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New Record of Aphytis melinus Debach, 1959 as a Parasitoid on the Oriental Yellow Scale Aonidiella orientalis in Iraq and its Seasonal Occurrence in Al-Suwaira Region and Al-Qanat Region

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Abstract

The oriental yellow scale insect (*Aonidiella orientalis*) (Newstead, 1894) was studied as a harmful pest affecting citrus trees in the Al-Qanat (Baghdad) and Al-Suwaira (Wasit) during the 2023–2024 seasons. This study reports the first record of the external parasitoid *Aphytis melinus* DeBach, 1959 in Iraq, based on morphological characterization of adult traits and pigmentation patterns of *A. melinus* pupae, consistent with molecular identification results using the COI gene fragment. The genetic analysis revealed a 99% match with a globally recorded strain in France (GenBank accession number KX065209) compared to the local strain registered in GenBank under the accession number PP301406.The study also investigated the impact of climatic factors on the seasonal distribution of the pest and its parasitoid. The highest pest density was recorded between September and January, with the lowest densities observed in May and June. The peak parasitism rate was recorded in October (1.25%). Climatic factors showed significant effects, with a negative correlation between temperature and each pest and parasitoid densities, while the increase in the parasitoid numbers correlated positively with relative humidity

Keywords. *Aphytis melinus*, Oriental yellow scale, Iraq.

تسجيل جديد Aphytis melinus Debach, 1959 كطفيلي على الحشرة القشرية الصفراء الشرقية Aonidiella orientalis في العراق ودراسة تواجده الموسمي في منطقة الصويرة ومنطقة القناة

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الخلاصه

تمت دراسة الحشرة القشرية الصفراء الشرقية *Aonidiella orientalis* (Newstead, 1894) كآفة ضارة على أشجار الحمضيات في منطقتي القناة (بغداد) والصويرة (واسط) خلال موسمي 2023–2024. أظهرت الدراسة تسجيلًا جديدًا للطفيلي الخارجي *Aphytis melinus* DeBach, 1959 في العراق، اعتمادا على نتائج التشخيص المظهري لبعض الصفات المظهرية للبالغات ونمط تصبغ عذارى *A. melinus*.

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وكانت مطابقة مع نتائج التشخيص الجزيئي باستخدام تحليل القطعة الجينية COI، وأكدت النتائج تطابقًا بنسبة 99% مع ما مسجل عالميا في فرنسا. والذي يحمل الرقم الجيني (KX065209) من خلال مقارنتها بالنوع المحلي الذي سجل في بنك الجينات تحت الرقم (PP301406) تمت دراسة التأثيرات المناخية على الانتشار الموسمي للآفة والطفيلي، حيث لوحظت ذروة كثافة الآفة بين سبتمبر ويناير، بينما كانت أدنى كثافة في مايو ويونيو. سجلت أعلى نسبة تطفل في أكتوبر (1.25%). أظهرت العوامل المناخية تأثيرًا معنويًا، حيث كان للحرارة علاقة سلبية مع كثافة الطفيلي والآفة، بينما ارتبطت الرطوبة إيجابيًا بزيادة أعداد الطفيلي. هذه النتائج ضرورية لتطوير استراتيجيات مستدامة لحماية المحاصيل، والحفاظ على صحة البيئات الزراعية.

1. Introduction

Citrus orchards are one of the most important components in the economies of many countries of the world, including Iraq. Although citrus fruits are used as fresh fruits, they are used in many food industries Citrus fruits are considered a nutritional and healthy source, as they are rich in carbohydrates, organic acids, nutrients, and nutritional supplements such as vitamin C, which are important sources of potassium It is rich in antioxidants [1, 2]. The genus Citrus belongs to the Rutacea family [3]. Citrus trees are infested with many insect pests all over the world, such as citrus leaf miners, mealybugs, whiteflies, aphids, mites, and scale insects, including the oriental yellow scale*, Aonidiella orientalis* (Hemiptera: Diaspididae) [4]. The scale insect is a widespread pest, especially in the tropical and subtropical regions, and the Mediterranean basin [5]. *Aonidiella orientalis* is characterized by a wide host range [6]. Huge economic losses are caused by this pest, which is estimated to be billions of dollars annually (Figure 1) [7], as it significantly affects crop productivity. Studies have shown that armoured scale insects infect all aerial parts of plants, including leaves, branches, stems and fruits (Figure 2) [8]. Studies indicate that scale insects feed on plant sap using specialized long and thread-like mouthparts, forming waxy scales on the affected parts, including mature fruits, in the form of prominent scars. This is accompanied by yellowing and deformation of plant tissues due to the secretion of toxic saliva by the insect, which disrupts the plant's physiological processes [9]. Using chemical pesticides to control insect pests was undesirable due to their slow degradation and environmental risks [1]. Insecticides are ineffective in reducing the population of armored-scale insects due to the protective covering that shields their life stages. Additionally, insecticides often eliminate a significant number of natural enemies present in their environment [10]. Therefore, researchers resorted to using biological control due to its high effectiveness in controlling insect pests and making them below the economic threshold level [11].

The genus *Aphytis* (Hymenoptera: Aphelinidae) is one of the most important ectoparasitoids that attacked the armored scale [12]; it has been used successfully in many biological control programs in the world [13] because it is one of the most effective natural enemies and more successful than endoparasitoid and predators of armored scale insect, it is actively attracted to citrus fruits infected with the oriental yellow scale, *Aonidiella orientalis* [14].

The adult female wasp inserts its ovipositor through the scale cover and deposits one or various eggs on the dorsal (Figure 5). Adult parasitoids of the genus *Aphytis* measure 1 mm or less in length, are yellow in color, and include 110 species [15]. This genus originates from northern India and Pakistan but has also been reported in Spain, California, Mexico, Argentina, Australia, Cyprus, Morocco, Greece, South Africa, and Italy [16]. Adults of the parasitoid A. melinus primarily feed on nectar and honeydew, while females require proteins for egg maturation, which they obtain by feeding on their host [17]. The parasitoid *A. melinus* locates its host by detecting volatile compounds emitted by infected and non-infected citrus trees [18]. The life cycle of *Aphytis* begins when the female parasitoid penetrates the protective cover of the armored scale insect and lays one or more eggs. The female parasitoid deposits her eggs by penetrating the cover of the armored scale insect using her ovipositor, on the dorsal or ventral. The developmental duration of *Aphytis* is influenced by climatic conditions, including temperature and relative humidity [19].

This study aims to identify a new ectoparasitoid species that attacks the oriental yellow scale *Aonidiella orientalis* in Iraqi citrus orchards based on some Morphological diagnostics pigmentation pattern and the meconium pellets and other morphological structures used in separating between Aphytis species and using DNA barcoding approach.

