Studies of Fungi and Some

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Arabic.Summary Rice (Orayza sativa L.) is one of the most important grain crops in Egypt. It is used for human consumption, paper production, starch, alcohol, rice flour, manual fertilizer, poultry and animal feeding. The present investigation was aimed to isolate and identify the fungi associated with rice grains in Egypt, determine their ability to produce mycotoxins, investigate the antifungal effect by some spices and determine of the antimycotoxin. Studied the biological evaluation for the safety of Cinnamon (Cinnamomum burmannil L.) extract against the health hazards resulted from the consumption of mycotoxins (aflatoxin and fumonisin)-contaminated rice in laboratory animals were also studied, This can be summarized as follows : 1. sing two types of media1270 fungal isolates were isolated 651 on Czapek's-yeast extract agar medium (219 from fresh rough and polished rice and 432 from stored rough and polished rice) and 619 fungal isolates on Malt-extract agar medium (200 from fresh rough and polished rice and 419 from stored rough and polished rice). 2.Surface sterilized grains gave lower numbers of fungal colony, whereas, 126 fungal isolates were recorded with sterilized fresh grains and 259 isolates in the sterilized stored grains. However, the non sterilized surface recorded higher counts 293 isolates in the non sterilized fresh grains and 592 in the non sterilized stored grains. 3. The results showed that the isolated fungi from polished rice grains more less than those isolated from rough rice whereas; 272 isolates were recorded in rough fresh rice and 502 isolates were isolated from rough stored rice. In case of polished rice, the result showed that 147 were associated with polished fresh rice and 349 isolates were obtained from polished stored rice. 4- the fresh and rough rice samples collected from Sharkia recorded the higher total fungal count (TFC) 91 isolates. However, the rough samples collected from Kalyobia recorded the less TFC 56 isolates. On the other hand, the stored rough rice grains collected from Sharkia recorded the highest TFC 138 isolates and the lowest found in Dkahila 111 isolates. The fresh polished rice samples collected from Behira showed the highest TFC 74 isolates and Sharkia recorded the less TFC 15 isolates. however, the stored polished samples showed the highest TFC in Behira grains 152 isolates and the lowest recorded in Dkahlia 52 isolates. 5.eight fungal genera were isolated Aspergillus, Fusarium, Penicillium, Rhizopus, Mucor, Alternaria, Cladosporium and Curvularia spp. however, apergillus sp was the most isolated fungal genera and it recorded 37.9 and 43.0 % of the total fungi of fresh rice in Czapek's-yeast extract agar medium and Malt-extract agar medium, respectively and 36.34 and 37.23 % in stored rice in the two types of media followed, by Fusarium spp which represented 28.77 and 23.0 % in fresh rice and 31.02 and 25.06% in stored rice in the two

media, respectively, 6. All fresh and stored rough and polished rice samples were infected with the toxigenic fungi, A. flavus and A. parasiticus which gave the ability to produce aflatoxins also F. moniliforme which have the ability to produce fumonisins appeared only in the fresh rice grains collected from Sharkia 9 and Behira 13 isolates and in all stored samples from the four governorates. The higher total count of A. flavus was found in fresh and stored rice grains obtained from Behira 28, 41 isolates respectively. However, the total count of A. parasiticus revealed that this species was the prominent in the fresh and stored samples collected from Sharkia 13, 25 isolates respectively.7. All the fresh rough rice grains collected from the four tested governorates recorded the same moisture content 13.22%. But the stored rough rice grains from Sharkia for one year recorded the higher moisture content 12.3% followed by the samples collected from Kalyobia 11.29%. However, samples collected from Dkahlia were found to be less moisture content 11.12%. In case of fresh polished rice, the moisture content of all rice grains collected from the tested governorates were also the same 14.17%, whenever the moisture content of the stored polished rice grains collected from Behira showed the higher moisture content13.76% , and Kalyobia recorded the less moisture content 11.03%. 8.It was found that A. flavus has the ability to produce aflatoxin B1 (AFB1) and B2 (AFB2). The concentration of AFB1 determined by HPLC was 35.2 µg/L liquid media whereas; the concentration of AFB2 was 19.1µg / L On the other hand, A. parasiticus also had the ability to produce aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2). The concentration of AFB1 was 41.2µg/L and AFB2 was 12.1µg / L whereas; the concentration of AFG1 was 7.4 1µg / L and AFG2 was 7.2µg / L. Also F. moniliforme produce fumonisin B1 (FB1) in a concentration of 126.9 mg / L as determined by HPLC 9. It was noticed that the presence of the four aflatoxins AFG1, AFB1, AFG2 and AFB2 in the rough rice grains however ; AFG1 was absent in all polished rice grains and AFG2 was absent in the polished rice grains collected from Kalyobia. The highest aflatoxins contamination was found in the fresh rough grains collected from Behira 1.44 µg / kg followed by those obtained from Dkahlia, Sharkia and Kalyobia 1.28, 1.132 and 0.926µg / kg respectively. whereas, in the fresh polished samples, Behira was the higher aflatoxins concentrations 0.794µg / kg followed by Sharkia, Dkahlia and Kalyobia 0.745, 0.257 and 0.034µg/kg respectively. All the stored rice grains were contaminated with aflatoxin and the stored rough grains collected from Sharkia showed the highest contamination level 2.506 µg / kg followed by Behira, Dkahlia and Kalyobia 1.81, 1.594 and 1.580 µg / kg respectively - On the other hand, the stored polished rice grains collected from Behira recorded the highest aflatoxin contamination level 1.041 µg/kg followed by those collected from Sharkia, Dkahlia and Kalyobia 0.646 645 and 0.513 µg / kg respectively 10. Only FB1 was present in all samples,The fresh rough and polished rice grains collected from Sharkia showed the highest fumonisin contamination 26 and 8 mg / kg respectively, followed by Behira 3 mg / kg in both, however Dkahlia and kalyobia were fumonisin free. It is of interest to mention that the concentration of fumonisin in rough rice was higher than polished rice, also higher in stored than fresh. the stored rough rice grains collected from Shrakia was the highest FB1 concentration 214 mg / kg followed by Dkahlia, Kalyobia and Behira 134, 94, and 80 mg / kg respectively. however, the stored polished rice grains from Dkahlia showed

