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Ecological Studies on the Peach Green Aphid, *Myzus persicae* and its Natural Enemies

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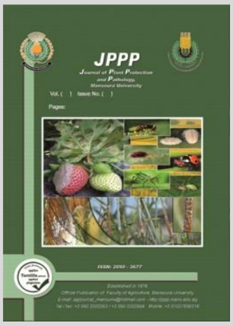

ABSTRACT

The population fluctuation, migration and flight activity of aphids vary during the year, because they are affected by various biotic and abiotic factors, among them being the occurrence of variations in temperature and natural enemies' composition. Field studies were carried out in peach-producing-area located in Aga region, El-Dakahlia Governorate during seasons 2019/2020 and 2020/2021. Population of aphids, predators and parasitoids were estimated. Person-product-moment simple correlation coefficients between number of living individuals of *Myzus persicae*, each of natural enemies or each of temperature or relative humidity were determined. Two peaks of aphid population were annually recorded in April and May-June. Larvae of *Aphidoletes aphidimyza*, *Metasyrphus corolla*, and *Chrysoperla carnea* had two peaks annually, whereas those of *Scymnus interruptus* had only one. adults of *Coccinella undecimpunctata* and *C. septempunctata* recorded two population peaks; and adults of *Coccinella novemnotata*, *Cheilomenes propinqua isis*, and *C. carnea* recorded one peak annually. The population of mummified aphids had two-three peaks annually of abundance in April, June, and July. among the aphidophagous predators, only *A. aphidimyza* larvae and *C. undecimpunctata* adults exhibited significantly positive responses to the increase in aphid populations during both seasons of study. In both seasons, parasitism contributed with the highest percentages in the total population mortality of *M. persicae*, whereas the unknown mortality contributed with the lowest fate. The average of annual mortality was 21.19 and 7.38%, 2.30 and 6.75%, and 1.58 and 1.4% for parasitism, predation, and unknown mortality in the first and second season, respectively.

Keywords: Aphidophagous, mortality, parasitism, population, predation, temperature

INTRODUCTION

Aphids consider one of the economic group of pests that infest various plant species mainly due to their high reproductive ability and rapid population increase. Sucking the plant sap and transmission of viral diseases from infected to healthy plants are the main damages caused by aphids (Ali *et al*., 2006). In addition, aphids secrete honeydew, which impedes photosynthesis and finally leads to wilt and death of the plants, resulting in qualitative and quantitative losses in crops. The green peach aphid, *Myzus persicae* (Sulzer) is among the major aphid species which infest a wide range of host plants in both outdoor and greenhouses, particularly in Egypt (Hemiptera: Aphididae) (Van Schett, 2003; Ali *et al*., 2006; Gissella *et al*., 2006).

The green peach aphid, *M. persicae* is attacked more than 300 host plants that belong to more than 66 plant families. It is considered one of the most serious pests of peach (*Prunus* spp.) trees. In some of peach-producing regions, it leads to wrinkle and deform the leaves (Gallo *et al*., 2002), as well as impact the development of the apical shoots, activate the growth of side branches and modify the plant architecture (Salles, 1998). In addition to its direct effect on plant, it indirectly transmits the non-propagative viruses (Namba and Sylvester, 1981; Isac *et al*., 1998; Gildow *et al*., 2004). These symptoms make it a severe threat to peach farms worldwide, and the situation getting worse year after year, especially because it shows resistant to many common insecticides (Unruh *et al*., 1996; Guillemaud *et al*., 2003; Cravedi and Mazzoni, 2004). In addition, this aphid species can survive on weeds and hardy plants during hot seasons and reproduce under unfavorable conditions resulting latter in problems for main crops (Tamaki, 1975; Tamaki and Fox, 1982).

The population fluctuation, migration and flight activity of aphids vary during the year, because they are affected by various biotic and abiotic factors, among them being the occurrence of variations in temperature, rains, winds, and lack of food (Kisimoto and Dyck, 1976; Lázzari, 1985; Sequeira and Dixon, 1997) as well as increasing natural enemy pressure, and declining in host plant quality (Crafton-Cardwell 1991; Karley *et al*. 2004). A number of natural enemies including ladybird beetles, *Coccinella septempunctata* (L.) and *C. undecimpunctata* (L.) (Coleoptera: Coccinellidae), Syrphid flies, *Episyrphus balteatus* (DeGeer) (Diptera: Syrphidae), lacewings, *Chrysoperla carnea* (Stephens) (Neuroptera: mainly Chrysopidae), and *Braconid* wasp, *Aphidius matricariae* Haliday (Hymenoptera: Braconidae) have been found associated with *M. persicae* populations.

