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ENTOMOPATHOGENIC FUNGI OF *SCHIZAPHIS GRAMINUM* (ROND.) (HOMOPTERA: APHIDIDAE) INFESTING WHEAT PLANTS IN ASSIUT, EGYPT

Asmaa H. M. Mohamed[1], S. M. A. El-Maraghy[2], M.A. A. Abdel-Rahman[3], Azza M. A. Awad[1] and Y. M. Omar[4]

[1]*zoology department, Faculty of Science, New Valley University, Egypt*
[2]*Botany and Microbiology Department, Faculty of Science, Assiut University, Egypt*
[3] *Zoology Department, Faculty of Science, Assiut University, Egypt*
[4]*Plant Protection Research Institute, A.R.C., Egypt*
[5]*Plant Protection Department, Faculty of Agriculture, Assiut University, Egypt*

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Eight species of entomopathogenic fungi were identified and recorded naturally infecting the greenbug infesting wheat plants during 2012-2013 and 2013-2014 wheat growing seasons in Assiut, Egypt. These fungi including six entomophthorales (*Conidiobolus coronatus*; *Entomophthora planchoniana*; *Neozygites fresenii*; *Pandora neoaphids*; *Paecilomyces lilacinus* and *Zoophthora radicans*) and two hyphomycetes (*Beauveria bassiana* and *Verticillium lecanii*). Data revealed that the aphid began to infest wheat plants at the beginning of January when wheat plants were in tillering stage. Aphid numbers increased gradually to reach a peak during the second half of February when the plants were in the booting stage. Then, the population declined till vanishing from the field during the end of March when the plants were in the ripening stage. Mortality rate with the fungal pathogens was recorded from the end of January up to the end of March. The numbers of cadavers increased gradually to reach the maximum level during the end of March and synchronized with aphid population.

Key words: Greenbug, *Schizaphis graminum* (Rond.), wheat, entomopathogenic, Assiut, Egypt.

INTRODUCTION

The greenbug *Schizaphis graminum* (Rond.) is a warm season perennial pest, causing substantial losses to cereal crops and wheat in particular. It is a serious pest having a wide host range of at least 60 plant species including wheat, barley, sorghum and corn (Kindler *et al*., 1984; Bowling *et al*., 1998). It sucks the sap and injects the toxin into the plant and interferes with the grain formation (Kannan, 1999). Yellow to red lesions surrounded by a large chlorotic area can be readily identified on leaf surface, which turn necrotic with time. Increasing aphid resistance to

common insecticides (Foster and Devonshire, 1996; Harrington and van Emden, 2007) has stimulated interest in developing alternative methods of control in an effort to move towards sustainable cropping practices (Carter, 1989; Gurr *et al*., 2004; Pell, 2007; Thomas, 1999). An attractive alternative method to chemical pesticides is the microbial biocontrol agents as the natural enemies of the pest population devastate pests with no hazard effects on human health and environment. Fungal pathogens are the most important pathogens of aphids as they infect insects by breaching the host cuticle. They are reported as major factors suppressing populations of cereal aphids (Feng *et al*., 1991 and Wells *et al*., 2000) and epizootics, particularly those caused by Entomophthorales are frequently observed and often rapidly reduce aphid populations (Dedryver, 1983 and Steinkraus 2006). 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 and Humber 1981; Car-Ruthers and Soper 1987; Hajek and St. Leger 1994; Bateman and Chapple 2001; Wraight *et al*. 2001; Barta and Cagan 2006). The key biological attributes for a mycoinsecticide are generally considered to be virulence toward the target insect(s) and limited pathogenicity to non-target organisms (Goettel *et al*., 1990; Wraight and Carruthers, 1999). Therefore, the aim of this work is to identify the incidence of the entomopathogenic fungi infecting the greenbug *S. graminum* inhabiting wheat plants in Assiut, Egypt.

MATERIALS AND METHODS

The present study was carried out at Experimental Farm of Faculty of Agriculture, Assiut University throughout the period from 2013-2014 wheat growing seasons. An area of about half feddan (2100m^2) was cultivated with wheat, cultivar (Sids1) during mid-November in both 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.

Regular samples consists of 100 tillers from wheat were randomly collected and brought back to the laboratory for counting aphid numbers. Four replicates each were taken weekly from the beginning of January when the migration of aphids onto wheat crop, at the stage of tillering, from the overwintering sites began and continued till aphid population declined to low or undetectable levels. At the same time, number of infected aphids was also counted and recorded.

