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Use of auxiliaries in biological control against the citrus leafminer *Phyllocnistis citrella* S. (Lepidoptera, Gracillariidae) in the region of El Tarf (Northeastern Algeria)

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Abstract

Citrus cultivation in Algeria, covering 45,000 hectares, faces challenges such as aging orchards, inadequate labor, limited water resources, and pest infestations.The citrus leaf miner (*Phyllocnistiscitrella*) is a significant pest affecting citrus orchadsin Algeria. First detected in 1994, it has since caused significant damage to young citrus plantations, particularly lemon trees. The aim of the present study is to assess the effectiveness of biological control methods against *P. citrella* in two lemon orchards in El Tarf province: a 16-year-old untreated orchard and a 3-year-old orchard treated with systemic insecticides. Weekly leaf sampling was conducted from October to December 2019 to assess infestation rates, larval populations, and parasitism rates.Results indicated that the treated orchard had significantly lower larval counts and infestation rates compared to the untreated orchard. Specifically, the treated orchard showed reduced numbers of larvae in early developmental stages and higher parasitism rates by *Semielacherpetiolatus* and *Pnigalio* sp. The statistical analysis revealed significant differences in infestation and parasitism rates between the two orchards and across the sampling months. These findings highlight the effectiveness of biological control in managing *P. citrella* and suggest that it can be a viable alternative to chemical control, offering potential benefits for sustainable citrus pest management.

Key words: Citrus fruits, *Phyllocnistiscitrella* S., biological control, *Semielacherpetiolatus*, *Pnigalio* sp.,El Tarf-Algeria.

Introduction

In Algeria, citrus cultivation covers 45,000 hectares, representing 16% of the total tree area (Anonymous, 2002). Despite its significant economic value, the citrus industry has stagnated over the past decade, and current production levels are insufficient to meet national demand. This decline can be attributed to several factors: the aging of orchards, a shortage of skilled labor, inadequate water resources, and the emergence of various diseases, compounded by the lack of an effective control strategy.In addition to these challenges, citrus orchards are threatened by numerous pests, many of which are highly deleterious. Notable among these are the Mediterranean fruit fly (Ceratitis), scale insects, mites, and the citrus leaf miner, which has emerged as a significant concern over the past decade (Khfif, 2022). This microlepidopteran pest poses a serious threat, affecting the tree's vigor, fruit yield, and quality.*Phyllocnistiscitrella* Stainton (Lepidoptera: Gracillariidae) has been extensively studied worldwide, with research primarily focusing on developing efficient and ecological control methods for managing larval populations. The endophytic nature of this pest complicates chemical control, which remains the predominant method employed by citrus growers (Boualem, 2009).Since the introduction of *Phyllocnistiscitrella* in Algeria, several studies have been conducted to investigate the pest's population dynamics and its impact on citrus crops across different growing regions. These studies have assessed infestation levels and identified natural regulatory factors affecting the phytophage (Berkani, 1995; 1999; 2003; Doumandji et al., 1999; Sahraoui et al., 2001; Boualem, 2009).

This study focuses on assessing the effectiveness of biological control methods against the citrus leaf miner, *Phyllocnistiscitrella*, in the wilaya of El Tarf, specifically within the Bounamoussa perimeter, a recognized citrus-growing zone. By implementing biological control, the study aims to reduce dependence on chemical methods, which are often ineffective and harmful to beneficial insects. The goal is to evaluate the impact of these biological control strategies both at the regional plant protection station of El Kous and across various citrus orchards throughout the wilaya of El Tarf.

1. Materials and methods

1.1. Presentation of the study site

The study was conducted in two lemon orchards (Citrus limon) in the Ben M'Hidi and Zerizer districts of the El Tarf province. The first orchard, known as the Hemmeda-Ben M’hidi Station, is located in Hemmeda in Ben M'Hidi (36°46'36.5"N, 7°53'03.4"E), and covers an area of 2 hectares and is located at an altitude of 505 meters. It is bordered to the north by National Road No. 44 and is surrounded on the south, east, and west by agricultural land.The second orchard, namely the SidiAbd-Zerizer Station, is situated in SidiAbd (36°44'27.2"N, 7°55'04.4"E) within the Zerizer district. Covering an area of 3 hectares, the orchard is bordered to the north by the Lalaimiya Pilot Farm, to the south by agricultural land, to the east by the Zerizer wastewater treatment plant, and to the west by the Bounamoussawadi (Figure 1).

