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Research Article

Performance of *Aphytis lepidosaphes* Compere as a Biological Control Agent of *Lepidosaphes beckii* (New.)

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Abstract

Background and Objective: The purple scale, *Lepidosaphes beckii* (New.) (Hemiptera: Diaspididae) is considered one of the most important pests of citrus around the world. Biological control represents a sustainable alternative for save control of *L. beckii*. The present work was conducted to study the factors affecting the biological control of *L. beckii* by *Aphytis lepidosaphes* compere (Hymenoptera: Aphelinidae) in Egypt Valencia orange. **Materials and Methods:** The parasitism rates by *A. lepidosaphes* were determined throughout two years on the scales of *L. beckii* which attacks leaves (upper and lower surfaces) and fruits of Valencia orange. The effectiveness of 5 insecticides namely, dinotefuran, imidacloprid, pyriproxyfen, azadirachtin, abamectin also were tested against *L. beckii* as well as *A. lepidosaphes*. **Results:** It was found that all nymphal instars and adult of *L. beckii* were parasitized by *A. lepidosaphes*. However, percentage parasitization was higher in the 4th instar males and gravid and non-gravid females compared with the 2nd and 3rd instar of males and 2nd instar of females. Parasitism percentages of *L. beckii* on fruits were significantly lower than its on the leaves. The differences in the parasitization percentages with regards to the seasons were determined to be statistically significant. The tested insecticides showed high effectiveness against *L. beckii* and *A. lepidosaphes*. But a better effect, reflected in high reduction percentages in *L. beckii* compared with low reduction percentages in the parasite was found for the soil application with dinotefuran, imidacloprid. **Conclusion:** The host stage preference showed that the parasitoid had a significant preference for the older host stages. The soil application of insecticides was less harmful to the parasite.

Key words: *Lepidosaphes beckii*, *Aphytis lepidosaphes*, parasitism percentages, soil insecticides, Valencia orange

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Scale insects (Hemiptera: Coccoidea) are sap sucking hemipterous insect pests widely distributed in different worldwide ecosystems. The family Diaspididae is one of the most commonly encountered of scale insects families with the most number of genera (420 genera) of armored scale insects[1-3]. The adult females of family Diaspididae are wingless and have reduced antennae and legs and are often protected by waxy protective secretions while the adult males haven't mouthparts and have one pair of delicate wings, well-developed legs and antennae. The purple scale *Lepidosaphes beckii* (New.) is a cosmopolitan citrus insect pest; it infests citrus in all Mediterranean countries. The purple scale which also known citrus mussel scale attach citrus leaves, fruits and small branches. In addition to the direct injury of *L. beckii* that causing by sucking the trees parts sap, the presence of this insect on the citrus fruits reduces their commercial value[4] and consequently decrease the export demands on citrus fruits. The control of scale insects is still relying essentially on the applications of synthetic insecticides. However, excessive use of insecticides for effective pest control has generated a lot of concerns about the insect pest's resistance for insecticide and also includes effects on the human health[5]. The use of resident natural enemies either alone or together with other control methods (integrated pest management) is a more sustainable approach to scale insects management. Both parasites and predators have potential to control the densities and alter the individual traits of hosts or prey[6]. Throughout the world, the Aphytis Genus (Hymenoptera: Aphelinidae) is an important parasite of several species of armored scale insects. The female of Aphytis species deposits its eggs beneath the cover of the scales insects and upon hatching, the larva feeds on the scale (Ectoparasite)[7-12]. Knowledge of the ecology and behavior of natural enemies of the scale insects is essential for the performance of biological control strategies. Keeping in view the above information, the present experiments were conducted to study the factors affecting the biological control of the purple scale, *L. beckii* by *A. lepidosaphes* in Valencia orange trees. These factors included effect of host stage preference; affect the host feeding sources and impact of prevailing climatic factors throughout the different seasons of the year. Also, 5 insecticides were tested for their effectiveness on the parasitism rates of *L. beckii* by *A. lepidosaphes*.

