EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

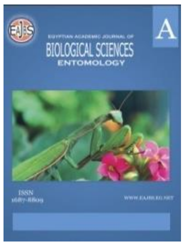
Vol. 14 No. 4 (2021)

**Citation**: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(4) pp.215-226 (2021)*
**DOI: 10.21608/EAJBSA.2021.216442**

Egypt. Acad. J. Biolog. Sci., 14(4):215-226(2021)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/




# Ecological Studies on *Parlatoria ziziphi* (Lucas) and Associated Parasitoid as Biological Control Agents on Mandarin Trees in Menufia Governorate

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ARTICLE INFO

**Article History**

Received:10/11/2021

Accepted:24/12/2021

**Keywords**:

Ecology, Parlatoria ziziphi (Lucas), *Aphytis* sp, Mandarin Trees

## ABSTRACT

Seasonal abundance, generations and horizontal distribution of *Parlatoria ziziphi* (Lucas) were carried out on mandarin trees at the Faculty of Agriculture in Shebin El-Kom, Menoufia Governorate during two successive years (2017-2018 and 2018-2019). The obtained results revealed that the total numbers of alive stages had two peaks of activity during the first year (2017-2018) they took place in September and January while, during the second year (2018-2019) three peaks of activity were recorded in September, January and March. Also, the insect had three annual generations on mandarin trees the generation duration varied from three to five months. The horizontal distribution showed that the insect and its associated parasitoids *Aphytis* sp. concentrated in the quarter of the trees' north eastern direction. The effects of some weather factors on *P. ziziphi* and its associated parasitoids were studied.

## INTRODUCTION

The citrus trees, *Citrus* spp. (Rutaceae) are one of the most popular fruits in Egypt. It contains a high percentage of vitamins C which prevents scurvy and essential nutrients at good levels and it plays an important role in food industrialization such as juices, which has increased in demand due to the quality of Egyptian varieties. Orange is one of the most cultivated in Egypt; however, the delta Governorates of Qalyoubia, Beheira, Sharqiya, Ismailia and Menufia are the main producing areas. Scale insects are the most unusual known insects. Their common name derives from the presence of a protective cover or scale, the cover or scale is produced by the insects. Scale insects or coccoids (Coccoidea) are sap-sucking hemipterans insects. They are the most important insect pests of perennial plants and can cause serious damage to a lot of fruit trees, woody ornamentals, forest vegetation, greenhouse plants, and house plants. These insects have the ability to transmit plant pathogens, injection of toxins and production of large quantities of honeydew which motivate the growth of sooty mold fungi that cover leaf surfaces and reduce photosynthesis. Also distortion of foliage, discoloration of flowers, galls and tumors formation, distorted blossoms, affecting in the general physiological processes of the trees, Thus, the effect on the quality and quantity of the resulting crop, dieback of twigs, and sometimes the death of the entire plant (Miller and Davidson, 2005, Mazzeo *et al*., 2014 and Nabil 2018).

**Citation**: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(4) pp.215-226 (2021)*
**DOI: 10.21608/EAJBSA.2021.216442**

Among many species of scale insects infesting citrus trees in Egypt, the diaspidid scales, the black parlatoria scale, *Parlatoria ziziphi* (Lucas). It attacks leaves, branches and fruits, the insect had two peaks of activity per year in April and October. Also, they mentioned that the scale had 2 to 3 generations per year (Tawfeek and Abu-Shall, 2010, Hassan *et al.*, 2012, Moustafa 2012 and Nabil *et al*., 2019).

So, the objective of the present work is to study the following points:

1- Seasonal fluctuations of different stages, number of annual generations and horizontal distribution of *P. ziziphi* on mandarin trees.

2- The associated parasitoid as well as its role as biological control agents in reducing the infestation of the pest.

3- Effects of climatic factors (temperature, relative humidity and sunshine) on both insect and their associated parasitoid.

## MATERIALS AND METHODS

Field experiments were carried out in the citrus farm, Faculty of Agriculture in Shebin El-Kom, Menoufia Governorate. The study was continued for two successive years, from June 2015 until May 2017. The farm received normal agricultural practices and no chemical control was applied.

**Seasonal Abundance:**

The study was started from June 2017 until May 2018, in an area about one feddan of mandarin trees, *Citrus reticulate* Blanco. Five trees were selected and labeled. These trees were nearly similar in size, age and vegetation. Each tree was divided into four main directions (east, west, north and south).

For sampling, five leaves were picked up at random twice a month from each direction, *i.e.* 100 leaves per sample (5 trees × 4 directions × 5 leaves). The samples were put in polyethylene bags and transferred into the laboratory for careful inspection.