Therefore, the current study aims to examine some ecological factors that more likely to affect the green peach aphid, *M. persicae* and its natural enemies.

MATERIALS AND METHODS

The field studies were carried out in peach-producing-area located in Aga region and in guava orchard located in the experimental farm at Faculty of Agriculture, Mansoura

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University, during seasons 2019/2020 and 2020/2021. Five homogenous trees from each host tree were selected to collect leaf samples. Samples were collected from the different directions of the tree (i.e. north, south, west, east, and center). Samples were collected weekly until the absence of aphid. Each sample consisted of 50 leaves (ten leaves/tree). Leaves were transferred gently to the laboratory in polyethylene bags and investigated in the same day by binocular microscope. Both surfaces of each leaf were investigated. Aphid individuals were classified as living, unknown dead, parasitized, and damaged individuals by predaceous-sucking insect predators. In respect to insect predators, the number of predatory larvae of each predator species on leaf samples were recorded. Before collecting each sample, the selected trees of each host plant was inspected visually in the field to record the number of different insect predatory species that inhibiting aphid colonies. Five minutes were given to each tree item in the field to wholly inspect each tree. Ladybird predators were collected in vials and then identified in the laboratory, whereas adult lacewings were recorded in the field. In each sample, aphid mummies were kept in Petri-dishes with small piece of saturated cotton wool until adult emergence. These emerged parasitoids were preserved in Eppendorf tubes having 90% alcohol until the time for identification.

Percentages of parasitism, predation, and unknown mortality in colonies of the green peach and cotton aphids within each sample and during the whole period of aphid activity were estimated as follows:

$$\textbf{\% Mortality factor} = \frac{\textbf{Targeted mortality factor}}{\textbf{(Living+ parasitized+ preyed+ unknown dead aphids)}} \textbf{\%100}$$

The relationship between numbers of living aphids and number of predacious larvae of each species, or number of predacious adults, or mummified aphid, were determined using the Person-product-moment simple correlation coefficient at 5% probability level.

Data of temperature and relative humidity during the period of investigations were obtained from the Metrological Station at Dakahlia Governorate. Person-product-moment simple correlation coefficient between number of living aphids and each of temperature and relative humidity was determined. The same was done for number of parasitoids and predatory individuals with each of both abiotic factors.

RESULTS AND DISCUSSION

Population estimates

Seasonal occurrence of *M. persicae*

The seasonal occurrence of the green peach aphid, *M. persicae* on 50 leaves of peach trees in Aga district is showed in Figure (1). In both seasons, the insect population had two peaks of abundance. In the first season (2019/2020), these peaks were occurred in April and May, whereas they were appeared in April and June in the second season (2020/2021). The density ranged from 0.36 to 252.2 aphids/leaf in the first season, and from 10.49 to 56.52 aphids/leaf in the second season. The highest densities were recorded in April and June in the first and second season, respectively.

Seasonal occurrence of aphidophagous larvae

The seasonal occurrence of aphidophagous larvae in the green aphid, *M. persicae,* colonies that inhabiting leaves of peach trees during the first (2019/2020) and second (2021/2021) seasons at Aga district are presented in Figure (2). Larvae of the dipterous predator, *Aphidoletes aphidimyza* had two peaks of abundance during each season. In the first season, these peaks were occurred in April and May, respectively.

