BIONOMICS AND LIFE HISTORY OF THE CITRUS LEAF ROLLER, *ARCHIPS ROSANUS* IN NATURAL AND LABORATORY CONDITIONS

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ABSTRACT

The citrus leaf roller (CLR), *Archips rosanus* (L.) (Lep.: Torticidae) is one of the most threatening pests of leaf buds and blossoms in fruit trees, which in recent years has caused considerable damage to citrus trees in some parts of Mazandaran province of Iran. The bionomics and life history of the pest was studied in natural and laboratory conditions in 2017. Based on the results obtained in vitro, five larval instars of the citrus leaf roller were found on both hosts of Thomson-Novel orange and Unshu mandarin trees. As the age of larvae increased, the width of the head capsule and the length of the larval body was expanded. The length of the body at the age of 4th instar larvae was between 9 and 11 mm in males and females. While this size was observed between 14 and 15 mm at the age of 5th instar. The longest period of larval instars was observed at age 5th, which was nearly between 6 and 7 days. The longevity of the adult insect was observed in laboratory conditions between 16 and 18 days. The longevity of the adult on the mandarin host was less than orange. The egg hatching time on the orange host was 8 days, while it lasted 10 days on the mandarin host. The results of experiments in field conditions were such that hatching all eggs on orange and mandarin hosts lasted 16 and 22 days, respectively. The activity of adult males captured by the pheromone traps containing the artificial pheromone capsule of the female was on January 15 to January 18 on Thomson-Novel orange and mandarin hosts in field conditions. The length of the flight period in the orange orchard was 33 days and, in the mandarin was 28 days. The length of the oviposition period on citrus cultivars lasted about 15 days. This moth has one generation per year and overwinters as the egg. The findings of this study can be useful in the Integrated Management Plan for citrus pest control, implemented by experts and practitioners.

KEYWORDS:
citrus leaf roller, *Archips rosanus*, thomson novel orange, Unshu mandarin, biology, Iran

INTRODUCTION

The citrus leaf roller (CLR), *Archips rosanus* (L.), is one of the world's lepidopteran pest of Torticiadae family [1]. This pest is also known by other scientific names, including *Cacoecia rosana*, *C. hewittana*, *A. rosana*, *Tortrix rosana*, *T. laevigana* and *T. hewittana* [2]. It is an important pest of fruit trees in Europe and North America [3]. It attacks a large number of plant species including apple [4-6], pear [7], pomegranate [8], rose [9-10], stone fruits including cherry [11-12], cranberry [13], citrus [14-15] and hazelnut [16]. In British Columbia, (from where it probably made the first entry to western North America), it has been reported from 12 species of plants; apple, rose, privet and dogwood are the primary hosts [17]. It is also a pest of blueberry [18], cranberry and raspberry [19] in that province.

This insect is considered as an initial pest and occasionally a secondary pest of cherry orchards, it depends on time and space [20]. For example, its damage to the fruit trees in Greece [21], Sicily [22] and Italy [20] on citrus fruits are important as well as in Europe and North America. The rate of damage of this pest in an organ in North America and on the hazelnut is estimated to be 80-70% [23].

Citrus is one of the most important tropical and semi-tropical fruits in the world. All fruits called citrus are from the Rotaceae family and the Aurantioideae subfamily. Citrus production is of great importance in today's world and is one of the most important sources of wealth generation, trade and employment of residents of about 125 citrus-growing countries around the world. Citrus is a great source of vitamins, minerals and energy. Orange after apple is the second most commonly used fruit in the world [15]. In recent years, citrus leaf roller has caused a lot of damage to leaf buds and blossoms of citrus trees in Mazandaran province of Iran, which has led to the lack of flower buds and fruit. Observations indicate that the pest attacked to orange trees, mandarins, oranges, peaches, nectarines, green tomatoes, pomegranates, ornamental plants, Bergen shrubs, spruce and bush bean in recent years, and resulted in wrapped leaves and bad Formation of leaf buds [15]. According to personal observations of citrus orchards in Qaym-Shahr region, where control operations were not carried out, most of the leaves were attacked by the pest and were wrapped.

