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Mycological and molecular studies on some fungi isolated from meat products and spices.

A thesis presented by

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7- SUMMARY

A total of 120 random samples of luncheon, basterma, kofta and burger (30 of each) and 99 samples of spices were collected from different markets in Gharbia governorate. These samples were subjected to mycological examination for isolation and identification of different mould species.

The results revealed that the mold could be detected in (18) 60%, (27) 90%, (21) 70%, (27) 90%, (30) 90.9%, (33) 100% and (30) 90.9% in examined luncheon, kofta, basterma, burger, luncheon spices, kofta spices and burger spices, respectively.

The most frequently isolated mould species from samples was *Aspergillus* spp. with prevalence of 24 (88.88%) in luncheon, 36 (85.71%) in kofta, 35 (85.36%) in basterma, 46 (58.23%) in burger, 60 (66.66%) in luncheon spices, 86 (55.48%) in kofta spices and 66 (57.89%) in burger spices followed by *Penicillium* spp. 3 (11.12%), 6 (14.29%), 3 (7.32%) and 12 (15.18%), 9 (10.03%), 24 (15.48%) and 21 (18.44%), respectively. Other identified mould isolates were *Acremonium*, *Cladosporium*, *Geotrichum*, *Mucor*, *Claveolaria*, *Emericella.nudulans*, *Eurotium*, *Fusarium* and *Scorulopsis*.

A.flavus was the most isolated *Aspergillus* spp in luncheon, kofta, basterma and burger; it was presented as 15 (62.5%), 12 (33.33%), 18 (51.44%) and 15 (32.61%), respectively. followed by *A.niger* 9 (37.5%), 24 (66.67%), 9 (25.71%) and 15 (32.61%), respectively. While *A. clavatus* 6 (17.14%), 9(19.57%) and *A. sydowii* 2(5.71%), 4 (8.69%) detected only in basterma and burger samples and *A. Ochraceus* 3 (6.52%) also detected only in burger samples

A.niger was the most isolated *Aspergillus* spp. in luncheon spices, kofta spices and burger spices; it was presented as 18 (30.0%), 30 (34.88%) and 24 (36.36%), respectively. followed by *A.flavus* 21 (35.0%), 30 (34.88%) and 18

(27.26%), respectively. then *A. ochraceus* 3 (5.0%), 9 (10.47%) and 9 (13.64%), respectively. then *A. carbonareus* 3 (5.0%), 6 (6.98%) and 9 (13.64%), respectively. then *A. fumigatus* 9 (15.0%) , 6 (6.98%) detected in luncheon spices and kofta spices, respectively. *A. clavatus* 2(2.33%), 3 (4.55%) detected in kofta spices and burger spices, respectively. on the other hand *A. candidus* 3 (5.0%) and *A. parasiticus* 3 (5.0%) detected only in luncheon spices, while *A. sydowii* 3 (3.49%) and *A. ustus* 3 (4.55%) detected in kofta spices and burger spices, respectively.

The prevalence of *Penicillium* spp. in meat products were presented as *P. citrinum*, *P. aurantigreum*, *P. chrysogenum* were the most predominant species isolated from burger samples, their frequencies were 6 (50%), 3 (25%), 3 (25%), respectively. While from kofta samples, *P. Paxilli* and *P. restrictum* were the most predominant isolated species, its frequency were 3 (50%), 3 (50%), respectively. 3(100%) *P. citreognigrum* and 3 (100%) *P. carneum* only detected in luncheon and basterma samples, respectively.

The prevalence of *Penicillium* spp. in spices were presented as *P. aurantigreum*, *P. chrysogenum*, *P. citrinum*, *P. crustosum* and *P. implicatum* *P. simplicissimum* were the most predominant species isolated from kofta spices samples, their frequencies were 6 (25%), 3 (12.5%), 3 (12.5%), 6 (25%), 3 (12.5%) and 3 (12.5%), respectively. While from burger spices samples, *P. aurantigreum*, *P. flavigenium* and *P. simplicissimum* were the most predominant isolated species, its frequency were 9 (42.85%), 3 (14.3%) and 9 (42.85%), respectively. 6 (66.67%) *P. citreognigrum* and 3 (33.33%) *P. citrinum* detected in luncheon spices.

The aflatoxin B1 could be detected in 1, 1, 2, 6 samples of Luncheon, kofta, basterma and spices, respectively. The minimum detected level of aflatoxin B1 in Luncheon and kofta was 3.3µg/kg and 2.6µg/kg, respectively. While in basterma samples, the minimum detected level of aflatoxin B1 was

5.1µg/ kg and maximum was 7.2µg/kg. On other hand in spices samples, the minimum detected level of aflatoxin B1 was 4.7µg/ kg and maximum was 13.1µg/kg with a mean value of $9.6 \pm 2.2 \mu\text{g/kg}$. Aflatoxin B1 could not be detected in examined burger.

The ochratoxin A could be detected in 5, 1, 6, 6, and 4 samples of luncheon, kofta, basterma, burger and spices, respectively. The minimum and maximum detected level of ochratoxin A in luncheon and kofta, basterma, burger and spices were 3.2-11.3µg/kg, 2.8 µg/kg, 2.1-7.6 µg/kg, 2.6-7.8 and 4.6-7.1µg/kg, respectively. A mean value of ochratoxin A in these samples were $5.1 \pm 2.0 \mu\text{g/kg}$, -, $4.2 \pm 1.1 \mu\text{g/kg}$, $4.0 \pm 1.1 \mu\text{g/kg}$ and $5.2 \pm 0.7 \mu\text{g/kg}$, respectively.

The results was shown that *A. flavus*, *A. niger*, *A. ochraceus*, *A. clavatus*, *A. sydowii*, *A. candidus*, *A. parasiticus*, *A. fumigatus*, *A. ustus*, *F. verticillioides*, *P. aurantigreum*, *P. Paxilli*, *P. citrinum*, *P. restrictum*, *P. carneum*, *P. simplicissimum*, *P. chrysogenum* and *P. crustosum* were positive for proteolytic activity on modify Czapek agar (MCA) medium, while *A. carbonarius*, *P. implicatum*, *P. flavigenium* and *P. citreognigrum* were negative.

Two *Asperigillus* (*A. clavatus* and *A. sydowii*), three *Penicillium* isolates (*P. chrysogenum*, *P. carneum* and *P. restrictum*) from meat products, one *Penicillium* isolate (*P. crustosum*) and one *F. verticillioides* isolate from spices were examined by molecular methods polymerase chain reaction (PCR) with using primer ITS. PCR products of strain were positive on agarose gel electrophoresis. ITS was amplified and sequenced successfully for the positive tested samples. The obtained sequences were deposited at NCBI under Gene bank accession number: MK643344 *A. clavatus* isolate AYMA1, MK643345 *A. sydowii* isolate AYMA2, MK643347 *P. restrictum* isolate AYMA4, MK643348 *P. chrysogenum* isolate AYMA5, MK643349 *P. carneum* isolate

AYMA6, MK643350 *P.crustosum* isolate AYMA7, and MK643346
F.verticillioides AYM3.