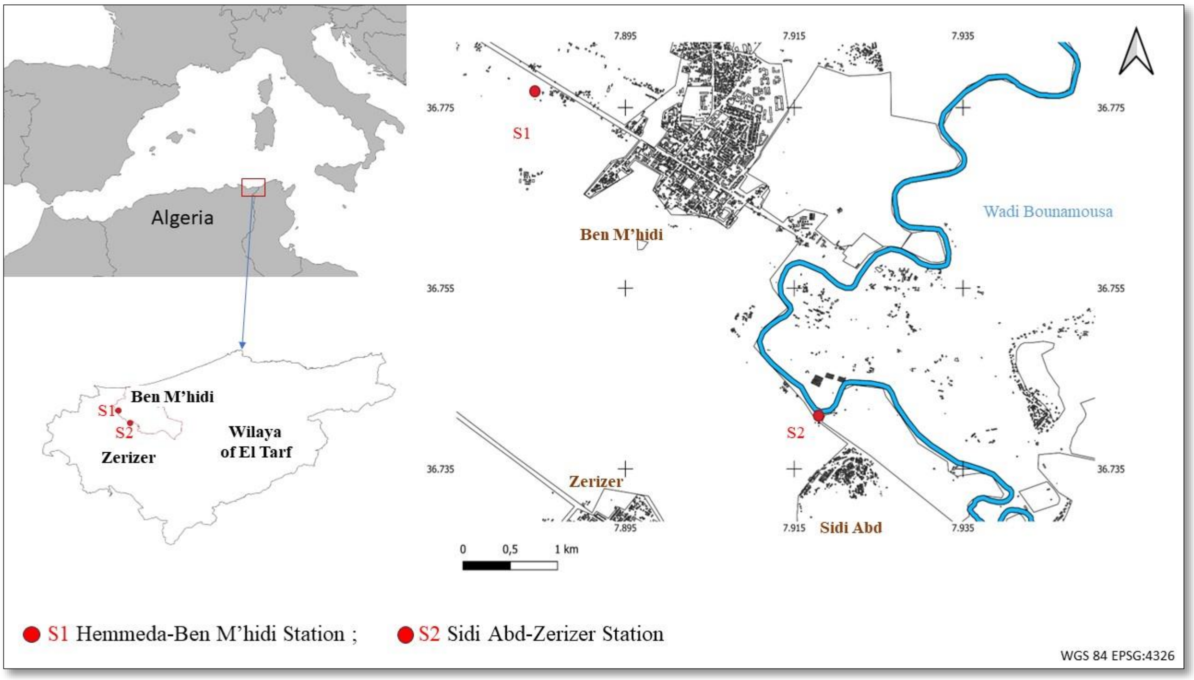

Figure 1. Location of the studied area in Edough Peninsula (Northeastern coast of Algeria).

1.2. Samplingand experimentaldesign

To limit the damage caused by the citrus leafminer, a pest with significant proliferation and destructive power (Mechelany and Matny, 1998), we conducted a comparative study between biological and chemical control methods during the autumn harvest, which coincides with the third sap flush. This study was carried out in two lemon orchards: one that is 16 years old and untreated, and the other that is 3 years old and treated with systemic insecticides.

