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Natural Occurrence of Entomopathogenic Fungi on Cereal Aphids at Assiut: A Comparison Study between Field and Laboratory Observations

M.A. A. Abdel-Rahman[*], A. Y. Abdel-Mallek[**], S. A. Omar[**] and G. H. A. Hamam[**]

[*]Plant Protection Research Institute, Agriculture Research Center, Giza, Dokki, Egypt
[**]Botany Department, Faculty of Science, Assiut University, Egypt

ABSTRACT

The present investigation was carried out during two successive wheat-growing seasons (2000 and 2001). The impact of entomopathogenic fungi on cereal aphid populations was evaluated under natural conditions. The aphid began to appear on wheat plants, early in the season, on 22nd January when the plants were in the stem-extension stages. Thereafter number of aphids increased gradually to reach a peak of abundance, when the plants were at flowering stage, during the first half of March. During the next three weeks the number of aphids declined sharply. The insect was observed in few numbers during the third week of March, when the plants were at the ripening stage. In the field, mortality rate with the fungal pathogens (cadavers count) was observed from January 22nd up to March 22nd. The number of cadavers increased gradually to reach the maximum level during the second week of March coincided with the peak of cereal aphids. Number of infected aphids developed in the laboratory from alive aphids collected from wheat field fluctuated in mortality rates from the first week of February up to the third week of March. The maximum level of parasitism coincided with the collapse of aphid population. At the peak of aphid populations, the rates of parasitism in the field were 24.67% and 12.01% compared to 58.67% and 42.00% in the laboratory during 2000 and 2001 seasons, respectively. The infection of cereal aphids with the entomopathogenic fungi (laboratory observation), was always much higher than the infection rate in field observation, being 2.35 and 3.50 times higher during population peak of cereal aphids during 2000 and 2001, respectively. Based on the regression model the effectiveness of entomopathogenic fungi on cereal aphids and the mortality rate can be predicted by looking for aphid cadavers on wheat plants.

Key Words: Entomopathogenic Fungi, Cereal Aphids, Field and Laboratory Observations, Assiut.

INTRODUCTION

Four species of cereal aphids have been found infesting wheat plants in Assiut. These species are Oat bird-cherry aphid, *Rhopalosiphum padi* L.; greenbug, *Schizaphis graminum* (Rondani); corn leaf aphid, *R. maidis* (Fitch) and the English grain aphid, *Sitobion avenae* (F.). The first two species, *R. padi* and *S. graminum*, were more abundant than others (El-Hariry 1979, Abdel-Rahman 1997, El-Lathy 1999, and Ali and Abdel-Rahman 2000).

Entomopathogenic fungi are considered by some entomologists to be the best candidates for the biological control of aphids (Latge and Papierok, 1988, Wraight *et al.*, 1993 and Hatting *et al.*, 2000), and numerous accounts of cereal aphids killed by entomophthoralean fungi have been documented in Europe (Dean and Wilding 1971, 1973; Dedryver 1983; Papierok and Havukala 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). Several species of entomopathogenic fungi can cause fatal disease in aphids, including *Conidiobolus obscures* (Hall & Dunn) Remaudiere & Keller, *Erynia neoaphidis* Remaudiere & Hennebert, *Verticillium lecanii* (Zimmerman) Viegas, various species of *Beauveria*, and *Paecilomyces farinosus* (Roberts and Yendol 1971, Samson *et al.* 1988 and Hayden *et al.* 1992).

In Egypt, few studies revealed the effect of entomopathogenic fungi on the population dynamics of some pests (Sewify, 1989 & 2000; Abdel-Rahman, 2001, and Abdel-Rahman & Abdel-Mallek, 2001,). In Assiut little is known about the role of entomopathogenic fungi in controlling cereal aphids. Therefore, the present investigation aimed to evaluate the impact of entomopathogenic fungi on the cereal aphid population. Also, this research is indispensable for providing certain basic information on entomopathogenic fungi-cereal aphid interaction in wheat fields under natural conditions.

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, 1999-2000 and

2000-2001. An area of ca. 4200 m^2 (one feddan) was cultivated with wheat (cultivar Giza 164) by mid-November. 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.

