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SEASONAL INCIDENCE OF ENTOMOPATHOGENIC FUNGI INFECTING CEREAL APHIDS OF WHEAT PLANTS IN ASSIUT, EGYPT

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ABSTRACT: The present investigation was carried out during two successive wheat growing seasons (2002/2003 and 2003/2004). The seasonal incidence of entomopathogenic fungi infecting cereal aphids was evaluated. Regardless of the aphid host and the season, the present results indicated that various entomopathogenic fungi caused aphid mycoses. The incidence of each pathogen or pathogen group was estimated from 2100 cadavers of three aphid species examined microscopically during 2003 and 2004 wheat growing seasons. There are twelve identified species of entomopathogenic fungi infecting cereal aphids. The first case of mycosis was observed at the beginning of February. In addition, the number of diseased aphids increased towards end of March.

The results indicated that *Pandora neoaphidis*, *Beauveria bassiana* and *Zoophthora radicans* seem to be the most abundant entomopathogenic fungi as biological control agents. However, *Beauveria brongniartii*, *B. alba*, *Condiobolus coronatus*, *C. obscurus*, *C. thromboids*, *Entomophthora planchoniana*, and *Paecilomyces farinosus* could be of economic importance, if the environmental conditions changed in their favour. Meanwhile, the species of *Verticillium lecanii* and *Basidiobolus* sp. are expected to be of little economic importance.

Regarding seasonal incidence of these fungi species, *Beauveria* spp, *Zoophthora radicans* and *Paecilomyces* were observed from the beginning of February up to March 15. *Pandora neoaphidis* and *Conidiobolus* spp. were detected one week later of the previous species till March 15. Incidence with *Entomophthora planchoniana* lasted for one month from February 22 to March 15. *Verticillium* was scarce where it was observed two times only at the beginning of the season from February 1 to 8.

Key words : Wheat plants, cereal aphids, entomopathogenic fungi.

INTRODUCTION

Entomopathogenic fungi are now being considered as biological alternatives to chemical control. Several species of entomopathogenic fungi can cause fatal diseases in aphids, including *Conidiobolus* spp. *Verticillium leacanii* (Zimmerman) Viegas, various species of *Beauveria*, *Paecilomyces farinosus* (Holm ex S.F. Gery) Brown & Smith (Roberts & Yendol 1971, Samson *et al.* 1988).

Entomopathogenic fungi are frequently reported as major factors suppressing populations of cereal aphids and can cause sudden decline of dense populations (Feng *et al.* 1991). Three entomopathoralean fungi species killed 65-80% of common cereal aphids (Dean & Wilding 1973). Drastic reduction in the populations of various cereal aphids due to infection with Entomopathorales fungi was observed by Dedryver (1983).

Aim of the present study is to monitor seasonal incidence of the indigenous entomopathogenic fungi infecting cereal aphids at Assiut Governorate.

MATERIALS AND METHODS

The present investigation was carried out at the Experimental Farm of Assiut University (Faculty of Agriculture - Agronomy Department) throughout two successive wheat-growing seasons, 2002-2003 and 2003-2004. An area of ca. 4200 m^2 (one feddan) was cultivated with wheat (cultivar Giza 164) normally at mid-November in all cultivated seasons. Regular conventional agricultural practices were normally performed and no chemical control (insecticides or fungicides) was used during the study period. Weeds were removed by hand.

During the two seasons, weekly samples each consisted of 150 living late instars and apterous adults of cereal aphids (*Rhopalosiphum padi*, *R. maidis* and *Schizaphus graminum*) were randomly collected from the field and transferred to the laboratory. Fifty individuals of which were selected and reared individually on 5-cm leaf sections in 65- mm Petri dishes. Moistened cotton was placed over the ends of the leaf sections in the dish to maintain relative humidity near saturation. Petri dishes containing alive aphids were incubated for 10 days at 20°C with a photoperiod of 16:8 (L:D). Leaves remained fresh for several days and were replaced twice a week. Dead aphids were recorded, placed in 1x5 cm vials and stored at 5°C. Aphid cadavers were

examined under a dissecting microscope as soon as possible after death to observe external symptoms and fungal reproductive structures if produced in situ on the plant. Desiccated and fresh cadavers were placed in a moist chamber for about 20 hrs to allow hyphae and reproductive structures to develop. Individual aphids were mounted in cotton blue or aceto-orcein and observed under a compound microscope. Identification of fungi was based on external symptoms and the morphology of spores and sporulating structures (Waterhouse and Brady, 1982), and new revision of the classification of Entomophthorales (Humber 1989) was followed. Fungi identified as known aphid pathogens were considered to be the cause of death of their host.