Field sampling involved collecting lemon leaves from Citrus limon trees once a week from 03/10/2019 to 19/12/2019, with 10 randomly selected trees at each observation post, and 5 leaves from each tree, oriented east, west, north, south, and center, were placed in plastic bags. In the laboratory at the regional plant protection station (S.R.P.V) of El Kous - El Tarf, the samples were examined under a binocular magnifying glass. Three parameters were calculated including: (1) the infestation rate, calculated as the number of infested leaves (either side) relative to the total number of leaves using the formula TI = (Number of infested leaves / Total number of leaves) × 100. (2) Population assessment, which is the count of the different developmental stages of the leafminer on each leaf surface, including live larvae (L1, L2, L3), prenymphs, and chrysalises. In addition, (3) the parasitism rate, was determined by calculating the number of parasitized larvae, prepupae, and nymphs relative to the total number of individuals, using the following formula:

Tp = (Number of parasitized individuals / Total number of individuals observed) × 100.

We calculated the relative abundance of different stages of *Phyllocnistiscitrella* in two types of orchards (treated and untreated) as a percentage of the total number of individuals in each group.

Figure 2. Harvesting citrus leaves and analyzing samples using a binocular microscope

1.3. Data analysis

A chi-squared test was conducted to explore the association between orchard types and larval stages in terms of abundance.Two beta regression analyses were conducted using the betareg function from the R package betareg (Zeileis et al., 2016) to test whether month and orchard type have effects on infestation and parasitism rates. A PERMANOVA test was performed using the adonis function from the vegan package (Oksanen et al., 2007) to assess the effects of month and orchard type on the composition of *Phyllocnistiscitrella* stages. Additionally, pairwise PERMANOVA was conducted to examine differences in the composition of *Phyllocnistiscitrella* stages between months.

2. Results

In the treated orchard, the counts of larvae and other developmental stages are relatively low compared to the untreated orchard. For instance, in December, the counts are 11 for Larva1, 12 for Larva2, and 7 for Larva3, with only 5 individuals in the Prenymph stage and 2 in the Chrysalis stage. In contrast, the untreated orchard shows significantly higher counts across all stages. For example, in November, abundances are 139 for Larva1, 191 for Larva2, and 96 for Larva3, along with 13 and 8 individuals in the Prenymph stage and Chrysalis stage respectively (Figure 3).