Figure 1: Orchards Affected by Scale Insects in the Regions of Al-Suwaira and Al-Qanat

Figure 2: Leaf, stem, fruit infested with scale insect *Aonidiella orientalis*

Figure 3: Fruit mummification due to infestation by the scale insect *Aonidiella*

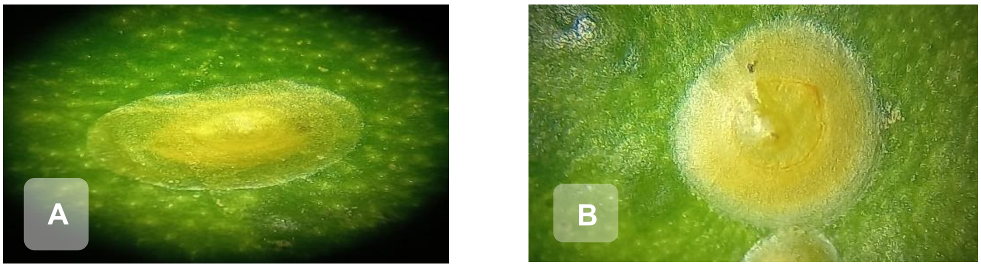

Figure 4:(A) body of a male pupa (B) cover and body of female of Aonidiella orientalis

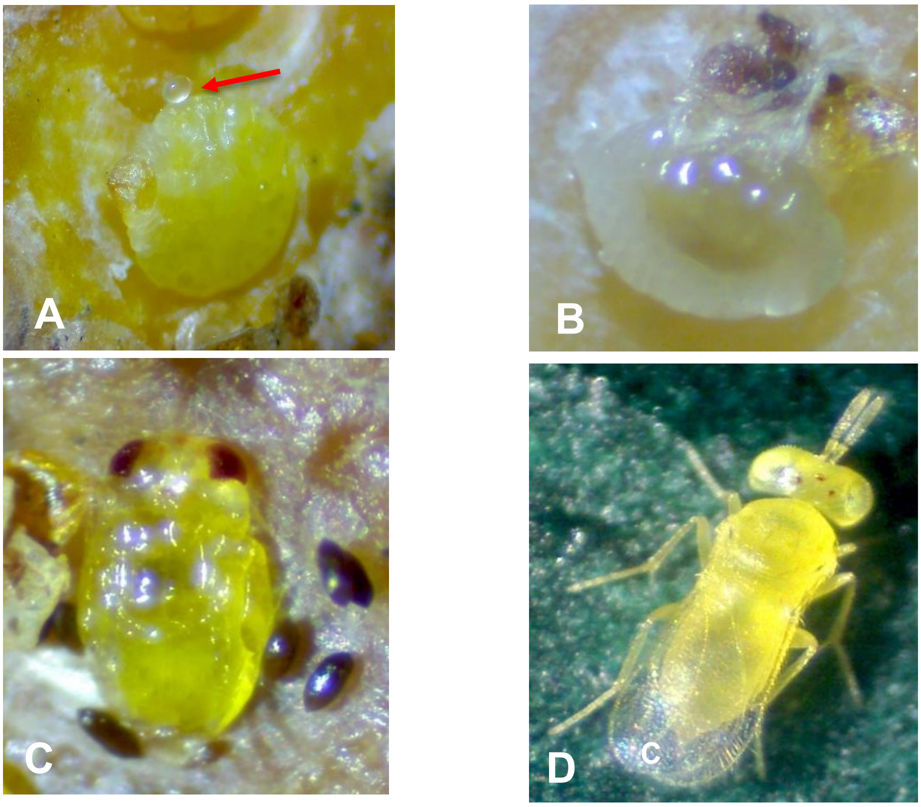

Figure 5: (A) egg (B) larva (C) pupa(D) adult of *Aphytis melinus*

1. Materials and methods

1.1. Sample Collection

Samples of citrus leaves and fruits infected with *Aonidiella orientalis* were collected from citrus orchards of Baghdad /Al-Qanat (33°24'55.8" N 44°21'29.2" E) and Wasit / Al-Suwaira (32°59'08.0" N 44°47'03.5" E) (Figure 6). These samples were then placed in plastic cages with dimensions of 40*40*40 cm under laboratory conditions of 25 ± 2 C and 65 ± 5% relative humidity. Adult parasitoids were collected from the plastic cages by a mouth aspirator. Pupae were separated from infected leaves and fruits.

1.2. Morphological Identification

Samples of live parasitoids adults have been sent to Iraq Natural History Research Center and Museum for all places in the research. to identify the specimen collected according to the morphology of the species belonging the *Aphytis* genera such as the pupal pigmentation pattern, the meconium pellets and Y-shaped structure on the mesosternum [20, 21, 22, 16].

Figure 6: Map of study areas Baghdad Al- Qanat (33°24'55.8" N 44°21'29.2" E) and Wasit - Al-Suwaira (32°59'08.0" N 44°47'03.5" E).

2.3 Molecular Identification

2.3.1 DNA Extractions and PCR Amplification

Genomic DNA was extracted using A gSYNC DNA extraction kit (Geneaid Biotech Ltd. New Taipei City, Taiwan) following the manufacture and protocol provided by the company after crushing single parasitoids adult. After DNA extraction, the Polymerase Chain Reaction (PCR) technique was conducted using 25 µl of reaction mixture containing 12.5 µl of Mastermix (2X), 10.5µl of nuclease-free water, and 1 µl (Mention the concentration) from each primer (forward the forward primer was 5′-GGTCAACAAATCATAAAGATATTGG-3′ and reverse primer was 5′-TAAACTTCAGGGTGACCAAAAAATCA-3'.

The PCR conditions are provided in Table 1. After the PCR reaction, the samples were checked using electrophoresis by loading the sample in the wells made in agarose (1%) with 2.5 µl of green viewer mixed with 0.5 of TBE buffer, and finally, the samples with the band were delivered to Macrogen Co. (Korea) for sequencing. The chromatogram for the quality of the sequence was checked out, and the consensus of the sequence was created to make an assembler for sequences by using DNA Baser Assembler (DNA Sequence Assembler v4 2013, HeracleBioSoft, www.DnaBaser.com). After that, the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to blast and compare with other species in this database.

Table 1: The PCR cycle program for reproduction of COI gene of local *Aphytis* species.