MATERIALS AND METHODS

This study was conducted from January, 2018 until December, 2019 at Nubaria district, Beheira Governorate, Egypt in a Valencia orange orchard infested by *L. beckii*. The trees were 15 years old and spaced 5 by 5 m apart. During the period of this study no chemical treatments were applied and for higher yield, all the horticultural practices were followed as per the package of practices. The orchard was sampled twice a month and the samples which consisted of 50 leaves and 50 fruits (when available) were randomly collected from ten trees, packed in polyethylene bags and transferred directly to the laboratory. Scales on the leaves (in upper and lower surface) and fruits were counted, examined using stereoscopic microscope binocular after turned over the cover and the stage specific parasitism recorded for 2nd instar males, 2nd instar females, prepupal and pupal males, non-gravid and gravid females. Parasitism was indicated by the presence of eggs, larvae, prepupae or pupae of *A. lepidosaphes*. Also, unparasitised scales for each stage were counted and the data were recorded separately for each of leaves (lower and upper surface) and fruits.

Effect of different insecticides on the purple scale insect and its parasitoides: Five insecticides were tested against the purple scale insect, *L. beckii* and its parasite, *A. lepidosaphes*. The used insecticides were dinotefuran, pyriproxyfen, imidacloprid, abamectin and azadirachtin. Details of these insecticides are listed in Table 1. Two methods were used to apply each of dinotefuran and imidacloprid, the first was throughout the soil and the second was the foliar spray as the other three treatments. In foliar spray, to ensure complete coverage of all parts of the tree, the trees were sprayed with a rate of 5-6 L/tree with conventional hand knapsack sprayer.

Table 1: Evaluated insecticides with their formulations, common names and rates of application

		Recommended rates		
Common name	Trade name	Foliar application	Soil application	Company
Dinotefuran	Oshin 20% SG	200 mg L^{-1}	0.5 g L^{-1}	Sumitomo Chemical Co., Japan
Imidacloprid	Confidor 35% SC	0.5 mL L^{-1}	1 mL L^{-1}	Bayer Crop Science, Germany
Pyriproxyfen	Admiral 10% EC	0.25 mL L^{-1}	-	Formuulat or Sumitomo, Japan
Azadirachtin	Nimbecidine 0.03% EC	5.00 mL L^{-1}	-	T. Stanes Co.
Abamectin	Agromec 1.8% EC	100 mL/fed	-	Syngenta Co.

The soil insecticides were applied with a rate of 4 L/tree. The specific dose was diluted with four liters of water in a container and drenched around the Valencia orange tree within 20-50 cm at 4 points of the tree trunk (cardinal directions) and then irrigated. Insecticides were distributed in a randomized complete block design (RCBD) in five treated replications and untreated control. Water was used as control treatment. The applied of the tested insecticides was in two dates, the 1st date was set to target 1st nymphal instar (crawlers) of the purple scale and the 2nd to target gravid and non-gravid females. Samples consisted of forty Valencia orange leaves infested with *L. beckii* were randomly collected from each treatment immediately before spraying and after 1, 2, 3, 4 and 5 weeks of application. After collection, the samples were packed in paper bags, taken to the laboratory and divided into two parts. In the 1st part, the live and dead scales of *L. beckii* were recorded. Dead scales were visually distinct (shriveled, discolored and can separate easily from the leaves). In the 2nd part, except *L. beckii* the other scales were removed from the leaves by a needle and the leaves were placed inside a plastic jars (15×20 cm). The jars covered with muslin cloth and tight with rubber bands for securing the emerging parasitoids. The weekly emerging parasitoids from each of the seven treatments and/or control were recorded. The reduction percentages of *L. beckii* and its parasite, *A. lepidosaphes* were calculated according to Henderson and Tilton equation[13] as following:

$$\text{Reduction (\%)} = 1\text{-}\frac{\text{Cb}\times\text{Ta}}{\text{Tb}\times\text{Ca}}\times 100$$

where, Cb is the mean of population density of *L. beckii* or *A. lepidosaphes* in control plots before treatment, Ta is the mean of population density of *L. beckii* or *A. lepidosaphes* in treated plots after treatment, Tb is the mean of population density of *L. beckii* or *A. lepidosaphes* in treated plots before treatment and Ca is the mean of population density of *L. beckii* or *A. lepidosaphes* in control plots after treatment.

Statistical analysis: Thus, all the gathered data were subjected to the one way analysis of variance (ANOVA) and the mean values compared with LSD test[14].

