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Biodiversity of entomopathogenic fungi infecting wheat and cabbage aphids in Assiut, Egypt

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Abstract: A total of 4600 samples of aphids were collected from wheat (3200 individuals) and cabbage (1400) plantations in Assiut Governorate during February and March of the years 2006 and 2007. Two species of cereal aphids were found infesting wheat plants namely, the oat bird-cherry aphid, *Rhopalosiphon padi* and the green bug, *Schizaphis graminum*. The cabbage aphid *Brevicoryne brassicae* was found infesting canola plants. During the period of rearing and incubation, fungal growth was observed on 26.7%, 30.4% and 33.0% of *R. padi*, *S. graminum* and *B. brassicae* respectively. Six species of entomopathogenic fungi were identified of which *Pandora neoaphidis* and *Neozygites fresenii* were the most dominant (42.4% and 24.3% of total infected cadavers matching 12.7% and 7.3% of total collected aphids respectively). Each of *Entomophthora planchoniana* and *Zoophthora radicans* were found infecting 10.8% of total cadavers representing 3.2% of total aphids. *Conidiobolus coronatus* and *Verticillium lecanii* were less frequently encountered (5.6% and 5.9% of cadavers representing 1.7% and 1.8% of total aphids respectively). These fungi could be regarded as promising candidates for application in the biocontrol of insect pests.

Key words: Entomopathogenic fungi, wheat aphids, cabbage aphids

Introduction

Fungal diseases in insect populations are common and widespread. They can often destroy insect populations in spectacular epizootics and thus attract man's attention. Many of these are considered an important factor regulating insect pest populations. Until now at least 90 genera and more than 700 species of entomopathogenic fungi have been identified as closely associated with invertebrates, predominantly insects, but only 10 of them have been or are currently being developed for insect control (Roberts & Humber 1981, Car-Ruthers & Soper 1987, Hajek & St. Leger 1994, Bateman & Chapple 2001, Wraight *et al*. 2001, Barta & Cagan 2006).

Aphids can be attacked by entomopathogens of Zygomycetes and Hyphomycetes, but the entomophthoralean fungi of the class Zygomycetes are the major fungal pathogens of aphids (Humber 1991). Entomopathogenic Hyphomycetes include hundreds of species, but just a few of them are specific to aphids. *Verticilium lecanii* is one of the most important Hyphomycetes parasites of aphids. It can however attack a broad spectrum of insects both in tropical and temperate regions, but it is distributed mainly in tropical regions (Hall 1984, Humber 1991). The fungus can rarely affect aphids under field conditions (Feng *et al*. 1990, Humber 1991, Hatting *et al*. 1999).

Crop damage by aphids is largely due to virus transmission but direct feeding damage can also cause significant yield losses in outbreak years. There is increasing economic and environmental pressure to develop alternative control strategies to replace chemical control for use in integrated pest management strategies. These include the exploitation of beneficial organisms such as entomopathogenic fungi.

Little effort has been made to survey fungal pathogens infecting cereal and canola aphids in Upper Egypt. This study was oriented to identify and isolate entomopathogenic fungi from cereal and cabbage aphids infesting wheat and canola plantations in Assiut.

Materials and Methods

The present study was carried out during the growing seasons of 2005-2006 and 2006-2007 at the Experimental Farm of Assiut University. An area of about one feddan (4200m^2) was cultivated with wheat, *Triticum vulgare* L. (cultivar Giza 164) and canola, *Brassica napus* L. (cultivar Pactol). Wheat plants were normally planted in the middle of November and canola plants during the second half of October. Regular conventional agricultural practices were normally performed and no chemical control was used during the study period. Weeds were removed by hand.

Collection and incubation of alive aphids:

In each inspection date, during the two seasons of 2006 and 2007, 100 living late instars of each cereal aphid species (*Rhopalosiphum padi* L. and *Schizaphis graminum* Rondani) and cabbage aphid (*Brevicoryne brassicae* L.) were collected regularly and transferred to the laboratory. Aphids were reared individually on 5 cm leaf sections in 65-mm Petri dishes. Moistened cotton was placed over the ends of the leaf sections to maintain relative humidity near saturation. Petri dishes containing live aphids were incubated for 10 days at 20ºC with a photoperiod of 16:8 (L: D). Leaf sections remained fresh for several days and were replaced twice a week. Dead aphids were collected and placed in vials for fungal identifications.