1- Sampling of aphids and the associated cadavers (Field observations):

Weekly samples consisted of 100 tillers (four replicates each contains 25 tillers) were randomly collected and brought back in transparent polyethylene bag to the laboratory for counting aphid species and cadavers. Samples were taken weekly during the two seasons from the 3rd week of January, when the migration of aphids onto the wheat crops, at the stage tillering or early stem-elongation from over-wintering sites began, and continued through the time when aphid population and cadavers declined to low or undetectable levels. The number of cereal aphid species "*Rhopalosiphum padi* L., *Rhopalosiphum maidis* (Fitch) and *Schizaphis graminum* (Rondani)" and cadavers within each species was counted and recorded at each inspection date.

2- Real fungus-infection (Laboratory observations):

In each inspection date, during the two seasons, 50 living aphids 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. 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). 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.

Percentage of infection (mortality %) caused by entomopathogenic fungi in each aphid species as well as total aphid was calculated in each sampling date from field and laboratory data according to Feng *et al.* (1992) as follow:

$$\text{Mortality (\%)} = \frac{\text{Number of cadavers}}{\text{(sum of alive aphids and cadavers)}} \times 100$$

Temperature (maximum and minimum) and relative humidity (maximum and minimum) were obtained from a metrological station located at 500 m away from the experimental site.

RESULTS AND DISCUSSION

1-Population of cereal aphids and fungal infection:

Data obtained in Tables (1 and 2) and illustrated in Figures (1 and 2) show the seasonal occurrence of alive cereal aphids in the field and those infected (cadavers) with entomopathogenic fungi in both field and laboratory during 2000 and 2001 wheat growing seasons.

Aphid population

In 2000 season, the aphid began to appear on wheat plants (2.57 aphids / tiller) on 22nd January when the plants were in the stem-extension stage (Table 1). Thereafter, the number of aphids increased gradually to reach a peak of 15.05 aphids / tiller, when the plants were at flowering stage in the second week of March. During the next three weeks the number of aphids declined sharply. The insect was observed in few numbers during the third week of March, when the plants were at the ripening stage. In 2001 season, (Table 2) the population's densities of aphids were detected early in the season (third week of January). Thereafter the population increased gradually reaching a peak of 73.61 aphids / tiller on March 1st. On 22nd of March a decline in the population was observed, reaching 1.32 aphids / tiller.

The population fluctuation of cereal aphids in wheat fields in southern Egypt was previously described (Ali and Rizk 1980, Ali and Darwish 1990 and Abdel-Rahman 1997). They generally mentioned that cereal aphids usually show their population peak around the middle of March. It seems that low temperatures early in the season prevent build up of populations during January. In this period, minimum temperature ranged from 2° to 8°C (night temperature) and the maximum temperature ranged from 18° to 24°C (daytime temperature). The increase of aphid populations to the highest levels occurred during the period of rapid wheat development and increasing temperatures. During the end of February the minimum temperature ranged from 5° to 18°C, whereas the maximum ranged from 18° to 32°C. These conditions seem to be the optimum range for the development and multiplication of cereal aphids. Previous studies confirmed the present results. Cartier and Painter (1956) found that the high temperature, longer period of sunlight and

better growing conditions of the plants favored the reproduction of aphids. Jones (1972) also found that the weather conditions affect cereal aphid population. (Dean (1974) found that *R. padi* population peaked in late August and early September when temperatures were in optimum range (20-25°C). Ali and Rizk (1980) reported that temperature ranged from 17-19°C and R.H. within the range of 44% and 52% are the most favorable conditions for the activity of cereal aphid species. The decline in aphid population later in the season could be attributed mainly to the role of natural enemies (predators, parasitoids and entomopathogenic fungi). Several authors have pointed out that natural enemies may also contribute to population decline (Ali and Morsy 1983, Abdel-Rahman *et al.* 2000, Ali and Abdel-Rahman 2000 and Ali *et al.* 2001).