Statistical Analysis

Data were statistically analyzed using analysis of variance (F test) and means were compared according to Duncan's multiple range test.

Dominance (%) and abundance (%) degrees of the identified fungi species were evaluated according to the formula of Facylate, 1971.

Percentage of infection (mortality %) caused by entomopathogenic fungi was calculated in each sampling date according to Feng *et al.* (1992) as follow:

$$\text{Mortality (\%)} = \frac{\text{Number of infected aphids}}{\text{(Total number of alive aphids)}} \times 100$$

RESULTS AND DISCUSSION

Data in Tables 1, 2 and 3 show the seasonal incidence of fungi infecting cereal aphid complex regardless of the aphid species during two successive wheat-growing seasons (2002-2003 and 2003-2004).

2002 - 2003 season

Data in Table 1 indicate that 365 individuals of cereal aphids were infected with fungi out of a total of 1050 alive individuals constituting 34.73% mortality rate during the whole season. Eleven species belonging to seven genera of entomopathogenic fungi, have been identified infecting cereal aphids in the field. These species are *Beauveria bassiana*, *B. brongniartii*, *B. alba*, *Conidiobolus coronatus*, *C. obscurus*, *C. thromboides*, *Entomophthora planchoniana*, *Paecilomyces farinosus*, *Pandora neoaphidis*, *Verticillium lecanii* and *Zoophthora radicans*.

Statistical analysis showed that there are significant differences among the occurrence of these fungi species.

Table 1. Seasonal incidence of entomopathogenic fungi identified from alive cereal aphid complex collected from wheat plants during 2002 / 2003 season

Fungi species	Number and percentage (%) of infected aphids									
	Inspection date									
	Jan	Feb.				March				Total
	22	1	8	15	22	1	8	15	22	
Beauveria bassiana	-	3(2.00)	*7(4.67)	12(8.00)	12(8.00)	9(6.00)	8(5.33)	10(6.67)	-	61b
B. brongniartii	-	2(1.33)	0	0	0	2(1.33)	9(6.00)	7(4.67)	-	20d
B. alba	-	3(2.00)	6(4.00)	8(5.33)	5(3.33)	7(4.67)	3(2.00)	1(0.67)	-	33c
Conidiobolus coronatus	-	0	2(1.33)	4(2.67)	5(3.33)	8(5.33)	9(6.00)	7(4.67)	-	35c
C. obscurus	-	0	1(0.67)	3(2.00)	4(2.67)	7(4.67)	9(6.00)	7(4.67)	-	31c
C. thromboides	-	0	1(0.67)	2(1.33)	3(2.00)	5(3.33)	5(3.33)	5(3.33	-	21d
Entomophthora planchoniana	-	0	0	0	2(1.33)	4(2.67)	9(6.00)	4(2.67)	-	19d
Paecilomyces farinosus	-	3(2.00)	6(4.00)	3(2.00)	2(1.33)	0	0	0	-	14d
Pandora neoaphidis	-	0	1(0.67)	6(4.00)	9(6.00)	14(9.33)	19(12.67)	28(18.67)	-	77a
Verticillium lecanii	-	2(1.33)	0	0	0	0	0	0	-	2e
Zoophthora radicans	-	0	0	1(0.67)	7(4.67)	14(9.33)	17(11.33)	13(8.67)	-	52b
Total number of infected aphids	-	13	24	39	49	70	88	82	-	365
Number of alive aphids / sample	-	150	150	150	150	150	150	150	-	1050
(%) of infection	-	8.67	16.00	26.00	32.67	46.67	58.67	54.67		34.73

Values followed by the same latter within column are not significantly different at <0.05 level of probability.
-Samples where there no aphids.
* Number of infected individuals / 150 alive aphids.
Figure in parentheses refer to the percentage of infection (%).