RESULTS

Mean population density of various developmental stages of *L. beckii*: Densities of all developmental stages of *L. beckii* on Valencia orange leaves (upper and lower surface) throughout two successive seasons (2018 and 2019) at Nubaria district, Beheira Governorate, Egypt are illustrated in Fig. 1a-b. Statistical analysis of data revealed that during the 1st season the gravid female was the highly dominant stage (26.99 and 28.17% of the total population). The 2nd instar female comes in second place (21.18 and 23.53%), followed by non-gravid female (17.93 and 32.21%), 2nd instar males (16.38 and 13.4%), 4th instar males (pupa) (12.81 and 10.17%) and finally 3rd instar males (pre-pupa) (4.72 and 4.08%) in upper and lower leaf surfaces, respectively. Similar results were obtained in the 2nd season, whereas the general means of gravid female reach 27.29% and 26.11 of the total population in the upper and lower surface of leaves, respectively. The 2nd instar females recoded 23.53 and 16.74% followed by non-gravid females 18.51 and 31.62%, 2nd instar males (17.01 and 13.02%), 4th instar males (pupa) (10.29 and 9.23%) and finally 3rd instar males (pre-pupa) (3.37 and 4.3%).

Parasitoid's host stage preference: The parasite, *A. lepidosaphes* oviposited on the various nymphal instars and adults of *L. beckii*. The mean rates of parasitism by *A. lepidosaphes* on the adults and various nymphal instars of *L. beckii* are shown in Fig. 2a-b. There were significant differences between the numbers of parasitized scales on the different stages of *L. bekcii* (parasitism rates) in both the upper and lower leaf surfaces throughout 2018 and 2019 seasons. However, the highest parasitism percentages were observed on the gravid females of *L. bekcii* in both upper and lower surfaces. While the lowest rates of parasitism were recorded on the 2nd male and female instars. On the other hand, there was no significant difference in the parasitism percentages between the third and fourth instars of the male nymphs and non-gravid females of *L. beckii*.

Effect of feeding source of the host, *L. beckii* on the parasitism rates by *A. lepidosaphes*: In spite of the significantly increasing presence of scales of *L. beckii* on the Valencia orange fruits (as a measurement unit) comparing with its on the leaves. The parasitism rates on the scales which attack fruits were significantly lower ($p<0.05$) than that obtained in case of the scales on leaves. When the *L. beckii* developmental stages feed on leaves, the parasite, *A. lepidosaphes* attack significantly more scales than when they were feed on fruits (df = 28, $p<0.05$, t = 4.465, f = 8.979 for 1st season and t = 7.55, f = 7.759 in the 2nd season). Data of the rates of parasitism on the immature and mature stages of *L. beckii* by *A. lepidosaphes* in both of leaves and fruits showed the same trend of the two years of study (Fig. 3a-b).