MATERIALS AND METHODS

This research was carried out in 2017 in the laboratory and field conditions. Two plots of Thomson Novel orange and Unshu mandarin orchard in Qaym-Shahr region, Mazandaran province, which had been oviposited by the citrus leaf roller, *A. rosanus* were selected for this study. Geographic characteristics of orange orchard: Latitude 36° 35'41.24" N and longitude 52° 46' 32.03" E and −10.02 m from the sea level. And the geographical characteristics of mandarin orchard: Latitude 36° 35' 57.83" N and longitude 52° 48' 6.94" E, and -8.39 m from the sea level. The area of each two gardens was 1 hectare. Operation of irrigation, fertilization, pruning and weed control in these two orchards was carried out. No spraying was done to control the citrus leaf roller, *A. rosanus*.

In order to start the experiment to obtain *A. rosanus* larvae, the overwintering egg masses of the pest in the second half of March were collected from the trunk and branches of orange and mandarin trees and place in the plastic petri dishes (0.5 × 5 cm) with wet cotton, individually. At the door of each petri dish, one-millimeter holes were created for air conditioning. Petri dishes containing eggs were transferred to a growth chamber (25±2°C, 75±5% RH, and 14L:10D h photoperiods).

Number of larval instars. To determine the number of larval instars of *A. rosanus*, two methods were used: 1- direct observation; 2- measurement of head capsule width. For this purpose, the larvae were collected by brush and transferred individually on the fresh citrus leaves to feed them into the glass tubes (10 × 1 cm). Daily, after examining the infested leaves, the width of the head capsule of 20 larvae samples for each age was determined using a stereomicroscope with a visualized lens and the number of different larval instars was determined according to the Dyar rule [24]. According to the law, the size of a sclerotized body, especially the head capsule, is increasing in the ages, and the exponential increase is linear, and it is a constant proportion that varies from 1.3 to 1.7.

Larval developmental period and their body length. Larvae were observed daily and their appearance was recorded to determine the length of larval period and size of the body length of the pest. In this way, having the size of the head capsule corresponding to each larval age that was obtained according to the previous test, the length of the developmental period and the size of the body length of each larval instar of the pest were determined.

Pupal developmental period and their body length. In order to determine the length of pupal development and measurement of their body length, the conversion time of the last instar larva to the pre-pupa and pupa and the time of conversion of pupae to adult insects and the size of the body length of each stage were recorded.

Adult longevity. In order to find out the adult longevity, after the pupae were converted to adult insects, they were immediately confined in transparent plastic containers (10 × 10 × 10 cm) in 10 replicates. The door of the dishes was blocked with white net. 10% water and honey was used to feed the adults. Containers were examined on a daily basis.

Percentage of daily eggs hatching in laboratory conditions. Fifteen egg masses were collected from orange trees and 15 egg masses from the mandarin trees and transferred to the laboratory and their daily hatching percentage was recorded.

Percentage of daily eggs hatching in field conditions. Fifteen egg masses on the orange trees and 15 egg masses on mandarin trees were randomly selected from different parts of each orchard and their daily hatching percentage was recorded.

Length of the flight period in orange and mandarin orchards. For this purpose, in each orchard, three delta traps, with the sex pheromone of *A. rosanus* from Russell UK Company with the PH-104-1RB code, were installed in the orchards. The distance from each trap was 30 meters. Adhesives in each trap were replaced weekly. The number of catches per trap was recorded daily.

Length of oviposition period in orange and mandarin orchards. A daily observation of the orchards was made to obtain the length of the oviposition period. Ten trees were randomly selected for each site and the total number of eggs laid on the trunks and branches of all trees in each garden was recorded daily. Information about the average ambient temperature during hatching, the period of flight and the oviposition period were recorded by Data Logger.