Collected cadavers of aphid species with fungal pathogen were collected with a small amount of plant materials and placed in plastic vials and transferred to laboratory for identification of fungus. Species was examined under a compound microscope as soon as possible 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 a compound microscope. Fungus identification was based on external symptoms and the morphology of spores and sporulating structures as described by Waterhouse and Brady (1982), new revision of the classification of Entomophthorales (Humber, 1989) and help by Assiut University Mycology Center (AUMC). 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

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

$$\text{Mortality \%} = \frac{\text{Number of infected aphids}}{\text{Total No. of alive aphids + No. of cadavers}} \times 100$$

Results and Discussion

1-Entomopathogenic fungi identified from the greenbug aphid

From the survey studies through 2013 and 2014 wheat growing seasons, eight species of entomopathogens, including six entomophthorales and two hyphomycetes were identified from the greenbug *S. graminum* infesting wheat plants. Entomophthorales was represented by six species belonging to four families: Ancylistaceae was represented by one genus, *Conidiobolus coronatus*, Entomophthoraceae by *Entomophthora planchoniana*, *Pandora neoaphids* and *Zoophthora radicans,* Neozygitaceae by *Neozygites fresenii* and Trichocomaceae by *Paecilomyces lilacinus*. The hyphomycetes were represented by *Beauveria bassiana* and *Verticillium lecanii* both from order Moniliales. These fungi mentioned above are surveyed worldwide as they are well-known species as biological control agents of cereal aphids (Feng *et al*.,

1990; 1991 and 1992; Abdel-Rahman, 2001; Hammam, 2003; 2009 and Moubasher *et al*., 2010). Mycopathogens are considered to be the best means of biological control of aphids (Latge and Papierok, 1988), and numerous accounts of cereal aphids killed by entomophthoralean fungi were documented in Europe (Dean and Wilding, 1971; 1973; Dedryver, 1983 and Papierok and Havukkala, 1986) and South America (Lazzari, 1985). Regional lists of aphid pathogenic fungi have been published in Australia (Milner *et al*., 1980) and Finland (Papierok, 1989). Five entomopathogenic fungi were reported from 34 aphid hosts in eastern Canada and the United States (Remaudiere *et al*., 1978 and Humber and Soper, 1986).

2- Incidence of recorded entomopathogens

Data in Table 1 show the relative incidence of eight entomopathogens which infect the greenbug namely; *C. coronatus*, *E. planchoniana*, *N. fresenii*, *Pand. neoaphids*, *Z. radicans*, *Paeci. lilacinus*, *B. bassiana* and *V, lecanii* during 2013 and 2014 wheat growing seasons.

In 2013 season, data indicate that 307 cadavers were collected from the greenbug. Statistical analysis shows that there are significant differences among the fungi recovered. These species could be arranged descendingly as follows: *C. coronatus*, *E. planchoniana* and *Pand. neoaphids* were the predominant species indicating 22.80, 18.57 and 14.66 % of the grand total of fungi recorded. *Paeci. lilacinus*, *V. lecanii* and *Z. radicans* inflecting 12.38, 10.41 and 8.47 % of the total fungi recorded respectively. *N. fresenii* and *B. bassiana* showed the least occurrence (6.51 and 6.20 % respectively).

Similar results were obtained during 2014 season, whereas, *C. coronatus*, *E. planchoniana* and *Pand. neoaphids* were the predominant species with 28.37, 23.51 and 13.01 % of the total fungi recorded. *V. lecanii*, *Paeci. lilacinus* and *B. bassiana* had 9.25, 8.15 and 6.58 %. *Z. radicans* and *N. fresenii* had the least percentage (6.27 and 4.86 %).

3- Seasonal incidence of *S. graminum* and Entomopathogenic fungi:

Data in Table (2) show that in 2013 season, the greenbug were began to appear on the wheat plants in a relatively low level (20 aphids / 10 tillers) during the first week of January. Its population reached a peak of 285 aphids / 10 tillers during the end of February, then the number of aphids showed a sharp decrease and approximately vanished from the field during the end of March.

During 2014 season, the aphid started to appear on wheat plants in extremely low numbers (8 aphids / 10 tillers) in the first week of January. The population reached a peak of 265 aphids / 10 tillers during the second half of February. The population continued in relatively high numbers in the next week and vanished from the field during the end of March

Table (1): Numbers and percentage of mycopathogens recovered naturally infecting the greenbug aphid in the wheat field during 2013 and 2014.

Fungi species	2013		2014		Total	
	No.	(%)	No.	(%)	No	(%)
Beauveria bassiana (Balsamo) Vuill.	19d	6.20	42d	6.58	61d	6.45
Conidiobolus coronatus Batko (1964)	70a	22.80	181a	28.37	251a	26.56
Entomophthora planchoniana Cornu	57b	18.57	150a	23.51	207b	21.71
Neozygites fresenii (Nowak.) Remaud. & S. Keller 1980	20d	6.51	31e	4.86	51d	5.41
Pandora neoahidis (Remaudierel & Hennerbert) Humber	45b	14.66	83b	13.01	128b	13.55
Paecilomyces lilacinus (Thom) Samson	38c	12.38	52c	8.15	90c	9.52
Verticilium lecanii (Zimmermann) A.W. Viegas	32c	10.41	59c	9.25	91c	9.62
Zophthora radicans (Bref.) A. Batko	26c	8.47	40d	6.27	66d	6.98
Total	307	100.00	638	100.00	945	100.00

Means vertically followed by the same letter are not significantly different < 0.05 level of probability.