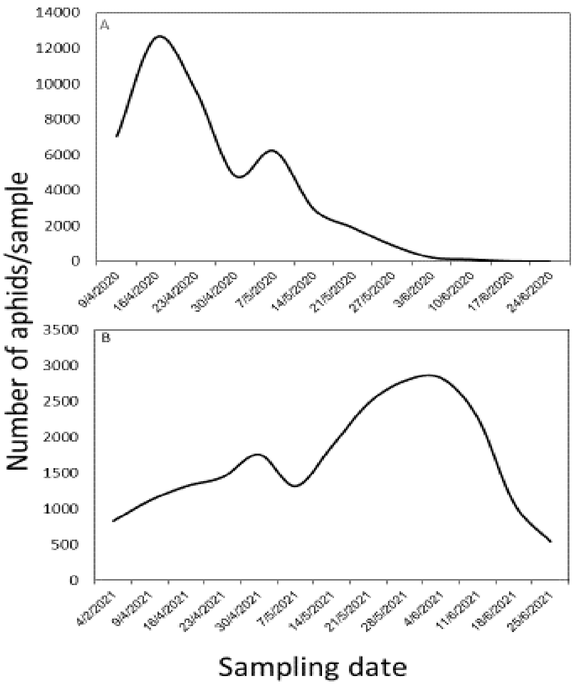

Fig. 1. Seasonal occurrence of the green peach aphid, *Myzus persicae* on 50 leaves of peach trees during 2019/2020 (A) and 2021/2021 (B) seasons at Aga district.

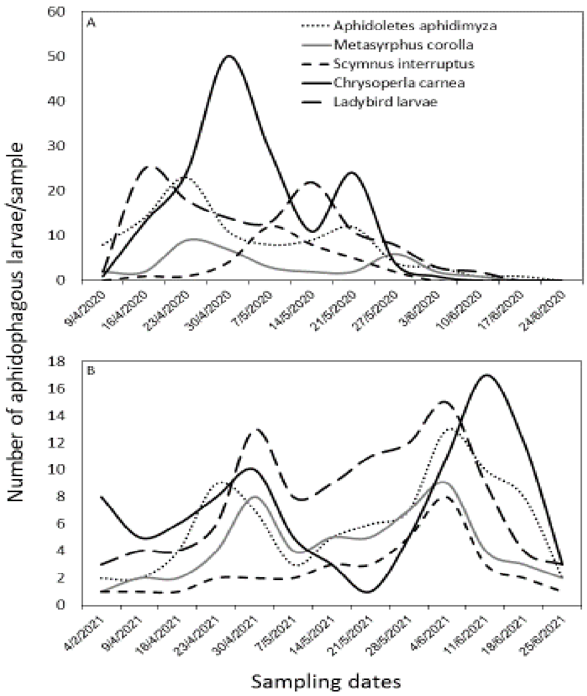

Fig. 2. Seasonal occurrence of aphidophagous larvae in the green peach aphid, *Myzus persicae* colonies that inhabiting leaves of peach trees during 2019/2020 (A) and 2021/2021 (B) seasons at Aga district.

In the second season, these peaks were occurred in April and June, respectively. In respect to larvae of the hoverfly *Metasyrphus corolla*, it showed two peaks of abundance in colonies of *M. persicae* during the first and second seasons.

These peaks were appeared in April and May in the first season, and in April and June in the second season, respectively. Regarding larvae of the ladybeetle predator *Scymnus interruptus*, it had only one peak of abundance in colonies of *M. persicae* per each season. This peak was in May and June in the first and second season, respectively. Larvae of the green lacewing, *Chrysoperla carnea* showed two peaks during April and May in the first season, while they were during in April and June in the second season. Larvae of coccinellid ladybirds exhibited two peaks of abundance in *M. persicae* colonies during each season of study. In the first season these peaks were occurred during April and May, while in the second season they occurred during April and June (Fig. 2).

Seasonal occurrence of aphidophagous adults

Numbers of aphidophagous adults that visually counted in peach trees that harboured the green peach aphid, *M. persicae* colonies during the first (2019/2020) and second (2021/2021) seasons at Aga district are appeared in Figure (3). Adults of the eleven-spotted ladybeetle *Coccinella undecimpunctata* had two peaks of abundance during each season. These peaks were occurred in April and May in each season. Adults of the seven-spotted ladybeetle *Coccinella septempunctata* had one and two peaks of abundance during the first and second seasons. In the first season, the highest abundance was during April, while in the second season, the highest abundance was during April and June. Regarding the nine-spotted ladybeetle, *Coccinella novemnotata*, it showed relatively higher abundance in the second season than the first season with the highest activity was in June during the second season. In respect to adults of *Cheilomenes propinqua isis*, it appeared in relatively few numbers during both season with only one peak during May in the first season and during June in the second season. Adults of the green lacewing, *C. carnea* appeared in relatively few numbers with highest numbers occurred in April during the first season and in May and June in the second season. (Fig. 3).

