatocystin-free Cracked Corn. The moisture level of the Corn was adjusted to 35% with dist. water before autoclaving. Each flask was inoculated with a 1.0 ml spore suspension prepared by adding 5 mg of dist.H_2O to a 7-day-old malt extract agar slant of each *A.versicolor* isolate and agitating.

Flasks were incubated at 25°C as static cultures for 10 days. Isolates of *A.versicolor* were also cultured in duplicates in 250 ml Erlenmeyer flasks containing 50 ml of 2% yeast extract-4% sucrose liquid medium. Cultures were inoculated and incubated as described above.

Sterigmatocystin in each corn and liquid cultures, as well as, in the original feed samples (three analyses/10 samples, 50g each) was determined by TLC (Shannon and Shotwell, 1976). The procedure involved the extraction of fermented cracked corn or feed (50 g) or liquid culture media in a blender for 3 min with methanol, 4% KCl (180 and 120 ml, respectively) followed by partial purification, then eluted with hexan followed by acetone: methylene chloride (5:95 vol./vol.). The eluates were examined by TLC developed in benzene-ethanol-acetic acid (90:5:5 vol./vol./vol.). The plates were air dried and sprayed with 20% ethanolic aluminum chloride and heated 10 min in an oven at 90°C. Sterigmatocystin was visualized as a yellow fluorescent spot under short wave-length UV light (245 nm).

Irradiation of feed samples and *V.versicolor* isolates: Twenty five grams in polyethylen bages of the dairy cattle feed samples as well as five toxi-genic isolates (producing sterigmatocystin) of *A.versicolor* (10^7 conidia/ml) were exposed to increasing doses of gamma irradiation 1 up to 10 KGY (One kGy= 100 Krad). Cobalt-60 Egypt's Industrial Mega-Gamma I Irradiation

NCRRT Nasr City, Cairo, Egypt was used for the irradiation procedure. After each treatment the samples as well as the isolates were subjected to serial dilutions. Viable counts were made on Czapek-Dox medium. Also, irradiated samples and isolates were analyzed for detecting the presence of sterigmatocystin.

RESULTS AND DISCUSSION

The feed samples contained approximately 10^{-2}-10^{-4} fungal propagules per gram of feed as determined by dilution plating. *Aspergillus versicolor* was the dominant mould, accounted for 80% of the total propagule density. Other fungi of lower densities included: *A. candidus* (0.2-6.0%), *A.wentii* (3.0-0.0%), *Penicillium oxalicum* (0.0 - 4.0%), *P.implicatum* (0.0 - 2.0%) and *Verticillum species* (8.0 - 3.0%).

Twelve isolates of *A.versicolor* were tested for sterigmatocystin production on autoclaved cracked corn and synthetic medium. Three isolates produced 7 to 56 ug/g of sterigmatocystin on cracked corn and trace amounts (less than 0.12 ug/ml) on liquid midium **(Table 1)**. On the other hand, sterigmatocystin (1.23 to 9.23 ug/g) detected in five feed samples associated with acute diarrhoea in dairy cattle. In recent studies by Vesonder and Horn (1985), sterigmatocystin.
(7.75 ug/g of feed) and high-propagule- density of *A.versicolor* were detected in feed associated with acute clinical symptoms of bloody diarrhoea and death in dairy cattle, the authors detected nine isolates of *A. versicolor* produced 13 to 89 ug of sterigmatocystin per gram on cracked corn. Sterigmatocystin has been found in wheat (0.3 ug/g, in Canada, Scott et al., 1972), corn (0.15 ug.g, in India, Devi and Polasa, 1982) and rice (0.8 to 16.30 ug, in Japane, Manabe and Tsuruta, 1975).

Table (1): Detection of Sterigmatocystin from dairy cattle feed samples and Aspergillus versicolor isolates.