RESULTS AND DISCUSSION

The results of this study showed that the citrus leaf roller, *A. rosanus* on both Thomson-Novel orange and Unshu mandarin host trees had five larval instars, and with increasing larval age, the head capsule width and the larval length were extended. The size of the head capsule of the female larvae, other than the larvae of the first instar, which was equal to the male larvae, was slightly larger than male larvae in the field conditions.

In the both host trees, the 1st instar larvae were very small, elegant with bright green color, and the color of the head capsule was dark brown. The second instar larvae, like the first instar, had a bright green color and dark brown head capsule. The width of the head capsule at this age was slightly wider than the previous one. The third instar larvae, had also a larger dark brown head capsule, but unlike the body color of the previous ages, which was bright green, it was dark green at this age. Body color and head capsule at 4th and 5th larval instars were similar to 3rd instar. The size of body length at the 4th instar larvae in males and females was between 9 and 11 mm, while this size was approximately observed 14 to 15 mm at the 5th instar.

In all stages of the larvae, and the pupal and adult stages of insect, the size of the female was larger than the male insect. The stages of pre-pupa are green, but in a short period of time, the color changes to dark brown and at the end of this stage the color becomes dark brown. The size of the pupa is smaller than that of the pre-pupa stage, and the pre-pupa stage is less than 5th instar larvae. The length of the pre-pupa is between 13 and 15 mm. The length of the period of different stages of larvae, as well as pre-pupa and pupae on the mandarin was longer than the orange. The longest period of larval instars was observed at 5th instar, which was roughly between 6 and 7 days.

The adult longevity was between 16 and 18 days in the laboratory condition. Also, the longevity of the adult insect on the mandarin host was less than orange. Among the developmental stages, the lowest period was observed in the 3rd instar larvae of the female, fed from orange leaves, with 3.58 ± 1.17 days, as well as the highest period in the 5th instar larvae, fed from the mandarin leaf, with 7.12 ± 1.50 days.

According to previous studies, the citrus leaf roller, *A. rosanus* has five larval instars [16, 23], which is similar to the results of this study.

Based on the theory of [25], each larval instar has a steady amplitude of the head capsule width, which by measuring the width of the head capsule can determine the number of larval instars of the lepidopterans. In the present study, the measurements performed for each larval instar, were well characterized by different instars and, in addition, the results of the measurements were adapted by the Dayar [24] rule, and the validity of the results have been proven. According to Dayar's law, which is basically true for lepidopteran, the size of the larval capsule at each larval stage varies with a constant ratio, with head growth at different stages increased ranging from 1.3 to 1.7 times, and this increase is for each species is constant. However, in some cases, this rate is not constant in some cases and may change with each molting.

TABLE 1

Mean ± Standard Error (SE) in body length, head capsule width and duration of different developmental stages of the citrus leaf roller, *Archips rosanus* reared on Thomson-Novel orange in laboratory conditions.

Developmental stage	Thomson-Novel Orange					
	Male			Female		
	Body length (mm)	Head Capsule Width (mm)	Duration (day)	Body length (mm)	Head Capsule Width (mm)	Duration (day)
1st instar larva	2.01±0.01	0.25±0.00	3.86±0.58	2.22±0.00	0.25±0.00	3.75±0.41
2nd instar larva	3.83±0.12	0.37±0.11	3.72±1.03	4.05±0.14	0.39±0.09	3.78±0.96
3rd instar larva	6.71±0.23	0.61±0.17	3.61±0.85	6.92±0.16	0.63±0.13	3.58±1.17
4th instar larva	10.68±0.64	1.01±0.34	4.51±1.23	10.89±0.71	1.03±0.37	4.55±1.08
5th instar larva	14.94±0.58	1.42±0.41	6.29±1.34	15.27±0.39	1.44±0.35	6.18±1.54
Pre-pupa	14.23±0.27	-	2.17±0.94	14.41±0.22	-	2.19±0.90
Pupa	13.11±0.35	-	11.13±2.61	13.27±0.42	-	10.75±3.19
Adult	9.67±0.18	-	17.24±2.74	10.22±0.29	-	17.91±2.61

TABLE 2

Mean ± Standard Error (SE) in body length, head capsule width and duration of different developmental stages of the citrus leaf roller, *Archips rosanus* reared on Unshu mandarin in laboratory conditions.