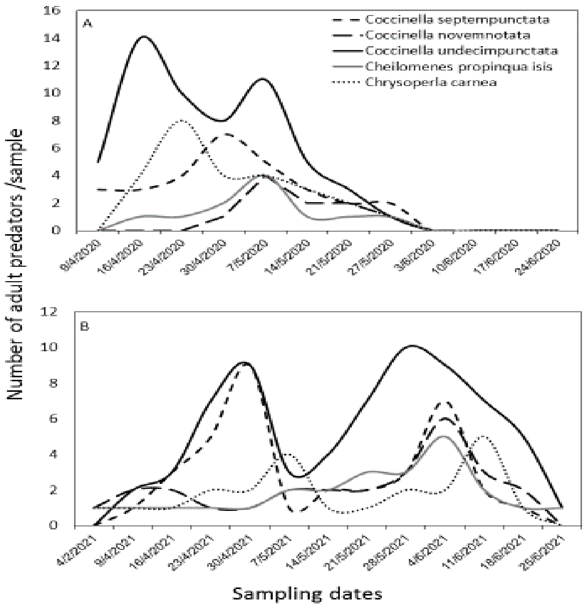

Fig. 3. Seasonal numbers of adult predators visually counted in peach trees that harboured the green peach aphid, *Myzus persicae* colonies during 2019/2020 (A) and 2021/2021 (B) seasons at Aga district.

Population density of parasitized aphids (mummified aphids)

Seasonal abundance of the green peach aphid, *M. persicae* parasitized with the pupal stage of the parasitoid (mummified aphids) during the first (2019/2020) and second (2021/2021) seasons at Aga district are presented in Figure (4). The population of mummified aphids had two peaks of abundance in April and June during the first season, while it had three peaks of abundance during April, early June, and late June in the second season.

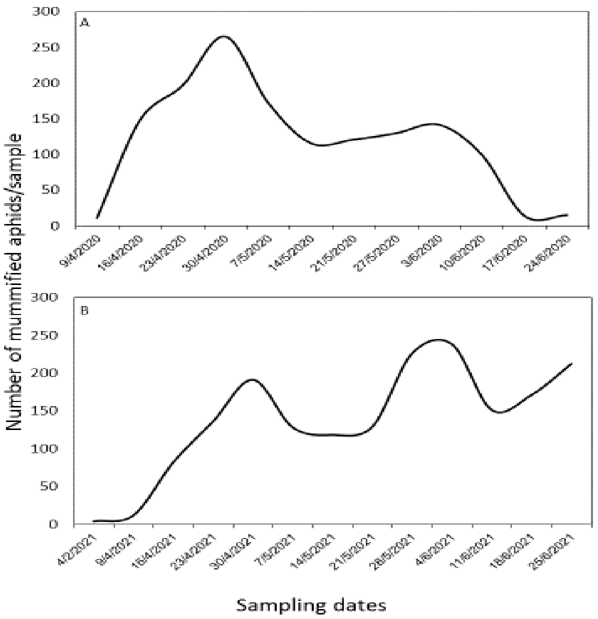

Fig. 4. Seasonal occurrence of the parasitized green peach aphid, *Myzus persicae* with the pupal stage of *Aphidius* spp. (i.e. mummified aphids) during 2019/2020 (A) and 2021/2021 (B) seasons at Aga district.

Ibrahim (2017) in Egypt recorded 3-4 peaks of abundance for *M. persicae* on pepper. In addition, he recorded two peaks for Coccinella spp. during the period of study. These peaks were during December, January, April, and May. Kavallieratos (2004) in Greece found that high *M. persicae* populations on tobacco plants were recorded in July and August, while most coccinellid individuals were recorded in June and July. In agreement with this study, he found that the mummification rate exhibited a specific increasing trend late in the season (August – September). Lampert (1989) recorded the maximum density of aphids per tobacco plant in early July**.** In Washington, Ro and Long (1998) recorded one peak of abundance for *M. persicae* on potato plants in June.

2. Population composition of aphidophagous species

Immature stages

The population composition of immature stages of the aphidophagous species inhibiting peach trees that harboured colonies of the green peach aphid, *M. persicae* during the first (2019/2020) and second season (2020/2021) is given in Figure (5). The aphid parasitized by *Aphidius* spp. constituted the highest percentage of the aphidophagous community in the first (76.59%) and second (83.2%). Green lacewing larvae came in the second rank with a percentage of 8.39% in the first season, while ladybeetle larvae occupied the second rank (4.67%) in the second season.

