SUMMARY

Biological evalution of efficiency of some isolates of Lactobacillus spp. of aflatoxins B1 associated with local wheat seed Iraq

By

Faleh Mahdi Saleh AL-Moussawi

The study comprised the sampling of wheat grain from the Iraqi provinces ,which covered the agricultural plan for growing season of 2015 (including Naynawa, Dahuk, Arbil, Sulaymaniyah, Kirkuk, Salah Din, Baghdad, Wasit, Babil, Qadisiyah, The holy Karbala, The holy Najaf, Al-Muthanna, Dhi Qar, Maysan and Basrah), , to isolate fungi producing aflatoxin B1 and estimate the amount of toxin in the local wheat. Also, lactic acid bacteria especially *Lactobacillus* spp. were isolated from various sources including cow's milk, buffalo milk, AL- Thafier cheese, local soft white cheese, local yoghurt, Iranian cheese, Saudi cheese and pickles. The results were as the following:

1. Five different genera of fungi that were identified after growing on culturing media were obtained including *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus niger*, *Penicillium* spp., *Trichoderma* spp., *Mucor* spp. and *Fusarium* spp.
2. The percentage of Occurrence and frequency of fungi in Local wheat grain for both species *Aspergillus flavus* and *Aspergillus parasiticus* were studied. The provinces: Dahuk, AL-Sulaymaniyah, Kirkuk, The holy Karbala, Dhi Qar, and Maysan were significantly higher (p <0.05) than the rest of other provinces with an occurrence ratio of 14.28% for *Aspergillus parasiticus*. However, no occurrence of this fungus was recorded in AL-Muthanna province. In the same provinces except the holy Karbala, occurrence ratio of *A. flavus* was significantly higher recorded at 14.28%. Concerning frequency ratio of *A. parasiticus*, Dahuk governorate was significantly highest at 46.66%. With Regard to frequency of *A. flavus*, it was the highest value in Baghdad governorate reached to 42.79%.

3. The fungal isolates that produced aflatoxin B1 were detected using ammonia vapor and ultraviolet ray for *Aspergillus flavus* and *Aspergillus parasiticus*. The ability of these isolates to produce aflatoxin B1 were 92.59 and 60.97% respectively.
4. The amount of aflatoxin B1 was identified in wheat grain marketed of growing season of 2015, which was estimated by using HPLC. It was found that the highest percentage of aflatoxin B1 was in the Naynawa governorate and the lowest percentage was in wheat grain of the holy Karbala governorate at 10 and 0.2 ppb respectively. The ability of fungal isolates to produce aflatoxin B1 was tested on three types of culturing media including Potato Dextrose Ager (PDA), Yeast Extract Sucrose Agar (YES) and Czapek Yeast Agar (CZAPK). The isolate (HF_1) of *A. flavus* isolated from wheat grain of Naynawa province produced the highest amount of Aflatoxin on CZAPK medium, which was 26 ppm; however, isolate (HF_{85}) of *A. flavus* isolated from Babil governorate wheat had the lowest production of aflatoxin B1 on PDA medium, which was 0.2 ppm.
5. The *aflR* gene responsible for the production of aflatoxin B1 was detected by using PCR technique. As a result, the size of the amplified gene was 800 bp. In addition, the gene appeared in all studied isolates.
6. *Lactobacillus* spp were identified by API50 CHL technique after using the biochemical tests. As a result, the isolates $AKF_{(1,3,6,8)}$ belonged to *Lactobacillus casei* while the local isolates $AKF_{(2,4,7,10,11,12,13,14)}$ related to *Lactobacillus plantarum*. Moreover, isolate AKF_5 belonged to *Lactobacillus jonsonii*; whereas, the last isolate AKF_9 related to *Lactobacillus bulgaricus*.
7. The fungi were inhibited *invitro* by using of local isolates of lactobacilli ($AKF_{1, 2, 3, 4, 5}$). It was found that viable and heat-killed isolate of bacteria was the highest inhibitory effect on fungi, cells and metabolic compounds of the isolate (AKF_4) at 97, 62.33 and 97% respectively. While the isolate

(AKF_3) had the lowest inhibitory effect on fungi, cells and its metabolic compounds were 59, 14.33 and 59% respectively.

8. Quantitative estimation of four metabolites produced from bacterial isolates ($AKF_{1, 2, 3, 4, 5}$) was carried out by using GC-mass technique. Those metabolites were oleic acid, palmitic acid and 2,4-ditertbutyl phenol. On the other hand, lactic acid was estimated by HPLC technique.
9. Aflatoxin was chemically bound by isolates ($AKF_{1, 2, 3, 4, 5}$) in MRS liquid medium with 200 ppm of Aflatoxin B1. The highest binding of aflatoxin was obtained with viable cells of ($AKF_{4)}$ isolate reaching to 99.9%; the volume of inoculum had $1X10^8$ cfu/ ml for 48 hours. The concentration of heat-killing cells was 500 pb for isolates ($AKF_{1, 2, 5}$) for 48 hours giving highest binding percentage 100 % at 37° C and pH 6.5. In addition, (AKF_4) had highest binding percentage 100% when it was incubated for 24 and 48 hours at the same conditions.
10. A laboratory system with high performance was designed to operate for binding mycotoxins (which contaminate grains) with viable bacterial cells, heat-killed cells and their metabolic products. The design of system was depend on several factors: numbers of viable bacterial cells, concentration of heat-killed cells, concentration of metabolic products of cells, temperature and period of incubation in order to use this system in grain silos for treating wheat grain contaminated by mycotoxins.
11. Aflatoxin was bound with *Lactobacillus* spp by using the designed system. The binding ratio was reached to 100% for isolate (AKF_4), which was *Lactobacillus plantarum*, after heat-killing at a concentration of 500 pb. at 37° C; whereas, the lowest binding ratio was when using its metabolic products at a concentration of 0.2 ml / ml at 45° C for 48 hours.
12. The results showed the binding efficiency of heat-killed cells was 100%, whereas the binding ratio of the viable cells were between 99.87% and 100%

when they were washed by using water, acetonitrile and phosphate buffered saline.

13. Biological estimation test of the effect of *L. Plantarum* on inhibition of aflatoxin B1 was applied by using laboratory rats through testing of several criteria such as weight, blood and histological anatomy. It showed that T2 and T3 treatments were significantly higher than other treatments undertaken to all tests mentioned above.