Developmental stage	Unshu Mandarin					
	Male			Female		
	Body length (mm)	Head Capsule Width (mm)	Duration (day)	Body length (mm)	Head Capsule Width (mm)	Duration (day)
1st instar larva	1.95±0.03	0.23±0.00	3.99±0.62	2.17±0.02	0.22±0.01	3.86±0.58
2nd instar larva	3.85±0.15	0.35±0.08	4.02±0.97	3.92±0.21	0.37±0.13	4.12±1.17
3rd instar larva	6.51±0.27	0.59±0.12	3.86±0.84	6.70±0.22	0.61±0.15	3.79±0.71
4th instar larva	9.83±0.59	0.99±0.27	5.02±1.42	10.07±0.78	1.01±0.31	5.14±1.29
5th instar larva	13.95±0.43	1.35±0.39	7.12±1.50	14.13±0.47	1.38±0.38	6.84±1.36
Pre-pupa	13.05±0.29	-	2.71±1.19	13.81±0.20	-	2.69±1.14
Pupa	12.62±0.38	-	12.28±2.66	13.01±0.35	-	11.19±2.49
Adult	9.16±0.21	-	16.59±1.87	10.07±0.19	-	16.93±2.25

In the present study, this ratio was calculated for 1st to 5th female larval instars on the Thomson-Novel orange leaves from 1.56, 1.61, 1.63 and 1.39 and for male larvae from 1.48, 1.64, 1.65 and 1.40, respectively. Similarly, the ratio for female larvae fed from Unshu mandarin leaves for 1st to 5th larval instars was measured 1.68, 1.64, 1.65, 1.36 and for male larvae 1.52, 1.68, 1.67 and 1.36, respectively.In the previous studies, the mean width of head capsule of *A. rosanus* male larvae on hazelnut in laboratory conditions for 1st to 5th instars larvae were 0.26, 0.36, 0.59, 0.93 and 1.47 mm, respectively, and for female larvae, 0.26, 0.37, 0.61, 1.01 and 1.64 mm [16], indicating that the mean head capsule width in female is much larger than male larvae. In the present study, in both tree hosts, the mean width of the head capsule of female larvae is greater than that of the male larvae. A slight difference in the width of the head capsule in this study with [16], could be due to the difference in host type. AliNiazee [16] reported the larval development period at 26°C on hazelnut leaves of 36.15 days, and AL-Zabidi [23], this time on artificial diet 16.8 days, while this period in the present study for the males and females fed on Thomson-Novel Orange, were 21.99 and 21.84, respectively, and were 24.00 and 23.75 days for males and females fed on Unshu mandarin. It is likely that the difference in experimental conditions, such as the difference in the type and quality of foods, is the reason for disagreement.

As Figure. 1, shows it is estimated that the first egg hatching on the Thomson Navel orange host in field conditions took place on March 19th. On this date, 6% of the eggs were hatched and the temperature was 11oC, while hatching date of the eggs on the Unshu mandarin host was the first one day after orange, with 7%. By increasing the temperature of the environment, the percentage of egg hatching was increased. The highest percentage of egg hatching on the mandarin host was on March 28th, when the temperature reached the highest during the course of the day (19ºC). The highest percentage of egg hatching on the orange host was 8 days after the first hatch, which was more than 20%. The last eggs were hatched on the orange host on April 4th, while the last egg hatching date on the mandarin host was on April 10th. Overall, all eggs were hatched on orange and mandarin hosts at 16 and 22 days, respectively.