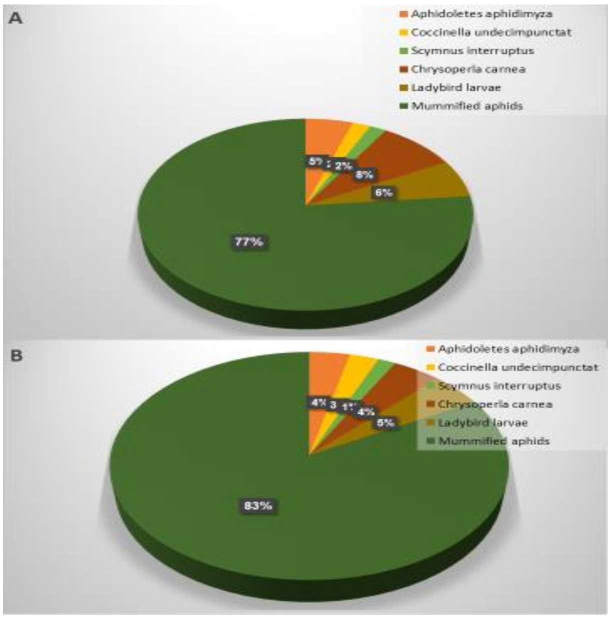

Fig. 5. Population composition of immature stages of aphidophagous species that coexisting with the green peach aphid, *Myzus persicae,* colonies in peach trees during the first season (A) and the second (B) seasons at Aga district.

Adult stages

The population composition of adult stages of the aphidophagous species inhibiting peach trees that harboured colonies of the green peach aphid, *M. persicae* during the first (2019/2020) and second season (2020/2021) is presented in Figure (6). The eleven-spotted ladybeetle, *C. undecimpunctata* constituted the highest percentage of the aphidophagous adult community in the first (42.86%) and second (37.85%) seasons. The eleven-spotted ladybeetle, *C. septempunctata* adults came in the second rank with percentage of 21.80 and 20.34 % in the first and second seasons, respectively.

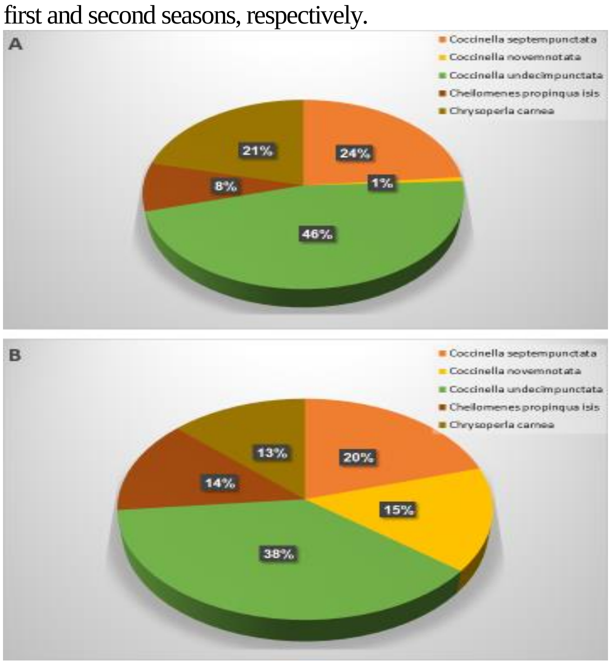

Fig. 6. Population composition of adult stages of aphidophagous species that inhibiting the peach trees infesting with green peach aphid, *Myzus persicae,* colonies during the first season (A) and the second (B) seasons at Aga district.

Thakur and Chandla (2013) examined species composition and abundance of natural enemies of *M. persicae* in potato agro-ecosystem in India. They found that coccinellid predators were the predominant predators that represented 33% of the total natural enemies. Ibrahim (2017) also reported *Coccinella* spp. as predominant predators of *M. persicae*.

3. Relationship between population of *Myzus persicae* and its natural enemies

Correlation analysis between the total numbers of the green peach aphid, *M. persicae* and larvae of the aphidophagous species that colonized aphid colonies during the first (2019/2020) and second (2020/2021) seasons is presented in Table (1). The relation between larval numbers of aphidophagous species and those of aphid varied from season to season. Only, the aphid midge, *A. aphidimyza* larvae exhibited significantly positive response to the increase in aphid populations during both seasons of study. In contrast, the green lacewing *C. carnea* larvae did not response significantly to the increase in aphid populations during both seasons. The predators' larvae of *M. corolla*, S. *interruptus*, and ladybirds significantly responded, in a positive way, to the increase in aphid population only during the first season (Table 1).