Table 2. Seasonal incidence of entomopathogenic fungi identified from alive cereal aphid complex collected from wheat plants during 2003 / 2004 season

Fungi species	Number and percentage (%) of infected aphids Inspection date									Total
	Jan	Feb.				March				
	22	1	8	15	22	1	8	15	22	
Basidiobolus sp.	0	0	*1(0.67)	3(2.00)	4(2.67)	2(2.00)	1(1.00)	0	-	11c
Beauveria bassiana	0	3(2.00)	6(4.00)	8(5.33)	10(6.67)	6(6.00)	3(3.00)	1(1.00)	-	37b
B. brongniartii	0	1(0.67)	2(1.33)	3(2.00)	4(2.67)	6(6.00)	8(8.00)	5(5.00)	-	29b
B. alba	0	0	2(1.33)	5(3.33)	4(2.67)	0	0	0	-	11c
Conidiobolus coronatus	0	0	0	2(1.33)	4(2.67)	4(4.00)	5(5.00)	4(4.00)	-	19c
C. obscurus	0	0	0	0	3(2.00)	4(4.00)	4(4.00)	3(3.00)	-	14c
C. thromboides	0	0	0	0	2(1.33)	1(1.00)	2(2.00)	2(2.00)	-	7d
Entomophthora planchoniana	0	0	0	0	0	2(2.00)	4(4.00)	6(6.00)	-	12c
Paecilomyces farinosus	0	0	0	0	0	1(1.00)	2(2.00)	4(4.00)	-	7d
Pandora neoaphidis	0	0	1(0.67)	4(2.67)	9(6.00)	7(7.00)	9(9.00)	8(8.00)	-	38b
Verticillium lecanii	0	0	1(0.67)	0	0	0	0	0	-	1d
Zoophthora radicans	0	1(0.67)	0	4(2.67)	10(6.67)	9(9.00)	12(12.00)	11(11.00)	-	47a
Total number of infected aphids	0	5	13	29	50	42	50	44	-	233
Number of alive aphids / sample	150	150	150	150	150	100	100	100	-	1050
(%) of infection	0	3.33	8.67	19.33	33.33	42.00	50.00	44.00		22.19

Values followed by the same latter within column are not significantly different at <0.05 level of probability.
-Samples where there no aphids.
*Number of infected individuals / 150 alive aphids.
Figure in parentheses refer to the percentage of infection (%).

Table 3. Seasonal incidence of entomopathogenic fungi identified from alive cereal aphid complex collected from wheat plants regardless of season

Fungi species	Number and percentage (%) of infected aphids										Dominance (%)	Abundance (%)
	Inspection date											
	Jan	Feb.				March				Total		
	22	1	8	15	22	1	8	15	22			
Basidiobolus sp.	0	0	*1(0.33)	3(1.00)	4(1.33)	2(0.80)	1(0.40)	0	-	11d	1.84	62.50
Beauveria bassiana	0	6(2.00)	13(4.33)	20(6.67)	22(7.33)	15(6.00)	11(4.40)	11(4.40)	-	98a	16.39	87.50
B. brongniartii	0	3(1.00)	2(0.67)	3(1.00)	4(1.33)	8(3.20)	17(6.80)	12(4.80)	-	49b	8.19	87.50
B. alba	0	3(1.00)	8(2.67)	13(4.33)	9(3.00)	7(2.80)	3(1.20)	1(0.40)	-	44b	7.36	87.50
Conidiobolus coronatus	0	0	2(0.67)	6(2.00)	9(3.00)	12(4.80)	14(5.60)	11(4.40)	-	54b	9.03	75.00
C. obscurus	0	0	1(0.33)	3(1.00)	7(2.33)	11(4.40)	13(5.20)	10(4.00)	-	45b	7.53	75.00
C. thromboides	0	0	1(0.33)	2(0.67)	5(1.67)	6(2.40)	7(2.80)	7(2.60)	-	28c	4.68	75.00
Entomophthora planchoniana	0	0	0	0	2(0.67)	6(2.40)	13(5.20)	10(4.00)	-	31c	5.18	50.00
Paecilomyces farinosus	0	3(1.00)	6(2.00)	3(1.00)	2(0.67)	1(0.40)	2(0.80)	4(1.60)	-	21c	3.51	87.50
Pandora neoaphidis	0	0	2(0.67)	10(3.33)	18(6.00)	21(8.40)	28(11.20)	36(14.40)	-	115a	19.23	75.00
Verticillium lecanii	0	2(0.67)	1(0.33)	0	0	0	0	0	-	3d	0.50	25.00
Zoophthora radicans	0	1(0.33)	0	5(1.67)	17(5.67)	23(9.20)	29(11.60)	24(9.60)	-	99a	16.56	75.00
Total number of infected aphids	0	18	37	68	99	112	138	126	-	598	100	100
Number of alive aphids / sample	150	300	300	300	300	250	250	250	-	2100		
(%) of infection	0	6.00	12.33	22.67	33.00	44.80	55.20	50.40	-	28.48		