Phase	Tm(°C)	Time	No. of cycle
Initial	95	5 min.	1
Denaturation	95	45 Sec.	
Annealing	55	45 Sec.	35
Extension	72	45 Sec.	
Final- Extension	72	7 min.	1
Colling	4	10 min.	—

2.3.2 Molecular Analysis of COI Region

The cytochrome oxidase subunit 1 (COI) is commonly used to identify and classify different biological materials to arrange them at the species level, which could be done in DNA barcoding studies. The evolutionary analysis of the genus *Aphytis* sp. comprised 12 valid nucleotide sequences contained our sequence conducted out; first, the BioEdit software, DNA Bazar, and MEGA 7 software program were used for this purpose [23]. The history of evolution was deduced using a neighbor joining (NJ) approach based on the K2 + G [24]. For the *Aphytis* genre, DNA sequences of Ablerus perspicuous were used as an outgroup taxon (accession number JQ268912) [25]. Branch support was determined by bootstrap analysis with 1100 replicates [26]. In addition, this program was performed for phylogenetic analyses, using the Maximum Composite Likelihood method [27] with 1000 replications of bootstrap [28]. Then, the nucleotide distance was calculated by Clustal W and Clustal X version 2 [29].

2.4 Seasonal presence

Field survey of the oriental yellow scale *Aonidiella orientalis* and its parasitoids *Aphytis melinus* was conducted from 1/ 9/ 2023 to 1/ 9/ 2024 on the citrus tree at Al-Suwaira, Wasit Governorate, (32°59'08.0" N 44°47'02.9" E). All trees received the same routine horticultural practices. Samples of citrus leaves and fruits were chosen to take samples from them biweekly throughout the years of the study. Selected trees were similar in size, shape, and height and were as homogenous in infestations as possible.40 infested leaves and fruits, and from cardinal directions (north, south, east and west) and the tree core of each selected tree were picked up using a garden scissor. The collected samples were packed in plastic bags, then in cool boxes transferred to the laboratory and examined with a needle using a stereoscopic microscope at the laboratory of the directorate of Authority of Scientific Research, Agricultural Research Center. A live stage of insects and Parasitoids (larvae, pupa) was categorized and counts were recorded. Population fluctuations of the scale insects and their parasitoids were estimated during one year. The main weather factors maximum temperature (Max.Temp.), and relative humidity (%R.H), were recorded to determine their effects on the population densities of the common surveyed scale insects. Simple correlation (r) and linear regression were calculated to determine the relationship between the mean number of individuals and the mean records of the three tested weather factors. The obtained results were statistically analysed using the SPSS software program.

3.Results and Discussion

3.1 Morphological Identification

The results showed that the parasite samples sent to the Iraqi Natural History Research Center and Museum belong to the species *Aphytis melinus* Debach, 1959 (Hymenoptera: Aphelinidae) which is considered a new record in Iraq. Where some morphological and biological differences of the species collected that can be useful for the separation of certain closely related species of *Aphytis* such as the pupal pigmentation pattern, the meconium pellets, and the Y- shaped structure of the mesosternum are similar to these known in *Aphytis melinus*. species [16] (Figure 7). Which is characterized by dark brown pigmentation only on the thoracic sterna of the pupae [30] (Figure 8), and the meconia are less elongated, more spindle-shaped than in lignanensis, not nearly parallel-sided and noticeably grooved longitudinally (Figure 8).

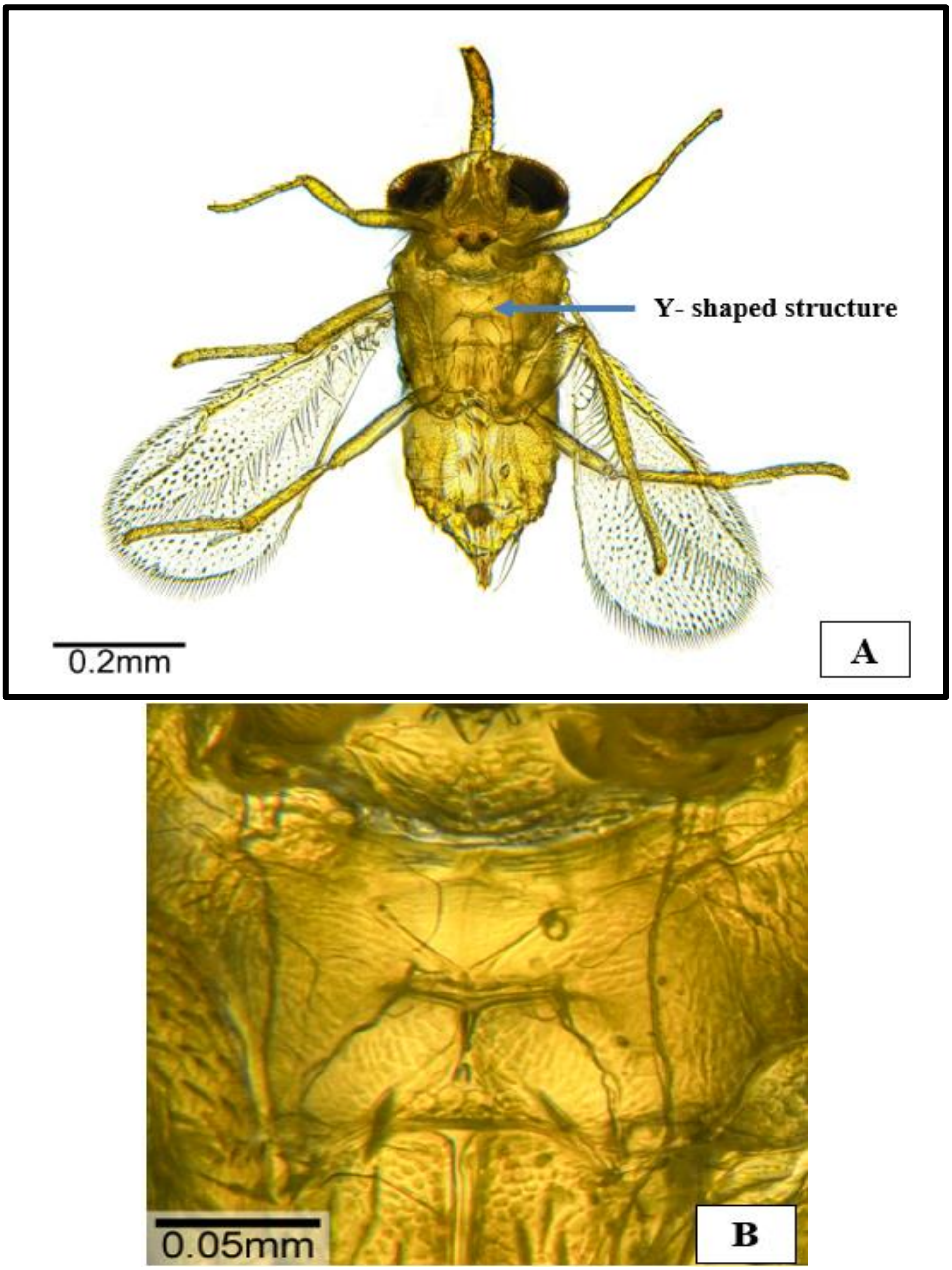

Figure 7: (A) The Y-shaped structure on the mesosternum 0.2mm. **(B)** The Y-shaped structure on the mesosternum of *Aphytis melinus* 0.05mm**.**

Figure 8: The pupal pigmentation pattern and the meconian pellets of *Aphytis melinus*.