Table 1. Person Product moment correlation coefficient (r) between the densities of the green peach aphid, *Myzus persicae* and the different stage populations of the aphidophagous species that colonized peach trees infesting with aphid populations during the first (2019/2020) and second (2020/2021) seasons at Aga district.

Species	**2019/2020**		**2020/2021**	
	r	***p***	***r***	***p***
Larval stage				
Aphidoletes aphidimyza	0.80	0.002**	0.70	0.007*
Metasyrphus corolla	0.42	0.18^{ns}	0.81	0.001**
Scymnus interruptus	0.13	0.67^{ns}	0.84	<0.001**
Chrysoperla carnea	0.41	0.19^{ns}	0.15	0.64^{ns}
Coccinellid ladybirds	0.71	0.01*	0.89	<0.001***
Pupal stage				
Aphidius spp.	0.39	0.22^{ns}	0.49	0.09^{ns}
Adult stage				
Coccinella undecimpunctata	0.94	<0.001***	0.85	<0.001***
Coccinella septempunctata	0.62	0.03*	0.49	0.08^{ns}
Coccinella novemnotata	0.03	0.93^{ns}	0.75	0.003**
Cheilomenes propinqua isis	0.37	0.24^{ns}	0.83	<0.001**
Chrysoperla carnea	0.72	0.008*	0.39	0.19^{ns}

^{ns} = non-significant at 0.05 level, *= significant at 0.05 level, ** = significant at 0.01, and *** = significant at 0.001 probability level

Statistical analysis revealed that the population of *Aphidius* spp. pupae (i.e. mummified aphid) did not coincide with the populations of the green peach aphid *M. persicae* during both seasons of study (Table 1).

The relation between numbers of living aphids and aphidophagous adults varied from season to season. Only, *C. undecimpunctata* adults exhibited significantly positive response to the increase in aphid populations during both seasons of study. Each of *C. septempunctata* and *C. carnea* adults significantly correlated with aphid populations during the first season, whereas *C. novemnotata* and *C. propinqua isis* adults positively synchronized, in a significant way, with aphid populations during the second season (Table 1). The population dynamics of *M. persicae* of coccinellid beetles revealed that their population increased gradually with the increase in aphid populations and vice-versa (Kumar and Paul, 2017).

Thakur and Chandla (2013) found that the seasonal abundance of predators synchronized with that of *M. persicae*, being maximum during July to August. Ibrahim (2017) revealed that there was non-significant correlation between aphids and mummies populations since the peaks of parasitism were not in timing with those of aphids. Further, he found that the peaks of predators were congruent in time with those of aphids

4. Total population mortality by different mortality factors acting *Myzus persicae* populations

The different mortality factors acting the population of the green peach aphid, *M. persicae* on peach trees in Aga district are presented in Figure (7). In both season, unknown mortality exhibited two peaks of contribution. In both seasons, these peaks were occurred during April and June. Predation exhibited two and three peaks of contribution in the first and second seasons, respectively. In the first season, these peaks were occurred during April and May. While in the second season, they were occurred in April, May, and June. In both seasons, parasitism contributed with the highest percentages in the total population mortality of *M. persicae*. It exhibited two and three peaks of contribution in the first and second seasons, respectively. In the first season, these peaks were occurred during April and May. While in the second season, they were occurred in April, early June and late June.

