

A Comparative Abundance of Entomopathogenic Fungi of Cereal Aphids in Assiut, Egypt

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ABSTRACT. The comparative abundance of entomopathogenic fungi of cereal aphids, *Rhopalosiphum padi* L., *Rhopalosiphum maidis* (Fitch.) and *Schizaphis graminum* (Rondani) was examined during two successive wheat growing seasons, 1999-2000 and 2000-2001. From 2100 alive aphid individuals collected during the two seasons, 598 individuals were proved to be infected by fungi. Twelve species of entomopathogenic fungi could be identified.

According to the percentage frequency of occurrence, regardless of aphid species, gathered fungal species could be arranged into three groups. The first one those of high occurrence ($\geq 10\%$) include = *Beauveria bassiana*, *Pandora neoaphidis*, and *Zoophthora radicans*. The second group of moderate occurrence ranged between 5% - 9% was represented by five species viz: *Beauveria brongniartii*, *B. alba*, *Conidiobolus coronatus*, *C. obscurus* and *Entomophthora planchoniana*. The third group of low occurrence ($\leq 5\%$) contains four species: *Basidiobolus* sp., *C. thromboides*, *Paecilomyces farinosus* and *Verticillium lecanii*.

Introduction

Cereal aphids are considered one of the most serious pests attacking wheat plants in Assiut, Egypt. They damage the plants roughly through loss of sap by sucking, reaction of plant tissues stimulated by aphid saliva; excreting liquid, viscous honeydew, that is very harmful and on which sooty-moulds usually develop and, finally aphid transmission of viral diseases to plants. Insecticides are in general used to control these pests. However, several serious problems arose from the extensive application of these chemicals. The routine application of common pesticides has two well-known adverse features: firstly, they are mostly non-selective and kill a lot of aphid natural enemies, which cause further disturbance to the cereal crop ecosystem; secondly, as in other species, resistant strains of aphids can be expected to develop soon (Doutt and Smith, 1971).

Several species of Entomopathogenic fungi were found infecting cereal aphids in the field under natural conditions (Dean and Wilding 1971, 1973; Dedryver 1983; Papierok and Havukkala 1986; Feng *et al.* 1991, 1992; Hatting *et al.* 1999, 2000; Abdel-Rahman 2001 and Abdel-Rahman and Abdel-Mallek 2001). Therefore several authors have considered fungi as promising natural enemies for applied aphid biological control to be

included in an integrated pest management programs (IPM) (Latge and Papicrok, 1988; Humber, 1991; and Milner, 1997).

The present study aimed to evaluate the dominance degrees and host preference of entomopathogenic fungi infecting cereal aphids.

Materials and Methods

The present investigations were carried out at the Experimental Farm of Assiut University (Faculty of Agriculture - Agronomy Department) throughout two successive wheat-growing seasons, 1999-2000 and 2000-2001. An area of ca. 4200 m² 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 of 50 living individuals from each of *R. padi*, *R. maidis* and *S. graminum* were randomly collected from the field and transferred to the laboratory. Aphids were reared individually on 5-cm leaf sections in 65- mm petri dishes. Moist 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). Leaf sections 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 produced in situ on the plant. Desiccated and fresh cadavers were placed in a moist chamber for about 20 h. to allow hyphae and reproductive structures to develop. Individual aphids were mounted in cotton blue and observed under a compound microscope. Fungal identification was based on external symptoms, 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.

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

Results

Data in Tables 1-3 demonstrate the relative abundance of twelve species of Entomopathogenic fungi on three species of cereal aphids throughout 2000 and 2001 wheat growing seasons.

In 2000 season, data in Table 1 revealed that 365 individuals of aphids were infected from 1050 alive individuals from the three-cereal aphid species consisted of 34.76% mortality rate during the whole season due to mycosis. Data showed that the frequency of fungal infection varied between the different species of aphid host. *R. padi* exhibited much higher mortality from fungal pathogens (37.81%) followed by *S. graminum* (31.78%), while *R. maidis* seemed to be least commonly infected (30.41). Eleven fungal species were

identified infecting those aphid species. These fungal species were *Beauveria bassiana*, *B. brongniartii*, *B. alba*, *Conidiobolus coronatus*, *C. obscurus*, *C. thromboides*, *Entomophthora planchoniana*, *Paecilomyces farinosus*, *Pandora (Erynia) neoaphidis*, *Verticillium lecanii* and *Zoophthora radicans*.

