

Thesis Title PRODUCTION, DETECTION AND DETOXIFICATION
OF TRICHOHECENES FROM FUSARIUM
SPOROTRICHIOIDES ITFRC T-592 (ATCC48019,
MCH 7452)

Name Srisurang Tantimavanich

Degree Master of Science (Microbiology)

Thesis Supervisory Committee Samaniya Sukroongreung, M.D.
Vithaya Meevootisom, Ph.D.
Timothy William Flegel, Ph.D.
Tongtavuch Anukarahanonta, M.D., Ph.D.

Date of Awarding the Degree May 7, 1986.

ABSTRACT

Fifteen kilograms of Sorghum bicolor grain infected with Fusarium sporotrichioides ATCC 48019 were cool shocked at 4°C for 1 week and further incubated at 25°C for 3 weeks. Toxic activities of the infected grain were biologically tested by the method of yeast screening for trichothecenes and highly potent, heat stable antifungal toxin(s) were detected. By semiquantitative yeast bioassay, the toxin(s) in the grain were estimated at approximately equivalent to the potency of 350 µg/g of standard T-2 toxin. Chemical analysis by TLC showed fluorescent spots at the same R_f values of T-2 toxin and HT-2 toxin in two solvent systems. Confirmatory GLC revealed 112.43 µg/g of T-2 toxin and 77.18 µg/g of HT-2 toxin in the infected grain. This toxin(s) containing grain was ground and used in the detoxification experiments.

For detoxification, chemical agents (2-10 volumes) used were H_2SO_4 , HCl, NH_4OH , NaOCl, NaOCl+NaOH, NaOH, $NaHSO_3$, ascorbic acid and formaldehyde. Treatment conditions were RT and 60°C for 18h and autoclaving at 121°C for 15 min. The most effective detoxifying agents were acids (H_2SO_4 , HCl) and bases (NH_4OH ,

NaOH) at 60°C for 18 h. GLC-analysis revealed that only small amounts of residual T-2 and HT-2 toxin remained in the samples after H₂SO₄ treatment (0.213 µg/g T-2 toxin, undetectable HT-2 toxin) and NaOH treatment (0.303 µg/g T-2 toxin, 0.258 µg/g HT-2 toxin), whereas none of these these toxins was observed in the 5% NH₄OH treated sample. At the same time, the toxic activities of these treated samples toward Kluyveromyces marxianus BBK7 (semiquantitative yeast bioassay) were markedly decreased (>99%-100% reduction of toxic activities).

Because of excellent detoxifying results, preliminary prefield trials were performed. Various concentrations of NH₄OH, H₂SO₄ and NaOH were used to treat ground moldy sorghum containing various amounts of toxins (10-350 ppm) at 60°C for 18 h, and various amounts of ground substrate containing 350 ppm of toxins at 60°C and 50°C for 18, 24 and 48 h. The results demonstrated that factors affecting chemical detoxification of trichothecenes are type and concentration of chemical agent, temperature and duration of treatment, and level of toxin in the substrate. NH₄OH (5%) was found to be the most effective detoxifying agent against trichothecenes (350 ppm).

In a secondary prefield trial, grains contaminated with 30-200 ppm of toxins were treated with 1%-5% NH₄OH both at 50°C and 60°C for 1, 3 and 5 days. The results showed that NH₄OH could completely detoxify the grains within 1-3 days depending on the concentration of this agent and the temperature of treatment.

These experiments demonstrated that glassware and lab equipment contaminated with trichothecenes, and particularly with T-2 toxin, should be decontaminated with these bases or acids. A possible detoxification procedure for field grains is proposed using aqueous ammonia. However, it should not be used on the farm without animal experiments.