Journal of Environmental Sciences Vol. 9, No. 2, pp. 215—220, 1997 ISSN 1001—0742

Detoxifying moniliformin in grains and water*

Zhang Hong**, Li Jilun

College of Biological Sciences, China Agricultural University, Beijing 100094, China

Abstract—The method to determine moniliformin content was established in our laboratory. The recovery rate is 97.7% while the moniliformin content in the sample is 8.8 mg/g. In comparison with different methods of detoxifying moniliformin in water, the best antidote is chlorinated lime. 1.5 mg active chlorine in chlorinated lime was required to detoxify 1 mg moniliformin. 5% H_2O_2 spray was the best way for detoxifying moniliformin in grains. These two methods are convenient, economic and with no secondary pollution. They can be used for preventing human and livestock from the toxicity of moniliformin.

Keywords: moniliformin; detoxifying; Keshan disease.

1 Introduction

Moniliformin is a kind of mycotoxin highly toxic to the heart of animals. It is soluble in water and exists in nature as Na^+ or K^+ salt (springer, 1974). Its LD_{50} for 7-day-old Beijing duckling is 3.65 mg/kg (Kriek, 1977).

The toxic fungus *Fusarium subglutinans* was isolated from moldy maize in Keshan disease (KSD) areas, and the toxicology was tested with purified moniliformin from this fungus by Zhang *et al*. (Zhang, 1989). On the basis of these studies, it was proposed that moniliformin in grains or in water might be the major causative agent of KSD in China, which has attracted more attention of domestic medical experts (Chen, 1990; Qiao, 1993). Generally, KSD is more likely to be prevalent in mountainous area or marshland. The ecological condition in such areas is favorable for growth of the toxin-producing fungus resulting in contamination of the grains and water source. Ji *et al*. (Ji, 1991) demonstrated moniliformin in rice from Yunnan and maize from Shaanxi in China where KSD is prevailing. However, nothing has been found in rice from Beijing where no KSD occurs. These results suggest that moniliformin exists naturally in grains produced in KSD areas.

Recently the prevalent rate of KSD has dramatically declined due to the improvement of living standard and the quality of drinking water; but moniliformin is still a potential toxin to cause the disease of man and livestock in some rural regions. Therefore, it is significant to carry out some convenient methods with lower cost and free of secondary pollution for detoxifying moniliformin in the grains, especially in maize, foodstuff and water. However, there are no such methods available either domestically or abroad.

* This research was supported by United Nations Environmental Programme (UNEP) and National Natural Science Foundation of China.

** Corresponding author

2 Materials and methods

2.1 Materials

Autoclaved maize was inoculated with conidial suspension of *Fusarium subglutinans* isolated from the maize sample collected from KSD area in the north of Shaanxi Province in China, and incubated at 25℃ in dark room for 21 days. The culture was dried at 50℃. The sodium salt crystal of moniliformin was purified from the culture (Zhang, 1989).

2.2 Methods

2.2.1 Detecting, detoxifying and bioassay of moniliformin in water

(1) Detecting moniliformin in water

The quantity of moniliformin was determined by UV spectrum scan from 210 to 270 nm. The maximum absorption is at 229 nm at which measurement was made.

(2) Detoxifying with chlorinated lime

The water solution of 0.4 g/L chlorinated lime was made by using chlorinated lime containing 37.2% active chlorine as determined by iodimetry (Liu, 1987). The solution was diluted by 10 times after centrifuged at 300r/min. The solutions containing moniliformin at 2.5, 5.0, 7.5, 10.0 and 12.5 μg/ml were mixed with different volumes of the above-mentioned chlorinated lime, respectively, and placed under room temperature for 2 hours. The minimum active chlorine for detoxifying moniliformin was measured at 229 nm.

(3) Bioassay of detoxification of moniliformin in water solution

30 of one-day-old Beijing ducklings (50 to 60 g in body weight) were randomly divided into three groups, 10 ducklings per group. The first group was fed with no toxin, the second group with pure moniliformin in water solution at 8 mg/kg body weight, and the third group with the same amount of toxin as the second group but detoxified by chlorinated lime with 1 mg toxin treated by 1.5 mg active chlorine. The survivors from each group were reared and observed for three days.