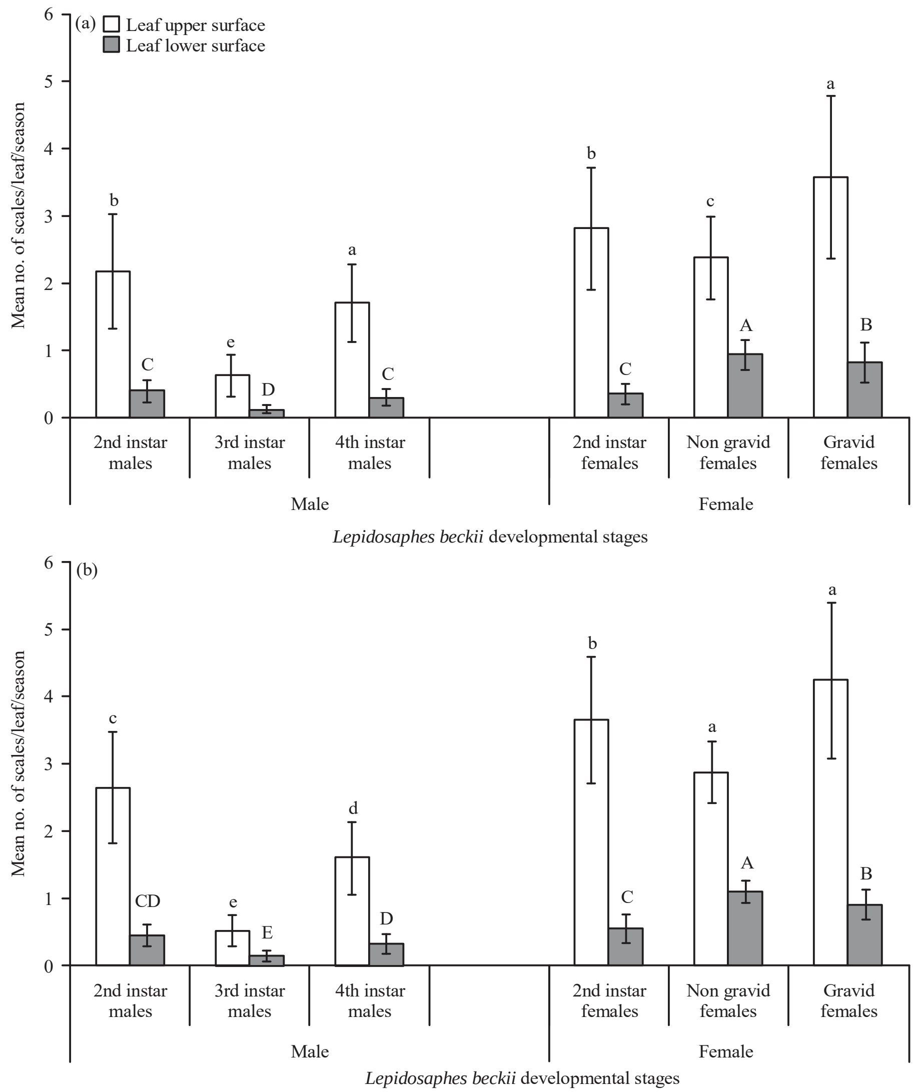

Fig. 1(a-b): General means of different developmental stages of *Lepidosaphes beckii* per a Valencia orange leaf throughout, (a) 2018 season on upper (F = 38.37, LSD = 0.4521) and lower surface (F = 70.974, LSD = 0.10535) and (b) 2019 season on upper and lower surface (F = 107.794, LSD = 0.0974)

Means followed by the same letter are not significantly different ($p \leq 0.05$), small letters for upper surface and capital letters lower surface

Effect of the prevailing climatic factors throughout the different seasons of the year: Regarding the rates of parasitism by *A. lepidosaphes* during the different seasons, results illustrated by Fig. 4 clarify noticeable variations in the parasitism rates from season to another. These differences may be due to the prevailing climatic factors throughout the considered seasons. The rates of parasitism reach maximum in summer season (39.83 and 42.29% in 2018 and 2019, respectively) at 31.74, 27.93 and 24.13 as an average of maximum, mean and minimum temperature of summer of 2018 and 31.37, 27.83 and 24.29 for the summer of 2019. In winter and fall seasons, the general means of parasitism rates significantly decreased to 10.31 and 30.51% in 2018 year and 27.08 and 33.75% in 2019 year, respectively. In general, results of the two consecutive years on the means of parasitism rates reveal that environmental conditions prevailing throughout summer are the most favorable for the development of parasites and its population increase.