Table (1). Abundance of entomopathogenic fungi infecting cereal aphid species during 2000 season.

Fungi species	Number and abundance				
	Aphid species			TNI	% & OR
	<i>R. maidis</i>	<i>R. padi</i>	<i>S. graminum</i>		
<i>Beauveria bassiana</i>	25 (40.98)a	36 (59.02)a	0	61b	16.71 H
<i>B. brongniartii</i>	0	11 (55.00)b	9 (45.00)c	20d	5.48 M
<i>B. alba</i>	25 (75.76)a	6 (18.18)c	2 (6.06)d	33c	9.04 M
<i>Conidiobolus coronatus</i>	8 (22.86)b	6 (18.18)c	21 (60.00)a	35c	9.59 M
<i>C. obscurus</i>	10 (32.26)b	3 (9.68)c	18 (58.06) b	31c	8.49 M
<i>C. thromboides</i>	8 (38.9)b	0	13 (61.91)b	21d	5.75 M
<i>Entomophthora planchoniana</i>	0	0	12 (63.16)b	19d	5.21 M
<i>Paecilomyces farinosus</i>	0	14 (100.0)b	0	14d	3.84 L
<i>Pandora neoaphidis</i>	13 (16.88)b	40 (51.95)a	24 (31.17)a	77a	21.09 H
<i>Verticillium lecanii</i>	0	0	2 (100.00)d	2c	0.55 L
<i>Zoophthora radicans</i>	22 (42.31)a	15 (28.85)b	15 (28.85)b	52b	14.25 H
Total	111c	138a	116h	365	100
(%)	30.41	37.81	31.78	100	-

Values followed by the same letter vertically, are not significantly different at <0.05 probability.

* Number of infected individuals / 50 alive aphid.

** Figure in parenthesis refer to the percentage of infection (%) within a row from the total.

TNI - total number of infected individuals out of 1050, % = percentage frequency & OR = occurrence remark; H = High occurrence ($\geq 10\%$); M = moderate occurrence (between 5-9 %); L = low occurrence ($\leq 5\%$).

Pandora neoaphidis was the most common species and occurred in 21.09% of infected aphid samples examined. It was recovered from 16.88, 51.95 and 31.7% of infected *R. maidis*, *R. padi* and *S. graminum*, respectively. *Beauveria bassiana* and *Zoophthora radicans* followed *P. neoaphidis* and recovered in high frequency of occurrence (16.71% and 14.25%, respectively). Three fungal species were identified from all aphid species. They were: *B. alba*, *C. coronatus*, and *C. obscurus* were represented in 9.04%, 9.59% and 8.49% of aphid examined. *B. brongniartii*, *C. thromboides* and *E. planchoniana* infected at least one type of aphid species but recovered in moderate frequency of occurrences. *P. farinosus* infected only *R. padi* while *V. leccenii* recovered from *S. graminum* and both fungal species occurred in rare frequency.

In season 2001, (Table 2) 233 individuals were infected from 1000 individuals alive from the three aphid species consisted of 23.30% mortality rate during the whole season. Twelve species of fungi were identified during this season. *R. padi* (42.49%) was the most common species infected with fungal pathogens followed by *S. graminum* (38.63%) and then *R. maidis* (18.88%), which seemed to be least commonly infected.

Statistical analysis showed that *Zoophthora radicans* was the most dominant and common species composting 20.17% of the total aphid cadavers followed by *Pandora neoaphidis*, *Beauveria bassiana* and *B. brongniartii* composting 16.31%, 15.88% and 12.45% of the total aphid cadavers, respectively. *C. coronatus* (8.16%), *C. obscurus* (6.01%) and *E. planchoniana* (5.15%) were recovered in moderate frequency of occurrence. The remaining five species listed in Table (2) were of low frequency of occurrence ranged between 0.43% to 4.72%.