2.2.2 Detecting and detoxifying moniliformin and bioassay of detoxification in grains

(1) Detecting moniliformin in grains

The qualitative analysis was performed by thin layer chromatography (TLC) and spraying with 0.4% 2, 4-dinitrophenylhydrazine in 2 mol/L HCl. The content of moniliformin in maize culture was determined by standard adding method described as follows: 1g sample of *Fusarium subglutinans* culture was extracted with 5 ml of methanol, and centrifuged for 5 min at 3000 r. 10 μl of the supernatant was subjected to TLC on GF silica gel plate and developed with the solvent system of chloroform and methanol (3/2, v/v). Under UV the spot that has the same

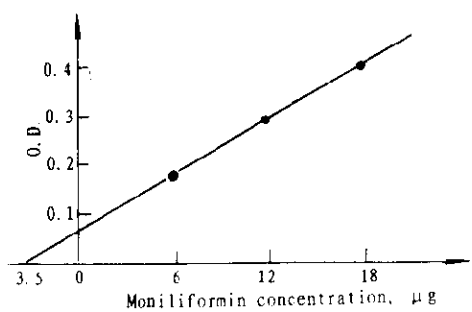

Fig. 1 Curve for measurement of moniliformin in culture by using standard sample adding method. 3.5 μg is presumably *X*

Rf value (0.5) as that of pure moniliformin sodium salt was scraped out and mixed with 5 ml of deionized water in a centrifuge tube. After centrifugation, the supernatant was made as diluted sample. 7 ml of deionized water and 1 ml of diluted samples were accurately mixed in each of four test tubes with 0, 30, 60 and 90 μl of 0.2 mg/ml pure moniliformin sodium salt solution respectively. The OD value was measured at 229 nm. Data were plotted as toxin content vs. OD value on lower and left scales. From the standard curve the moniliformin concentration was obtained. The experiment was triplicate and the average is shown in Fig. 1. Based on the dilution factor, the moniliformin content was calculated with the following equation: toxin content $= 5 \times 500$ μg/g (sample) $\times X$, where X being the toxin content got from the standard curve.

(2) Detoxifying moniliformin in grains

10 g of moniliformin containing maize culture was treated under 27℃ as shown in Table 1. After drying at 50℃ for three days, pH test, moniliformin content determination and detoxification evaluation were made. The experiment was also triplicate.

Table 1 Experiments of detoxifying moniliformin in grains

Treatments	Samples of contaminated grains	Operation
Spraying with 5 ml of 5% H_2O_2	Moniliformin moldy maize kernel (MMMK) or powder	Spreading the kernel or powder and spraying
Spraying with 20 ml of 5% NH_3	Moniliformin moldy maize powder (MMMP)	Spreading, spraying and placing the sample at room temperature for 4 hours for removing residual NH_3
Spraying with 20 ml of 10% $NaClO_3$	MMMP	Spreading and spraying
Washing with 550 ml of water	MMMP	Spreading and spraying
Fumigating with 3 g of gaseous disinfectant (mainly Cl_2) produced by Fujian Ke Da Hygienic Products Co. Ltd.	MMMP	Putting the MMMP into Petridish in a sealed container ($0.19 \times 0.10 \times 0.16$ m^3), igniting and fumigating for 15 hours.
Radiating with 500 Krad γ-ray	MMMP	Putting the MMMP into the 15×150 mm glass tube and radiating

(3) Bioassay for detoxifying moniliformin in grains

30 of one-day-old Beijing ducklings were divided average into 3 groups. No moniliformin was fed in the first group. The second group was fed with maize culture powder at 14.7 mg moniliformin /kg body weight, which was 4 times of the LD_{50} of moniliformin in water solution. During 7 days rearing period every duckling was fed with 0.4 g of maize culture powder every day. The ducklings in the third group were fed with the same amount of maize culture powder pretreated with 5% H_2O_2.

3 Results and discussions

3.1 Bioassay of detoxifying moniliformin in water

3.1.1 Determination of moniliformin in water

The maximum UV absorption wave length of moniliformin is 229 nm, and there is also a small

absorption at 260 nm (Fig.2). A standard curve can be drawn based on the OD value at 229 nm. The content of toxin, if it is less than 7 mg/ml, is directly proportional to OD value. The coefficient of correlation r is 0.9998.