In both seasons, the initial infestation by aphids was correlated with the appearance of cadavers in few numbers and was observed at the beginning of February. The number of cadavers progressively increased to exhibit a peak after the aphid population reached a peak.

Several species of entomopathogenic fungi are known to cause fatal diseases in aphids, including *Conidiobolus* sp., *V. lecanii* and various

species of *Beauveria* and *Paecilomyces* (Roberts and Yendol, 1971 and 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 entomopathogenic fungi species killed 65-80% of common cereal aphids in eastern England (Dean and Wilding 1973). Drastic reduction in populations of various cereal aphids due to infection with Entomophthorales fungi was observed by Dedryver (1983). Members of order Entomophthorales are excellent candidates for biological control of aphids (Latge and Papierok, 1988). Worldwide, *P. neoaphidis* is the most common and frequently the dominant pathogen of aphids (Waterhouse and Brady, 1982). This fungus can cause collapse of unmanaged aphid population within few weeks of the onset of disease (Feng *et al*., 1990).

Table (2): Number (10 tillers) of the greenbug on wheat plants and natural infection rate with entomopathogens in the field during 2013 and 2014 wheat growing seasons.

Inspection date	Growth stage (ZGS)*	2013 season			2014 season		
		No. / 10 tillers		Infection (%)	No. /10 tillers		Infection (%)
		Aphids	Cadavers		Aphids	Cadavers	
Jan. 6	Tillering	20	0	0.00	8	0	0.00
13		21	0	0.00	19	1	5.00
20	Stem extension	33	0	0.00	23	3	11.53
29		56	2	3.44	48	16	25.00
Feb. 5		57	4	6.55	78	28	26.41
11		253	21	7.66	243	95	28.10
18	Booting	285	28	8.94	265	131	33.08
24	Head emergence	275	56	16.91	210	175	45.45
Mar. 4		155	78	33.47	64	125	66.13
11	Ripening	75	62	45.25	41	53	56.38
18		35	50	58.82	22	9	29.03
26		11	6	35.29	8	2	20.00

*(ZGS) = A decimal code for growth stage of cereal (Zadoks *et al*., 1974).

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الفطريات الممرضة لحشرات البق الأخضر التي تصيب نباتات القمح بأسيوط – مصر

أسماء حنفي محمود محمد١، سعد شحاتة محمد المراغى٢، محمد علاء الدين احمد عبد الرحمن٣، عزة عبد المنعم عوض4، يوسف محمد عمر5

١- *قسم علم الحيوان والحشرات – كلية العلوم – جامعة الوادي الجديد*
٢- *قسم النبات والميكروبيولوجى – كلية العلوم – جامعة اسيوط*
٣- *معهد بحوث وقاية النباتات – مركز البحوث الزراعية*
٤- *قسم علم الحيوان والحشرات – كلية العلوم – جامعة أسيوط*
٥- *قسم وقاية النبات – كلية الزراعة – جامعة اسيوط*

تم تعريف ثمانية أنواع من الفطريات الممرضة لحشرات البق الأخضر التي تصيب القمح خلال مواسم٢٠١٢-٢٠١٣، ٢٠١٣-٢٠١٤ من مواسم زراعة القمح بأسيوط.

اتضح من الدراسة أن هناك خمسة أنواع تتبع مجوعة الفطريات الإجبارية التطفل Entomophthorales وهم :

Conidiobolus coronatus,
Entomophthora planchoniana,
Pandora neoaphids,
Paecilomyces lilacinus
Zoophthora radicans)

بالإضافة إلى نوعين من الفطريات الناقصة Hyphomycetes وهما :

Beauveria bassiana
Verticillium lecanii

اتضح من الدراسة أن حشرات من البق الأخضر تبدءا في إصابة نباتات القمح مع بداية شهر يناير وتكون نباتات القمح في مرحلة التفريع ، ثم بعد ذلك يأخذ تعداد حشرات المن في الزيادة حتى تصل إلى اقصي تعداد وتحدث هذه المرحلة مع منتصف شهر فبراير وتكون نباتات القمح في مرحلة انتفاخ الساق. يأخذ تعداد حشرات المن في الانخفاض بعد ذلك حتى يختفي جميع أفراد المن مع نهاية شهر مارس حيث تكون معظم النباتات في بداية مرحلة تكون الحبوب. أوضحت الدراسة

أن الموت الحادث بالمسببات المرضية الفطرية تبدءا مع نهاية شهر يناير وتستمر حتى نهاية شهر مارس. تعداد الأفراد الميتة بالفطريات الممرضة يزداد تدريجيا حتى يصل إلى أعلي عدوى مع نهاية شهر مارس كما أظهرت النتائج وجود علاقة تلازمية مع تعداد حشرات المن على نباتات القمح.