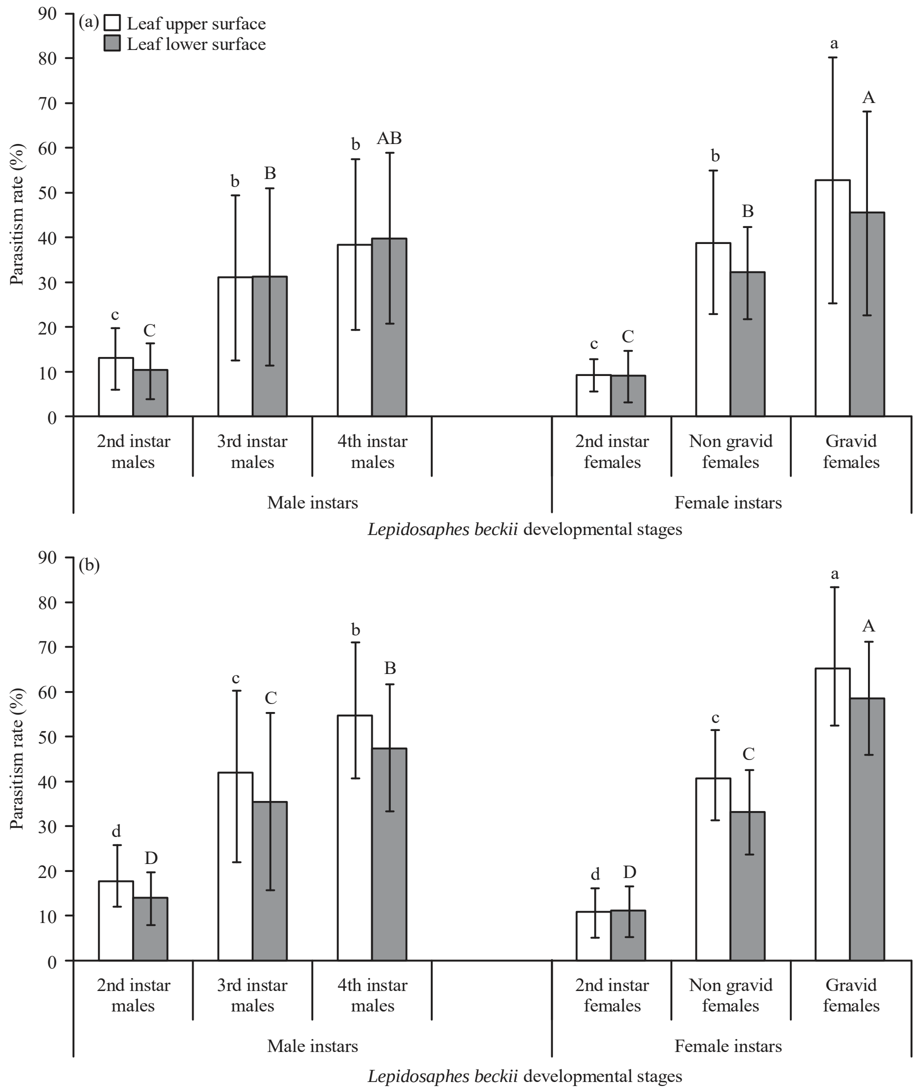

Fig. 2(a-b): Host stage preference of different life stages of *Lepidosaphes beckii* parasitized by *Aphytis lepidosaphes*, (a) 2018 season in upper (F = 22.62, LSD = 9.79) and lower surface (F = 22.78, LSD = 8.85) and (b) 2019 season in upper (F = 55.005, LSD = 7.8828) and lower (F = 54.125, LSD = 7.0251) Valencia orange leaves

Means followed by the same letter are not significantly different ($p \leq 0.05$)

Effect of different insecticides on *L. beckii* and its parasite, *A. lepidosaphes*: The effect of the first spray which achieved in mid-March to target 1st nymphal instar (crawlers) is illustrated in Fig. 5a. Among the foliar insecticide sprays, the overall mean of reduction percentages of *L. beckii* at five weeks post treatment can be arranged in descending order as follows; imidacloprid (85.36%), pyriproxyfen (80.56%), dinotefuran (69.98%), abamectin (59.33%) and azadirachtin (49.73%). In all treatments, the parasite, *A. lepidosaphes* was more affected by the tested insecticides than its host (*L. beckii*) whereas the reduction percentages of *A. lepidosaphes* were 86.77, 83.9 and 76.52% for imidacloprid, pyriproxyfen and dinotefuran, respectively. The differences in influence were statistically significant in case of abamectin (75.93%) and azadirachtin (72.77%). In contrast, in case of soil applications of imidacloprid and dinotefuran, the purple scale, *L. beckii* was more affected than its parasite whereas the reduction percentages recorded 66.39 and 56.2%, respectively for *L. beckii* and 58.42 and 49.82%, respectively for *A. lepidosaphes*.