Values followed by the same latter within column are not significantly different at <0.05 level of probability.
-Samples where there no aphids.
*Number of infected individuals / 150 alive aphids.
Figure in parentheses refer to the percentage of infection (%).

Concerning seasonal incidence of these fungi, data showed that the first case of diseased insects was observed from the beginning of February with a percent mortality of 8.67% up to March 15 with a percent mortality of 54.67. Maximum mortality (58.67%) was detected on March 8. *Beauveria bassiana* and *B. alba* were observed from the beginning of February (mortality 2.00% for each) up to half of March (mortality 6.67% and 0.67%, respectively), maximum mortalities of 8.00% and 5.33% were observed during February 22 and 15, respectively. *Conidiobolus* spp. (*C. coronatus*, *C. obscurus* and *C. thromboides*) and *Pandora neoaphidis* were observed after one week of *Beauveria* spp. appearance. Mortalities caused by these species fluctuated from 1.33%, 0.67%, 0.67% and 0.67%, respectively during February 8 to 4.67, 4.67, 3.33 and 18.67%, respectively. *Zoophthora radicans* was detected from February 15 with a percentage mortality of 0.67% up to March 15 with 8.67%. *Entomophthora planchoniana* was detected from February 22 to March 15. *Beauveria brongniartii* was present during the last month of the season (three first weeks of March). *Verticillium* was detected one week only on February 1.

2003 - 2004 season

Data indicate that 233 individuals were infected out of collected 1050 alive aphids constituting 22.19% mortality rate during the whole season. Twelve species belonging to eight genera of fungi were found infecting cercal aphids regardless of aphid species, i.e; *Basidiobolus* sp., *Beauveria bassiana*, *B. brongniartii*, *B. alba*, *Conidiobolus coronatus*, *C. obscurus*, *C. thromboides*, *Entomophthora planchoniana*, *Paecilomyces farinosus*, *Pandora neoaphidis*, *Verticillium lecanii* and *Zoophthora radicans*.

Statistical analysis showed that *Zoophthora radicans* followed by *Pandora neoaphidis* and *Beauveria bassiana* were the most dominant species encountered 16.31%, 15.88% and 12.45%, respectively. The species *Basidiobolus* sp., *Beauveria alba*, *Conidiobolus coronatus*, *C. obscurus*, *C. thromboides* and *Entomophthora planchoniana* showed a moderate level of infection while *C. thromboides*, *Paecilomyces farinosus* and *Verticillium lecanii* were scarce.

