7. Summary

The present study deals with the phylogenetic analysis of *A. nidulans* group and assay of how much *A. aegyptiacus* belongs to *A. nidulans* group or *A. versicolor* group.

The revealed results can be summarized as follows:

1- The eight fungal isolates used in this investigation were identified as two isolates of *Aspergillus aegyptiacus* (AUMC 6122 & 3603),*E. nidulans* (AUMC152), *E. rugulosa* (AUMC164), *E. variecolor* (AUMC165), *A.caespitious* (AUMC4289), *A. sydwoii* (AUMC 4221), *A.versicolor* (AUMC 92),by Assiut University Mycological Center (AUMC) whichwere isolated during the present study; using dilution Plate method from different sites in Fayoum Governorate and were employed in the present study.

2- The best mycelial growth for subsequence studies was obtained on liquid Czapek's-Dox medium supplemented with 1 g/l yeast extract under dark conditions at 28°C for one week. In absence of yeast extract, the fungi grow faintly after two weeks of incubation under the same conditions.

3- Large quantities of DNA were extracted from the eight isolates of *Aspergillus spp.* DNA yields appeared as clear, intense and sharp bands using CTAB extraction method.

4- RAPD analysis was used to investigate the polymorphism for all isolates under study using six arbitrary primers A1, A2, A3, B1, B2 and B4. The six random primers recorded variation between the four isolates. The total number of

bands was **(142)** for all six primers. Primer **(A1)** produced the highest number of bands **(32)**, while the lowest number of bands was **(18)**produced by primer **(B2)**.The size of the amplified bands varied with the different primers. The largest band was at **3,000-bp**, which was amplified by primer **B4** of isolate No. 2 (*E. variecolor*), while the smallest one was at **100 bp**.amplified by primer **B1**of isolate No. 3 *(E. rugulosa).*

5- The degree of similarity was determined and dendrogram was established for the *Aspergillus* isolates using UPGMA program. The *Aspergillus* isolates were collected into two main clusters (Cluster I, Cluster II). Cluster II included the two isolates of *Aspergillus aegyptiacus* only while Cluster I was splitted into two subclusters (Cluster A, Cluster B). Cluster A, included: *E. nidulans; E. rugulosa* and Cluster B, included only *E. variecolor*

6- The results obtained by partial sequencing of ITS gene showed only one unique band range from 250- 270bp in ITS1 region and 320-350 bp in ITS2 region.

7- Purification and sequencing of the PCR products for the isolates under investigation were performed in Genetic Analyzer Unit, Egypt. DNA sequences were obtained using Dye Terminator Cycle Sequencing. Blast program (www.ncbi.nlm.gov/blast) which was used to assess DNA similarities of eight isolates sequenced with already recorded sequences on NCBI Genebank. Multiple sequence alignment and molecular phylogeny were performed using BioEdit software, the phylogenetic tree was displayed using the TREEVIEW program (Mega4 and ClastalW2).

8- Based on the length of ITS1 of the eight *Aspergillus* spp. could be divided into two clusters. The first cluster consisted of *E. nidulans, E. variecolor and E. rugulosa* at value 1.2 and subcluster consisted of *E. nidulans, E. variecolor* at value 0.5. The second cluster included the two isolates of *Aspergillus aegyptiacus* (AUMC 6122 & 3603),*A. sydowii, A.versicolor and A. caespitious*at value 2.4 and subcluster included two isolates of *Aspergillus aegyptiacus* (AUMC 6122 & 3603) and *A. sydowii, A. versicolor* at value 1.7. Depicting the genetic relationship among *Aspergillus* spp. revealed that *E. nidulans* is closely related to *E. variecolor* and two isolates of *Aspergillus aegyptiacus* (AUMC 6122 & 3603) closely related to *A. sydowii, A. versicolor* in phylogeny.

9- Based on the length of ITS2 sequences, the five *Aspergillus* spp. could be divided into two clusters. The first cluster consisted of *Emericella* species and one isolate of *Aspergillus aegyptiacus* (AUMC 3603) at value 0.58; the subcluster at value 0.25 included *E. nidulans, E. variecolor and E. rugulosa*. The second cluster included only one isolate of *Aspergillus aegyptiacus* (AUMC 6122) at value 0.75. Depicting the genetic relationship among *Emericella* species revealed that *E. variecolor* and *E. rugulosa* are closely related at value 0.1 in phylogeny.

10- The above results lend further support to the recommendation that *A.aegyptiacus* is closely related to *A. versicolor* group, rather than *A. nidulans* group.