Examination of aphid cadavers:

Aphid cadavers were examined under 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 hrs to allow hyphae and reproductive structures to develop. Individual aphids were mounted in cotton blue or aceto-orcein and observed under microscope.

Identification of fungi:

Fungus identification was based on external symptoms and the morphology of spores and sporulating structures according to Waterhouse and Brady (1982) and Humber (1989) and checked in Assiut University Mycological Center (AUMC). Fungi identified were considered to be the cause of death.

Isolation of aphid-infecting fungi:

Aphid cadavers were incubated in a moist chamber for 24 hrs. and a sterilized glass needle was used to transfer a small amount of inocula from the cadaver onto an agar plate. Sabouraud dextrose agar medium (SDAY) of the following components (gm/L): glucose or maltose 40 gm; peptone, 10gm; yeast extract, 10gm; and tween 80, 1ml; and agar, 15gm was employed for the isolation and counting of fungi.

Data analysis:

Dominance (%) and abundance (%) degrees of the identified fungal species were calculated according to the formula of Facylate (1971).

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

Results

Fungi infecting cereal and cabbage aphids:

Two species of cereal aphids were found infesting wheat plants namely, the oat bird-cherry aphid, *R. padi* and the greenbug, *S. graminum*, while the aphid species infesting canola plants was the cabbage aphid, *B. brassicae*. Six species of fungal pathogens were collected from wheat and canola aphids, including five Entomophthorales and one Hyphomycete during 2006 and 2007 seasons (Tables 1-7). Entomophthorales was represented by five species belonging to 3 families. Ancylistaceae was represented by one species *Conidiobolus coronatus*, Entomophthoraceae was represented by three species, *Entomophthora planchoniana*, *Pandora* (=*Erynia*) *neoaphidis* and *Zoophthora radicans* and Neozygitaceae was represented by *Neozygites fresenii*. The Hyphomycete was identified as *Verticillium lecanii*.

Incidence of mycopathogens identified:

Data in Tables (1-7) show the incidence of fungi recorded from 1600 alive aphids of each of *S. graminum*, and *R. padi* and 1400 of *B. brassicae* during two successive seasons (2006 & 2007).

On wheat aphids:

In 2006 season, 220 out of 800 individuals of *S. graminum* were infected by fungi representing 27.50% mortality (Table 1).

Statistical analysis shows that there are significant differences among the degrees of dominance of the fungi recovered. The dominance of these species could be arranged descendingly as follows: *P. neoaphidis* and *N. fresenii* were the most dominant species inciting 45.45% and 27.27% mortality followed by *Z. radicans* and *V. lecanii*, inflicting 10.91 and 7.27% mortality respectively. *E. planchoniana* and *C. coronatus* showed the least dominance causing 5% and 4.09% mortality respectively.

Data in Table (1) show that *P. neoaphids* seems to be the most important serious pathogen for this aphid species, *S. graminum*, as indicated by the highest values of dominance as well as abundance (45.45% & 100% respectively). However, the high abundance degrees of *Z. radicans* followed by *N. fresenii*, which had low dominance degrees, indicate that these species could be of economic importance, if the environmental conditions change for their favour. Meanwhile the species of *C. coronatus*, *E. planchoniana* and *V. lecanii* which had low values of dominance and abundance are expected to be of little economic importance as they may cause little role as natural enemies. There was a marked regular increase in the number of aphids infected with *P. neoaphids* till the third week of March 2006 when the maximum mortality was recorded (25%).

As for the oat-bird cherry aphid, *R. padi* during 2006 wheat growing season, data show that 186 individuals out of 800 were infected representing

23.25% mortality. Statistical analysis shows that *P. neoaphidis* was the most dominant species inducing 35.48% mortality followed by *N. fresenii* (29.57%). *C. coronaus*, *E. planchoniana*, *Z. radicans* and *V. lecanii* accounted for 6.99%, 10.75%, 9.14% and 8.06% mortality respectively.