These samples were examined on the same day with the aid of a stereomicroscope. The stages of the black parlatoria scale, *Parlatoria ziziphi* (Lucas) and their associated natural parasitoids were counted and recorded. The annual generations of scale insects were determined according to Audemard and Milaire (1975) and amended by Jacob (1977).

**Estimation of Parasitism Ratios:**

To study the parasitism ratios of scale insects the previously collected samples for seasonal abundance were carefully inspected and preserved in glass jars covered with muslin cloth by the aid of rubber bands and kept under laboratory conditions until parasitoids emergence. The percentage of total parasitism for each sample was estimated. All emerging parasitoids were mounted in Canada balsam and photo magnified under a stereomicroscope camera.

The parasitoid was identified with helping of Prof. Dr. S. Abd-Rabou, Chief Researcher, Scale Insects and Mealybugs Department, Plant Protection Research Institute, Agricultural Research Center, Egypt.

**Effect of Climatic Factors on The Insect Population and Parasitism Ratios:**

The prevailing means of air temperature (ºC), relative humidity (RH%) and solar radiation ($MJ/m^2$) in the experimental area during the periods of the present study were obtained from the Central Laboratory for Agricultural Meteorology, Agricultural Research Center, Ministry of Agriculture.

The relationships between climatic factors and each of population density of scale insect and parasitism ratios were studied. Simple correlation, partial regression values and explained variance (E.v%) were calculated using COSTAT Computer Program (2005).

**The Preferable Direction for The Insect Stages and Natural Enemies:**

To detect the effect of the cardinal directions on the distribution of scale insect and its associated parasitoid, the following formula was used.

$$H = \sqrt{F_1^2 + F_2^2 + 2F_1F_2 \cos Q}$$

This angle was calculated by dividing $F_2 / F_1$, Mahmoud (1981) and Nabil (2003 and 2010)

H = Powers summation

$F_1$ = The population on the east minus the population on the west if the first is higher and reversed it if the latter is higher.

$F_2$ = The population on the north minus the population on the south if the first is higher and the reverse is applied if the population on the south is higher.

The figure obtained represents the tangent, the corresponding values of which were obtained from the mathematical.

$F_1 = E – W$ $F_2 = N – S$ $\text{Tan } Q = F_2 / F_1$

## RESULTS AND DISCUSSION

**Seasonal Abundance:**

**Total Number of Alive Stages:**

As shown from obtained data in Figures (1 and 2) the total number of alive stages showed two peaks of activity during the first year (2017-2018) they took place in September and January. While during the second year (2018-2019) the total number of alive stages showed three peaks of activity there were in September, January and March. These results were in agreement with Helmy (2000) and Moustafa (2012) who noticed that the peaks of activity occurred in April, August and October.

**Total Number of Dead Stages**:

Data presented in Figures (1 and 2), the total number of dead stages showed three peaks during the first year there were in September, November and February. While during the second one the total number of dead stages recorded two peaks occurred in November and February.

**Percentages of Parasitism:**

During the study period, one hymenopterous species was recorded as a parasitoid of *P. ziziphi*. There was *Aphytis* sp. (Aphelinidae). The seasonal abundance of the parasitoid was represented as percentages of parasitism.

Data presented in Figures (1 and 2) showed that the percentages of parasitism occurred three peaks of parasitism during the first year (2017-2018) and two peaks of parasitism were recorded in the second year (2018-2019).

The peaks took place in November, February and April during the first year and in December and February during the second one.