3.2 Molecular Identification

From different regions, samples were randomly collected for categorization and identification based on molecular techniques using PCR and sequencing of a target gene (460 bp) that was the predicted site of the target gene. The outcome of the molecular analysis of the collected specimens was conducted using two primers, LCO1490 and HCO2198. The obtained outcomes agreed with the results documented by [31], who reported that the primers above were considered appropriate genes for the characterization and identification of various insects, and they have been used widely for taxonomical purposes and can provide an accurate phylogenetic tree. The analysis of obtained sequence analysis by BLAST software, which is a part of GenBank of NCBI (National Center for Biotechnology Information), showed that more than 99% of compared species followed the *Aphytis* genus. The closest specimen was *Aphytis melinus* (KX065209), collected and identified in France, which showed 98% similarities, and the query coverage was 99% with the species collected in this study (PP301406). In addition, the multiple alignments of a 460bps segment of the COI gene for 15 taxa showed that 11 sites were conserved, 471 sites were variable, 88 sites were singleton, and 381 sites were parsimony informative from 653 sites of all sequences. The phylogenetic tree of this group was reassembled based on the COI region, using maximum likelihood analysis, and reported that the *A. melinus* IRQ. A1 forms a monophyletic group with other species of *Aphytis* (Figure. 9). The mean inter-specific distance for mtDNA COI sequences was 0.88 % (range 0.00 – 1.81), calculated by the Tamura 3-parameter method. The difference between samples collected from Iraq and other species collected from areas around the world was 0.01 and 0.08 (Figure. 10).

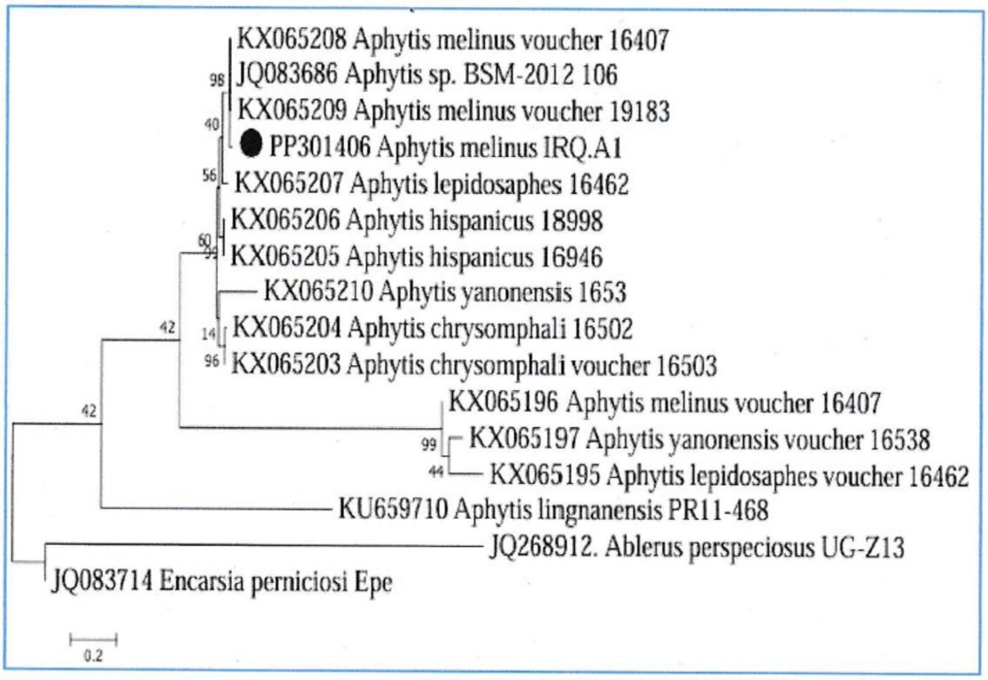

Figure 9: Molecular Phylogenetic analysis using the Maximum Likelihood method and the Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered is shown next to the branches. The analysis involved 16 nucleotide sequences. All positions containing gaps and missing data were eliminated. The difference between samples collected from Iraq and other species collected from areas around the world Evolutionary analyses were conducted in MEGA7.

Aphytis species	Pairwise distance among the species of Aphytis									
PP301406_Aphytis_melinus_IRQ.A1										
KX065208_Aphytis_melinus_voucher_16407	0.010									
KX065209_Aphytis_melinus_voucher_19183	0.013	0.003								
KX065207_Aphytis_lepidosaphes_16462	0.059	0.052	0.056							
KX065206_Aphytis_hispanicus_18998	0.063	0.052	0.056	0.046						
KX065205_Aphytis_hispanicus_16946	0.063	0.052	0.056	0.046	0.000					
KX065204_Aphytis_chrysomphali_16502	0.080	0.076	0.080	0.069	0.070	0.070				
KX065203_Aphytis_chrysomphali_voucher_16503	0.076	0.073	0.076	0.066	0.066	0.066	0.003			
KX065210_Aphytis_yanonensis_1653	0.210	0.202	0.206	0.197	0.181	0.181	0.189	0.189		
KX065197_Aphytis_yanonensis_voucher_16538	1.872	1.949	2.040	1.949	2.087	2.087	1.769	1.714	1.736	
KU659710_Aphytis_lingnanensis_PR11-468	1.629	1.598	1.569	1.737	1.670	1.670	1.603	1.603	1.538	1.750

Figure 10: Pairwise comparison of nucleotide significances among some *Aphytis* species with local species based on COI gene.

Furthermore, the molecular characterization of parasitoids *A. melinus* collected in Corsica, France, from infested orchard infested by armored scales showed variation in the number of nucleotide sequences (Figure. 10). One haplotype in the COI region was detected among more than 100 species of *A. melinus*. One sequence has been deposited in NCBI GenBank's database with accession number KX065209. According to the comparison and alignment of

COI sequences of Iraqi *A. melinus*, there were around 595 nucleotide sites, including the gaps, and there were more than 14 nucleotide sites between query and subject.

The results agreed with some research papers according to molecular weight and phylogenetic analysis [22], which illustrated the genetic relationship between Aphytis based on COI sequencing, and their results showed that there was more than 90% similarity with reference sequencing in NCBI blast. In addition, the COI genetic region is considered quite important for distinguishing between very close species in morphologic aspects, and this gene shows high and precise homology among very close species [31, 32]. It reported that the size of the band could show between 600 and 700bp when amplified. The sequencing gene was a host; a host-specificity was shown to be very strong among *Aphytis* species considered primary parasitoids. Consequently, according to the current results of phylogenetic analysis, the species of *A. melinus* is considered the first recorded species in Iraq based on molecular characterizations.