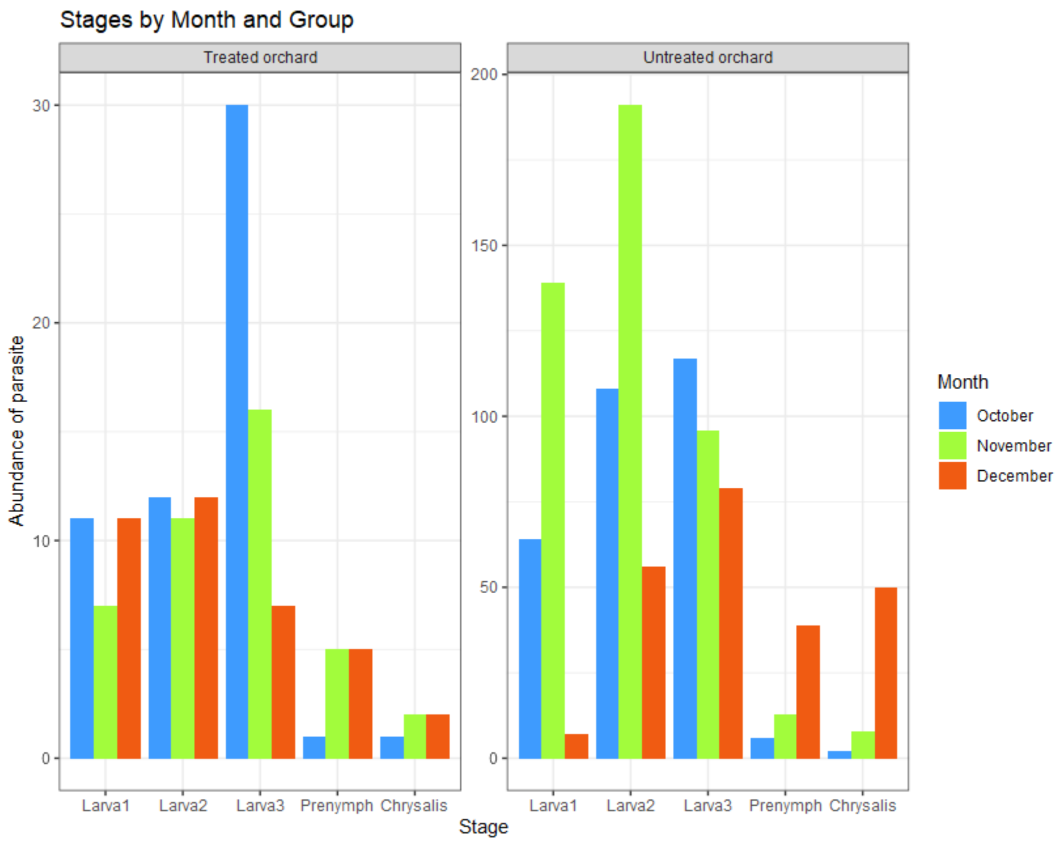

Figure 3. Abundance of different developmental stages of *Phyllocnistiscitrella* larvae in untreated and treated orchards over three months

The treated orchard exhibits a greater proportion of Larva.3 individuals (39.85%), whereas the untreated orchard has a higher percentage of Larva.2 (36.41%). While both orchards show comparable distributions in the earliest (Larva.1: ~21.8% vs 21.54%) and latest

(Prenymph: 8.27% vs 5.95%, Chrysalis: 3.76% vs 6.15%) developmental stages, they diverge significantly in the intermediate larval phases.

This difference is most pronounced in Larva.3 (39.85% treated vs 29.95% untreated) and Larva.2 (26.32% treated vs 36.41% untreated), suggesting the treatment may influence larval development progression (Figure 4).

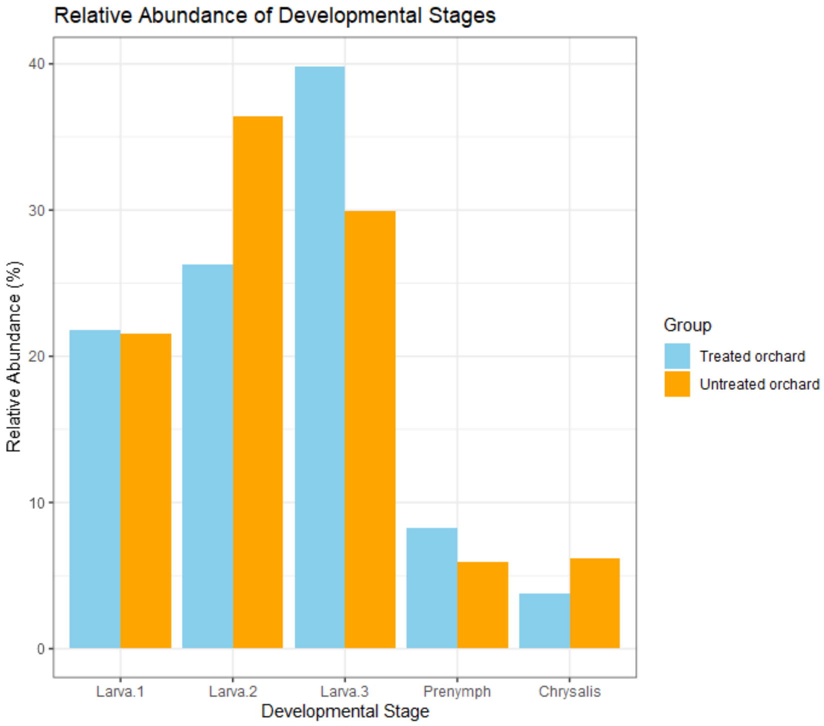

Figure 4. Relative abundance of *Phyllocnistiscitrella*across developmental stages in treated vs. untreated citrus orchards

The abundance of *Phyllocnistiscitrella* larvae per 600 leaves is significantly higher in the untreated orchard compared to the treated orchard across all larval stages (Table 1). The Pearson's Chi-squared test result (X-squared = 7.072, df = 2, p-value = 0.02913) shows a statistically significant association between the number of *Phyllocnistiscitrella* larvae and orchard habitats. In the untreated orchard, the number of larvae increases progressively from Larva 1 to Larva 3. In contrast, the treated orchard exhibits a peak in larval numbers at Larva 2, followed by a subsequent decline.