Table (2). Abundance of entomopathogenic fungi infecting cereal aphid species during 2001 season.

Fungi species	Number and abundance				
	Aphid species			TNI	% & OR
	<i>R. maidis</i>	<i>R. padi</i>	<i>S. graminum</i>		
<i>Basidiobolus sp.</i>	0	0	11 (100.00)c	11c	4.72 L
<i>Beauveria bassiana</i>	14 (37.84)a	23 (62.16)a	0	37b	15.88 H
<i>B. brongniartii</i>	1 (3.45)c	22 (75.86)a	6 (20.69)d	29b	12.45 H
<i>B. alba</i>	9 (81.82)b	2 (18.18)d	0	11c	4.72 L
<i>Conidiobolus coronatus</i>	3 (15.57)c	5 (26.32)c	11 (57.89)c	19c	8.16 M
<i>C. obscurus</i>	1 (7.14)c	4 (28.85)d	9 (64.29)c	14c	6.01 M
<i>C. thomboides</i>	2 (28.85)c	2 (28.57)d	3 (42.86)d	7d	3.00 L
<i>Entomophthora planchoniana</i>	0	6 (50.00)c	6 (50.00)d	12c	5.15 M
<i>Paecilomyces farinosus</i>	0	1 (14.29)d	6 (85.14)d	7d	3.00 L
<i>Pandora neoaphidis</i>	7 (18.42)b	16 (42.11)b	15 (39.47)b	38b	16.31 H
<i>Verticillium lecanii</i>	0	1 (100.00)d	0	1d	0.43 L
<i>Zoophthora radicans</i>	7 (14.89)b	17 (36.17)b	23 (48.94)a	47a	20.17 H
Total	44c	99a	90b	233	100
(%)	18.88	42.49	38.63	100	-

Values followed by the same letter vertically, are not significantly different at <0.05 probability.

* Number of infected individuals / 50 alive aphid.

** Figure in parenthesis refer to the percentage of infection (%) within a row from the total.

TNI = total number of infected individuals out of 1050, % = percentage frequency & OR = occurrence remark: H = High occurrence ($\geq 10\%$); M = moderate occurrence (between 5-9 %); L = low occurrence ($\leq 5\%$).

Table (3). Abundance of entomopathogenic fungi infecting cereal aphid species regardless seasons.

Fungi species	Number and abundance				
	Aphid species			TNI	% & OR
	<i>R. maidis</i>	<i>R. padi</i>	<i>S. graminum</i>		
<i>Basidiobolus sp.</i>	0	0	11 (100.00)c	11d	1.84 L
<i>Beauveria bassiana</i>	39 (39.79)a	59 (60.21)a	0	98a	16.39 H
<i>B. brongniartii</i>	1 (2.04)d	33 (67.35)b	15 (30.61)c	49b	8.19 M
<i>B. alba</i>	34 (77.27)a	8 (18.18)c	2 (4.56)d	44b	7.36 M
<i>Conidiobolus coronatus</i>	11 (20.37)c	11 (20.37)c	32 (59.26)a	54b	9.03 M
<i>C. obscurus</i>	11 (24.44)c	7 (15.56)d	27 (60.00)b	45b	7.52 M
<i>C. thomboides</i>	10 (35.71)c	2 (7.14)e	16 (57.15)c	28c	4.68 L
<i>Entomophthora planchoniana</i>	0	13 (41.94)c	18 (58.06)c	31c	5.18 M
<i>Paecilomyces farinosus</i>	0	15 (71.43)c	6 (28.57)d	21c	3.51 L
<i>Pandora neoaphidis</i>	20 (17.39)b	56 (48.69)a	39 (33.91)a	115a	19.23 H
<i>Verticillium lecanii</i>	0	1 (33.33)e	2 (66.67)d	3d	0.50 L
<i>Zoophthora radicans</i>	29 (29.29)b	32 (32.32)b	38 (38.38)a	99a	16.56 H
Total	155	237	206	598	100
(%)	25.92	39.63	34.45	100	-

Values followed by the same letter vertically, are not significantly different at <0.05 probability.