3.3 Seasonal presence

Population dynamics of the oriental yellow scale Aonidiella orientalis in citrus orchard revealed Al-Suwaira, Wasit, (32°59'08.0" N 44°47'02.9" E) permanent throughout the year with a clear effect of climatic conditions. The highest peak of the population density of the pest was in the months from Sep. to Jan. (1585, 1316, 1426, 1433, 1418, 1487, 1478, 1482, 1440 and1369) insects per 40 leaves, while the lowest population density of the pest occurred in May (745 and 681) and first half of June (546). The occurrence of parasitoids was between the second half of October and May, and its peak was in the second half of October (18). the highest percentage of parasitism was recorded in the second half of October. (1.25%). Table (2) and (Figure 11).

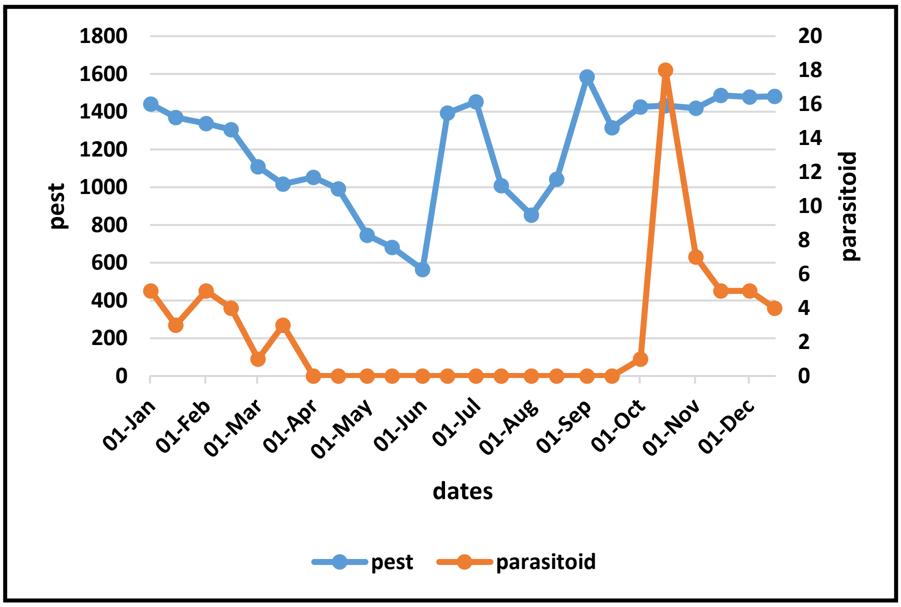

Figure 11: Means of half monthly counts of the parasitoid, *Aphytis melinus*, on the oriental yellow scale *Aonidiella orientalis* Al-Suwaira, Wasit.

Table 2: Mean numbers of half-monthly counts of different stages of the parasitoid, Aphytis melinus on the oriental yellow scale Aonidiella orientalis, with climatic factors affecting Al-Suwaira.

Season	Data of inspection	Parasitic stage			Non Parasitized	Total	Parasitism(%)	Climatic Factors		
		larvae	Pupa	Total				Max,temp.°C	Min.tem,°C	RH(%)
Autumn										
Sep	1 - 9	0	0	0	1585	1585	0	45.66	26.26	19.4
	15 - 9	0	0	0	1316	1316	0	41.71	22.42	18.8
Oct	1 - 10	0	1	1	1425	1426	0.07	37.73	20.8	26.4
	15 - 10	12	6	18	1415	1433	1.25	32.26	17.33	40.7
Nov	1 - 11	4	3	7	1411	1418	0.49	30.86	16.66	48
	15 - 11	1	4	5	1482	1487	0.33	22.35	10.35	65.8
Average		17	14	31	8634	8665	0.356666667	35.095	18.97	36.516667
Winter										
Dec	1 - 12	3	2	5	1473	1478	0.33	23.86	8	58.8
	15 - 12	3	1	4	1478	1482	0.26	19.13	6.86	75
Jan	1 - 1	4	1	5	1435	1440	0.34	21.53	6.6	62.4
	15 - 1	0	3	3	1366	1369	0.21	20.26	7	55.6
Feb	1 - 2	4	1	5	1332	1337	0.37	21.46	8.33	59.5
	15 - 2	0	4	4	1302	1306	0.3	19.84	6.46	61
Average		14	12	26	8386	8412	0.301666667	21.01333333	7.2083333	62.05
Spring										
Mar	1 - 3	0	1	1	1108	1109	0.09	26	9.26	40
	15 - 3	1	2	3	1014	1017	0.29	24.8	12.73	55
Apr	1 - 4	0	0	0	1052	1052	0	34.6	16.2	37.9
	15 - 4	0	0	0	991	991	0	35.92	20.14	34.3
May	1 - 5	0	0	0	745	745	0	35.6	19.8	44.4
	15 - 5	0	0	0	681	681	0	40.13	23.33	18
Average		1	3	4	5591	5595	0.063333333	32.84166667	16.91	38.266667
Summer										
Jun	1 - 6	0	0	0	564	564	0	47.6	26.86	18
	15 - 6	0	0	0	1394	1394	0	46.78	27.64	14.9
Jul	1 - 7	0	0	0	1453	1453	0	48.86	28.46	17.4
	15 - 7	0	0	0	1009	1009	0	45.86	29	19.5
Aug	1 - 8	0	0	0	853	853	0	42.6	26.9	18.5
	15 - 8	0	0	0	1042	1042	0	45.7	28.2	18
Average		0	0	0	6315	6315	0	46.23333333	27.843333	17.716667

The relationship between the population density of the host and its parasitoid *A. melinus* (Figure 12) was a positive correlation r= 0.44 due to the similarity of their influence on climatic conditions, in addition in addition to the fact that the abundance of the host for the parasitoid provided an opportunity to increase parasitism according to the linear regression equation $y = 0.006x - 4.6793$.