Table 1. Number of *Phyllocnistiscitrella* larvae at different stages in untreated and treated orchards.

Larval stage	UntreatedOrchard	TreatedOrchard
Larva1	210	29
Larva2	355	35

Larva3	292	53
Number of sampledleaves	600	600

Concerning the evaluation of the prenymph stage, 58 individuals of *Phyllocnistiscitrella*were recorded for 600 leaves sampled from the untreated orchard compared to 11 individuals for the same number of leaves sampled from the treated orchard. Additionally, the number of individuals of *Phyllocnistiscitrella* at the chrysalis stage per 600 leaves sampled was greater in the untreated orchard than in the treated orchard with 60 and 5 individuals respectively.

Phyllocnistiscitrella infestations in the two lemon orchards

The bar chart (Figure 5) shows that in the untreated orchard, infestation rates of the citrus leaf miner *Phyllocnistiscitrella* are consistently high, starting at 83.6% in October, rising to 98.0% in November, and slightly decreasing to 90.7% in December. In contrast, the treated orchard exhibits significantly lower infestation rates, with 24.8% in October, 24.0% in November, and 12.7% in December.

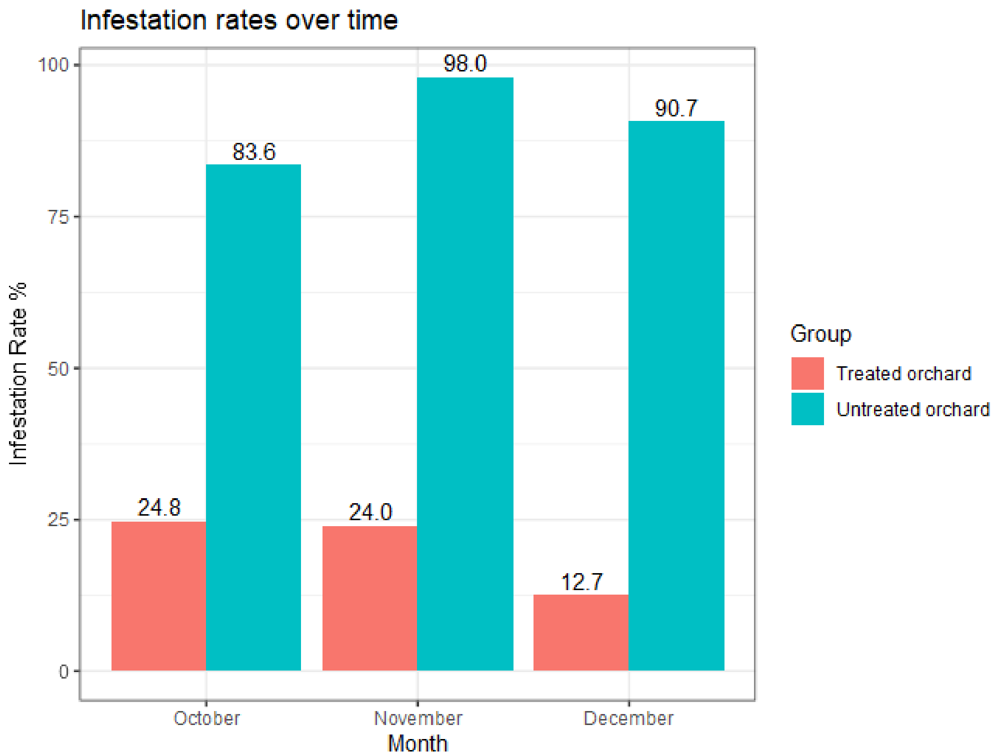

Figure 5. Monthly comparison of infestation rates between treated and untreated lemon orchards

Parasitism rate in the two lemon orchards

The parasitism rates of *Semielacherpetiolatus* and *Pnigalio* sp. on *Phyllocnistiscitrella* during the autumn period indicate that the treated orchard experienced a higher rate of parasitism (43%) compared to the untreated orchard (28%).