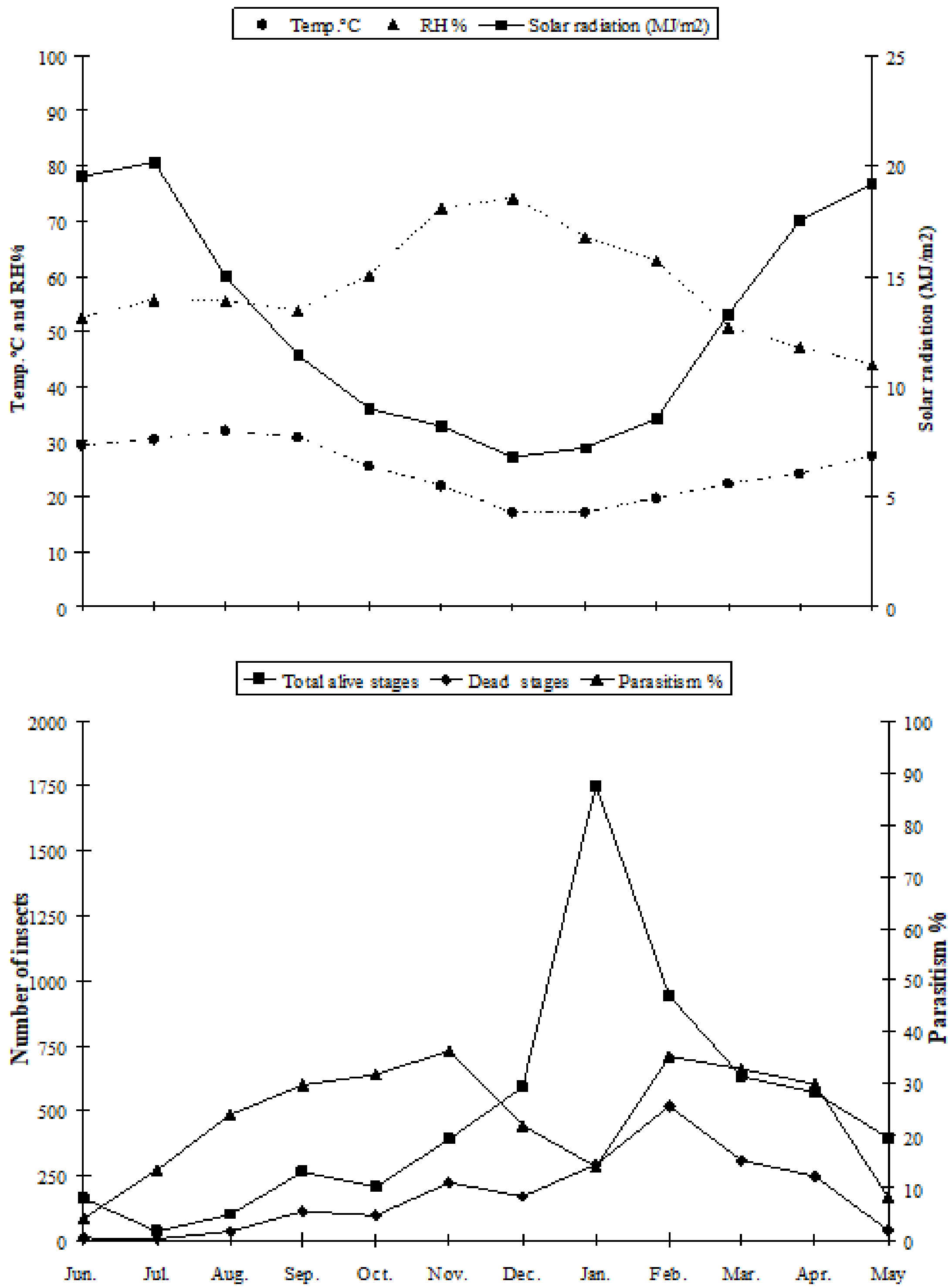


**Fig.1:** Seasonal abundance of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees in Shebin El-Kom district, Menoufia Governorate during the first year (2017-2018).

