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# PAUESIA SILANA TREMBLAY, 1969: BIOLOGICAL CONTROL AGENT FOR THE MAGHREB PINE APHID CINARA MAGHERBICA MIMEUR, 1934 ON PINUS HALEPENSIS MILL., 1768 IN ALGERIA

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### **Abstract**

The presence of *Pauesia silana* Tremblay, an aphid parasitoid (Hymenoptera: Braconidae: Aphidiinae), has been recorded in Algeria. This parasitoid was found in association with *Cinara pini* Linnaeus, which infests *Pinus nigra* Arn. and *Pinus halepensis* Mill. This study, conducted from 2019 to 2021, aimed to identify the natural enemies of the Maghreb pine aphid, *Cinara magherbica* Mimeur, in the Aleppo pine forests (*Pinus halepensis* Mill.) in the Khenchela province. Specimens were collected from mummified aphid colonies, randomly sampled across three forest areas. The study analyzed the correlation between aphid densities and ecological parameters, including mummy counts, parasitism rates, and the number of emerged parasitoids. The results revealed that while the parasitism of *P. silana* showed a slight increase, there was a concurrent decline in adult emergence. Secondary parasitoid species identified include *Asaphes vulgaris* Walker, *Pachyneuron aphidis* Bouché, and *Syrphophagus aphidivorus* Mayr (Hymenoptera: Chalcidoidea, Pteromalidae).

Keywords: Antagonist, Braconidae, conifers, pest aphid

## INTRODUCTION

In Mediterranean regions, the Aleppo pine (*Pinus halepensis* Mill., 1768) covers approximately 6.8 million hectares (Le Houerou, 1980). The Maghreb, as described by Quézel and Barbero (1992), offers the most conducive environment for its proliferation, with its presence spanning across all mountainous terrains.

In Algeria, *P. halepensis* constitutes the predominant forest species, covering (800 000 ha), or 35% of the forested land (Bentouati *et al.*, 2005), and is a principal choice in reforestation initiatives. Thriving primarily in the semi-arid bioclimatic zone, it adapts to various soil types (Louni, 1994). According to Kadik (1983), *P. halepensis* is prevalent across the mountainous regions, from the coastal

areas to the Saharan Atlas, notably in the western (Sidi Bel Abbès, Saida, Ouarsenis), central (Médea, Boghar, Bibans Mountains, Ouled Nail Mountains), and eastern (Aurès, Tébessa) parts of the country. The species faces threats from climatic adversities such as drought and anthropogenic disturbances like fires. The pine processionary caterpillar (*Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775)) is a significant biotic stressor and is partially responsible for the setbacks in Algeria's reforestation efforts initiated in the 1970s, which covered one million hectares (Bachir *et al.*, 2017).

Among the various insect groups affecting Aleppo pine, aphids hold a significant position. Blackman and Eastop (2023) have identified approximately 170 pine-feeding aphid species associated with









76 pine hosts. Severe infestations can cause considerable damage, leading to up to 30% loss in annual tree volume growth. For instance, in 1990, Pineus pini (Goeze, 1778) infestations in Malawi caused economic losses estimated at USD 5.2 million (Morris, 2006). Notably, Eulachnus agilis (Kaltenbach, 1843), Eulachnus brevipilosus (Börner, 1940), and Eulachnus rileyi (Williams, 1911) are recognized as destructive pests on both young and mature pine trees, causing symptoms like needle discoloration (yellow or white), deformation, premature needle drop, and potentially leading to stunted growth or branch death (Halperin, 1986; Binazzi, 1989; Alford, 2012).

In the Maghreb region, aphid species associated with Aleppo pine are relatively understudied. In Tunisia, recorded species include E. agilis, Eulachnus nigricola (Pašek, 1953) (Ben Halima Kamel, 2012; Kanturski et al., 2017), Eulachnus tuberculostemmatus (Theobald, 1915), Cinara palaestinensis (Hille Ris Lambers, 1948) and Cinara maghrebica (Mimeur, 1934) (Ben Halima Kamel et al., 2019). Algerian reports have identified C. maghrebica, Cinara pini (Linnaeus, 1758), E. tuberculostemmatus (Laamari et al., 2009; Aroun, 2015; Benhamacha et al., 2017; Hedjam et al., 2017; Aggoun et al., 2022). Remarkably, C. maghrebica was first documented in Morocco by Mimeur in 1034. Aphids from the Cinara genus are known to feed on the foliage and woody structures of Pinaceae and Cupressaceae families, including genera such

as *Pinus*, *Abies*, *Larix*, and *Picea*, affecting trunks, branches, and roots, and potentially causing severe damage by constricting and desiccating plant parts (Blackman and Eastop, 2023).