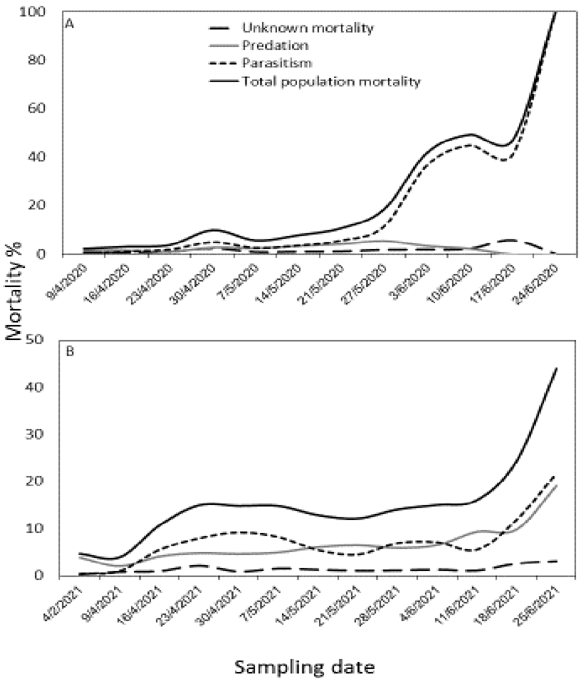

Fig. 7. Unknown mortality, parasitism, predation, and total population mortality of the green peach aphid, *Myzus persicae* on peach trees during 2019/2020 (A) and 2021/2021 (B) seasons at Aga district.

The simple correlation coefficients between the total population mortality rates of *M. persicae* and each of the main mortality factors acting aphid populations during the first (2019/2020) and second (2020/2021) seasons are given in Table (2). In the first season, only parasitism (x) had a significantly positive correlation with total population mortality (y) of *M. persicae*, but not for predation and parasitism. In the second season, each (x) of the three mortality factors (i.e. unknown mortality, predators, and parasitoids) and total population mortality (y) had a significantly positive correlation with total population mortality (y).

Table 2. Person Product moment correlation coefficient (r) between the total population mortality rates of the green peach aphid, *Myzus persicae* and each of the main mortality factors acting aphid populations during the first (2019/2020) and second (2020/2021) seasons at Aga district.

Mortality factors	2019/2020		2020/2021	
	r	***p***	***r***	***p***
Predation	-0.42	0.17^{ns}	0.88	$<0.001^{***}$
Parasitism	0.99	$<0.001^{***}$	0.96	$<0.001^{***}$
Unknown mortality	0.15	0.64^{ns}	0.98	$<0.001^{***}$

^{ns} = non-significant at 0.05 level, *= significant at 0.05 level, ** = significant at 0.01, and *** = significant at 0.001 probability level

Although the number of mummified aphids did not coincide with aphid population, parasitism significantly affected the total population mortality of aphids. This may indicate that parasitism is an inverse-dependent mortality force.

5. Effect of temperature and relative humidity on population of *Myzus persicae* and its natural enemies

The effect of temperature and relative humidity on populations of the cotton aphid, *M. persicae* and different stages of their natural enemies are given in Table (3). Correlation analysis revealed that temperature affected inversely, in a significant way, on population of *M. persicae* during the first season of the study. In the second season, neither temperature nor relative humidity affected population of *M. persicae*. Both temperature and relative humidity had no significant effects on larval populations of the midge aphid, *A. aphidimyza*, the hoverfly, *M. corolla*, *S. interruptus*, green lacewing, *C. carnea*, and ladybeetles during both seasons of the study.

Neither temperature nor relative humidity affected the pupal stage populations of *Aphidius* spp., i.e. mummified aphids, during both seasons of the study (Table 3).

The effects of temperature and relative humidity on populations of aphidophagous adults varied from season to season. In the first season, temperature inversely affected, in a significant way, populations of *C. undecimpunctata* and *C. septempunctata* adults, while relative humidity exhibited significantly positive effect on populations of *C. undecimpunctata* adult. In the second season, temperature or relative humidity did not change populations of any aphidophagous species (Table 3).

Temperature in the current study had no effect on population of *Aphidius* spp. However, Van Driesche *et al.* (2008) found that aphid suppression was poor when the greenhouse temperature exceeded 28 °C because such temperature is favorable to aphids and unfavorable to *A. colemani* (Goh *et al*., 2001; Kim and Kim, 2003). Kumar and Paul (2017) reported a significant negative correlation between aphid population and maximum temperature, while no significant correlation was found between aphid populations and minimum temperature or relative humidity.

In agreement with Thakur and Chandla (2013), correlation between parasitoids and relative humidity showed a non-significant correlation (Thakur and Chandla, 2013).

Table 3. Person Product-moment correlation coefficient (r) between temperature (°C) or relative humidity and either the population of the green peach aphid, *Myzus persicae* or each of the main natural enemies acting aphid populations during the first (2019/2020) and second (2020/2021) seasons at Aga district.