Temperature and photoperiod are the two prominent environmental factors that have a marked influence on the biology of an insect species. Many studies have attempted to describe the effect of temperature on insect development [26-30]. In a research conducted by AliNiazee [16] in orchard conditions, it showed that the egg hatch of *A. rosanus* is spread over an extended period. Detailed studies in 1976 indicated that hatching from 31 egg masses was spread over a 16-day period, with a sharp peak on the 12th day. In another study, the researchers proved that from 1993 to 1996, caterpillars began to hatch in the last ten days of April. In 1997, they hatched later, as of May [1]. In the present study, the length of the egg hatching period in the orange and mandarin orchards, was 16 and 23 days, respectively which was the oviposition peak in these two gardens 9 days after the first hatch. The results of this research were somewhat similar to the AliNiazee [16] study, and the slight difference could be due to differences in weather conditions. In the current study, it has been shown that the egg hatching decreases with decreasing temperature and cooling the environment. In the present study, egg hatching period on different hosts in laboratory conditions, on the orange and mandarin, was 8 and 10 days, respectively, which halved in comparison with orchard conditions, and this indicates that if temperature conditions are favourable for hatching, the length of the period is also reduced.

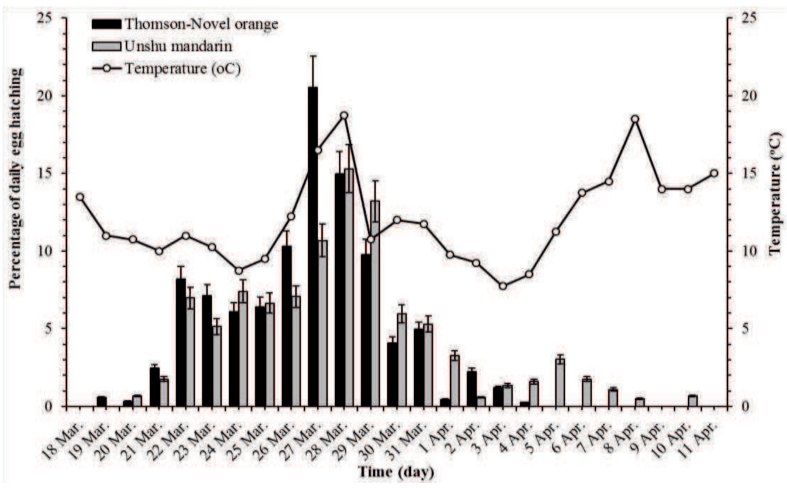

FIGURE 1

Relationship between percentage of daily egg hatching and the different time (2017) and temperature of the citrus leaf roller, *Archips rosanus* in field conditions.

Based on the results presented in Figure 2, which shows the percentage of egg hatching on two orange and mandarin hosts in laboratory conditions, the egg hatching time on the orange host was 8 days, while the value on the mandarin host lasted 10 days. The highest percentage of egg hatching was on the orange host, three days after the first hatching, which was 29%. The highest percentage of egg hatching on the mandarin was five days after the first hatch, which occurred on March 17th, and was 22%.

The seasonal cycle of the filbert leaf roller, *A. rosanus* has been described by Chapman and Lienk [31], Chapman [32] and AliNiazee [16] from different geographic regions. All investigators indicated that this species overwinters in the egg stage, eggs hatch in early spring depending on temperature and can be predicted by using a degree day's accumulation model. Using this approach, the beginning egg hatch was predicted to occur at about 40 days over the basic threshold of 8°C starting Jan 1st. The egg hatch continues for about 3 weeks [16]. Field-collected, overwintered eggs started to hatch 6 days after they were placed in rearing chambers. They continued to hatch for one week, with a peak on the 8th day and about 60% within a 3-day period (7-9th day). A detailed examination of egg hatching pattern of the 15 selected egg masses indicate that all eggs from a single egg mass do not hatch at the same time. Approximately 30% of the eggs from an egg mass hatch during the 1st day, while other 40% hatch on days 2-4, and the remaining eggs may take one week.