Table (3) shows that *P. neoaphids* and *N. fresenii* were the most serious pathogens infesting *R. padi*, as indicated by the highest values of dominance and abundance. However, the high abundance degree of *V. lecanii* followed by *E. planchoniana,* indicates that these species could be of economic importance, if the environmental conditions change for their favour. On the other hand, *C. coronatus*, and *Z. radicans,* which had low values of dominance and abundance, are expected to be of little economic importance as natural enemies.

Concerning the periods of prevalence of these fungi, the data show that *P. neoaphidis* and *N. fersenii* were firstly detected on February 7th and lasted till March 28th. Mortality rate at the beginning was 1% reaching maximum (18% and 15%) in the second and third weeks of March respectively. *V. lecanii* was recorded one week behind the above-mentioned species inciting its highest infection at the end of March. *Conidiobolus*, *Entomophthora*, and *Zoophthora*, were encountered in relatively low incidence (Table 3).

In general, 406 individuals during 2006 were recorded infected from 1600 alive individuals from the two aphid species causing 25.37% mortality during the whole seasons due to mycoses. Six species were identified. The abundance of these fungi could be arranged descendingly as follows: *P. neoaphidis* represented the most frequent species with a rate of occurrence reaching 40.89% followed by *N. fresenii* (28.32%). *Z. radicans*, *E. planchoniana* and *V. lecanii* represented the second group with a percentage occurrence of 10.09% and 7.64%, respectively. The third position was occupied by *C. coronatus* with percentage of 5.42%.

During 2007 season, the entomopathogenic fungi were found to attack 266 out of 800 individuals of *S. graminum* causing 33.3% mortality (compared to 27.5% mortality in 2006). As shown in Table (2), *P. neoaphidis* and *N. fresenii* were also the most prevalent fungal species showing the highest dominance and abundance degrees followed by *E. planchoniana*, *Z. radicans* and *V.lecanii*, inflicting 6.77, 6.39 and 4.13% mortality of the total alive aphids respectively. *C. coronatus* was found attacking 5.26% of *S. graminum* collected during 2007 season.

As for the oat-bird cherry aphid, *R. padi*, during 2007 wheat growing season, data in Table (4) show that 238 out of 800 individuals were infected (29.75% mortality). Statistical analysis showed that *P. neoaphidis* and *E. planchoniana* were significantly the most dominant being recovered from 33.61% and 31.09%, of cadavers respectively. *N. fresenii*, *Z. radicans* and *V. lecanii* were encountered on 18.91%, 7.98%, and 5.46% of the dead aphids respectively.

It is worthy to mention that *E. planchoniana*, *N. fresenii* and *P. neoahidis* can be excellent candidates for controlling *R. padi* in wheat fields. These species were responsible for 75.80% and 83.61% of mortality during 2006 and 2007 respectively.

According to seasonal prevalence of these fungi, data exhibit that *P. neoaphidis* and *N. fersenii* were firstly detected on February 7th and lasted till March 28th. Mortality rate was 2% at first, reaching 16% and 22% during the first and second weeks of March respectively. *C. coronatus* and *V. lecanii* were recorded one week behind the above-mentioned species to the end of March. *Entomophthora planchoniana* was recorded in the third week of February, increased regularly and sharply during the successive weeks of sampling recording its climax by the third week of March (38 infected aphids), then it declined by the end of March 2007.

On cabbage aphids

In 2006 season 216 out of 700 individuals of *B. brassicae* were found infected by the entomopathogenic fungi representing 30.86% mortality (Table 5).

Statistical analysis shows that there are significant differences among the fungal species recovered. *P. neoaphidis*, *Z. radicans* and *N. fresenii* were the most dominant species with the rate of 41.67%, 21.76 and 18.05%, respectively. *C. coronatus*, *E. planchoniana* and *V. lecanii* showed the least percentage of dominance <10%.