Species	2019/2020				2020/2021			
	Temperature		Relative humidity		Temperature		Relative humidity	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Larval stage								
Aphis gossypii	-0.51	0.01^{**}	-0.57	0.005^{**}	-0.59	0.006^{**}	0.24	0.32^{ns}
Aphidoletes aphidimyza	-0.05	0.81^{ns}	-0.35	0.10^{ns}	0.78	$<0.001^{***}$	-0.12	0.61^{ns}
Metasyrphus corolla	-0.07	0.74^{ns}	-0.01	0.95^{ns}	0.39	0.08^{ns}	-0.28	0.24^{ns}
Scymnus interruptus	0.09	0.68^{ns}	0.00	0.99^{ns}	0.64	0.002^{**}	-0.14	0.56^{ns}
Chrysoperla carnea	0.13	0.56^{ns}	-0.44	0.04^{*}	0.49	0.03^{*}	0.02	0.92^{ns}
Coccinellid ladybirds	-0.12	0.59^{ns}	-0.05	0.81^{ns}	0.54	0.01^{**}	-0.16	0.49^{ns}
Pupal stage								
Aphidius spp.	0.21	0.33^{ns}	0.22	0.31^{ns}	0.13	0.57^{ns}	0.69	0.001^{***}
Adult stage								
Coccinella undecimpunctata	0.01	0.96^{ns}	-0.24	0.28^{ns}	0.73	$<0.001^{***}$	-0.14	0.56^{ns}
Cheilomenes propinqua isis	0.39	0.06^{ns}	0.47	0.02^{*}	0.45	0.04^{*}	-0.07	0.76^{ns}

^{ns} = non-significant at 0.05 level, *= significant at 0.05 level, ** = significant at 0.01, and *** = significant at 0.001 probability level

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دراسات إيكولوجية علي منْ الخوخ الأخضر وأعدائه الحيوية

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تختلف التذبذبات العددية والهجرة ونشاط الطيران لحشرات المنْ خلال السنة وذلك لأن حشرات المنْ تتأثر بالعديد من العوامل الحيوية وغير الحيوية ومن بينها التغيرات في عوامل الطقس والأعداء الحيوية. تم إجراء دراسات حقلية في مزرعة إنتاج خوخ توجد في منطقة أجا بمحافظة الدقهلية خلال موسمي 2020/2019 و 2022/2021 . حيث تم تقدير تعداد كل من حشرات المنْ والمفترسات والطفيليات. كما تم تحديد معامل الأرتباط البسيط بين عدد الحشرات الحية لمنْ الخوخ الأخضر وكل من الأعداء الحيوية أو كل من الحرارة والرطوبة. ومن خلال الدراسة تم تسجيل عدد ذروتين لتعداد المنْ سنوياً في شهر أبريل وخلال مايو – يونيه. و قد سجلت يرقات مفترسات *Aphidoletes aphidomyza* ، *Metasyrphus corolla* وأسد المنْ الأخضر ذروتين للتعداد سنوياً، في حين أن يرقات مفترس الأسكمنس سجلت ذروة واحدة للتعداد. أما الحشرات الكاملة لمفترس أبو العيد 11 نقطة وأبو اليعد 7 نقط سجلت ذروتين للتعداد سنوياً، أما الحشرات الكاملة لمفترس أبو العيد 13 نقطة ومفترس أبو العيد الأسود وأسد المنْ الأخضر فقد سجلت ذروة واحدة للتعداد سنوياً. كما سجل عدد المنْ المتطفل عليه (الموميأت) من 2-3 ذروة سنوياً في أبريل ويونيه ويوليو خلال موسمي الدراسة. ومن ضمن مفترسات المنْ فإن يرقات مفترس *A. aphidimyza* وأبو العيد 11 نقطة أظهرت أستجابات معنوية للزيادة في تعداد المنْ خلال موسمي الدراسة. في كلا موسمي الدراسة فإن التطفل أسهم بالنسب العالية في معدلات الموت الكلية لتعداد منْ الخوخ الأخضر، في حين أن الموت الناتج عن أسباب غير معروفة أسهم بالنصيب الأقل. حيث سجل متوسط الموت السنوي 21.19 و 7.38%؛ 2.30 و 6.75 %؛ 1.58 و 1.4 % لكل من التطفل والافتراس والموت الناتج عن أسباب غير معروفة في السنة الاولى والثانية علي التوالي .