No. of isolates tested	11
No. of toxigenic isolates	3
Amounts of toxins	
on cracked corn (ug/g)	7-56
on YES Medium (ug/ml)	0.08-0.12
No. of tested feed samples	10
No. of positive samples	5
Amount of toxin (ug/g feed)	1.23-9.23

Ionizing radiation is characterized by a very high energy content, great penetrating power and lethality due to action at the cellular level. Until recently, the main applications of ionizing radiation have been in industrial processes and as tools to examine the genetic, structure and function of micrroorganisms. Practical application of ionizing radiation to destroy microorganisms on commerical products has been developed by Ingram and Roberts (1980). In our study, by increasing the dose of gamma irradiation the number of viable counts of moulds in the dairy cattle feeds as well as sterigmatocystin concentrations decreased greatly **(Table, 2)**. At 4 kGy, all samples were found to be free completely from mould and at 18 kGy sterigmatocystin was not isolated. Aspergillus versicolor which was isolated from the dairy cattle feed samples was very sensitive to gamma irradiation. The colony formation decreased greatly by increasing the irradiation doses until complete inhibition at 4 kGy **(Table 3)**. This observation is in agreement with that recorded by Aziz (1982) and Kume et al., (1983). There is a good relationship between the colony formation and the concentration of sterigmatocystin on cracked corn decreased from 56 ug/g (0 kGy) to 12.50 ug/g (3 kGy) and the toxin could not be more detected at 4 kGy . On the other hand, on synthetic medium the production of sterigmatocystin was very poor (less than 0.10 ug/ml). Change and Markakis (1982) reported that, in general, increasing the radiation dose at the range of 0 to 4 kGy resulted in decreasing aflatoxin formation in barley. On the other hand, El-Hadi (1986) reported that when the two strains of *A.flavus* producing aflatoxins were exposed to increasing doses of gamma irradiation up to 5 kGy the aflatoxin-quantities decreased and finally was completely inhibited. In another study made by Hassanein (1987) it was reported that upon exposure of *A. flavus* to low doses of gamma rays (0-2.0 kGy) both the fungal

Table (2): Effect of gamma irradiation on the total fungal counts and Sterigmatocystin concentration in dairy cattle feed. (Three analysis/sample).

Irradiation Doses (kGy)	Total Counts of Molds	Sterigmatocystin Concentration (ug/g)
0	3.40×10^4	9.23
1	2.39×10^3	9.23
2	1.17×10^2	7.50
3	3.50×10^1	4.21
4	0.00	1.30
5	0.00	0.13
6	0.00	0.10
7	0.00	0.04
8	0.00	0.00

Table (3): Effect of gamma irradiation on the colony formation of *A.versicolor* and Sterigmatocystin production.

Irradiation Doses (kGy)	Total Viable Counts/ml	Sterigmatocystin Production [a]	
		Corn Craked (ug/g)[b]	YES Medium (ug/ml)[c]
0	2.21×10^7	56	0.10
1	3.10×10^6	27.11	0.07
2	5.20×10^4	20.7	0.05
3	2.50×10^1	12.50	ND
4	0.00	0.00	0.00
5	0.00	0.00	0.00
6	0.00	0.00	0.00

(a) : Incubated for 10 days at 25°C

(b): Mean of three flasks.

(c): Mean of two flasks. YES: 2% yeast -4% sucrose.

ND : Not detected.

growth and aflatoxin production increased to a maximum value and followed by complete inhibition of growth and aflatoxin production at dose level of 3 kGy. It therefore seemed that doses needed to affect toxin-production can vary according to the propagules tested. So, irradiation may affect the pathway of toxin production rather than inducing alterations in genetic systems related to these toxins (Paster et al., 1985).

Sterilization by gamma radiation has proved to be suitable method for the treatment of a variety of food and feed products, the radiation dose of 25 kGy has proved effective for the control of the contamianting organisms (ley et al., 1969). Gamma radiation has been widely reported to prevent or delay food spoilage. In our investigation, no adverse effects on rats receiving the irradiated diets (8 kGy) and the animals have been noted to be healthy and the irradiated diets appeared to be nutritionally satisfactory. Additional studies must be carried out in future on cattles receiving the irradiated diets. The dose, 8 kGy was noticed to be the best dose for the preservation of animal diets, (Mossel, 1967).

SUMMARY

Ten dairy cattle feed samples collected from different farms in Egypt were tested for the presence of sterigmatocystin and *Aspergillus versicolor*. Sterigmatocystin (9.23 ug/g feed) and high contamination by *A. versicolor* were detected from five samples. Three from twelve isolates of *A. versicolor* produced 7 to 56 ug of sterigmatocystin/g on cracked corn medium and very traces (less than 0.12 ug/ml) on synthetic liquid medium. The application of gamma irradiation reduced the growth of *A. versicolor* (10^7 conidia/ml) greatly as well as the concentration of sterigmatocystin. Gamma irradiation at 4 kGy decontaminated

completely the dairy cattle feed from *A.versicolor* and at 8 kGy the samples were observed to be freed from sterigmatocystin.