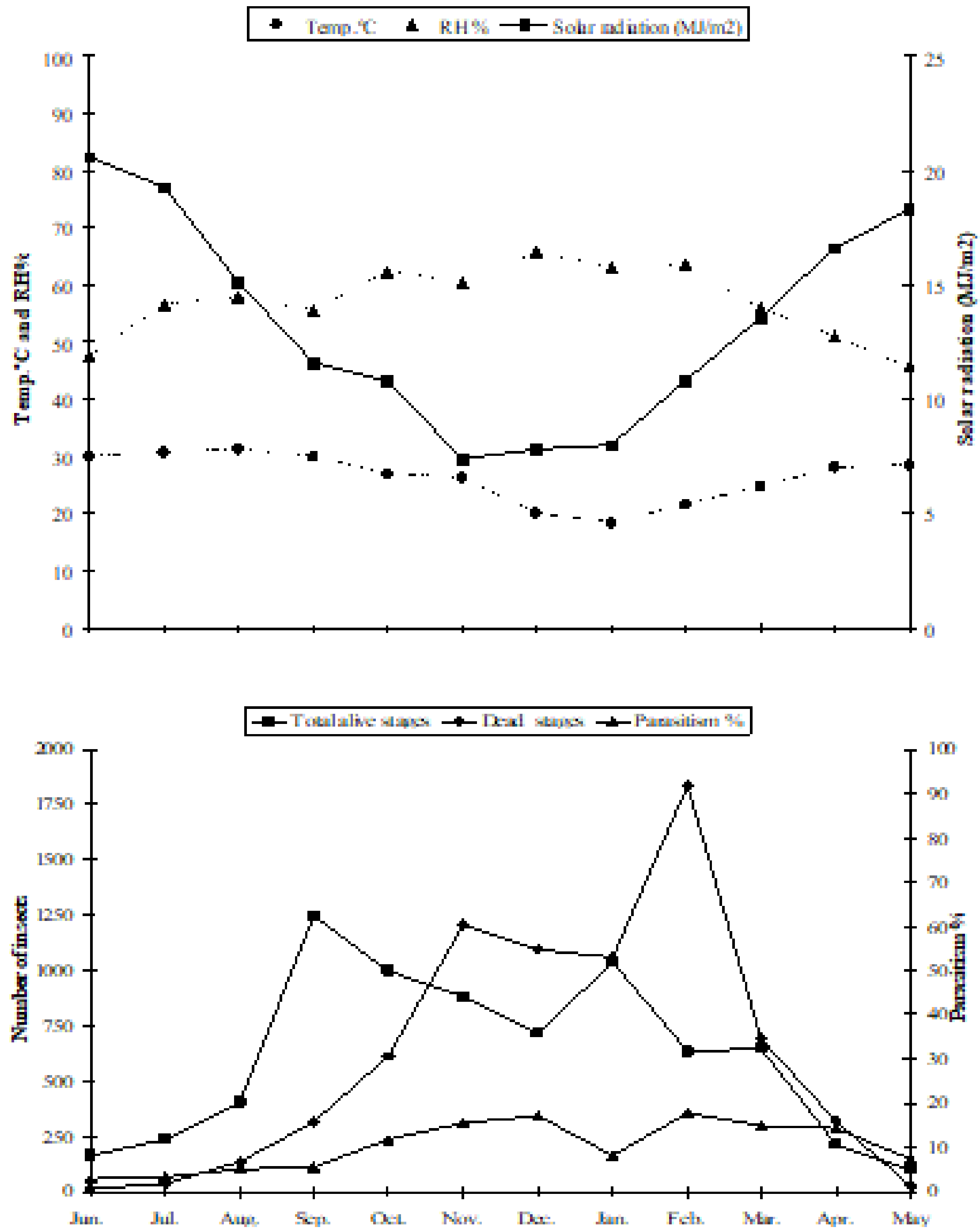


**Fig. 2:** Seasonal abundance of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees in Shebin El-Kom district, Menoufia Governorate during the second year (2018-2019).

These results were in agreement with those of Rosen (1986) who recorded parasitic hymenopterous species mainly Aphytis spp. and showed that this species is quite useful for controlling the diaspidids population. Also, Kamel et al. (2003) showed that the parasitism rates of Aphytis species were between 0.8 and 14.6%. Darwish (2016) revealed that Aphytis lingnanensis and Encarsia citrine (Aphelinidae), were recorded as parasitoids of P. ziziphi. The mean parasitism rate was 12.69 % and 14.8 % for A. lingnanensis in 2014 and 2015 years, respectively.

## 1.4. Effect of Climatic Factors:

### 1.4.1. On A Total Number of Alive Stages:

Data presented in Table (1) showed that during the first year (2017-2018), there was a negative highly significant effect between the total number of alive stages and temperature whereas r = 0.788**. While, during the second year (2018-2019) relative humidity and solar radiation showed positive significant and negative highly significant effects on the total number of alive stages whereas r = 0.670* and -0.830**, respectively. E.v.% affected this stage by 67.23 and 75.14% during the first and second years, successively.

**Table 1:** Statistical analysis based on correlation coefficient (r) and explained variance percentage (E.v.%) indicating the effects of climatic factors on different stages of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees, in Shebin El-Kom district, Menoufia Governorate during two successive years (2017-2018 and 2018-2019).

| | | Temp. (ºC) | RH (%) | Solar radiation (MJ/m²) | Explained variance (E.v. %) | Combined effect | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | RH (%) | Solar radiation (MJ/m²) |
| 2017-2018 | Total number of alive stages | -0.788** | 0.352 | -0.538 | 67.23 | Temp. (ºC) | -0.634* | 0.718** |
| | Mortality % | -0.268 | 0.278 | -0.567 | 44.75 | RH (%) | | -0.815** |
| | Parasitism % | -0.001 | -0.399 | 0.426 | 39.47 | | | |
| 2018- | Total number of alive stages | -0.426 | 0.670* | -0.830** | 75.14 | Temp. (ºC) | -0.662* | 0.722** |
| | Mortality % | -0.753** | 0.605* | -0.686* | 60.90 | RH (%) | | -0.839** |
| | Parasitism % | -0.641* | 0.516 | -0.629* | 47.23 | | | |

### On Percentages of Total Mortality:

Data given in Table (1) cleared during the first year no significant effects were recorded on this stage. While, during the first year temperature, relative humidity and solar radiation showed negative highly significant, positive significant and negative significant effects on the percentage of total mortality whereas r = -0.753**, 0.605* and -0.686*, respectively. Statistical analysis showed that E.v.% affected percentages of total mortality by 44.75 and 60.95% during the first and second years, consecutively.