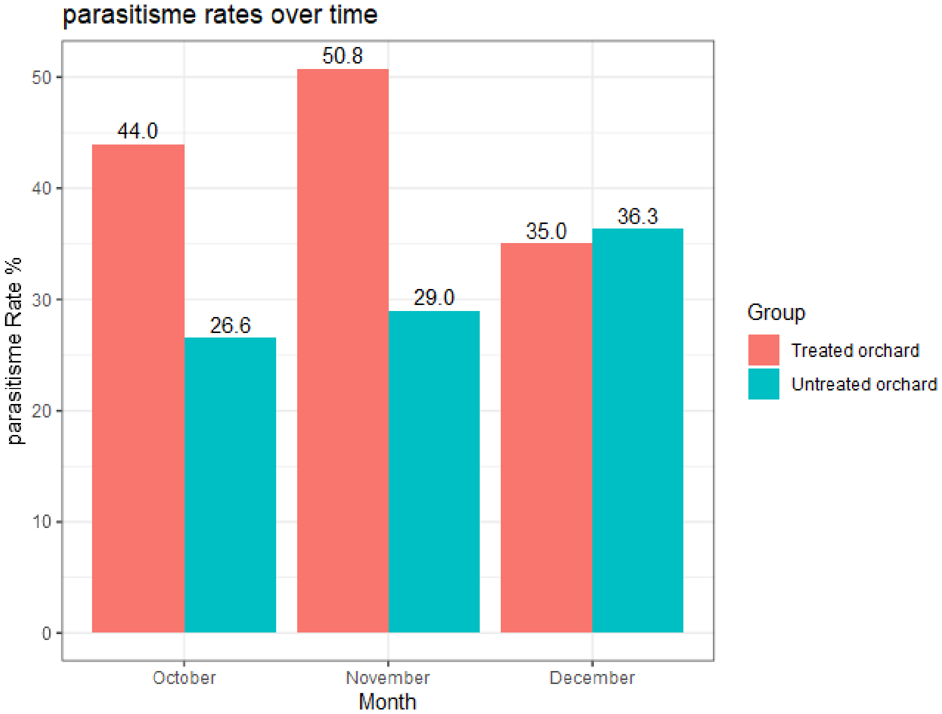

Figure 6. Monthly comparison of parasitim rates between treated and untreated lemon orchards

The *Semielacherpetiolatus*was the dominant parasitoid compared to *Pnigalio* sp. In the untreated orchard, *Semielacherpetiolatus* had a parasitism rate of 22%, whereas *Pnigalio* sp. had a parasitism rate of only 6%. In the treated orchard, *Semielacherpetiolatus* achieved a parasitism rate of 31%, compared to 12% for *Pnigalio* sp. (Table 2).

Table 2. Parasitism rate of *Semielacherpetiolatus* and *Pnigalio*spin the two lemon orchards

	UntreatedOrchard		**TreatedOrchard**	
	Semielacherpetiolatus	***Pnigaliosp***	***Semielacherpetiolatus***	***Pnigaliosp***
Parasitism rate	**22%**	**6%**	**31%**	**12 %**

Table 3 shows that in the untreated orchard, the infestation rate (90%) significantly exceeds the parasitism rate (28%). In contrast, the treated orchard has a lower infestation rate (22%) but a higher parasitism rate (43%).

Table 3. Relationships between infestation and parasitism rates in the untreated orchardand the treated orchard

UntreatedOrchard		**TreatedOrchard**	
Infestation rate	Parasitism rate	Infestation rate	Parasitism rate
90%	28%	22%	43%

The beta regression analysis (Figure 7 A) shows that the untreated orchard significantly increases the infestation rate compared to the treated orchard (estimate = 3.6476, $p < 2e-16$). Relative to the baseline month (October), November shows a significant increase in the infestation rate (estimate = 0.5777, $p = 0.0371$), whereas December does not have a statistically significant effect (estimate = -0.2640, $p = 0.3898$).The beta regression analysis of parasitism rates (Figure 7 B)showed that the untreated orchard significantly decreases theparasitism rate compared to the treated orchard (estimate = -0.59715, $p = 0.0391$). However, November and December do not show significant effects on the parasitism rate compared to October (estimates = 0.15031 and -0.07699, with p-values of 0.6519 and 0.8337, respectively).