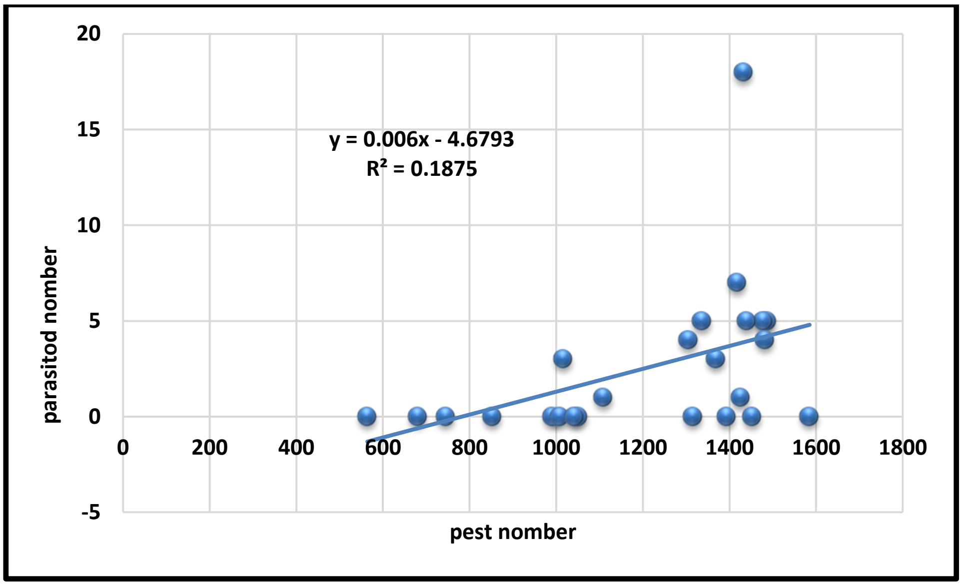

Figure 12: Relation between population density of the oriental yellow scale *Aonidiella orientalis* and the population of the parasitoid, *Aphytis melinus* Al-Suwaira

The effect of climatic factors on the seasonal occurrence of the pest showed the increase in maximum temperatures produces a decrease in population density, y = -10.099x + 1549.1 (Figure 13).

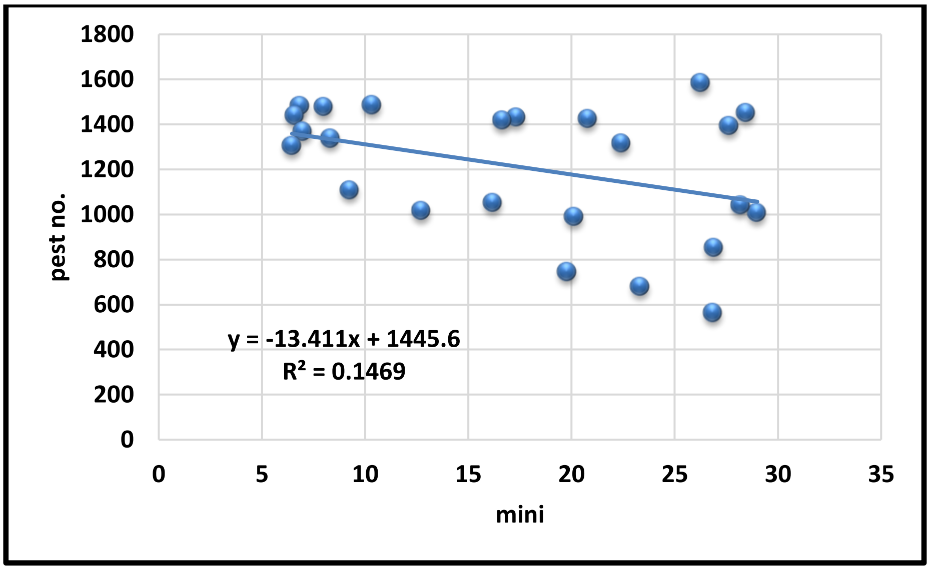

Figure 13: Effect of maximum temperatures on the population density of the oriental yellow scale *Aonidiella orientalis* Al-Suwaira

In the same way, the minimum temperatures affected by the population density, decreasing the population density with the increase of minimum temperatures; y = -13.411x + 1445.6 (Figure 14).

The population density of pests increased with an increase in relative humidity according to the linear equation y= 5.8456x + 981.93 (Figure 15).

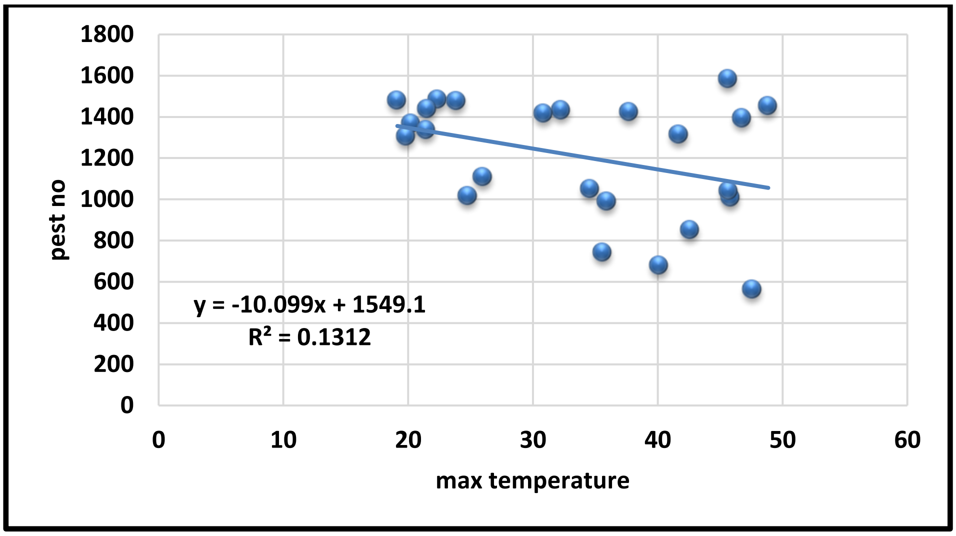

Figure 14: Effect of Minimum temperatures on the population density of the oriental yellow scale *Aonidiella orientalis* Al-Suwaira

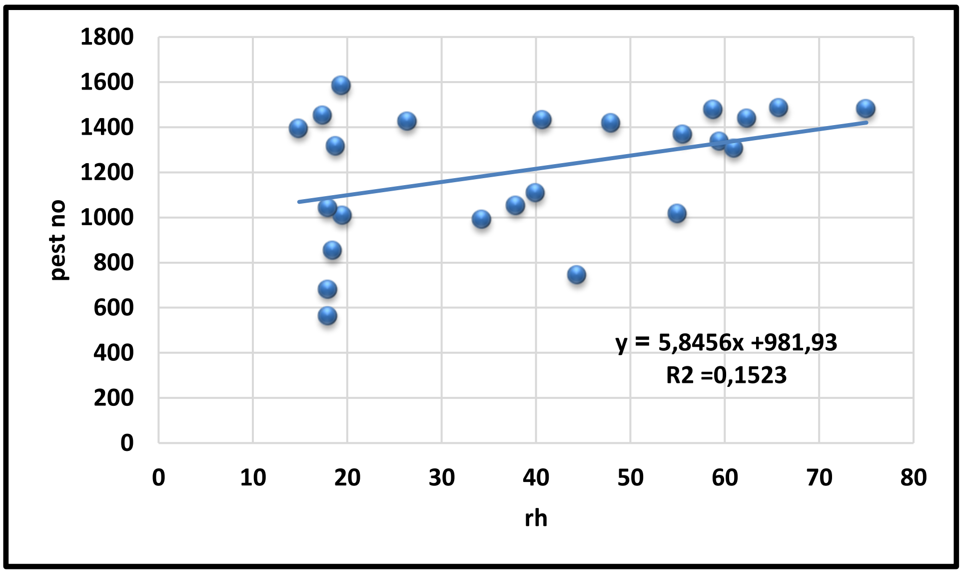

Figure 15: Effect of relative humidity on the population density of the oriental yellow scale *Aonidiella orientalis* Al-Suwaira