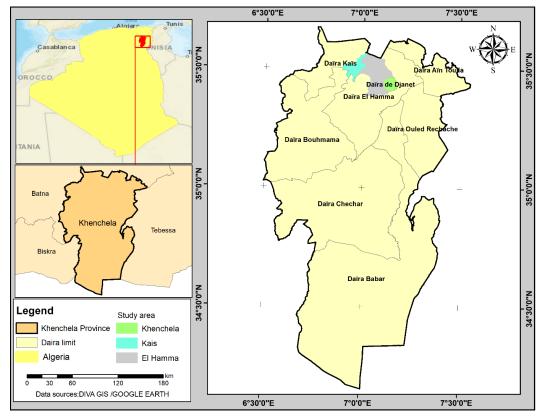
In Algeria, *Pauesia silana* (Tremblay, 1969) has been noted as a parasitoid of *C. maghrebica* on *P. halepensis* (Aroun *et al.*, 2016; Aggoun *et al.*, 2022) and *C. pini* on *Pinus nigra* (Arnold, 1785) (Benhamacha *et al.*, 2017). Similarly, on *P. halepensis*, it was reported on *C. palaestinensis* in Malta (Mifsud and Starý, 2009) and Tunisia (Ben Halima Kamel *et al.*, 2022). It also parasitized *C. palaestinensis* on *Pinus pinea* (Linnaeus, 1753) in Greece (Kavallieratos *et al.*, 2016). Additionally, its presence was documented in Lithuania on *C. pini* on *P. halepensis* (Havelka *et al.*, 2021). *P. silana* is characterized as a solitary endoparasitoid (Starý, 1970; Kavallieratos *et al.*, 2001; Kavallieratos *et al.*, 2016; Havelka *et al.*, 2021; Das and Chakrabarti, 2023).

This study aims to assess the biotic potential of *P. silana* as a parasitoid on *C. maghrebica* across three distinct *P. halepensis* stands representing natural, suburban, and urban environments.

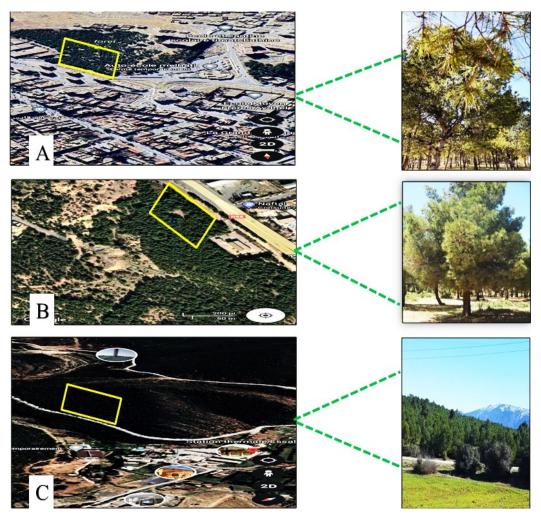
#### MATERIALS AND METHODS

## **Study Region**

The study was conducted in the Khenchela province, located in northeastern Algeria, which is part of the Aurès massif. Here, the Aleppo pine (*Pinus halepensis*)



1: The geographical location and specific areas studied in the Khenchela province, Algeria



2: Geomorphology and habitats of the studied areas in Algeria (2022) A: Kais, B: Khenchela, C: El Hamma

I: Geographical coordinates, sea level altitude, and the climate of the study's three regions

Localities	Coordinates	Altitude	Nature	
Kais	35° 29'41" N6° 55′ 27"E	902 m	Urban habitat	
El Hamma	35°26'14" N 7°05'2" E	1290 m	Natural habitat	
Khenchela	35°26'8" N 7°08'35" E	1 128 m	Sub urban habitat	

is notably abundant here, especially within the extensive stands of Béni-Imloul (75,000 hectares) and Ouled-Yakoub (20,000 hectares) (Bentouati and Bariteau, 2005). This region, nestled in the Saharan Atlas, experiences a predominantly Saharan climate. For this research, three distinct forest localities were selected, as depicted in Figs. 1, 2.