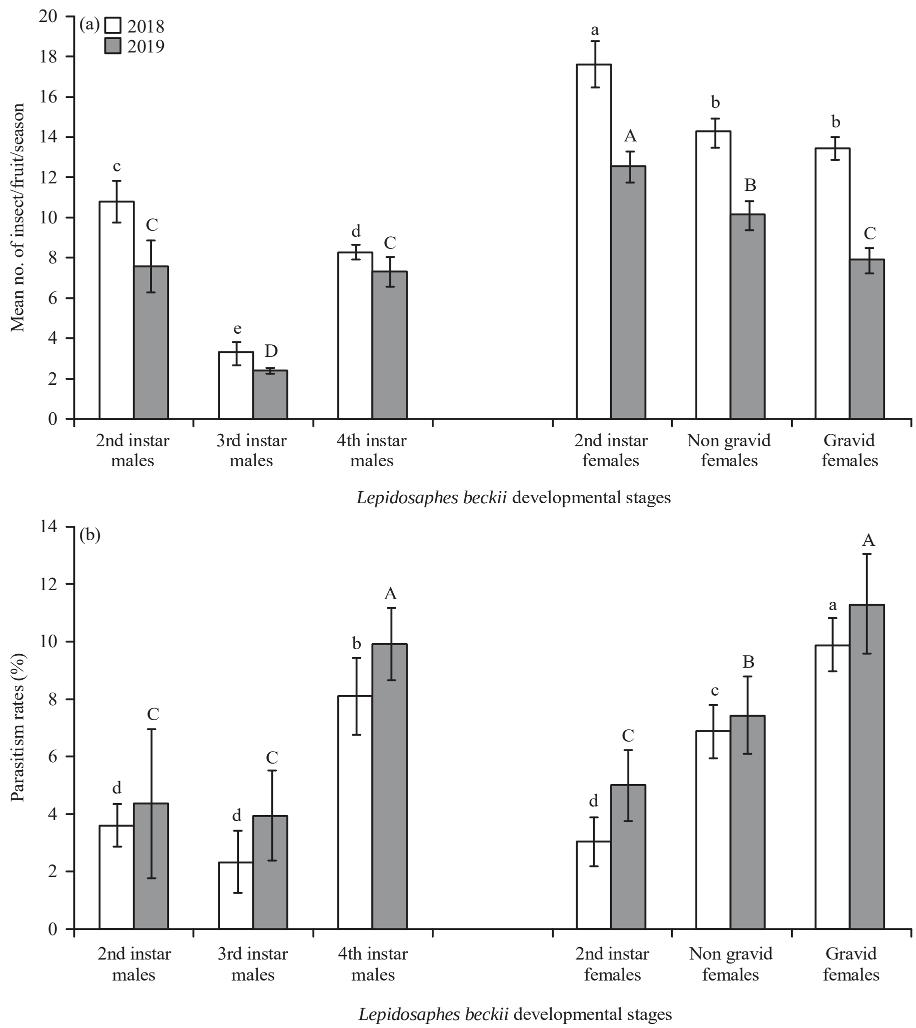

Fig. 3(a-b): Host stage preference of different life stages of *Lepidosaphes beckii* parasitized by *Aphytis lepidosaphes* on Valencia orange fruits throughout two successive seasons, 2018 and 2019, (a) General means of population density of different stages in 2017 (F = 52.247, LSD = 2.0306) and 2018 (F = 23.218, LSD = 2.0421) and (b) Rates of parasitism on different stages in 2017 (F = 49.410, LSD = 1.27495) and 2018 (F = 16.642, LSD = 2.2068)

Means followed by the same letter are not significantly different ($p \leq 0.05$)

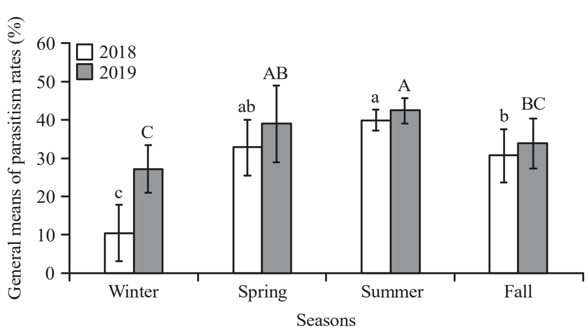

Fig. 4: General means of parasitism percentages by *Aphytis lepidosaphes* on *Lepidosaphes beckii* during the different seasons of years of 2018 (F = 20.782, LSD = 8.0706) and 2019 (F = 4.908, LSD = 8.8155)

Means followed by the same letter are not significantly different ($p \leq 0.05$), small letters for 2018 season and capital letters 2019