### On Percentages of Parasitism:

As shown from obtained data in Table (1) during the second year, temperature and solar radiation showed positive significant effects whereas r = - 0.641* and -0.629*, consecutively. Statistical analysis showed that E.v.% affected parasitism percentage by 39.47 and 47.23% during the two successive years, respectively.

### The Combined Effect of Climatic Factors:

Data are given in Table (1) indicated that there were negative significant effects between temperature and relative humidity whereas (r) values were -0.634* and -0.662* during the first and second years, respectively. Also, temperature showed positive highly significant effects with solar radiation whereas (r) values were 0.718** and 0.722** during the first and second years, respectively. During the first- and second-years relative humidity showed negative highly significant effects with solar radiation where r = -0.815** and -0.839**, consecutively.

Generally, it was clear that temperature and solar radiation had positive significant effects in all cases.

These findings were in agreement with those of the following investigators. Hassan (1998) and Hassan *et al.* (2001) noticed that the combined effect of temperature and sunshine period was a highly positive significant effect. While, relative humidity percentage and sunshine period showed a negative significant effect on all stages of *H. lataniae* on fig trees in El-Khattara region, Egypt. Also, stated the combined effect of climatic factors such as temperature, relative humidity and light intensity on *H. lataniae* population. They found that a highly positive significant effect of temperature and sunshine were detected, while relative humidity and sunshine had a negative significant effect on all stages of *H. lataniae* on pear trees in Sharkia Governorate, Egypt. El-Dash *et al.* (2002) revealed that the mean temperature correlated significantly and positively with the nymphs population, but it has a negative effect on the adults' population. The relative humidity showed an insignificant effect and mostly positive on most tested hosts. The mean temperature seemed to be the most effective factor in population activity. Nabil (2003) revealed that there were highly positive significant effects between each of sunshine, outer light intensity and temperature in the outer and inner zone of guava trees. Shahein *et al.* (2004) in Egypt, revealed that the total effects of biotic factors (Temp. ºC, RH % & sunshine hrs.) on the total number of alive stages of *P. psidii* in outer and inner zones of guava trees were 82.4, 53.4 & 22.1 % and 79.3, 81.3 & 17.1 %, successively. Temperature and both sunshine & outer light intensity had positive significant effects in whole cases. Also, Nabil (2010) mentioned that there were positive highly significant effects between temperature and light intensity in the top and bottom levels of mango trees.

**Number of Generations:**

As *P. ziziphi* is known to have overlapping generations, it was necessary to utilize the formula proposed by Audemard and Milaire (1975) and amended by Jacob (1977) for estimating the number of generations and their annual durations. Data of monthly counts of the nymphal stage were indicated on millimeter paper.

**Table (2):** Annual generations and durations of *Parlatoria ziziphi* (Lucas) on mandarin trees, in Shebin El-Kom district, Menoufia Governorate during the two successive years (2017-2018 and 2018-2019).

| Months | Number of insects / 200 leaves during the first year (2017-2018) | | | | Number of insects / 200 leaves during the second year (2018-2019) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Accumulated days of investigation | Monthly counts of . | Accumulated monthly counts | Accumulated insects % | Months | Accumulated days of investigation | Monthly counts of nymphs | Accumulated monthly counts | Accumulated insects % |
| **Jun.** | 30 | 22 | 22 | 2.20 | **Jun.** | 30 | 14 | 14 | 0.98 |
| **Jul.** | 61 | 9 | 31 | 3.10 | **Jul.** | 61 | 30 | 44 | 3.07 |
| **Aug.** | 92 | 13 | 44 | 4.41 | **Aug.** | 92 | 82 | 126 | 8.79 |
| **Sep.** | 122 | 39 | 83 | 8.31 | **Sep.** | 122 | 431 | 557 | 38.86 |
| **Oct.** | 153 | 25 | 108 | 10.81 | **Oct.** | 153 | 290 | 847 | 59.11 |
| **Nov.** | 183 | 19 | 127 | 1271 | **Nov.** | 183 | 192 | 1039 | 72.50 |
| **Dec.** | 214 | 132 | 259 | 25.93 | **Dec.** | 214 | 119 | 1158 | 80.81 |
| **Jan. 2016** | 245 | 488 | 747 | 74.77 | **Jan. 2017** | 245 | 139 | 1297 | 97.49 |
| **Feb.** | 274 | 118 | 865 | 86.59 | **Feb.** | 273 | 41 | 1338 | 93.37 |
| **Mar.** | 305 | 35 | 900 | 90.09 | **Mar.** | 304 | 62 | 1400 | 97.70 |
| **Apr.** | 335 | 61 | 961 | 96.20 | **Apr.** | 334 | 15 | 1415 | 98.74 |
| **May** | 366 | 38 | 999 | 100.00 | **May** | 365 | 18 | 1433 | 100.00 |