Mortality rate of pathogenic fungi was observed from the beginning of February up to the third week of March. According to seasonal incidence of these fungi

species, data showed that *B. bassiana* was detected from the first week of February to the second week of March with a peak during the end of February. Mortality rate being 2%, 6.67% and 1% for the first detection, epizootic and the last record, respectively. *B. brongniartii* and *Zoophthora radicans* were firstly detected on February 1 and lasted till March 15. Mortality rate by these species was 0.67% at occurrence of their first infection and 8% and 12% at their epizootic and last record periods, respectively. *Pandora neoaphidis* was detected one week later after *Beauveria*. This species caused mortality that fluctuated from 0.67% during February 8 to 8% during March 15 with a peak on March 8 (9%). *B. alba* was detected from February 8 to the end of this month. *Conidiobolus coronatus* was observed from February 15 up to March 15 with a percentage mortality of 1.33% and 3%, respectively. *Paecilomyces* was found from the first week of March to March 15. *Verticillium* was observed only one time during February 8. *C. obscurus* and *C. thromboides* were detected from February 22 to March 15.

Generally, it could be concluded that, regardless of the aphid host and the season, the present results indicated that various entomopathogenic fungi caused aphid mycoses. The incidence of each pathogen or pathogen group was estimated from 2100 cadavers of three aphid species examined microscopically during 2003 and 2004 wheat growing seasons. There are twelve species of entomopathogenic fungi identified infecting cereal aphids. The first case of disease incidence was observed at the beginning of February. In addition, the number of diseased aphids increased by end of March (Table 3).

Also, data in Table 3 and Figure 1 show that *Pandora neoaphidis*, *Beauveria bassiana* and *Zoophthora radicans* seem to be the most important entomopathogenic fungi as biological control agents due to their highest values of dominance and abundance degrees. However, the high abundance degrees of *Beauveria brongniartii*, *B. alba*, *Condiobolus coronatus*, *C. obscurus*, *C. thromboids*, *Entomophthora planchoniana*, and *Paecilomyces farinosus* which had low dominance degrees indicate that these species could be of economic importance, if the environmental conditions changed in their favour. Meanwhile, the species of *Verticillium lecanii* and *Basidiobolus* sp. which had low values of dominance and abundance degrees are expected to be of little economic importance.

Regards of seasonal incidence of these fungi species, data presented in Table 3 and illustrated in Figure 2 show that *Beauveria bassiana* and *Zoophthora radicans* were observed from the beginning of February (mortality 2.0 and 0.3 %) up to March 15 (mortality 4.4 % and 9.6%, respectively). Maximum mortality caused by these species were observed during February 22 and March 8, respectively. *Pandora neoaphidis* was detected after one week later, post occurrence of the previous species and lasted till March 15. Mortality rate by this species was shown to be 0.67% and 14.40% according their first infection and their epizootic, respectively. *Beauveria brongniartii* and *B. alba* were recorded from the first week of February to the second week of March with a peak during March 8th and February 15, respectively. In respective to the genera, *Conidiobolus* app. and *Basidiobolus* infected cereal aphid species on February 8 , one week after the genus *Beauveria*. These genera, however, showed its peak of activity on March 8 and February 22. Mortality rates recorded by these genera were 0.67, 0.33, 0.33% & 0.33% and 5.60, 5.20, 2.80% & 1.33% at first infection and epizootic period, respectively. *Entomophthora planchoniana* was found for one month from February 22 to March 15 with a maximum incidence during March 8. *Paecilomyces* appeared from the first week of February to March 15 with epizootic period during February 8. *Verticillium* was scarce and found for two weeks only at the beginning of the season i.e. from February 1 to 8.

From the foregoing results, it could be mentioned that entomopathogenic fungi appeared to be of a significant value in the natural control of cereal aphids in the study area. Results of the present study are in general agreement with those previously reported by Dean & Wilding 1973, Dedryver 1983 and Feng *et al.* 1991, who found that entomopathogenic fungi are frequently reported as major factors suppressing populations of cereal aphids and can cause sudden decline of dense populations.