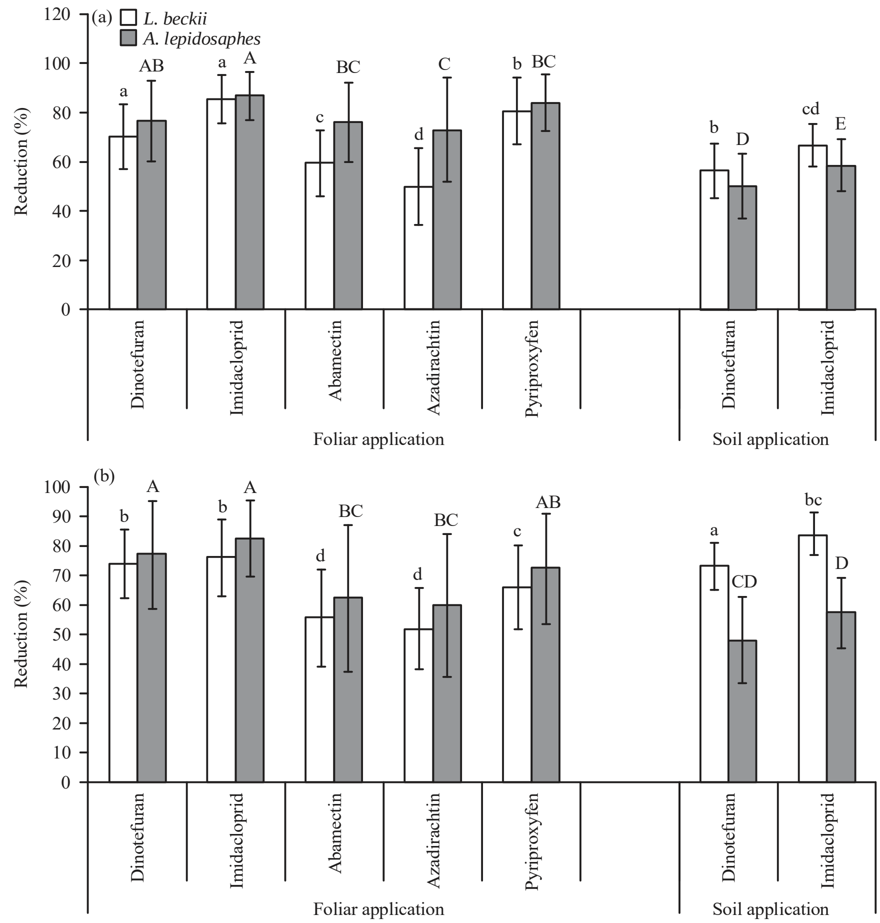

Fig. 5(a-b): Reduction (%) of the purple scale, (a) *Lepidosaphes beckii* (F = 26.097, LSD = 7.071) and its parasite, *Aphytis lepidosaphes* (F = 20.210, LSD = 8.3072) after 1st spray which set to target 1st nymphal instar (crawlers) and (b) *Lepidosaphes beckii* (F = 20.327, LSD = 7.06975) and its parasite, *Aphytis lepidosaphes* (F = 10.209, LSD = 10.5475) after 2nd spray which set to target gravid and non-gravid females

Small letters for *L. beckii* and capital letters for *A. lepidosaphes*

Considering the extracted general mean of reduction percentages after the 2nd application (achieved in mid-July to target the gravid and non-gravid females) of the different performed insecticides treatment (Fig. 5b). It is noticed that the treatment of imidacloprid was the most effective one in reducing the number of *L. beckii* and increased their reduction percentages up to 76.01% (foliar spray) and 83.56% (soil application). The treatment with dinotefuran gave a reduction percentage of 73.73% (foliar spray) and 72.94% (soil application) after 35 days post 2nd spray. The growth hormone, pyriproxyfen ranked the third and gave a reduction percentage of 65.96%. On the other hand, application of abamectin and azadirachtin gave the least reduction percentages of *L. beckii* (55.51 and 51.89%, respectively) (Fig. 5b). The detected effect of applied treatments in the 2nd spray against the parasite, *A. lepidosaphes* confirmed or revealed again that the foliar spray of imidacloprid and dinotefuran were proved to be the superior treatments achieving reduction (82.32%) and (76.9%) all over the inspection periods post-spraying followed by 72.09, 62.17 and 59.74% reductions for pyriproxyfen, abamectin and azadirachtin, successively. Meanwhile, the soil application treatments with imidacloprid and dinotefuran gave low reduction values of 57.34, 47.93%. The tested insecticides significantly reduced the population of *L. beckii* for up to 5 weeks after treatment.