REFERENCES

1. Aziz, N.H. (1982): The microflora of poultry diet in relation to human and poultry diseases and control by gamma radiation. M. Sc. Thesis Ain Shamis Univ. Faculty of Sci. Dept. of Microb. Cairo, Egypt.

2. Chang, H. G. and markakis, P. (1982): Effect of gamma irradiation on aflatoxin production in barely. J. Sci. Food Agric. 33, 559-564.

3. Devi, R.G. and Polasa, H. (1982): Mycotoxins from fungi on maize. Cur. Sci, 51, 751.

4. El-Hadi, A. F. M. (1986): Studies on the microbial flora contaminated animal feed and its control by gamma irradiation. M. Sc. Thesis Zagazig Univ. Faculty of Sci., Botany Dept. Egypt.

5. Hassanein, Wesam, A.A. (1987): Studies of the microflora of some food products. M. Sc. Thesis Zagazig Univ. Faculty of Sci., Botany Dept.Egypt.

6. Ingram. M. and Roberts, T.A. (1980): Ionizing Radiation in "Microbial Ecology of Foods" Vol. I. Factors Affecting Life and Death of Microorganisms. By The International Commission on Microbiological Specifications, Academic Press, New York.

7. Kume, T., Ito, H.; Irzuka, H. and Takehisa, M. (19839: Radiosensitivity of Aspergillus versicolor isoalted from animal feed and destruction of sterigmatocystin by gamma irradiation, Agric. Biol Chem. 47, 1065-1069.

8 . Ley, F.J. Coates, M.E. and Paterson, J.S. (1969): Sterilization of laboratory animal diets using gamma radiation. Lab. Animal, 3, 221.

9 . Manabe, W. and Tsuruta, O. (1975): Mycological damage of domestic brown rice during storage in warehouse under natural conditions. II. Natural occurrence of Sterigmatocystin on rice during a long time storage Trans. Mycol. Soc. Jpn. **16**, 399-405.

10. Mohyuddin, T., Mirza, A.; Skoropad, I. and William, P. (1969): Radiation sensitivity of wheatgrain spoilage fungi. Nuclus Charchi., **6**, 151-154.

11. Mossel, D.A.A. (1967): Salmonella Radicidation of dry mixed feeds and feed ingredients. Report of a Panel on Microbiological Problems. in Food Preservation by Irradiation. IAEA, Vienna,15-25.

12. Paster, N., Barki-Golan, R. and padova, R. (1985): Effect of gamma radiation on ochratoxin production by the fungus A. ochraceus. J. Sci. Food Agric. **36**, 445-449.

13. Poisson, J., Cahagnier, B. and Guilbot, A.(1971): Gamma sensitivity of the main corn moulds Conidia-Incidence on the radurization of corn. Mycopathol. Mycol. Appl. **45**, 193-209.

14. Rabie, V.J., Steyn, M. and Van Schalkwyk, G.C. (1977): New Species of Aspergillus Producing Sterigmatocystin. Appl. Environ. Microbiol. **33**, 1023-1032.

15. Schroeder, N.H. and Kelton, W.H. (1975): Production of Sterigmatocystin by some species of the genus Aspergillus and its toxicity to chicken embryo. Appl. Microbiol. **30**, 589-591.

16. Scott, P.M., Van Walbeek, W., Kennedy, B. and Anyeti, D. (1972): Mycotoxins "Ochratoxin A, Citrinin and Sterigmatocystin" and toxigenic fungi in grains and othe agricultural products. J. Agric. Food Chem. **20**, 1103-1109.

17. Shannon, G.M. and Shotwell, O.L. (1976): Thin-Layer chromatographic determination of Sterigmatocystin in cereal grains and soybeans. J. Assoc. Off. Anal. Chem. **59**, 963-965.

18. Vsonder, R.F. and Horn, B.W. (1985): Sterigmatocystin in dairy cattle feed contaminated with Aspergillus versicolor. Appl. and Envir. Microbiol. J. **49**, 234-235.