According to the correlation coefficient between climatic factors and the degree of their influence on the seasonal occurrence of the pest, the results showed a significant effect for all factors, the coefficient correlation was negative (r= - 0.46 and - 0.49) for minimum and maximum temperatures respectively and positive correlation for relative humidity (r =0.50), and the p-value were 0.02, 0.014 and 0.013 for mentioned factors respectively (Table 3).

Table 3 : Correlation coefficients (r) between the population of the oriental yellow scale *Aonidiella orientalis* and various weather factors Al-Suwaira

Weather factor	Correlation coefficient	P value
minimum temperatures	- 0.46	0.02
Maximum temperatures	- 0.49	0.014
humidity	0.5	0.013

Regarding the degree of influence of each of the climatic factors on the seasonal occurrence of the pest according to the multiple linear regression equation, the results showed that the percentage of influence of the maximum temperature is 13%, followed by the influence of humidity percentage 3%, then the minimum temperature 3% (Table 4).

Table 4 : Impact of weather factors on the population of the oriental yellow scale *Aonidiella orientalis* Al-Suwaira.

Weather factor	R^2	$100 \times R^2$	Role of factor (%)
y = 1415-13x1	0.13	13	13
y= 1259+ 1.4x1 -19x2	0.16	16	3
y= --1191+57.7x1-41x2+22.9 x3	0.19	19	3
x1= Maximum temperatures x2 = minimum temperatures x3= humidity			

The effect of minimum, maximum temperatures and relative humidity on the population density of the parasitoid showed the following result: a gradual decrease and then disappearance of the parasitoid was recorded in April, May, June, July, August, and September, with an increase of maximum temperatures, and an increase in its numbers in October, November, December, January, February and March which coincided with a decrease in temperatures and an increase in relative humidity (Figure 16).

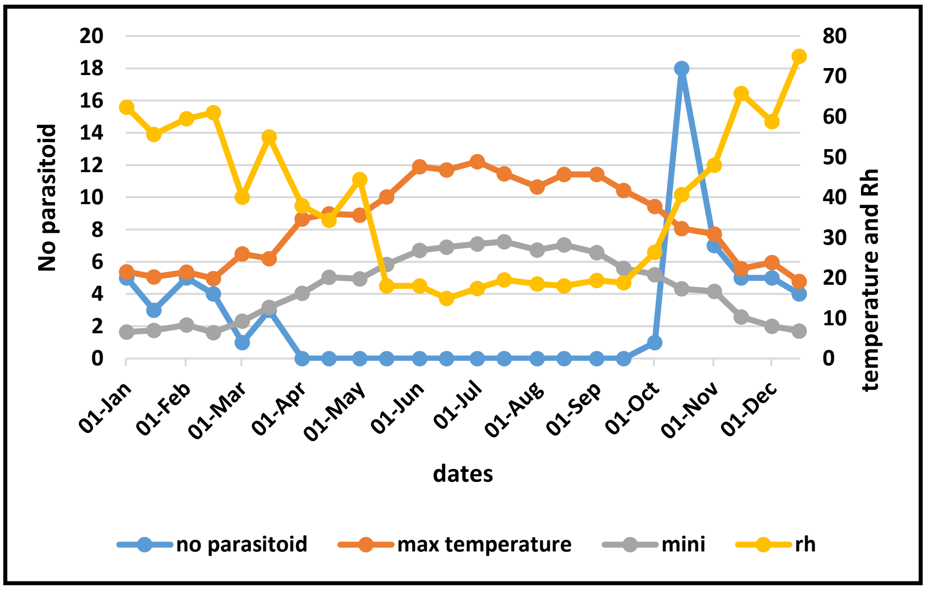

Figure 16: Means of half monthly counts of the parasitoid, *Aphytis melinus,* on the oriental yellow scale *Aonidiella orientalis* Al-Suwaira

The nature of the effect of maximum temperatures was recorded according to the linear equation y= -0.1859x + 8.8241 (Figure 17), and for the minimum temperature, y= -0.2172x + 6.3925 (Figure 18) and the relative humidity according to the equation y = 0.1025x - 1.4197 (Figure 19) The value of the correlation coefficient for the maximum temperature was - 0.48 with a significant effect (p=0.017) and for the minimum temperature -0.45, p value =0.017 and 0.50, 0.014 for relative humidity Tables (5).

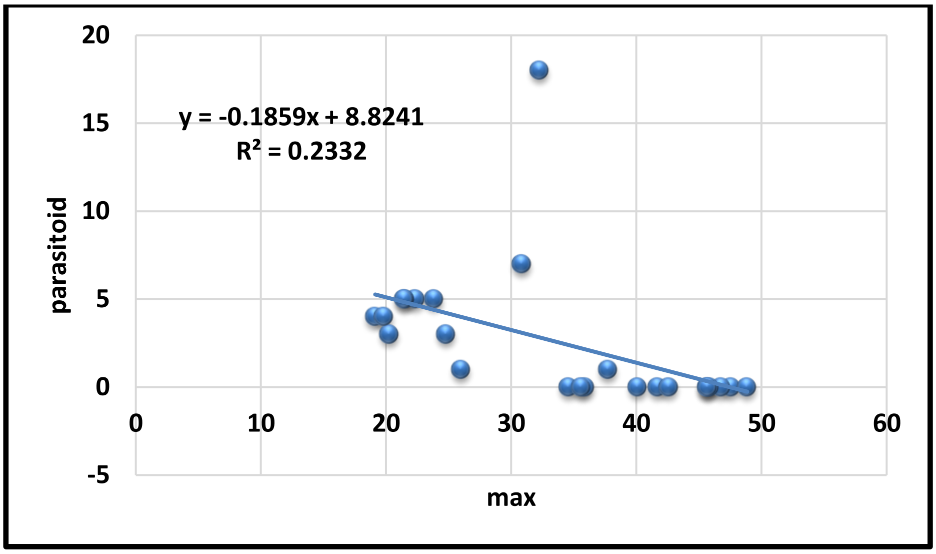

Figure 17: Effect of maximum temperatures on the population density of the parasitoid *Aphytis melinus* Al-Suwaira