Data in Table (5) show that *P. neoaphidis* can be a potential agent for biological control of the aphid species, *B. brassicae*, as indicated by the highest value of dominance and abundance (41.67% & 100% respectively) followed by *N. fresenii* (18.05% & 100% respectively). However, the high abundance percentage of *C. coronatus*, combined with low dominance percentage, indicate that this species could be of economic importance in biological control plan, if the environmental conditions change for their favour. Meanwhile *E. planchoniana* and *V. lecanii* recording low values of dominance and abundance are expected to be of little economic importance as biological control agents.

Sampling showed that *P. neoaphidis* and *N. fresenii* were observed from the first week of February (mortality 3% & 1% respectively). Maximum mortality (27% and11% respectively) by these species was observed during the second week of March. *C. coronatus* was observed on the 14th of February 2006 and persisted till the end of the experiment. *Z. radicans* and *V. lecanii* were observed three weeks behind *P. neoaphidis* in relatively low occurrence.

In 2007 season, 246 out of 700 individuals of *B. brassicae* were infected representing 35.14% mortality (Table 6). About 82% of the cadavers were killed by *N. fresenii* (24.8%), *P. neoaphidis* (46.8%) and *Z. radicans* (10.1%). This means that these entomopathogenic fungi are promising candidates for biocontrol of the canola aphid.

Discussion

The present results include information of the mycopathogens of cereal and cabbage aphids infesting wheat and canola plants under the dry conditions of Assiut area. The study creates better understanding of the role of entomopathogenic fungi infecting these aphids as natural mortality factors in the field and possible employment of one or more of these fungi for the biocontrol of the aphid.

Field survey of wheat and canola aphids generated data on six species of fungal pathogens, including five Entomophthorales and one Hyphomycete recovered from cadavers of aphids infesting wheat and canola plants during 2006 and 2007 seasons. The fungi were identified as *Pandora neoaphidis*, *Conidiobolus coronatus*, *Entomophthora planchoniana*, *Neozygites fresenii*, *Zoophthora radicans*, and *Verticillium lecanii*. These fungal pathogens are important natural mortality factors of many insects and other arthropods and frequently cause epizootics that significantly reduce host populations (Burges 1981, Car-Ruthers and Soper 1987, McCoy *et al*. 1988).

The obtained results demonstrate that *P. neoaphids* was the most prevalent species in populations of cereal and canola aphids. *N. fresenii* was second to *P. neoaphidis* in occurrence. The Hyphomycete, *V. lecanii* infected cereal and cabbage aphids in the field at low degree of occurrence compared with the Entomophthorales species. *Pandora neoaphidis* has a wide distribution, being recorded from Europe, Asia, Africa, North and South America, and Australia (Wilding and Brady 1984, Glare and Milner 1991). Unusually, for such a common species, its genus remains a subject of debate, with different authors variously assigning it to *Erynia* (Keller 1991), *Pandora* and *Zoophthora* but in this text it will be considered as *Pandora*. *P. neoaphidis* has been recorded from >10 species of aphids on annual and perennial crops, weeds and wild flowers (Wilding & Brady 1984). Infected aphids died at the end of the photophase, ensuring that sporulation occurs during the night, when conditions are humid, cool and free from ultraviolet radiation (Milner *et al*. 1984). Aphids killed by *P. neoaphidis* are typically pale brown or brick red in colour, depending upon the host species and adher to the plant substrate due to numerous rhizoids, each of which consists of a thin stalk ending in a disk lock terminal expansion (Feng *et al.* 1990).

With regard to the two cereal aphids, 910 (28.44%) out of 3200 individuals were killed due to mycoses. The abundance of these fungi could be arranged decsendingly as follows: *P. neoaphidis*, was also the most dominant species, followed by *N. fersenii*. *Z. radicans*, *E. planchoniana*, *V. lecanii* and *C. coronatus.*

As for mycopathogens of cabbage aphid infesting canola it was observed that 462 (33.0%) out of 1400 were parasitized by the entomopathogenic fungi. The abundance of these fungi could be arranged descendingly as follows: *P. neoaphidis*, *N. fersenii*, *Z. radicans*, *C. coronatus*, *E. planchoniana* and *V. lecanii*.