the highest FB1 concentration 98 mg/kg followed by Behira, Sharkia and Kalyobia 80, 53 and 46 mg / kg respectively 11. the extracts of Cinnamon, Thyme, Ginger, and Black seeds were found to increase the inhibition zone in the treated plates compared to the control. the ethanolic extract of Cinnamon was more effective and caused the highest inhibition zone 3.15 and 3.56cm, followed by Thyme 2.75 and 2.31cm, Ginger 1.96 and 1.56cm in case of both Aspergillus parasiticus and A. flavus respectively, in Fusarium moniliforme, the Cinnamon extract was the best 4.18cm followed by Ginger 2.4cm, thyme 2.35 Black seed 2.36cm. While the water extract of Cinnamon was the only one affected on the growth of A. parasiticus and A. flavus caused inhibition zones 2.85cm and 2.21cm. But in case of F. moniliforme, the Cinnamon was the more effective 2.55cm followed by Ginger 2.46 cm, Black seed 2.41cm and Thyme 2.28 cm compared to control 1.3 cm. 12. the addition of Cinnamon, Thyme, Ginger and Black seed powder at a concentration of 8% (w/v) inhibited the growth diameter of the three fungal species.Cinnamon had the ability to made complete inhibition to the growth of Aspergillus parasiticus, A. flavus and Fusarium moniliforme. However, ginger caused decreased in the growth diameter to 3.66 cm in A. parasiticus compared to control followed by thyme where the growth diameter recorded 4.35 cm. While in case of A. flavus, the effect of thyme was less than cinnamon and resulting in diameter measure 4.53 cm followed by ginger 4.56 cm, but black seed activated the growth 0.9 cm compared to control 7.83, 0.9 cm in both A. parasiticus and A. flavus. Both cinnamon and thyme extracts had the ability to induce complete inhibition of F. moniliforme however; ginger was less effective than both (4.4 cm) followed by black seed which was the less inhibitor 5.73 cm 13. Measuring the mycelia dry weight (biomass) of Aspergillus parasiticus, A. flavus and Fusarium moniliforme at 8% (w/v) of the spices powder revealed that, the cinnamon powder was the best in the three species due to the inhibition of their growth followed in there effect by thyme (0.37, 0.43 and 0.24 gm) and ginger (0.45, 0.54 and 0.38 gm), but black seed made growth activation to three species leading to greater biomass (0.69, 0.78 and 0.71 gm) compared to control (0.64, 0.72 and 0.67 g), 14. It was also showed that, the minimum inhibitory concentration (MIC) of thyme to Aspergillus parasiticus, A. flavus and Fusarium moniliforme were 16, 14 and 8 % (w/v) respectively. These concentrations were the minimum sufficient to stop the growth of the three species. Moreover, cinnamon was more active than thyme where MIC for A. parasiticus 2% and 1% w/v was sufficient to induce complete inhibition of the growth of A. flavus and F. moniliforme. 15. the cinnamon extract (200 mg/kg b.w) was further tested for its safety as antioxidants and for the protection against the health hazards of aflatoxin and fumonisin in rats using mature Sprague -Dawley male rats (100-120 g). 16. Animals received aflatoxin and / or fumonisin-contaminated diet 2 mg AFs and 125 mg FB / kg feed for three weeks showed a significant increase in liver enzymes activity and severe toxic effects on kidney function parameter, The extract alone 200 mg/kg did not show any significant effects. Moreover, animal fed the mycotoxins-contaminated diet and treated with the extract showed a significant improvement in the tested enzymes activity 17. the histological results cleared that the animals treated with the two mycotoxins singly or on combination showed severe histological changes in liver and kidney, these changes were more pronounced in the group received the

combined treatment. They seem to act synergistically with respect to cancer initiation as the number of hepatocytes nodular cirrhosis and foci was significantly increased 18. It could be concluded that the contamination of rice with the toxigenic fungi may induce risks to consumers and the treatment with cinnamon during storage and/or cooking may decrease or prevent these risks. Because cinnamon is chemically safe and acceptable to consumer, as synthetic chemical fungicides can cause adverse health affects consumers.