Fungal infections

In the field

In 2000 season, mortality with the fungal pathogens was observed from January 22nd up to March 22nd and the percentages of mortality ranged between 2.28% and 41.79%. The number of cadavers started with 0.06 cadaver / tiller (mortality 2.28%) on January 22nd (temperature was 15.4 °C maximum and 11.8°C minimum and relative humidity was 84.3% maximum and 40.9 % minimum). Then the number of cadavers increased gradually to reach the maximum level; 4.93 cadavers / tiller (mortality 24.67%) during the second week of March coincided with the peak of cereal aphids. At this time maximum & minimum recorded temperature and relative humidity were 17.6°C, 8.8°C and 91.3% and 34.1%, respectively. Maximum rate of mortality (41.79%) was recorded on March 22nd (Table 1 and Fig. 1). In 2001 season, numbers of cadavers appeared from January 22nd to the third week of March. Number of cadavers fluctuated from 0.10 cadaver/tiller (mortality 1.45%) on January 22nd to 10.53 cadavers / tiller (mortality 13.89%) on March 8th. Maximum mortality (41.33%) was observed on March 22nd. Maximum & minimum temperature and relative humidity on January 22nd were 16.7°C, 8.0°C and 90.7%, 38.3%; on March 8th 21.7°C, 13.5°C and 89.1%, 36.4% and 23.7°C & 14.9°C and 87.% and 34.9%, on March 22nd (Table 2 and Fig. 2).

In the laboratory

In 2000, mortality rate with entomopathogenic fungi increased from 8.67 % in the first week of February to reach the maximum during the third week of March (54.67%). The maximum level of infection (58.67%) coincided with the beginning of collapse of aphid population (Table 1 and Fig. 1). In 2001, fungus-infection steadily increased from 3.33% in February 1st to reach 44% during the third week of March. Maximum mortality was observed on March 8th (50%).

In general, the rates of mortality by entomopathogenic fungi in the field were always lower than those recorded from the aphid collected from the field and reared in the laboratory. At the peak of aphid populations, the rates of infection in the field were 24.67% and 12.01% compared to 58.67% and 42% in the laboratory during 2000 and 2001 seasons, respectively, being 2.35 and 3.50 times higher during peak population of cereal aphids during 2000 and 2001, respectively. It could be concluded that the laboratory rearing of alive aphids collected from the field is the more accurate method of assessing the intensity of entomopathogenic fungi infected cereal aphids.

When the number of cadavers observed in the field and those in the laboratory was taken into consideration, regression analysis was performed using the number of cadavers / 100 aphids on the wheat plant and the corresponding number taken from 100 alive aphids in the laboratory. Regression equation represented by:

$$Y = -2.69 + 2.70x$$

It seems that the linear regression model ($Y = a + bx$) provided the best fit of the data as indicating by the high values of correlation coefficient ($r = 0.99$ and $R = 0.98$). Based on this model the effectiveness of entomopathogenic fungi on cereal aphids and the real mortality rate can be predicted by looking for aphid cadavers on wheat plants.

The present study showed that the mortality estimated from observation of cadavers in the field was significantly less than that determined by rearing alive aphids. This might be attributed to the fact that infected aphids survived during the latent periods of the diseases (Feng *et al.* 1990, & Feng and Johnson 1991). Thus, counting cadavers in the field would tend to underestimate the infection rate as the mycosis would develop in a growing population of aphids. On the other hand, cadavers could be held on the plants by fungal rhizoids or proboscis (Feng *et al.* 1991), leading to overestimatation of the infection level, particularly in declining aphid populations. The effect of these phenomena would vary among aphid fungus systems.

Table (1): Population of alive cereal aphids and those infected (cadavers) with entomopathogenic fungi in field and laboratory, 2000 season.

Sampling date	No. aphids / tiller	Infection					Ratio**
		Field observations		Laboratory observations			
		No. cadavers / tiller	(%)	No. observations*	Infected		
					No.	(%)	
Jan. 22	2.57	0.06	2.28	-	-	-	-
Feb. 1	3.87	0.12	3.04	150	13	8.67	2.85
8	5.07	0.15	2.87	150	24	16.00	5.58
15	6.84	0.44	6.04	150	39	26.00	4.31
22	8.94	1.25	12.27	150	49	32.67	2.66
March 1	14.35	2.74	16.03	150	70	46.67	2.91
8	15.05	4.93	24.67	150	88	58.67	2.35
15	12.46	4.42	26.18	150	82	54.67	2.11
22	2.02	1.45	41.79	-	-	-	-

* Number of collected alive aphids.
** Mortality (%) in lab. / Mortality (%) in the field

Table (2): Population of alive cereal aphids and those infected (cadavers) with entomopathogenic fungi in field and laboratory, 2001 season.