As shown from the obtained data in Table (2) and Figure (3), *P. ziziphi* had three generations annually on mandarin trees during the two successive years. The first generation took about three months was during from the beginning of June till the end of August. While, the second generation lasted about four months, from the beginning of September till the end of December. The third generation occupied five months from the beginning of January till the end of May.

These results were in accordance with those of Helmy (2000) who reported that *P. ziziphi* had three generations a year on four citrus varieties (*i.e.* mandarin, Baladi orange, grapefruit, and trifoliate orange trees). Hassan *et al.* (2012) mentioned that the scale had 2 to 3 generations per year there were begin in June to October and the second started in April to May. Zaabta (2016) recorded that the scale had three generations per year the first in fall, the second in spring and the third during summer.

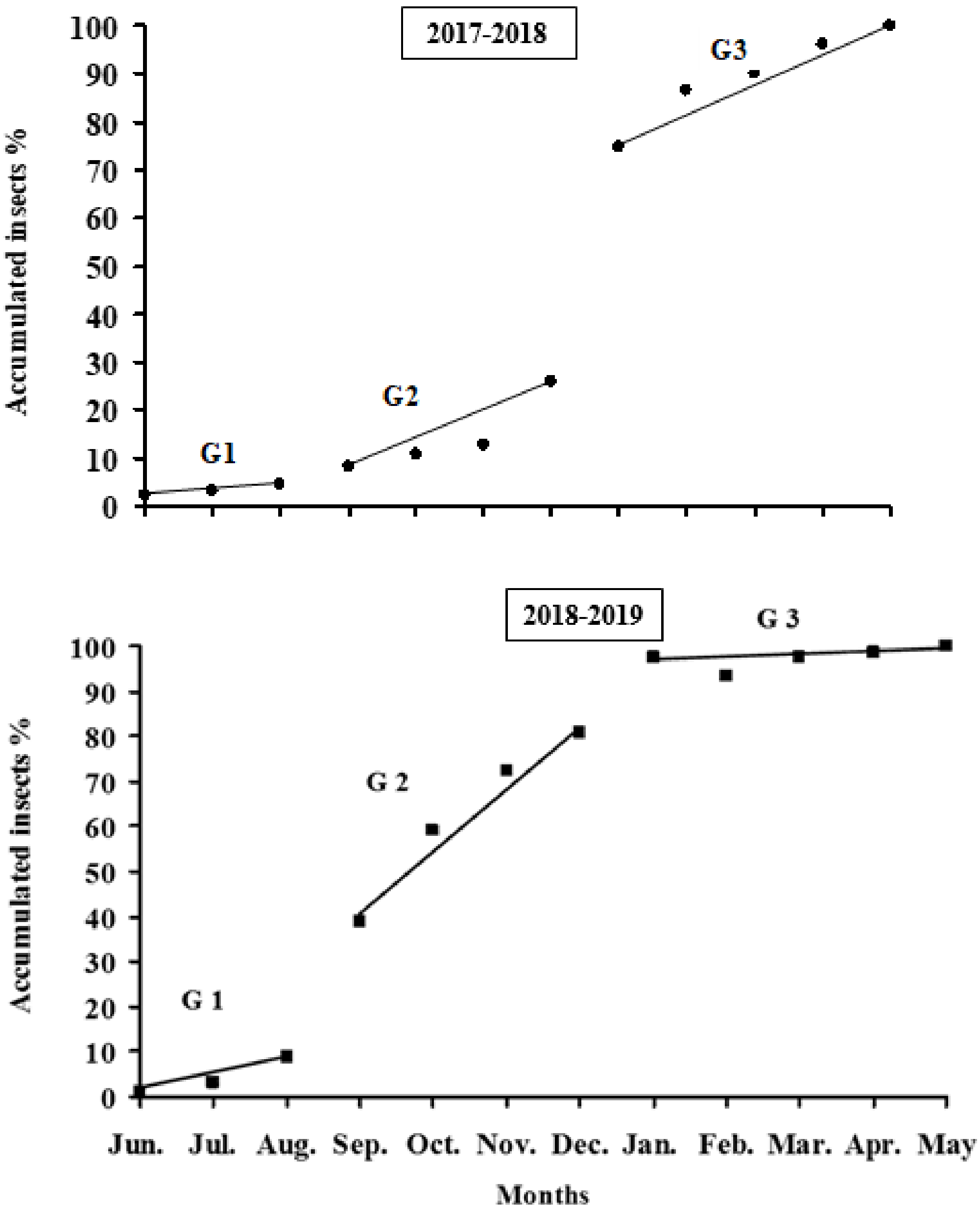


**Fig.3:** Annual generations and their durations of *Parlatoria ziziphi* (Lucas) on mandarin trees, in Shebin El-Kom district, Menoufia Governorate during the two successive years (2017-2018 and 2018-2019).

**Horizontal Distribution:**

Results illustrated in Tables (3 & 4) and Figure (4) showed that during the first and second years (2017-2018 and 2018-2019), the insect occurred in the north eastern side of the trees making angles 52° 43′ 36″and 76° 32′ 45″, respectively. Also, the parasitoid occurred in the northeastern side of the trees making angles 34° 30′ 31″and 43° 53′ 10″, consecutively.

Generally, illustrated data in Fig. (4) revealed that the armored scale insect, *P. ziziphi* and its associated parasitoid concentrated in the quarter of the trees' north eastern direction.