DISCUSSION

The results of the present study implied that, the general means of the parasitism rates throughout the 1st year of this study were 30.49 and 27.84%, while during the 2nd year these rates reach to 38.4 and 33.13% in upper and lower Valencia orange leaf surfaces, respectively. The parasitoid had a significant preference for the older host stages. Abdel-Fattah and El-Saadany[15] recorded different rates of parasitism throughout the different months of the year reach to 84% in October. Also, from these results, it's obvious that the parasitism percentages in the upper surface of the Valencia orange leave were superior to the lower surface. The results also revealed that, when the *L. beckii* stages feed on leaves, the parasite, *A. lepidosaphes* attack significantly more scales (about 5-6 times more) than when they were feed on fruits. These results are in conflict with the observations of Boyero *et al*.[16] who found higher levels of parasitism rates by *Aphytis melinus* and *A. chrysomphali* on California red scale, *Aonidiella aurantii* which recorded on the citrus fruits than in twigs. Environmental conditions particularly climatic factors prevailed during winter and autumn seasons were quite unfavorable for the parasite, *A. lepidosaphes*. On contrast of these results Hafez *et al*.[17,18] observed the highest percentages of parasitism on *L. beckii* by immature stages of *Aphytis* spp. during the winter season followed by spring, summer and finally autumn season while the period from March to August was the appropriate period for emerging the adults of the parasite. Also, these results are slightly different than those of Aly[19] who found that the rates of parasitism by *A. lepidosaphes* on *L. beckii* were low in summer and increased in autumn season but she didn't noticed the parasite during winter season.

The results also showed that, using the five insecticides (imidacloprid, dinotefuran, pyriproxyfen, abamectin and azadirachtin) in integrated pest management programs (IPM) reduced scale density on leaves but gives adverse side effect on the parasitoid of *L. beckii*. Meanwhile, use of imidacloprid and dinotefuran as a soil application can eliminate this defect. These results are in harmony with the results of Machigua[20] in their study on the calico scale (armored scale) and its natural enemies. They found that the soil application of the systemic insecticides, dinotefuran reduce the density of this scale on the leaves and did not reduce the population of its natural enemies. In this work, when the crawlers was the target (the 1st application in did-March) the insect growth regulator pyriproxyfen ranked the 2nd after imidacloprid. Meanwhile, dinotefuran was in the same rank when the females were the target. Quesada *et al*.[21] tested pyriproxyfen, chlorantraniliprole, spiromesifen and spirotetramat against *Chionaspis pinifoliae* and *Aspidiotus nerii* (armored scales) and two species of soft scales *Eulecanium cerasorum* and *Toumeyella pini*. They found that the four insecticides were killed armored scales when the crawler stage was the target of application. Also, these results are in inconsistent with the results of Sadof and Sclar[22] who found that the soil applications of imidacloprid did not effectively control euonymus scale because armored scales do not feed on plants in the same manner as other sucking insects that are easily killed by this pesticide. Dewer *et al*.[23] tested five insecticides azadirachtin, pyriproxyfen, acetamiprid, emamectin benzoate and summer mineral oil and their mixtures against *L. beckii* and its parasitoid *A. lepidosaphes* they found that each of acetamiprid and emamectin benzoate show less reduction percentages to the parasitoid while each of azadirachtin, pyriproxyfen and summer mineral oil have given the highest reduction (%) values for *L. beckii* and its parasitoid.

CONCLUSION

The main biological control agents of *L. beckk* are the parasitoid *A. lepidosaphes.* All nymphal instars and adult females of *L. beckii* were parasitized by the parasitoid, *A. lepidosaphes*. The parasitoid had a significant preference for the older host stages. The parasitism percentages of *L. beckii* which attacks the fruits of Valencia orang were significantly lower than its on the leaves. The tested insecticides (imidacloprid, dinotefuran, pyriproxyfen, abamectin and azadirachtin) showed high effectiveness against *L. beckii* and its parasite, but a better effect, reflected in high reduction percentages in *L. beckii* compared with low reduction percentages in the parasite was found for the soil application with dinotefuran, imidacloprid.

SIGNIFICANCE STATEMENT

This study discovers the performance of *Aphytis lepidosaphes* (Hymenoptera: Aphelinidae) as a biological control agent of *Lepidosaphes beckii* under field conditions. Also, the study provided that the soil application of systemic insecticides of *L. beckii* was less harmful to its parasite that can be beneficial for try to maximize the role of biological control of *Lepidosaphes beckii* in the integrated pest management programs.

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