3.1.2 Detoxification experiments of chlorinated lime

Different volumes of 0.4 g/L chlorinated lime solution were added into the water solutions of toxin containing moniliformin of 2.5, 5.0, 7.5, 10.0 and 12.5 μg/ml, respectively. The mixtures were placed under room temperature. The result is shown in Fig. 3. Theoretically, the amount of active chlorine can be determined by equation $(T_0 - T) \times 1.5/1.10$ μg/ml, where T_0 is the toxin concentration before detoxifying treatment, and T is the residual concentration after detoxicate. The equation can be simplified as active chlorine = $(T_0 - T) \times 1.5$ μg/ml for complete detoxification, it means that 1 mg of toxin can be detoxified by 1.5 mg of active chlorine in chlorinated lime. According to the "Stipulation for Drinking Water" decreed by the Ministry of Public Health of The People's Republic of China, the content of chlorine (Cl) in water must be lower than 250 mg/L. However, the chlorinated lime used in our experiment for detoxifying water containing toxin of 12.5 μg/L only contains 22.25 mg/L of chlorine (Cl) as determined by $AgNO_3$ titration. It is much lower than the stipulated content of chlorine. In addition, some other methods for detoxifying, such as heating, activated carbon absorption and treating with O_3, microwave or UV were also tested simultaneously in the present study. The results show that using chlorinated lime to detoxify moniliformin in water is the best method, because it is low in cost, easy to use, and free from secondary pollution.

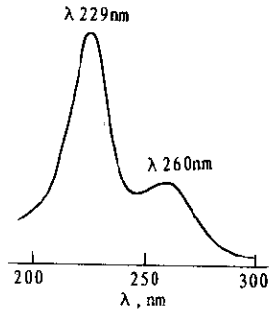

Fig. 2 The ultraviolet absorption spectrogram of moniliformin

3.1.3 Bioassay of detoxifying moniliformin in water

All of the 10 ducklings in the toxin-feeding group died within 2 hours after feeding. In contrast, the ducklings in the other two groups, fed without toxin and with toxin but pretreated with active chlorine respectively, all survived and remained normal for 3 days.

3.2 Detecting and detoxifying moniliformin and bioassay of detoxification in grains

3.2.1 The detecting of moniliformin in grains

There was a moniliformin spot on TLC plate at $Rf = 0.5$ by UV detection. When the plate was sprayed with 2, 4-dinitrophenylhydrazine in 2 mol/L HCl, a red-brown spot occurred due to the reaction with moniliformin. The content of moniliformin in maize culture was determined as 8.8 mg/g by using standard adding method. The result was verified by adding equal amount of standard toxin into non-contaminated maize powder. The recovery rate was 97.7%, and the coefficient of variation (CV) was 5.0. It is very difficult to detect moniliformin and to determine its quantity in maize culture, because *Fusarium subglutinans* may produce many other secondary metabolites, and maize contains methyl alcohol-soluble substances also. As a result, it is difficult to

separate moniliformin from such materials by TLC clearly. In the past, quantitative analysis can be done only by HPLC. But HPLC is more complicated, expensive and in convenient for rapid detection of moniliformin. Therefore, we established the standard adding method for quantitatively determining moniliformin in maize culture. It was revealed to be accurate and applicable.

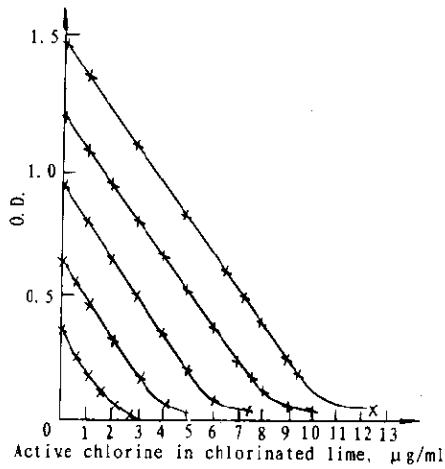

Fig. 3 Active chlorine in chlorinated lime, UV absorption spectrum at 229 nm of moniliformin solutions of different concentrations detoxified with chlorinated lime. Concentration in μg/ml: a:2.5, b:5.0; c:7.5, d: 10.0, e:12.5