Entomopathogenic fungi are now being considered as biological alternative to chemical control. Several species of Entomopathogenic fungi can cause fatal disease in aphids, including *Conidiobolus obscurus* (Hall & Dunn) Remaudiere & Keller, *Erynia neoaphidis* Remaudiere & hennebert, Verticillium lecanii (Zimmerman) Viegas, various species of *Beauveria*, and

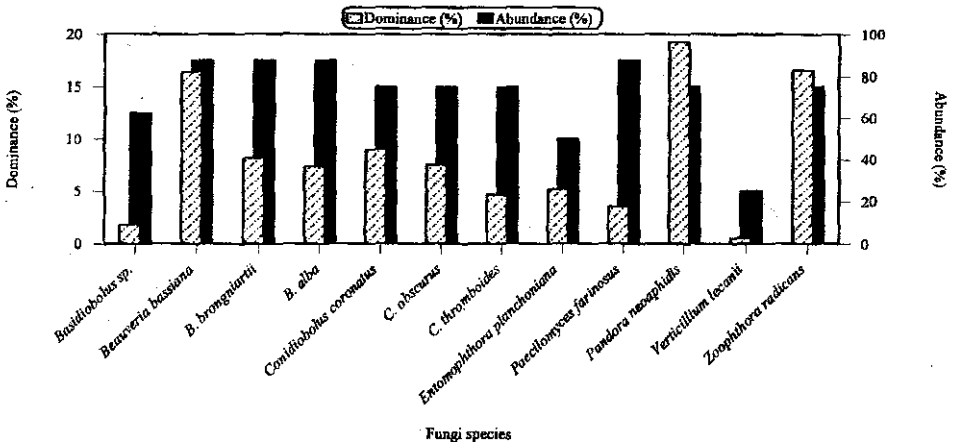

Figure 1. Dominance (%) and abundance (%) degrees of entomopathogenic fungi identified from alive cereal aphid complex collected from wheat field regardless of season.

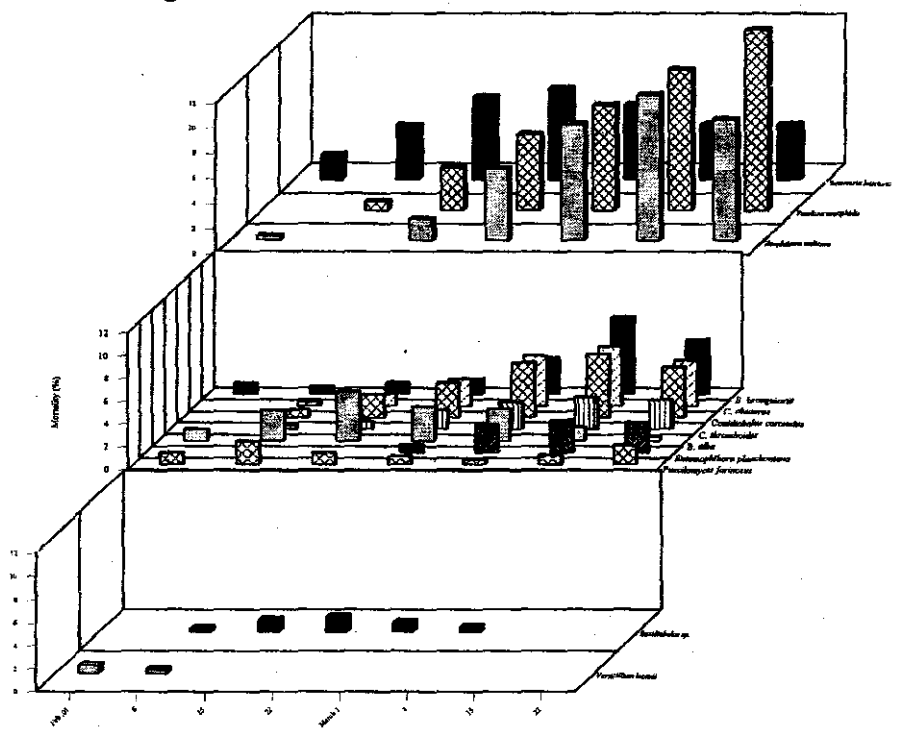

Figure 2. Seasonal incidence of enteomopathogenic fungi identified from alive ceral aphid complex collected from wheat field regardless of season.

Paecilomyces farinosus (Holm ex S.F. Grey) Brown & Smith (Roberts & Yendol 1971, Samson *et al.* 1988).