* Number of infected individuals / 50 alive aphid.

** Figure in parenthesis refer to the percentage of infection (%) within a row from the total.

TNI = total number of infected individuals out of 2100, % = percentage frequency & OR = occurrence remark: H = High occurrence ($\geq 10\%$); M = moderate occurrence (between 5-9 %); L = low occurrence ($\leq 5\%$).

In general, regardless of the growing season, data in Table 3 indicated that 598 individuals were infected from 2050 individuals alive from the three aphid species consisted of 29.17% mortality rate during the two seasons. There are twelve species of entomopathogenic fungi identified infecting cereal aphid species during 2000 and 2001 seasons. *R. padi* was the most aphid species infected comprised 39.63% of the total

mortality followed by *S. graminum* and *R. maidis* encountered 34.45% and 25.92%, respectively.

The dominance of entomopathogenic fungi recovered during the present investigation could be grouped into three categories viz: high, moderate and low frequency of occurrence. The first category includes the predominant fungi: *P. neoaphidis*, *Z. radicans* and *B. bassiana*. They occurred in 19.23%, 16.56% and 16.39%, respectively. The second category had moderate frequency of occurrence and includes five fungal species namely: *Beauveria brongniartii* (8.19%), *B. alba* (7.36%), *C. coronatus* (9.03%), *C. obscurus* (7.52%) and *E. planchoniana* (5.18%). The least category (recovered in low frequency of occurrence) includes: *Basidiobolus* sp., *C. thromboides*, *Paecilomyces farinosus* and *Verticillium lecanii*.

Discussion

Based on limited epizootiological literatures, factors determining the development of mycosis in aphid populations are complex and vary among crop-aphid-fungus systems. The occurrence of precipitation or heavy dews before the occurrence of epizootics among cercal aphid populations has been emphasized by several authors (Dean and Wilding 1971, 1973; Voronina 1971; Wilding 1975; Dedryver 1983). Missonnier *et al.* (1970) tried to quantify this relationship, postulating that there must be a minimum of 90% RH for ≥ 8 h/d for an enzootic and ≥ 10 h/d for three consecutive days for the development of an epizootic of *Entomophthora* sp. in an aphid population. In addition, Milner and Bourne (1983) detected no consistent effect of temperature on infectivity of *P. neoaphidis* primary spores to *Acyrtosiphon knodi* Shinij, but the final level of infection increased with increasing periods of leaf wetness up to 24 h after inoculation. However, a poor relationship was found between *Entomophthora* infection of populations of the bean aphid, *Aphis fabae* Scopoli, and any weather factors (Wilding and Perry 1980). However, Soper and Macleod (1981) concluded that inoculum levels, host density and, perhaps more abundant, the degree of host aggregation, were major factors controlling epizootics among *Schizolachnus piniradiatae* (Davidson).

One variable we did not measure was inoculum level, which was found to be of importance for *S. piniradiatae* epizootics (Soper and Macleod 1981). Additionally, the virulence of entomophthoralean fungi varies between host aphid species (Feng *et al* 1990, Feng and Johnson 1991). Thus, a complete explanation is impossible.

However, another factor that appears to affect mycosis development is the location each species of aphid typically occupies on the wheat plant. The most important seems to be the tendency to inhabit microhabitats with or without characteristics favourable to fungal infection. Such characteristics may influence the exposure of an aphid and its immediate surroundings to sunlight and wind. These, in turn, affect the aphids' exposure to pathogens and microclimates that influence the ability of the fungi to infect aphid hosts.

During our study we observed that *R. padi* and *S. graminum* typically resides on the undersurface of lower leaves. In this location, these aphids are exposed to fungal activity discharged or splashed from inocula possibly overwintered on the soil surface (Lateur and Godefroid 1983), which are shaded from ultraviolet radiation to increase their longevity. Simultaneously, the microhabitat is shaded and protected from wind, which tends to moderate temperatures and increase relative humidity, facilitating fungal infection.