Sampling date	No. aphids / tiller	Infection					Ratio**
		Field observations		Laboratory observations			
		No. cadavers / tiller	(%)	No. observations*	Infected		
					No.	(%)	
Jan. 22	6.81	0.10	1.45	150	0	0.00	0.00
Feb. 1	18.24	0.65	3.44	150	5	3.33	0.97
8	29.61	1.35	4.36	150	13	8.67	1.99
15	55.43	6.12	9.94	150	29	19.33	1.94
22	72.57	9.35	11.41	150	50	33.33	2.92
March 1	73.61	10.05	12.01	100	42	42.00	3.50
8	65.27	10.53	13.89	100	50	50.00	3.60
15	10.95	4.12	27.34	100	44	44.00	1.61
22	1.32	0.93	41.33	-	-	-	-

* Number of collected alive aphids.
** Mortality (%) in lab. / Mortality (%) in the field.

In conclusion, entomopathogenic fungi played a principle role in natural suppression of cereal aphid populations at Assiut, particularly at the time of their highest population level. It is necessary to take into account 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. The results reported herein support that the utilization of entomopathogenic fungi as a biological control agent could be complementary strategies in an integrated pest management program against cereal aphids in southern Egypt.

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التواجد الطبيعي للفطريات الممرضة لحشرات المَن النجيليات بأسيوط: دراسة مقارنة بين التطفل الحقيقي والتطفل المشاهد

محمد علاء الدين احمد عبد الرحمن* ، احمد يحيى عبد المالك ، شكري احمد عمر** ، جمال همام عبد العليم همام****

*معهد بحوث وقاية النباتات – مركز البحوث الزراعية

**قسم النبات – كلية العلوم – جامعة أسيوط

أجريت هذه الدراسة على محصول القمح خلال موسمى ٢٠٠٠، ٢٠٠١م. تم تقييم الدور الذى تلعبه الفطريات الممرضة لحشرات المَن النجيليات فى تنظيم التعداد تحت الظروف الطبيعية. بينت الدراسة أن حشرات المَن النجليات تبدأ فى إصابة نباتات القمح فى الأسبوع الثالث من شهر يناير حيث تكون النباتات فى مرحلة إستطالة الساق، ثم يأخذ التعداد بعد ذلك فى الزيادة التدريجية حتى يصل إلى أعلى كثافة عددية حين تكون نباتات القمح فى مرحلة التزهير وذلك خلال النصف الأول من شهر مارس، وبعد ذلك يقل التعداد حتى يصل إلى اقل كثافة عددية مع نهاية شهر مارس حيث تكون نباتات القمح فى مرحلة الطور اللبنى من نضج الحبوب. أوضحت النتائج إن الموت بالمسببات المرضية الفطرية أى التطفل المشاهد (عدد الأفراد الميتة) بدأ حدوثه مع نهاية شهر يناير وبداية شهر فبراير واستمر حتى الأسبوع الثالث من شهر مارس. كما كان تذبذب العدوى بالفطريات الممرضة متزامنة مع تعداد حشرات المَن. أما بالنسبة للتطفل المأخوذ من حشرات مَن حية إلى المعمل لمشاهدة التطفل عليها (التطفل الحقيقى) وجد انه أيضا يتذبذب مع بداية شهر فبراير حتى الأسبوع الثالث من شهر مارس مع حدوث أعلى تواجد للفطريات مع بداية إنخفاض تعداد حشرات المَن، اتضح من الدراسة أن نسبة الموت الحادث (التطفل المشاهد) منخفضة مقارنة بالتطفل الحقيقى. وأيضا فى مرحلة أعلى تعداد لحشرات المَن وجد أن نسبة التطفل المشاهد ٢٤،٦٧%، ١٢،٠٢% مقارنة بالتطفل الحقيقى وهى ٥٨،٦٧%، ٤٢،٠٠% خلال أعوام ٢٠٠٠، ٢٠٠١ على التوالى. اتضح من النتائج ظهور الموت بالمسببات المرضية الفطرية بصورة وبائية خلال النصف الثانى من شهر مارس ومتزامن مع قلة تعداد حشرات مَن النجيليات. أمكن من خلال معرفة تعداد الأفراد المتطفل عليها (التطفل المشاهد) وكذا تعداد الأفراد الميتة والتى ظهرت من أفراد المَن الحية والتى تم جمعها فى نفس التاريخ والمرباة فى المعمل عمل (نموذج) باستخدام معادلات الانحدار البسيط يمكن بواسطته معرفة مدى نشاط الفطريات حقليا عن طريق معرفة عدد الأفراد المتطفل عليها بالفطريات (المشاهد) لكل ١٠٠ فرد من حشرات مَن النجيليات.