Members of order Entomophthorales are excellent candidates for biological control of aphids (Latge and Papierok 1988). Worldwide, *Pandora neoaphidis* (Remaudiere & Hennebert) is the most common and frequently the dominant-pathogen of aphids (Wilding and Brady 1984). This fungus can cause collapse of unmanaged aphid population within few weeks of the onset of disease (Feng *et al.* 1990). Without management however, the fungus is not effective as control agent alone because it dose not attack until the aphid population has peaked and has already caused considerable damage (Feng *et al.* 1991).

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الظهور الموسمي للفطريات الممرضة لحشرات من النجيليات بأسيوط

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أجريت هذه الدراسة خلال موسمي ٢٠٠٢ ـ ٢٠٠٣ ، ٢٠٠٣ ـ ٢٠٠٤ من مواسم زراعة القمح ، وذلك بهدف تقييم الظهور الموسمي للفطريات الممرضة لحشرات من النجيليات.

اتضح من الدراسة بغض النظر عن أنواع حشرات من النجيليات المختلفة وكذا المواسم الزراعية أن هناك أنواع عديدة من الفطريات الممرضة تؤدى إلي حدوث موت لحشرات من النجيليات تحت الظروف الطبيعية. تم حساب الحدوث الموسمي من ٢١٠٠ فرد جثة (Cadavers) من حشرات من النجيليات خلال موسمي ٢٠٠٢ ـ ٢٠٠٣ ، ٢٠٠٣ ـ ٢٠٠٤. تم تعريف أثنى عشرة نوعا من الفطريات الممرضة لحشرات من النجيليات.

أظهرت الدراسة أن بداية العدوى بالفطريات الممرضة تم مشاهدته مع أوائل شهر فبراير ثم بعد ذلك تستمر نسبة الموت المتسببة عن الفطريات الممرضة حتى تصل إلي أقصى معدل مع نهاية شهر مارس.

وقد اتضح من الدراسة أن كل من ***Pandora neoaphidis*** ، ***Beauveria bassiana*** ، ***Zoophthora radicans*** تعتبر من أهم الفطريات الممرضة كعامل من عوامل المكافحة البيولوجية حيث اتضح ذلك من خلال زيادة قيم كل من السيادة والوفرة

النسبية لهذه الفطريات بالمقارنة بالفطريات الأخرى التي تم الحصول عليها من خلال هذه الدراسة. بينما أظهرت الدراسة أن كل من الفطريات الآتية: ***Beauveria brongniartii*** ، ***B. alba*** ، ***Conidiobolus coronatus*** ، ***C. obscurus*** ، ***C. thromboides*** ، ***Entomophthora planchoniana*** ، ***Paecilomyces farinosus*** من الممكن أن تكون ذات أهمية اقتصادية إذ توفرت الظروف البيئية لهذه الفطريات. بينما اتضح أن كل من الفطر ***Verticillium lecanii*** ، ***Basidiobolus*** sp كانت من الفطريات النادرة التواجد على حشرات من النجيليات بأسيوط.

وفيما يتعلق بالتذبذب الموسمي لهذه الفطريات اتضح أن كل من ***Beauveria*** spp. ، ***Zoophthora radicans*** وكذلك ***Paecilomyces*** تبدأ في العدوى لحشرات من النجيليات مع بداية شهر فبراير وتستمر العدوى بها حتى الأسبوع الثالث من شهر مارس ، أما فطر ***Pandora neoaphidis*** وكذا الأنواع التابعة للجنس ***Condidiobolus*** spp. فهي تبدأ في عدوى حشرات من النجيليات بعد الأنواع السابقة بأسبوع وتستمر حتى نهاية الموسم. أما الفطر ***Entomophthora planchoniana*** فقد وجد أن العدوى به تبدأ من ٢٢ فبراير وتستمر حتى ١٥ مارس. وتعتبر العدوى بالجنس ***Verticillium*** نادرة الوجود على حشرات من النجيليات حيث تم تسجيل هذه الملاحظة لمدة أسبوعين فقط مع بداية الموسم.