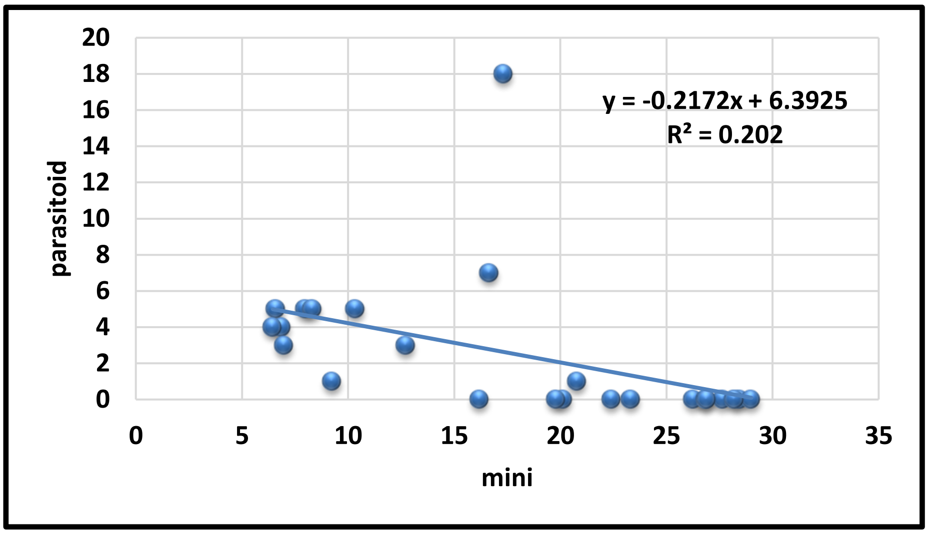

Figure 18: Effect of minimum temperatures on the population density of the parasitoid *Aphytis melinus* Al-Suwaira

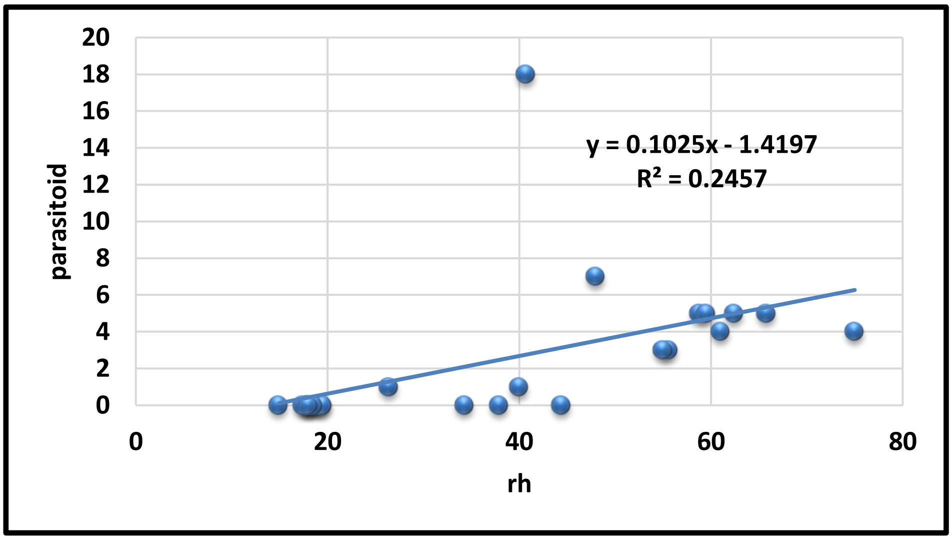

Figure 19: Effect of relative humidity on the population density of the parasitoid *Aphytis melinus* Al-Suwaira

Table 5 : Correlation coefficients (r) between the population of parasitoid *Aphytis melinus* and various weather factors. Al-Suwaira

Weather factor	Correlation coefficient	P value
minimum temperatures	- 0.45	0.028
Maximum temperatures	- 0.48	0.017
humidity	0.50	0.014

The degree of influence of each of the climatic factors on the seasonal occurrence of the parasitoid, according to the multiple linear regression equation, was 23% for the maximum temperature, followed by relative humidity 1%, then the minimum temperature 3% (Table 6).

Table 6 : Impact of weather factors on the population of parasitoid *Aphytis melinus* Al-Suwaira

Weather factor	R^2	$100 \times R^2$	Role of factor (%)
$y = 161 - 3.5x_1$	0.23	23	23
$y = -198 - 6.9x + 4.42\ x2$	0.26	26	3
$y = -0.001x1 + 0.14\ x2 + 0.121\ x3 - 4.46$	0.27	27	1
x1= Maximum temperatures x2 = minimum temperatures x3= Maximum humidity			

From the results above, it was found that the population of tested insects was affected by the studied weather factors. These results were in agreement with those obtained by [33], who found that the temperature hurt adults, and relative humidity had a positive correlation on most different hosts. [34], who mentioned that the weather had a significant influence on the spread and infestation of the scale insects which preferred high humidity and low mild temperature and light [35], showed that the correlation between the number of pests and their parasitoids and the maximum and minimum temperature and relative humidity were significant or highly significant [36], mentioned there was a strong correlation between some climatic factors and scale insect population and their parasitoids on navel orange trees. [37] reported the increase or decline of populations of the black parlatoria scale insect, *Parlatoria ziziph*i (Lucas) and its parasitoid is correlated with some climate factors such as temperature, relative humidity and light intensity and also influenced by some chemical components in navel orange trees. These results can be beneficial when developing an integrated program for controlling this pest without the use of pesticides that may pollute the environment.
Population fluctuations and spatial distribution of A. orientalis were studied by [38] in the south of Kerman, and the results showed the pest activity started in mid-March and increased gradually, depending on weather conditions, the peak of population happened in mid-April, and mid-May.

4. Conclusion

In conclusion, the *COI* gene is one of the most important genes that could effectively apply for measuring heterogeneity between very close species of the same genera and could be used sufficiently to evaluate the phylogenetic relationship among very similar taxa. This supported the morphological characterizations that were conducted in this study. Therefore, further work should be conducted, and more samples should be collected to categorize other species for current genera, depending on the molecular barcoding technique. According to the results obtained in this study, we find the period of disappearance of the parasitoid is suitable for implementing chemical control programs for the scale insect. According to the occurrence of the parasitoid, it can be adopted in applied biological control programs against this pest.

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