Consequently, it seems that *S. graminum* and *R. padi* are more likely to encounter viable spores of entomopathogenic fungi under circumstances suitable for establishing infection. It is also among the cereal aphid species that are very susceptible to such fungi, particularly *P. neoaphidis*.

R. maidis is relatively xerophilic, typically feeding on upper leaves. It is exposed to an environment less suitable for germination of the spores that are encountered. Thus, it is not surprising that *R. maidis* populations experience mycosis less frequently than populations of the more mesophilic species. This could also explain the greater correlation between precipitation and mycoses among *R. maidis* than in the other two species.

The occurrence of each fungal pathogen infecting the three aphid hosts provides further evidence for the postulation stated above. The majority of mycosed *S. graminum* and *R. padi* found in the field were infected with *P. neoaphidis*, whereas most diseased *R. maidis* were infected with *Conidiobolus* spp. Roberts and Campbell (1977) summarized that epizootics induced by *P. neoaphidis* usually require for more moisture than do those caused by *Conidiobolus* spp. Therefore, the relative abundance of these fungi in *S. graminum*, and *R. padi* cadavers seem to be related to the site each aphid species occupies on the host plant.

Dedryver (1983) observed Entomophthorales caused diseases to drastically reduce the populations of various cereal aphids in six of eight years. In the other years, the weather was extremely dry. He distinguished three types of mycoses in the populations of cereal aphids. An epizootic develops when the number of disease-killed aphids increases more quickly than does the number of living aphids and eventually leads to nearly local extinction of the aphids. An enzootic occurs when the proportion of mycoses aphids remains approximately stable during population growth and diminution. Such a level of mycosis contributes to the decline of host populations, but by itself does not bring it about. The third type of mycosis occurs when the activity of fungal pathogens lacks significant effect on the development of its host population. Other reports have also revealed that the control of cereal aphid by fungal pathogens is as important to control cereal aphids and agreement with the document (Lazzari 1985, Papierok and Havukkala 1986). The fungi are thus excellent candidates for biological of various aphids (Latge and Papierok 1988).

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مقارنة وفرة الفطريات الممرضة لحشرات من النجيليات بأسبوط - مصر

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المستخلص. أجريت هذه الدراسة خلال موسمين زراعيين متتاليين ١٩٩٩-٢٠٠٠ ، ٢٠٠٠-٢٠٠١ على الفطريات التي تتطفل على حشرات من النجيليات التي تصيب نباتات القمح بأسبوط بهدف دراسة وفرة وتفضيل هذه الفطريات لأنواع حشرات من النجيليات المختلفة ، وهي حشرات من الشوفان ، وحشرات من أوراق الذرة وكذا حشرات البق الأخضر.

تم تسجيل اثني عشر نوعا من الفطريات الممرضة التي تتطفل على أنواع حشرات المن السابقة بصورة طبيعية وتعتبر عاملا مهماً من عوامل المكافحة الحيوية. واتضح من الدراسة أن نسبة الموت الناتجة من الفطريات الممرضة لحشرات من النجيليات بصورة طبيعية تصل إلى حوالي ٣٠% من إجمالي التعداد. واتضح أيضا أن كلا من حشرات من الشوفان وكذا حشرات من الغلال من أكثر الأنواع إصابة بالفطريات الممرضة مقارنة بحشرات من أوراق الذرة.

أمكن من الدراسة تقسيم الفطريات الممرضة لحشرات من النجيليات إلى ثلاثة أقسام حسب درجة وفرتها بغض النظر عن أنواع حشرات المن وكذلك المواسم الزراعية وهي (١) المجموعة الأولى وهي تضم الأنواع الأكثر سيادة وهي *Beauveria bassiana* ، *Pandora neoaphids* ، *Zoophthora radicans*. (٢) الأنواع متوسطة التواجد وهي *Conidiobolus* ، *B. alba* ، *Beauveria brongniartii* وهي المجموعة الثالثة (٣) *Entomophthora planchoniana* ، *C. obscurus* ، *coronatus*. وهي تضم الأنواع الأقل تواجدا وهي *C. thomboides* ، *Basidiobolus* ، *Paecilomyces* ، *Verticillium lecanii*