The importance of entomogenous fungi as biological control agents has been reviewed by Latge and Moletta (1988), McCoy *et al.* (1988), McCoy (1990), Roberts and Hajek (1992), Tanada and Kaya (1993), Hajek and St Leger (1994), and Butt and Goettel (2000). Entomopathogenic fungi are principal pathogens among Homopteran piercing-sucking insects (Hajek & St. Leger 1994).

Thoizon (1970) and Dedryver (1978) reported that *N. fresenii* appear to be somewhat a typical entomopathogenic fungus because it has caused epizoon during relatively dry period. Steinkraus and Slaymaker (1994) mentioned that cotton aphid *A. gossypii* mortality caused by *N. fresenii* and its sporulation occurred mainly during night and early morning hours when humidity was much higher than during the daylight hours.

In Egypt few studies revealed the effect of entomopathogenic fungi on the population dynamics of some pests (Sewify 1989 and 2000, Abdel-Rahman 2001, Hammam 2003, Abdel-Mallek *et al.* 2003, Abdel-Rahman *et al*. 2004 and Hussein 2007). Results of the present study reflect the possibility of application of some common entomopathogenic fungi especially *E. planchoniana*, *N. fresenii*, *P. neoaphidis* and *Z. radicans* as biocontrol agents of wheat and cabbage aphids.

Table 1: Mycopathogens identified from alive *S. graminum* directly collected from wheat plants, 2006 season.

Fungal species	Number of infected / 100 alive aphids								Total	Dominance (%)	Abundance (%)
	Sampling date										
	Feb.				March						
	7	14	21	28	7	14	21	28			
C. coronatus	0	0	1	2	2	3	0	1	9d	4.09	62.50
E. planchoniana	0	0	1	2	3	4	1	0	11c	5.00	62.50
N. fresenii	0	1	6	11	10	12	16	4	60b	27.27	87.50
P. neoaphidis	2	4	8	15	17	21	25	8	100a	45.45	100.00
Z. radicans	1	1	2	5	6	5	2	2	24c	10.91	100.00
V. lecanii	0	0	2	3	4	2	5	0	16cd	7.27	62.50
Total	3	6	20	38	42	47	49	15	220	100.00	100.00
No. of alive aphids	100	100	100	100	100	100	100	100	800	-	-

Values followed by the same letter within a column are not significantly different at <0.05 level of probability.

Table 2: Mycopathogens identified from alive *S. graminum* directly collected from wheat plants, 2007 season.

Fungal species	Number of infected / 100 alive aphids								Total	Dominance (%)	Abundance (%)
	Sampling date										
	Feb.				March						
	7	14	21	28	7	14	21	28			
C. coronatus	0	1	2	3	4	2	1	1	14c	5.26	87.50
E. planchoniana	0	1	2	5	6	2	1	1	18c	6.77	87.50
N. fresenii	1	2	4	11	16	18	14	8	74b	27.82	100.00
P. neoaphidis	15	16	17	19	20	25	15	5	132a	49.62	100.00
Z. radicans	1	1	2	3	4	4	1	1	17c	6.39	100.00
V. lecanii	0	1	2	2	2	3	1	0	11c	4.13	75.00
Total	17	22	29	32	36	54	33	16	266	100.00	100.00
No. of alive aphids	100	100	100	100	100	100	100	100	800	-	-

Legends as in Table 1

Table 3: Mycopathogens identified from alive *R. padi* directly collected from wheat plants, 2006 season.

Fungal species	Number of infected / 100 alive aphids								Total	Dominance (%)	Abundance (%)
	Sampling date										
	Feb.				March						
	7	14	21	28	7	14	21	28			
C. coronatus	0	0	1	1	2	3	6	0	13bc	6.99	62.50
E. planchoniana	0	0	1	2	4	6	4	3	20b	10.75	75.00
N. fresenii	1	2	3	4	8	12	15	10	55a	29.57	100.00
P. neoaphidis	1	3	5	6	12	18	13	8	66a	35.48	100.00
Z. radicans	0	0	0	2	1	4	6	4	17b	9.14	62.50
V. lecanii	0	1	1	2	2	3	5	1	15b	8.06	87.50
Total	2	6	11	17	29	46	49	26	186	100.00	100.00
No. of alive aphids	100	100	100	100	100	100	100	100	800	-	-

Legends as in Table 1

Table 4: Mycopathogens identified from alive *R. padi* directly collected from wheat plants, 2007 season.