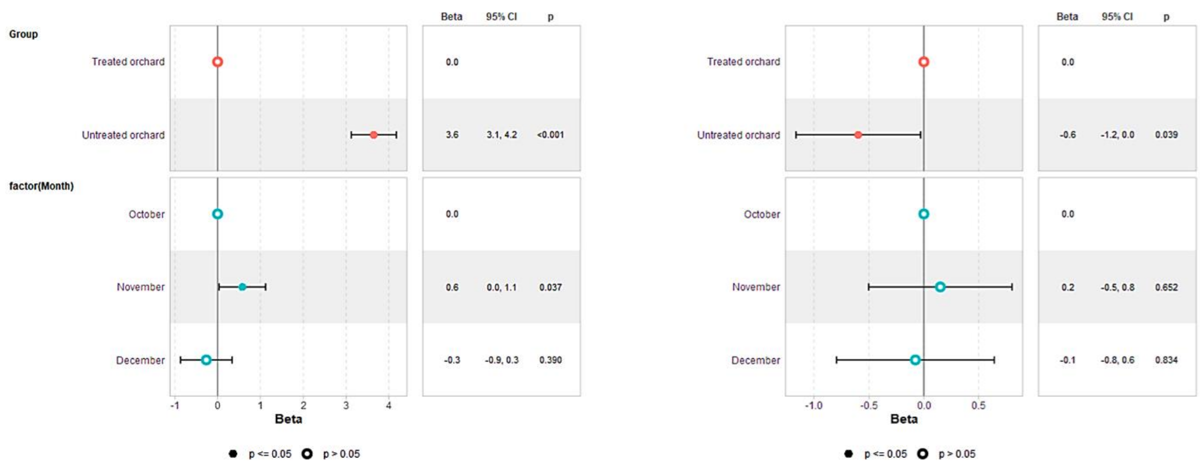

Figure 7. Beta regression results showing the effects of month and orchard group (treated and untreated) on infestation rates (A) and parasitism rates (B).

The PERMANOVA results indicate that both Month and Group (treated vs. untreated) significantly affect the community composition of *Phyllocnistiscitrella*. The Group factor explains 44.14% of the variation with a highly significant p-value of 0.001, while Month accounts for 13.86% with a significant p-value of 0.010. The pairwise PERMANOVA results show a significant difference in community composition between October and December (p-value = 0.025), but no significant differences between October and November (p-value = 0.503) or November and December (p-value = 0.246).

Discussion

Natural regulation is considered one of the most important factors in maintaining ecosystem balance. Insects, like all living organisms, are dependent on the environmental conditions in which they live. Mortality can be influenced by both abiotic factors (such as climate and habitat conditions) and biotic factors (such as parasitism, predation, and intraspecific competition). These biotic factors are intrinsic to the ecosystem; each pest species has a unique set of more or less specific natural enemies. These various antagonists, primarily predatory and parasitoid auxiliaries, mainly affect *Phyllocnistiscitrella*.

The infestation rates recorded during the autumn period were relatively high. The analysis of these results reveals a consistent trend in leaf miner populations throughout autumn. This observation corroborates the findings of Lo Pinto and Fucarino (2000), who reported that in Sicily, the citrus leaf miner remains active during both the summer and autumn months.

