



# Studies on the Entomopathogenic Fungi of Cereal Aphids (Homoptera: Aphididae) in Upper Egypt

By

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## THESIS

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#### SUMMARY

The results could be summarized as follows:

1- Owing to survey studies through 2014-2015 and 2015-2016 wheat growing seasons in three governorates (Assiut, Sohage and Quena) five entomopathogenic fungi were isolated and identified. These fungi are *Cladosporium cladosporioides, Beauveria bassiana, Paecilomyces variotii, Verticillium lecanii* and *Metarhizium anisopliae*.

2- The results also showed a great diversity of entomopathogenic fungi found attacking cereal aphids in southern Egypt. Five species of entomopathogenic fungi were surveyed and identified infecting cereal aphids which infesting wheat plants in southern Egypt.

3- Incidence of the entomopathogenic fungi recovered attacking cereal aphids on wheat plants in three governorates in Upper Egypt during two seasons 2014/2015 and 2015/2016 and that *C. cladosporioides* had great abundance in February at the three governorates on cereal aphids in the season 2014/2015. However, the largest abundance was reported in Sohage with 77.4 % frequency in the same season.

4- *B. bassiana* was only superior in its abundance on cereal aphids in February with 28.4 % frequency. Meanwhile, *P. variotii*, showed its infecting abundance superiority on these aphids with 35.6 % only in March at Assiut governorate. Meanwhile, its abundance has reached 31.1% and 27.6 % frequency in Qena and Sohage governorates respectively in February and March.

5- In case of *M. anisopliae* the highest abundance on the cereal aphids was recorded with great abundance 36.0 % of frequency that was happened at Assiut in March. However, abundance the same fungus

reached to 18.6 % of frequency in April at Sohage and 32.0 % in February at Qena governorate.

6- *M. anispoliae* and *B. bassiana* had a great abundance in March and April month by 32.7 % and 36.34 % at Assiut governorate on the cereal aphids 2015/2016 season. However, *M. anispoliae* was superior in its abundance at Qena governorate in March and April months by 46.96 and 54.6 % respectively. Meanwhile, the other entomopathogenic fungi which were superior in there spread in the 2014/2015 season on the cereal aphids such as *C. cladosporioides*, showed the least distribution on the cereal aphids in three governorates in Upper Egypt in 2015/2016 season.

7- Results indicated that various entomopathogenic fungi caused aphid mycoses according to seasonal incidence of these fungi on cereal aphid species.

8- Entomopathogenic fungi appeared to be of high value in the natural control of cereal aphids infesting wheat plants in southern Egypt.

9- In conclusion, Entomophthoralean fungi played a principle role in natural suppression of cereal aphid populations in Upper Egypt, particularly at the time of their highest population level. It is necessary to consider that the decline of cereal aphid population results from a combination of an increased population of alate adults, decline host plant quality and the action of aphid natural enemies. Thus, proper methods of pest management in winter wheat crops, particularly in wheat field, should be applied to protect populations of natural enemies against the damaging effect of unnecessary application of pesticides.

10- The utilization of entomopathogenic fungi as a biological control agent could be complementary strategies in an integrated pest management program against cereal aphids in Upper Egypt.

11- *M. anisopliae* was superior in its effect and induced the percentage of cereal aphid mortality by 62.67 % and followed by *C. cladosporioides* with 56.00 %, respectively. However, *V. lecanii and P. variotii* induced the percentage of cereal aphid mortality by 52.67% and followed by *B. bassiana*, which induced cereal aphid mortality by 49.33 % after 4 days from exposure.

12- *M. anisopliae* was superior in its effect and induced the percentage of cereal aphid mortality by 65.33% and followed by *B. bassiana* and *C. cladosporioides* with 61.33%. However, *V. lecanii* induced cereal aphids by 60.00 % and followed by *P. variotii*, which induced cereal aphid mortality by 57.33% after 6 days from spraying.

13- The sporulation of the tested entomopathogenic fungi was greatly affected by the type of culture medium. Modified culture medium of potato dextrose (PDA) agar, rice agar medium and Murashige &Skoog showed a tangible effect on fungal growth. Data showed also that modified Murashige & Skoog was the best culture medium which accelerated the fungal growth of the five tested entomopathogenic fungi.

14- The entomopathogenic fungi were greatly affected by different liquid culture media. Modified liquid culture media of potato dextrose (PDA) agar, rice agar medium and Murashige &Skoog showed a tangible effect on fungal bio-mass growth and sporulation. Data showed also that modified Murashige & Skoog was the best liquid culture medium which accelerates fungal biomass growth and sporulation of five tested entomopathogenic fungi.

15- The modified PDA, rice and Murasieghe and Skoog medium produced more conidial fungal spores when the tested entomopathogenic fungi grown on it. Results also showed that modified Murasieghe and

Skoog medium was superior in its effect on the all five tested entomopathogenic fungi and enhancement its sporulation

16- Significant differences were found between the five tested fungi and the interaction between them in case of the effect of the three tested culture media on its sporulation at 1%.

17- Data of Scanning electron microscopy (SEM) revealed that cereal aphid insects when subjected to artificial inoculation with spore suspension of five tested entomopathogenic fungi with concentration of  $(5x10^9 \text{ spores/ml plus } 0.03 \text{ ml /l of Tween 22})$  were sensitive to invasions and penetrations by the tested entomopathogenic fungi, and those fungi were able to induce mortality.

18- The close attachment as shown in the scanning electron micrograph of fungal mycelium to cereal aphid cuticle may be due to a secretion from insect host to fungal spores to accelerate its germination and conditions it for penetration as sensitive host. This may be considered as unique mechanism of the entomopathogenic fungus.

19- Scanning electron microscopy allowed us a tool to observe the mode of action of entomopathogenic fungi and to observe how they can colonize and infect the host insects.

20- Data obtained showed that by using primer A11 at 30° C there is no intraspecific variability between *M. anisopliae* isolates No. 1, 2, 3, and 4 of PCR amplified fragments. Meanwhile, when using primer Bo1 at the same temperature 30° C *M. anisopliae* isolates showed high level of similarity between 1, 2 and 3 isolates at 700, 400, and 300 and very little in case of isolate No. 4 at 700 base pairs. However, a little similarity has been found with the PCR-amplified fragment of *P. variotii* isolates No. 5 and 6 at 400 and 300 base pairs but not isolate No. 7.

21- Phylogenetic analysis showed close relation between M. *anisopliae* isolates 1, 2 and 3 when using primer Bo1 at the same temperature 30°C. However, a little similarity has been found with the PCR-amplified fragment of *P. variotii* isolates No. 5 and 6 at 400 and 300 base pairs but not isolate No. 7.