Fungal species	Number of infected / 100 alive aphids								Total	Dominance (%)	Abundance (%)
	Sampling date										
	Feb.				March						
	7	14	21	28	7	14	21	28			
C. coronatus	0	1	1	1	2	1	1	0	7e	2.94	75.00
E. planchoniana	0	0	1	2	10	18	38	5	74b	31.09	75.00
N. fresenii	2	2	4	8	16	6	4	3	45c	18.91	100.00
P. neoaphidis	2	5	9	10	17	22	11	4	80a	33.61	100.00
Z. radicans	0	0	0	3	0	6	8	2	19d	7.98	50.00
V. lecanii	0	1	1	2	3	2	3	1	13d	5.46	87.50
Total	4	9	16	26	48	55	65	15	238	100.00	100.00
No. of alive aphids	100	100	100	100	100	100	100	100	800	-	-

Legends as in Table 1

Table 5: Mycopathogens identified from alive *B. brassicae* collected directly from canola plants, 2006 season.

Fungal species	Sampling date							Total	Dominance (%)	Abundance (%)
	Feb.				March					
	7	14	21	28	7	14	21			
C. coronatus	0	1	2	4	6	2	1	16c	7.41	85.71
E. planchoniana	0	0	0	0	6	3	2	11c	5.09	42.86
N. fresenii	1	2	3	7	9	11	6	39b	18.05	100.00
P. neoaphidis	3	4	11	17	22	27	6	90a	41.67	100.00
Z. radicans	0	0	0	10	12	20	5	47b	21.76	57.14
V. lecanii	0	0	0	1	6	4	2	13c	6.02	57.14
Total	4	7	16	39	61	67	22	216	100.00	-
No. of alive aphids	100	100	100	100	100	100	100	700	-	-

Legends as in Table 1

Table 6: Mycopathogens identified from alive *B. brassicae* collected directly from canola plants, 2007 season.

Fungal species	Sampling date							Total	Dominance (%)	Abundance (%)
	Feb.				March					
	7	14	21	28	7	14	21			
C. coronatus	0	1	2	4	6	5	1	19d	7.72	85.71
E. planchoniana	0	0	1	0	4	6	4	15d	6.09	42.86
N. fresenii	2	6	11	13	18	8	3	61b	24.79	100.00
P. neoaphidis	4	12	17	20	24	27	11	115a	46.75	100.00
Z. radicans	0	0	0	6	5	12	2	25c	10.16	57.14
V. lecanii	0	0	0	2	4	2	3	11d	4.47	57.14
Total	6	19	31	45	61	60	24	246	100	-
No. of alive aphids	100	100	100	100	100	100	100	700	-	-

Legends as in Table 1

Table 7: Number of wheat and canola aphids (cadavers) killed by the entomopathogenic fungi and percentage of mortality calculated to total cadavers and total aphids collected during 2006 and 2007.

Fungal species	*R. padi* (n= 1600)		*S. graminum* (n= 1600)		*B. brassicae* (n= 1400)		Total infected aphids	% to total (1375) cadavers	% to total (4600) Aphids
	Total	%	Total	%	Total	%			
C. coronatus	20	4.7	23	4.7	35	7.6	78	5.6	1.7
E. planchoniana	94	22.2	29	6.0	26	5.6	149	10.8	3.2
N. fresenii	100	23.6	134	27.6	100	21.6	334	24.3	7.3
P. neoaphidis	146	34.4	232	47.4	205	44.4	583	42.4	12.7
Z. radicans	36	8.4	41	8.4	72	15.6	149	10.8	3.2
V. lecanii	31	7.3	27	5.6	24	5.2	82	5.9	1.8
Total cadavers	427	100	486	100	462	100	1375	100	30.0
% mortality	26.7		30.4		33.0		30.0		

n= total number of aphids collected during 2006 and 2007,
% mortality = Percentage of cadavers killed by fungi to total collected aphids

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