# Sampling

Field sampling was conducted twice a month from 2019 to 2021 in the Khenchela region, where three areas of different nature were selected: urban habitat, represented by the Kais area; suburban habitat, corresponding to Khenchela; and the natural habitat, represented by the El Hamma area.

Urban habitat refers to areas with high human density and developed infrastructure, while natural and suburban habitats are minimum disturbance and transition zones respectively (Tab. I). At each site, 40 young branches, each measuring 20 cm, were taken from 10 trees selected randomly, or four branches per tree, harvested in the four cardinal directions of Lambertian (north, east, south, west) relative to each tree, ensuring equal coverage of all orientations. This approach accounts for potential environmental variations (sunlight, wind exposure) and enhances the representativeness of the sampling, which could influence the distribution of aphids or their antagonists (Southwood and Henderson, 2009).

In the laboratory, viable aphids were counted and preserved in ethanol. Mummified aphids were placed in ventilated Petri dishes to allow parasitoid emergence. Daily observations ensured the comprehensive collection of newly emerged parasitoids, which were meticulously transferred into Eppendorf tubes filled with ethanol using a fine brush.

For the dissection of *P. silana* adult, individuals were immobilized at the thoracic region using an entomological pin. Dissection under a stereomicroscope (SFX-91D - Optika) involved the separation of the head, wings, first abdominal tergite, propodeum, and genitalia. These components were mounted on slides with Hoyer medium. Similarly, adult specimens of *C. maghrebica* were prepared and mounted for identification purposes. A reference collection of these specimens is maintained at the LATPPAM research laboratory, Batna 1 University, Algeria.

Identification keys used for determining primary parasitoids included those by Rakhshani *et al.* (2015); Rakhshani *et al.* (2019). Hyperparasitoids were identified using the keys of Gibson and Vikberg (1998), Gibson (2001), and Ferrer-Suay *et al.* (2017).

#### **Statistical Analyses**

Statistical analyses were performed using R software v. 4.2.1 (R Core Team, 2022) and SPSS v. 26.0 (IBM corp., 2019). The chi-square homogeneity test was employed to compare the counts of *C. maghrebica*, mummy counts, and *P. Silana* parasitism rates and number of emerged parasitoids, was analysed using regression analysis. Principal Component Analysis (PCA) was also performed in R Studio v. 2022) to explore relationships among variables, incorporating the sampling year as a categorical variable to provide a multidimensional view of the dataset variability.

#### **RESULTS**

# **Aphid Population Dynamics**

Throughout the research period, *C. maghrebica* was predominantly observed on Aleppo pines during the spring, from March to July. The highest aphid abundance was recorded in May 2019, with a count of 2,050 aphids, this abundance indicates the total aphids collected from all sample branches during the entire sampling effort for May 2019. In subsequent

years, there was a significant decline, with 548 aphids in 2020 and only 11 in 2021. These fluctuations corresponded to average monthly temperatures ranging from 14°C to 29°C. A notable decrease in the aphid population was observed during the summer, with their complete disappearance by autumn (Tab. II, Fig. 3). The year 2021, particularly from March to June, exhibited abnormal population dynamics when compared to the previous years, likely due to varying climatic factors.

The spatial distribution and significance of *C. maghrebica* varied across locations, influenced by environmental conditions, host plant availability, and the broader ecological interactions. The study emphasized the dominance of *C. maghrebica* at the Kais site, underscoring its substantial impact in that

Statistical analysis revealed a distinct lack of homogeneity in *C. maghrebica* populations between 2019 and 2020 ( $\chi^2$  (9) = 3293.10, p < 0.001), with a 40.91% decrease in aphid numbers in 2020 compared to 2019 (1952). The number of mummies also showed a significant decrease between 2019 and 2020 ( $\chi^2$  (7) = 44.26, p < 0.001), dropping by 45.45% from 198 in 2019 to 90 in 2020. However, there was no significant change in parasitism rate ( $\chi^2$  (7) = 1.37, p = 0.98) between these years.

A total of 6,903 indicates the total abundance of *C. maghrebica* individuals were recorded from all sample branches during the entire sampling effort at the Kais site from 2019 to 2021, with all specimens (100%) being collected exclusively from this location. This high concentration indicates potential diversity and vitality within the plant community, which suggests a healthier ecosystem (as shown in Tab. III). