ECOLOGICAL STUDIES ON NATURAL ENEMIES ASSOCIATED WITH APHIDS ON ALFALFA CROP AT FAYOUM GOVERNORATE, EGYPT.

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ABSTRACT

The presnt study was carried out during 2012/2013 and 2013/2014 alfalfa seasons. The aphid species, Aphis craccivora Koch, Therioaphis trifolii Mon. and Acyrthosiphon pisum Harris were found in both study seasons, Α. craccivora and T. trifolii were prevalent. The dominant predators were Coccinella undecimpunctata L. reaching 32.3% of predators population in the first season, Chrysopa carnea Stephens and Orius sp. which reached 80.1% in the 2nd season. Aphid fungal infection rate (8.4 to 8.7%) was higher than the rate of aphids parasitized (3.6 to 6.1%). The total death occurred by parasitism and fungal infection ranged from 12.3 to 14.5 % of the total collected aphids. *C. undecimpunctata* had 5 peaks per season with highest peaks in the 1st week. and last week of Dec for both study seasons, respectively. The highest peak of C. carnea was recorded in the 2^{nd} week of April, while the highest population density of Orius sp. was in spring. Aphid parasitism occurred during two periods of activity in both seasons. The highest peaks of fungal infection were 8.67 and 10.0 infected individuals / 10 stems of alfalfa in the 2^{nd} and 4^{th} weeks of Dec. The results obtained from this study showed that fungal infected aphid and C. undecimpunctata population were positively correlated with aphid population and %RH, while they negatively correlated with mean temperature. **Key words**: Alfalfa, Aphids, fungal infection, parasitoids, predators,

population fluctuations

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most used forage for animal feeding (Walton, 1983) for its highest feeding value and protein quantity for livestock (Hanson and Barnes, 1988). Also, it is an effective source of biological nitrogen and well known for its ability to improve soil structure (Summers, 1998).

Meanwhile, alfalfa is known as a good habitat for natural enemies. Harper, (1978) in Canada, mentioned that *Chrysopa oculata* Say was important predator of alfalfa aphids. Seven primary parasitoids were found associated with alfalfa aphids in the Mediterranean region (Aeschliman,1981). Hossian *et al.* (2001) observed higher numbers of *Coccinella transversalis* Fabricius in uncut strips compared with harvested alfalfa. In alfalfa, pea aphid is attacked by a specialized parasitoid wasp, *Aphidius ervi*, and a group of general predators such as Nabis bugs, Orius bugs and Coccinellid and Carabid beetles (Snyder and Ives, 2003).

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Weiser *et al.* (2003) found that the population of insect predators was higher in unharvested strips than in harvested alfalfa. Rakhshani *et al.* (2010) noted moving of aphid predators from harvested strips to adjacent unharvested strips as a refuge. Pons *et al.* (2013) recorded *Acyrthosiphon pisum* Harris, *Therioaphis trifolii* Mon. and *Aphis craccivora* Koch. in alfalfa crop, where *A. pisum* was the most common species.

The present study aims to survey the available biological control agents for aphids in alfalfa plantations to determine a) the seasonal abundance of predators and parasitoids, b) the rates of parasitism and fungal infection of aphids and c) the relationships between parasitism and fungal infection rates and aphid population growth.

MATERIALS AND METHODS Experimentation:

One feddan of alfalfa was cultivated in early October for two successive seasons 2012/2013 and 2013/2014 at Moqrany village, Yousef El-Sedik district, Fayoum governorate and received usual agricultural practices, excluding pesticides. Two sampling techniques were used for sampling. The first was by using a sweeping net, where 50 double strokes were weekly made a cross the field to determine the abundance of parasitoid adults and aphid predators, as well as aphid densities (Maiteki et al., 1986; Hufbauer, 2002). Such samples were placed in polyethylene bags and transferred to laboratory and invisitegated under stereomicroscope to separate and count both aphids and natural enemies. The 2ndtechnique was the selection of 30 plants per week at random from the field and as well transferred to the laboratory. These plants were shaken onto a white sheet of paper immediately where the dropped healthy, mummified and fungal infected aphids were counted. Thereafter, the plants were dissected and examined to count all remained aphids. Mummified aphids collected were incubated in the laboratory until adult parasitoids emergence. The collected insects were identified by the biological control specialists in the Plant Prot. Dept., Fay. Fac. of Agric. All dead aphids with external symptoms of fungal infection were removed from leaves to a piece of wet filter paper in a small Petri dish which was incubated at 25°C for three days, then investigated under microscope for characteristic fungal structures which confirm the infection. Fungi were identified by their external symptoms on host and the morphology of their sporulating structures. Percentages of parasitism and fungal infection were calculated as the number of mummified and fungal infected aphids of the total number of aphids collected according to Chen and Hopper, 1997. Weather data (daily maximum and minimum temperatures, and daily mean relative humidity were taken from the Agrometeorological Station in Fayoum. Data was subject to statistical analysis for the

simple correlation values between the number of insects and weather factors using the program **SPSS 2000**, version 11.

RESULTS AND DISCUSSIONS

1-Aphid species collected from alfalfa and their natural enemies

As shown in table (1) and fig. (1), the dominant aphid species were *Aphis* craccivora Koch, *Therioaphis trifolii* Mon. and *Acyrthosiphon pisum* Harris. In both seasons of study, *A. craccivora* and *T. trifolii* were prevalent. Three prevalent predators were present, namely *Coccinella undecimpunctata* L., *Chrysopa carnea* Stephens and *Orius* sp. The former was the major predator representing 32.3% of predators population in the first season, while the latter was dominant in the 2^{nd} season representing 80.1 of the population. The fungal infection rate which ranged 8.4 to 8.7% was higher than that of parasitism rate (3.6 to 6.1%). The total death that occurred by parasitism and fungal infection ranged 12.3 - 14.5 % of the total collected aphids.

2013/2014 seasons.									
	Whole plant	samples (10 sten	ns x 3reps)).					
parameters		2012/201	13	2013/2014					
	-	Mean±S.E	%	Mean/ ±S.E	%				
	Aphis craccivora	12.21±6.06	64.1	7.16±3.08	49.4				
Healty aphids	Therioaphis trifolii	3.41±0.93	17.9	4.37±1.37	30.2				
	Acyrthosiphon pisum	0.63 ± 0.34	3.3	1.17 ± 0.52	8.1				
	Total	16.25	85.5	12.70	87.7				
Parasitism	Parasitized aphids	1.16±0.43	6.1	0.52±0.21	3.6				
and fugal	Fungal infected aphids*	1.60 ± 0.44	8.4	1.26 ± 0.39	8.7				
infection	Total dead aphids	2.76±0.77	14.5	1.78±0.49	12.3				
Grand total of aphids		19.05±6.38		14.48±4.92					
Sweeping net samples (50 double strokes)									
Parameters		Mean±S.E	%	Mean±S.E	%				
Healthy	Aphis craccivora	9.59±3.09	24.7	13.07±3.29	30.8				
and dead	Therioaphis trifolii	9.76 ± 2.92	25.2	21.00±5.14	49.5				
aphids	Acyrthosiphon pisum	19.41±5.43	50.0	8.38 ± 2.78	19.7				
	Total	38.76	-	42.45	-				
Parasitoids**		17.1±4.19	-	7.07±1.82	-				
predators	C. undecimpunctata	8.0±1.69	32.3	6.55±2.07	15.4				
	C. carnea.	$7.20{\pm}1.29$	29.0	1.21±0.31	2.9				
	Orius sp.	6.45 ± 2.67	26.0	33.97±16.08	80.1				
	Syrhus sp.	2.38 ± 1.47	9.6	0.28 ± 0.22	0.7				
	Staphylinus sp.	0.76 ± 0.67	3.1	0.41±0.33	1.0				
	Total	$2\overline{4}\ 79+3\ 88$		12 1 + 16 12					

Table (1): Means and percentages of aphid species, parasitized aphids, fungal infected aphids, predators and parasitoids during 2012/2013 and 2013/2014 seasons

* Beauvaria bassiana **Aphidus sp. And Aphilinus sp.

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Fig.1. A) predators (*C. undecimpunctata*; *C. Carnea*; *Orius* sp.; *Syrphus* sp.; *Staphylinus* sp. B) Aphid species (*A. craccivora*, *T. trifolii*; *A. pisum*); C) redator aphids; D) fungal infected aphids

2- Population fluctuations of predators

Data in figs. (2 and 3) showed that *C. undecimpunctata* was the most abundant predatory species in the first season while *Orius* sp. was dominant in the 2^{nd} season. *C. undecimpunctata* recorded 5 peaks, the highest peaks were 45.0 and 53.0 individuals/ 50 double strokes in the 1^{st} and 2^{nd} weeks of Dec. during the 1^{st} and 2^{nd} seasons, respectively. The population of *C. carnea* in the 1^{st} season was

higher than that in the second season with highest peaks 23.0 and 5.0 individuals/ 50 double strokes, respectively in the 2nd week of April. Concerning Orius sp., in the 1st season, it was collected from the first week of Dec. and showed two activity periods. Population fluctuated with low numbers in the 1st period. In the 2^{nd} period, population density increased to reach the highest at the end of season with 59 individuals / 50 double strokes. Also, in the 2^{nd} season, the 1^{st} activity period extended from the 2nd week of Nov. to the 4th week of Dec. The 2nd period was from the 1st week of March to the end of season. Population recorded three peaks in last week of Nov., the 3rd week of Dec. and the 4th week of April with 15.0, 11.0 and 33.0 individuals / 50 double strokes. Besides, the total number of predators in the 1st season, showed five peaks in the 1st week of Dec., 4th week of Dec., mid-Jan., the 4th week of Feb. and the 1st week of April. The highest peak was in the 1st week of Dec. (67 individuals/ 50 double strokes). In the 2nd season, only four peaks occurred; in the 4th weeks of Nov. and of Dec., the end of Jan., and the 3rd week of March. Pons *et al.* (2009) stated that Heteropterous predators were the most prevalent group and adults of Orius sp. appear in mid April and reach their highest abundance in mid July and August. Ximenex-Embun et al. (2014) found that coccinellids represented 51% of the total predators. Statistically, the relationship between aphids on alfalfa plant samples and C. undecimpunctata population was positive in both two seasons, while it was positive in the first season and negative in the 2nd season for *C. carnea*. Ingawale and Tambe, (2007) reported that The population of aphids was positively correlated with Chrysopa larval and lady bird beetles population **3- Intensity of Parasitiods**

The population of parasitized insects was most abandunt in the 1st season and had six peaks in the 2nd week of Dec., 9th Jan., the 1st week of Feb.,the 3rd week of March, 1st week of April and the 4th week of April, with 12.0, 100, 15.0, 49.0, 48.0 and 30.0 individuals /50 double strokes. Concerning the 2nd season, parasitoids were appeared in sweeping net from the 1st week of Dec. to record two activity periods. The first period continued from the 1st week of Dec. to the 4th week of Jan., while the 2nd period was from the end of Feb. to mid-April. Population of parasitoids had four peaks in the 4th week of Dec., mid-Jan., 4th week of Feb. and mid April with 21, 11, 37 and 13 individuals/ 50 double strokes (figs. 2 and3).



Fig. (2): Weekly counts of dominant predators and parasitoids associated with alfalfa plants in Moqrany village, YousefElsedik district, Fayoum governorate, during the 2012/2013 season.



Fig. (3): Weekly counts of dominant predators and parasitoids associated with alfalfa plants in Moqrany village, Yousef Elsedik district, Fayoum governorate, during the 2013/2014 season.

4-Population of Parasitized aphids

As shown in figs. (4 and 5), data revealed that aphid parasitism occurred during two periods of activity per season. The first period in the 1^{st} season started from early to the end of Jan., and the 2^{nd} period from the1st week of March to the 2^{nd} week of April. The 1^{st} period in the 2^{nd} season from the 4^{th} week of Nov. to the 1^{st} week of Jan. and the second period from the 1^{st} to the 2^{nd} week of March. Also, three peaks occurred during the 1^{st} season with the highest peak (10.67)

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individuals/10 branches) in the 3^{rd} week of Jan. In the second season, two peaks were recorded in the 2^{nd} week of Dec. and March with 5.0 and 2.67 individuals / 10 branches, respectively. Rakhshani *et al.* (2009) stated that the highest percentage of parasitism was obtained in early and late growing season when aphid numbers were lower than 50/20 stems.

5- Intensty of Fungal infection

Fungal infection of aphids had three peaks in 1^{st} season and 4 peaks in the 2^{nd} season. The highest peaks were 8.67 and 10.0 infected individuals / 10 branches in the 2^{nd} and 4^{th} weeks of Dec. for the 1^{st} and 2^{nd} seasons, respectively. On the other hand, the total dead aphids had four peaks per season. For the 1^{st} season, the peaks were in the 2^{nd} week of Dec., the 3^{rd} week of Jan., the 1^{st} week of March and the 4^{th} week of March with 8.67, 18.0, 9.33 and 2.33 individuals / 10 branches, respectively. For the 2^{nd} season, the 2^{nd} and highest peak (11.0 individuals) was in the 4^{th} week of Dec. (figs. 4 and 5).



Fig 4. Weekly counts of parasitized , fungal infected and total phids on alfalfa plants in Moqrany village, Yousef Elsedik district, Fayoum governorate, during the 2012/2013 season.



Fig. 5. Weekly counts of parasitized , fungal infected and total phids on alfalfa plants in Moqrany village, Yousef Elsedik district, Fayoum governorate, during the 2013/2014 season.

6- Percentages of parasitism and fungal infection

The percentage of aphid parasitized in the 1st season was higher than that of the 2nd season, where percentage of parasitism was 6.1and 3.6% and fungal infection was 8.40 and 8.7% of the total number of aphids. Also, the percentage of fungal infection was higher than the percentage of parasitism in both study seasons (figs, 6 &7). Cagan and Barta, (2001) mentioned that relatively low infestation of alfalfa by aphids could be a reason for low fungal infection in the population. (Lopez Lastra *et al.*, 2006) stated that disregarding the aphids, fungal infection in populations of insects was more common during autumn-winter. Manfrino *et al.* (2014) showed that aphid's fungal infection occurred with a great extent between May and July



Fig. 6. Weekly percentages of parasitism , fungal infection and total mortality of aphids on alfalfa plants in Moqrany village, YousefElsedik district, Fayoum governorate, during the 2012/2013 season.



Fig. 7. Weekly percentages of parasitism , fungal infection and total mortality of aphids on alfalfa plants in Moqrany village, YousefElsedik district, Fayoum governorate, during the 2013/2014 season.

Data in table (5) demonstrated that the simple correlation between mean temperature, and numbers of parasitized aphids was insignificantly negative in both study seasons (r=-0.202 and -0.084for 1st and 2nd seasons, respectively), while the effect of % RH was insignificantly positive (r=0.310 and 0.58). Concerning the fungal infection, the relationship between mean temperature and numbers of fungal infected aphids was significantly negative(r= -0.469*) in the 1st season and insignificantly negative (r=-0.214) in the 2nd season. %RH had positive effect on the fungal infection but this effect was significant in the 1stseason (r= 0.622*).The positive correlation between an entomophthoralean infection and relative humidity was found by Feng *et al.* (1992); Elkinton *et al.*(1991).

Regarding the effect of weather factors on parasitoid and predator insect populations, it was found that the relationship between parasitoids population and mean temperatures was insignificantly negative in the 1st season and significantly negative in the 2nd season (r=-0.008 and -0.481*). For % RH, this relationship was insignificantly positive in the 1st season and insignificantly negative in the 2nd season. The simple correlation between *C. undecimpunctata*, and mean temperatures was insignificantly negative, while it was insignificantly positive with %RH. The effect of temperature on *C. undecimpunctata* was significantly positive, while the effect of %RH was insignificantly negative in the 1st season and significantly negative in the 2nd season.

Parameters	Simple correlation							
	2012/2013			2013/2014				
	Mean	%RH	Total	Mean	%RH	Total		
	temp. °C		aphids	temp. °C		aphids		
Parasitized aphids	-0.202	0.303	0.003	084	0.058	0.521*		
Fungal Infected aphids	-0.469*	0.622**	0.469*	-0.214	0.113	0.121		
Total dead aphid	-0.381*	0.526**	0.271	-0.208	0.115	0.323		
Parasitoids	-0.008	0.315	-0.117	-0.481**	-0.073	0.035		
C. undecimpunctata	-0.012	0.154	0.700**	086	0.068	0.013		
C. carnea	-0.184	0.277	0.257	0.193	-0.240	-0.265		
Orius sp.	0.583**	-0.259	-0.063	0.427*	-0.423*	-0.171		
Total predators	0.360	-0.027	0.361	0.419*	-0.415*	-0.174		

Table (2): Simple correlation (r) analysis of variance between population of parasitized, fungal infected aphids, parasitoids and predators, and mean temperature and % RH during 2012/2013 and 2013/2014 seasons.

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دراسات بيئية على الأعداء الإحيائية المرتبطة بالمن على محصول البرسيم في محافظة الفيوم ،مصر

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أجرى هذا البحث لدراسة تذبذبات التعداد و العلاقة بين بعض أنواع المن و أعدائها الإحيائية المرتبطة بمحصول البرسيم في محافظة الفيوم خلال موسمي الدراسة ٢٠١٣/٢٠١٢، ٢٠١٤/٢٠١٣. كانت أنواع المن الشائعة هي حشرة من البقوليات Aphis craccivora Koch وحشرة من البرسيم المنقط .Thrioaphis trifolii Mon و حشرة من البسلة Acyrthosiphon pisum Harris وقد وجد أنحشرات من البقوليات ومن البرسيم المنقط هما الأكثر سيادية . أما المفترسات السائدة فهي حشرة أبو العيد ذو ١١ نقطة Coccinella undecimpunctata وحشرة أسد المن Chrysopa carnea stephens وحشرة الأوريس .Orius sp . وقد وجد ان حشرة أبو العيد هي الأكثر تواجداً في الموسم الأول (٣,٣٢%) بينما سادت حشَّرة الأوريس في الموسم الثاني (١,٨٠%) من التعداد الكلي للمفترَّسات أماً بالنسبةُ لإصابةُ المن بالفطر والتطفل على المنَّ تراوحت النُّسبةُ من ٤ ٨-٧,٨ % للفطر و٦,٦-١,٣% للتطفل وقد تراوحت نسبة الموت الكلية نتيجة الفطر والتطفل معا من ٢٢٢-٥٤٦ %. من ناحية أخرى فقد وجد لحشرة أبو العيد خمسة قمم للنشاط كان أعلاها خلال الأسبوع الأول والأخير من ديسمبر لكل من موسمي الدراسة على التوالي، بينما سجل أعلى تعداد للأوريس خلال الربيع. أظهر التطفل على المن فترتين من النشاط خلال موسمي النشاط. أما بالنسبة للإصابة الفطرية فقد كانت أعلى قمم الإصابة ٨,٦٧، ٠.١٠ فرد مصاب /١٠ أفرع تخلال الإسبوع الثاني والرابع من ديسمبر لكل من موسمي الدراسة على التوالي، كما كانت نسبة العدوى الفطرية أعلى من نسبة التطفل. أيضاً أظهرت النتائج المتحصّل عليها أن تعداد المن المصاب بالفطر وكذلك تعداد أبو العيد ارتبط إيجابياً مع تعداد المن الكلي والرطوبة النسبية بينما ارتبط سلبيا مع متوسط در جات الحر ار ق