ABSTRACT

This thesis aims to recommend an *in vitro* enzymatic detoxification process for aflatoxin B_1 (AFB_1) with special reference to AFB_1 infected groundnut meal (GNM). Horseradish peroxidase (HRP) / guaiacol test in an *in vitro* assay system was used for investigation of the reactivity with AFB_1. AFB_1- HRP reaction in phosphate buffer was studied with respect to varied substrate and enzyme concentration, pH dependency, time course, cofactor specificity, kinetics, and inhibitor sensitivity. The reaction in buffered liquid medium as well as GNM was optimized by response surface methodology (RSM). The optimum enzymatic reaction occurred in 50 mM phosphate buffer at 45 °C temperature, pH 6, incubation time 45 min, 5 mM H_2O_2 and normal pressure using 4 U of HRP per mM of AFB_1 in liquid medium resulting 77.1 % detoxification. In GNM optimum reaction occurred at 8 U of HRP used per mM of AFB_1 for 1 day incubation period and 20 mM hydrogen peroxide. Moisture content of the reaction media was maintained between 12-15%. Calcium propionate 0.3 g/ 100g of GNM was used as fungistat. The reaction resulted in 69% detoxification. The determination of kinetic parameters of Michaelis -Menten equation showed that the V_{max} and K_m were 160 x 10^{-4} mM/ min and 0.975 mM in liquid medium and 50 x 10^{-5} mM/ min and 1.25 mM in GNM respectively. E_a for AFB_1- HRP reaction in liquid medium was 75.92 kJoule / mole. Overall nitrogen solubility of the enzyme treated GNM increased in the 2- 6 pH range. Polyacrylamide gel electrophoretic patterns of the protein did not show any remarkable change. Amount of protein nitrogen in the meal increased after the enzymatic treatment. Mortality rate of the young rats was high when they were fed AFB_1 containing meal. Resistance increased with the age. Among various organs of rats tested, liver was the most affected organ followed by kidney, spleen and heart. Toxicity test of the AFB_1-HRP reaction products was performed using *Bacillus megaterium* as the test organism. The test resulted in 3% growth inhibition when 200 U/ mg HRP was used to detoxify AFB_1 in liquid medium. The mutagenicity and carcinogenicity of AFB_1- HRP reaction products were tested and compared to standard AFB_1 using the Ames' *in vitro* microbial detection system using *Salmonella typhimurium* TA 100 as the tester strain. Purified AFB_1 judged mutagenic at a concentration of 0.9 μg/plate beyond which number of revertants/ plate increased steeply. Other fractions elicited much less mutagenicity as expressed by lesser number of revertants/ plate. Embryogenic callus of *S. album* could give rise to only 60 % somatic embryo in presence of 1 mM toxin with several abnormalities which is much less compared to 94 % conversion to distinct bipolar embryos in case of control set without toxin. Cell mass growth in suspension culture of *C. roseus* showed 34% and 77% increase in dry weight after 4 and 8 weeks of incubation at 25 °C when detoxified products were tested but in presence of toxin only 21 % and 43 % dry weight increase occurred in the same time interval. 71% *A. hypogaea* seeds germinated when grown in detoxified media, while only 49% germination occurred in the media containing 1 mM toxin. Several anomalies were predominant in pollen tube morphology and pollen germination in presence of AFB_1 used without enzyme treatment.

Key words: *Aflatoxin, Horseradish peroxidase, enzyme, kinetics, GNM, toxicity, mutagenicity*