These results were in accordance with those of Hammad *et al.* (2003 and 2004) who studied the horizontal distribution of scale insects that infested guava trees in Egypt. They reported that according to mathematical calculation the insect pests occur almost in the north-eastern direction during the second and third years, while during the first one it occurs in the north-western direction. Darwish (2016) studied some ecological and behavioral aspects of *P. ziziphi* and its parasites on mandarin trees during two successive years (2014 and 2015) in Nobaria district, Beheira Governorate, Egypt. Data reported that the insect prefers the existing leaves on the southern and eastern branches of mandarin trees.

**Table 3:** Monthly numbers of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees, in Shebin El-Kom district, Menoufia Governorate during the first year (2017-2018).

| Months | Number of insects / 50 leaves during the first year (2017-2018) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | East | | West | | North | | South | |
| | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number |
| **Jun.** | 38 | 2 | 38 | 0 | 38 | 2 | 47 | 2 |
| **Jul.** | 5 | 2 | 5 | 0 | 13 | 0 | 9 | 2 |
| **Aug.** | 22 | 2 | 21 | 0 | 22 | 0 | 32 | 2 |
| **Sep.** | 58 | 2 | 68 | 0 | 68 | 0 | 68 | 2 |
| **Oct.** | 52 | 5 | 41 | 2 | 58 | 3 | 53 | 4 |
| **Nov.** | 124 | 7 | 70 | 5 | 98 | 7 | 95 | 6 |
| **Dec.** | 169 | 9 | 119 | 3 | 168 | 4 | 131 | 8 |
| **Jan. 2016** | 441 | 11 | 421 | 9 | 460 | 8 | 427 | 10 |
| **Feb.** | 235 | 15 | 210 | 32 | 271 | 29 | 225 | 19 |
| **Mar.** | 155 | 19 | 177 | 24 | 156 | 27 | 140 | 30 |
| **Apr.** | 129 | 38 | 165 | 24 | 132 | 35 | 143 | 18 |
| **May** | 99 | 5 | 106 | 2 | 93 | 4 | 94 | 5 |
| **Total** | **1527** | **117** | **1441** | **101** | **1577** | **119** | **1464** | **108** |

**Table 4:** Monthly numbers of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees, in Shebin El-Kom distract, Menoufia Governorate during the second year (2018-2019).