Figure 3 shows the flight activity of the male moths captured by the pheromone traps containing the artificial pheromone capsule of the female from 15 May to 18 January on two hosts of Thomson Novel orange and Unshu mandarin in field conditions. It showed that the emergence of the moths from the pupae in the orange orchard was one day earlier than the mandarin, and it rises rapidly as the temperature increased, so that the flight peak of the male insects in the orange orchard was on the 29th of May when 23oC was recorded by Data Logger. According to the chart, the length of the flight period was 33 days in the orange orchard and in the mandarin, was 28 days. The population of citrus leaf roller, *A. rosanus* in both orchards in the first and second decades were more than the third decade.

In studies conducted by Pluciennik and Tworkowska [1] from 1993 to 1997 on the filbert leaf roller, *A. rosanus* they proved that the first moths were captured in the pheromone traps in June, and their flight lasted until the end of July. Sporadic single moths were caught even in August. The flight period ranged from 30 to 53 days. 80-90% of the moths were caught in the first and second ten days of July. In 1996, a year with an early and warm spring, most of the moths were caught in the third ten days of June. In the remaining years, the flight did not begin in earnest until July. Usually, the flight began when the sum of effective temperatures (above 10°C) reached 290°C. The results are consistent with the results presented in this study. In the present study, the first male moth was captured in mid-July, and the distance between the first capturing and the last catches by pheromone traps was 33 and 28 days in the Thomson Novel orange and Unshu mandarin, respectively. The first moth captured in the mandarin orchard, as well as its flight peak, occurred later than the Thomson-Novel orange orchard, which could be due to longer larval stages and the development of larval and pupa-fed of mandarin versus orange.

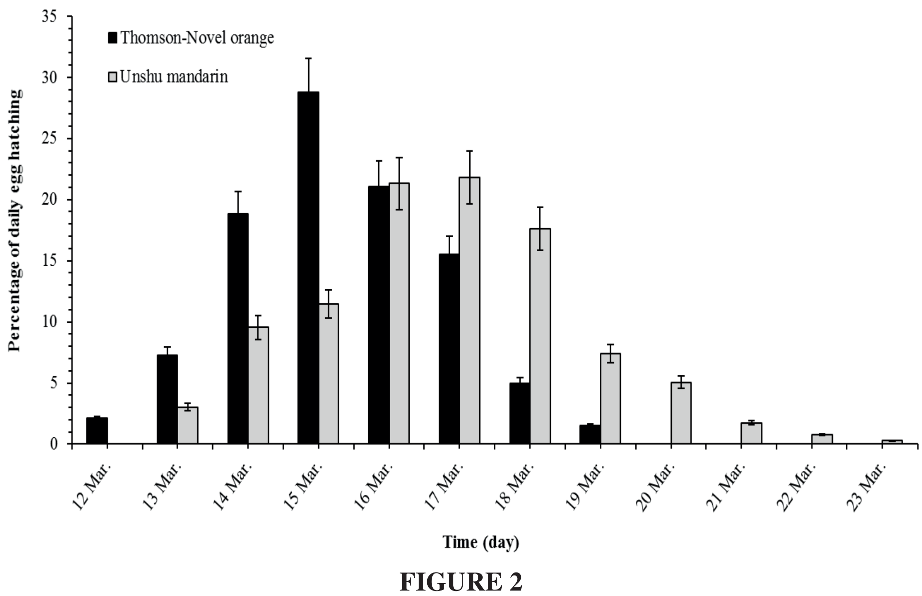

FIGURE 2
Relationship between the percentage of daily egg hatching in the different time (2017) of the citrus leaf roller, *Archips rosanus* in laboratory conditions.