In his study on the bioecology of *Phyllocnistiscitrella* and its parasitic complex in the Mostaganem region (north-west Algeria), Boualem (2009) suggests that the significance of infestations during the summer outbreak may be linked to the climatic conditions of that period. Specifically, the summer climate, characterized by relatively high and particularly favorable minimum temperatures, promotes the development of the phytophage, leading to faster growth of its various biological stages and a consequently shorter development period. Under natural conditions, the duration of the life cycle of *Phyllocnistiscitrella* varies according to local climatic conditions. For an average temperature of 30 ± 1°C, the pre-imaginal cycle of *Phyllocnistiscitrella* in Algeria lasts 12.57 days (Berkani, 1999). In contrast, on Réunion Island, it ranges from 13 to 15 days under temperatures between 27 and 30°C (Quilici et al., 1995). Likewise, it has been observed that the vegetative mass produced by trees in summer is lower compared to that in spring and autumn. Consequently, the number of infested leaves increases, leading to a situation where nearly all sampled leaves contain at least one leaf miner. During summer, the climatic conditions were favorable for the development of *Phyllocnistiscitrella*, falling within the pest's development threshold of 12.2 to 46°C as reported by Guerout (1974).

For this purpose, the female chooses young leaves from the apical part of the branch and abandons older leaves with more or less lignified parenchyma in order to optimize the chances of survival of her offspring. However, this choice can lead to overpopulation, resulting in increased competition for food in summer and autumn and an increase in larval mortality.

Khechna (2011) observed in his study on the parasitic incidence in *Phyllocnistiscitrella* populations that there is a perfect synchronization between the pest and its parasitic complex across the three citrus varieties studied. He noted that the rate of parasitism peaks during the summer and autumn seasons.In Tunisia at the SidiThabet site, Chermiti et al. (1999) report that in a treated orchard in the autumn of 1995 the activity of the parasitic complex evaluated by parasitized *Phyllocnistiscitrella*is relatively high, it becomes zero when it is associated with chemical control. To this end, the drop in the infestation rate could be caused by the first chemical application. However, the resumption of parasitic activity is probably linked to increased activity of parasitoids in autumn.The beta regression analyses further demonstrated that both month and orchard type significantly influence infestation and parasitism rates. Specifically, the treated orchard exhibited lower infestation rates and higher parasitism rates by *Semielacherpetiolatus* and *Pnigalio* sp., suggesting that biological control not only reduces pest abundance but also enhances natural enemy activity.

Amalin et al. (2002), indicates that biological control remains the most effective method for regulating*Phyllocnistiscitrella*populations. In our study, the most dominant species is *Semielacherpetiolatus*(ectoparasitic hymenoptera at the L2-L3 larval stage). This parasite exhibited the highest rates in the two orchards. In Algeria, *S. petiolatus* is the only one of the two introduced species that has survived in the Mostaganem region in the west of the country (Boualem, 2002; Boualemet al., 2007). It is also the only one of the four species released in the Mitijda (plain of the Algiers hinterland, in northern Algeria) to have acclimatized (Sahraoui et al., 2001).This finding is consistent with Boualem's (2009) study, where *S. petiolatus* was also identified as the most abundant exotic species, alongside the native species *Cirrospiluspictus*. In Tunisia, *S.petiolatus* therefore presents many attributes of a good biological control agent, such as high parasitism level, host-feeding, climatic adaptability and high dispersal capacity. Its biologicalcontrol action would supplement the action of the native parasitoids *Pnigalio* sp. and *C.pictus*, which had an average percentage of parasitism of 6% (Braham et al., 2006).

Conclusion

The citrus leaf miner (*Phyllocnistiscitrella*) poses a significant threat to young citrus plantations both in Algeria and globally. While chemical control methods have demonstrated effectiveness against this pest, they are often unsustainable due to their potential negative impacts on the environment and non-target species. In contrast, biological control, which leverages natural enemies such as predators and parasitoids, offers a more sustainable approach. This study highlights that biological control methods not only reduce *P. citrella* populations but also support the activity of beneficial insects, thereby contributing to a more balanced ecosystem. Given these advantages, biological control should be prioritized as a primary strategy in managing *P. citrella*, supplemented as needed by other methods. Ongoing research and adaptation of biological control techniques are essential for enhancing their efficacy and ensuring sustainable pest management in citrus orchards.

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