| Months | Number of insects / 50 leaves during the second year (2018-2019) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | East | | West | | North | | South | |
| | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number | Alive stages | Parasitoid Number |
| **Jun.** | 38 | 3 | 42 | 0 | 42 | 2 | 41 | 0 |
| **Jul.** | 38 | 2 | 44 | 0 | 110 | 7 | 45 | 0 |
| **Aug.** | 68 | 3 | 60 | 0 | 203 | 25 | 75 | 0 |
| **Sep.** | 252 | 21 | 282 | 16 | 433 | 35 | 279 | 15 |
| **Oct.** | 251 | 37 | 236 | 18 | 288 | 109 | 223 | 26 |
| **Nov.** | 244 | 97 | 200 | 58 | 204 | 74 | 236 | 97 |
| **Dec.** | 185 | 95 | 159 | 54 | 201 | 114 | 169 | 49 |
| **Jan. 2017** | 286 | 84 | 248 | 19 | 268 | 49 | 235 | 18 |
| **Feb.** | 169 | 133 | 153 | 103 | 161 | 115 | 150 | 91 |
| **Mar.** | 153 | 51 | 153 | 48 | 158 | 45 | 181 | 56 |
| **Apr.** | 52 | 36 | 49 | 18 | 48 | 9 | 65 | 13 |
| **May** | 18 | 3 | 28 | 1 | 26 | 4 | 25 | 1 |
| **Total** | **1754** | **565** | **1654** | **335** | **2142** | **588** | **1724** | **366** |

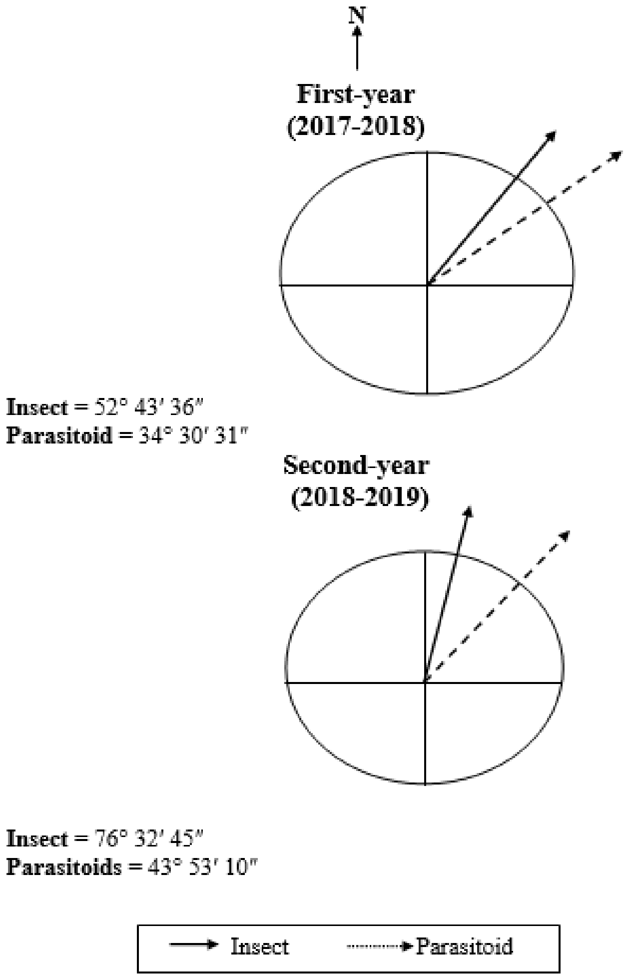


**Fig. (4):** The calculated directions of *Parlatoria ziziphi* (Lucas) and its associated parasitoid on mandarin trees, in Shebin El-Kom district, Menoufia Governorate during the two successive years (2017-2018 and 2018-2019).

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## ARABIC SUMMARY

**دراسات بيئية حول بارلاتوريا زيزيفي (لوكاس) والطفيلي المرتبط به كعناصر تحكم بيولوجية على أشجار اليوسفي في محافظة المنوفية**

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أجريت الدراسة لبيان النشاط الموسمي والأجيال والتوزيع الأفقي لحشرة البارلاتوريا السوداء *Parlatoria ziziphi* (Lucas) على أشجار اليوسفي بكلية الزراعة بشبين الكوم بمحافظة المنوفية خلال سنتين متتاليتين (2017-2018 و2018-2019). أظهرت النتائج المتحصل عليها أن الحشرة كانت لها ذروتان للنشاط خلال العام الأول (2017-2018) حدثا في شهري سبتمبر ويناير بينما تم تسجيل ثلاث ذروات للنشاط خلال العام الثاني (2018-2019) في سبتمبر ويناير ومارس. كما أن للحشرة ثلاثة أجيال سنوية على أشجار اليوسفي وتراوحت مدة الجيل من ثلاثة إلى خمسة أشهر. أظهر التوزيع الأفقي أن الحشرة والطفيليات المرتبطة بها Aphytis sp.