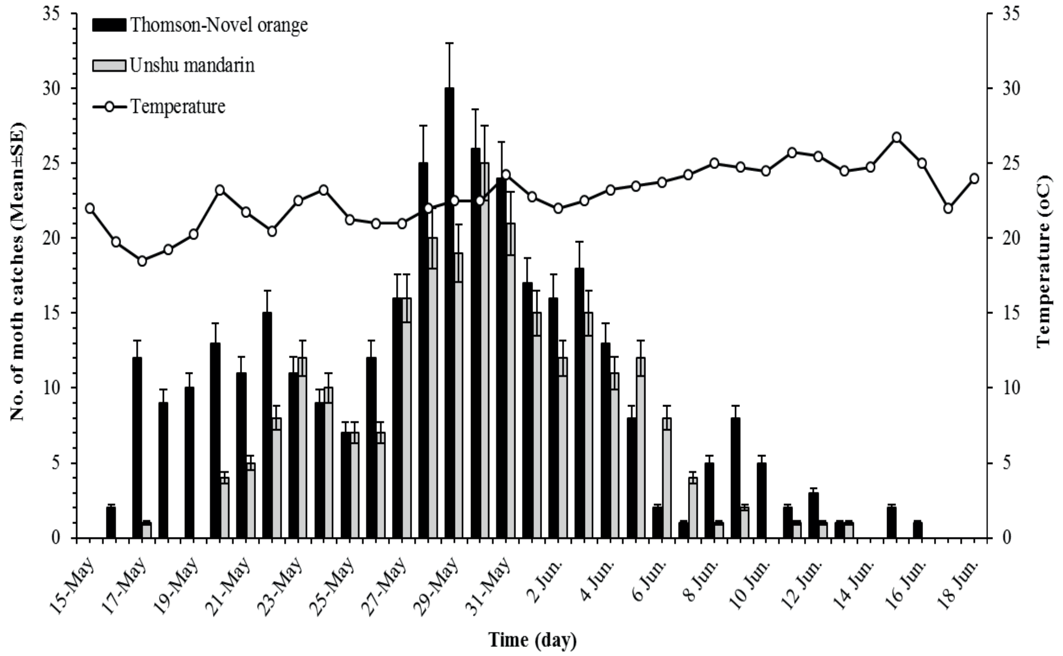

FIGURE 3

Relationship between a number of moth catches of the citrus leaf roller, *Archips rosanus* by pheromone trap in the different time (2017) and temperature in field conditions.

Information on the length of oviposition period for female insects in orange or mandarin orchards is shown in Figure 4. According to this information, the length of oviposition period on citrus varieties is about 15 days. This chart also shows the number of egg masses laid by the female moth on 10 citrus branches. The first oviposition on the orange tree branches was seen on May 28 and on the mandarin tree on May 29th. The oviposition peak on the orange trees was observed eight days after the first egg mass that was observed on January 4, while the oviposition peak on the mandarin tree branches was counted on January 4th. The eggs are initially greenish, but over time, the color changes to a dark brown. Most eggs are placed at low altitudes of the branch and on the thicker ones. The laid eggs will go through the winter and will hatch in the spring of next year with forming of tree buds. The result of the AliNiazee [16] showed that oviposition was spread over a 10-day period. Eggs were laid within a day after adult emergence by some adults, but most eggs were laid during days 2-6 of adult life. Females deposited 0-2 egg masses/night. Some females did not lay any eggs until the 10th day of their life, then deposited a few egg masses and died. Other females deposited a few eggs during the 2nd and 3rd day of their life and none afterwards, although they lived for about 10-15 days. The pre-oviposition period averaged 2.4 days, oviposition period 4.6 days, and post-oviposition period 3.4 days. In the present study, like the results of AliNiazee [16], the length of the oviposition period was 12 to 13 days. The first mass of eggs in citrus orchards was observed 12 days after the first capturing of the male moth. The oviposition peak was occurred 7-9 days after observing the first eggs. The oviposition of *A. rosanus* in the orchards was partly dependent on the temperature of the environment and increased with temperature.

The thermal threshold for egg laying was estimated to be 12.2°C. The optimum oviposition occurred at 20°C, while a slight reduction in fecundity was found at 16 and 24°C. Egg laying occurred over a period of 7-12 days. Over 86.1% of females oviposited at 20°C, while only 44.2% of females oviposited at 28°C. At higher temperatures, premature adult mortality increased and the number of females laying eggs drastically decreased. This indicates the major role of temperature in fecundity of *A. rosanus*. Many females began oviposition on the third day after emergence, and after a few days of oviposition, the females completely stopped laying the egg. Some females had eggs in the ovaries but did not oviposit during their entire life, while others did not produce any eggs [23]. In the present study, the oviposition of *A. rosanus* in the orchards was partly dependent on the temperature of the environment and decreased with increasing temperature, for example, from May 28 to January 3, the oviposition trend was ascending. However, on May 31, the gradient slope in the mandarin orchard declined, and even in the orange garden, the slope of the chart dropped, due to an increase in the air temperature to 24oC.

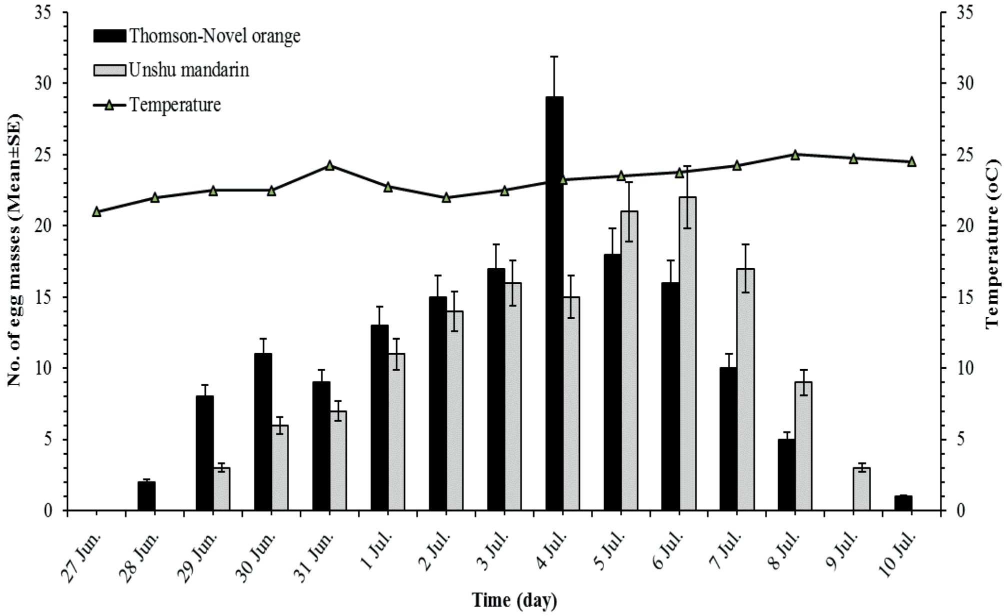

FIGURE 4
Relationship between a number of laid egg masses of the citrus leaf roller, *Archips rosanus* in the different time (2017) and temperature in field conditions.

CONCLUSION

Based on the results of this study, citrus leaf roller, *A. rosanus* in Iran has one generation per year on the orange and mandarin tree hosts. The larvae of the first instar are very small and after hatching they quickly move towards the buds and blooms and settle on them, and silk cords secrete from their mouths, and through these silk cords, the two edges of the leaf are transversely wedge-shaped and create a larval shelter and it feeds on it. The level of feeding in the 1st instar larvae is low, and with increasing age of larvae, the tufts of silk cords and the feeding rate of larvae are increased. The findings of this study can be useful in the integrated control program for the citrus leaf roller, *A. rosanus* control, which is being implemented by experts and practitioners.

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