3.2.2 Detoxifying moniliformin in grains

The maize culture was treated by spraying 5% H_2O_2, 5% NH_3 and 10% $NaClO_3$, washing with water, fumigating with gaseous disinfectant and radiating with γ-ray, respectively. The qualitative and quantitative analyses were made through TLC. Table 2 shows the details of the experiments. The main component of gaseous disinfectant is Cl_2. After treatment with Cl_2 the sample is seriously acidified (pH value decreased to 3), and the detoxifying effect of Cl_2 is not acceptable. After treating with NH_3 the color of the maize culture becomes brown and the detoxifying effect is not satisfied. The washing with water is unable to detoxify completely. The treatment with 10% $NaClO_3$ is good for removing 70% toxin, but the pH is a little bit higher (pH = 8.0—8.5) than that of the original (pH = 5). Radiating with 500 Krad γ-ray is not ideal because it can not detoxify completely. Moniliformin is unable to be detected after spraying with 5% H_2O_2, and the color of maize and moldy smell become obviously lighter. It is an ideal and applicable way for detoxifying moniliformin in maize.

Table 2 The evaluation of different treatments for detoxifying moniliformin

Treatments	Toxin content before treatment, mg/g	pH, appearance and smell after treatment	Toxin content after treatment, mg/g	Detoxicate efficiency, %
Spraying with 5 ml of 5% H_2O_2	8.8	pH unchanged (=5), color and smell lighter	Undetectable	100
Spraying with 20 ml of 5% NH_3	8.8	pH higher (6.0—6.5), darker, smell unchanged	6.5	26
Spraying with 20 ml of 10% $NaClO_3$	8.8	pH much higher, (8.0—8.5), color unchanged, smell lighter	1.9	78
Washing with 550 ml of water	8.8	pH and color no change, smell lighter	3.5	60
Fumigating with gaseous disinfectant	8.8	pH lower (3.0), color lighter Cl_2 smell strong	5.0	43
Radiating with 500 Krad γ-ray	8.8	pH unchanged, color darker	6.5	26

3.2.3 Bioassay for detoxifying moniliformin in grains

All(10) of the ducklings in the group treated by toxin died within 2 hours after feeding maize culture powder. However the ducklings in the groups treated without toxin or with toxin but pretreated with 5% H_2O_2 remained alive. This result indicates that 5% H_2O_2 has fully detoxifying action. Table 3 shows that there is no effect on the body weight increasing of the ducklings ($P > 0.05$) fed with maize culture pretreated by H_2O_2 for seven days.

Table 3 Body weight of 10 ducklings after feeding with maize culture powder pretreated with H_2O_2

Treatments	Average weight, g	Weight of 7 days later, g	Average weight increased/day, g	*P*
Control	57.6	91.8	4.9	
Pretreated maize culture powder	52.8	88.0	5.0	>0.05

4 Conclusion

Chlorinated lime is an ideal agent for detoxifying moniliformin in water. 1 mg of moniliformin is detoxified by 1.5 mg active chlorine contained in chlorinated lime. The best method to detoxify moniliformin in grains is to spray 5% H_2O_2. These two methods which are more convenient, economical and with no secondary pollution provide means to detoxify moniliformin.

References

Chen LY, Tian CL, Yang B. Mycopathologia, 1990; 110(2):119

Ji Z, Liu W, Liang XT. National Medical Journal of China (in Chinese), 1991; 71(1):14

Kriek NPJ, Marasa WFO, Steyn PS, Van Rensburg SJ, Syeyn M. Food Cosmet Toxicol, 1977; 15:579

Liu FL, Dai XJ. Physical and chemical analysis methods of food (in Chinese). Beijing Light Industry Publishing House. 1987:196

Qiao HL, Yu RP, Li QZ, Liang XM, Ma YX. Chinese Journal of Veterinary Medicine (in Chinese), 1993; 19(4):3

Springer JP, Clardy J, Cole RJ, Kirksey JW, Hill RK, Carlson RM, Isidor JL. J Amer Chem Soc, 1974; 96:2267

Zhang H, Li JL. Acta Microbiologica Sinica (in Chinese), 1989; 29(2):93

(Received March 18, 1996)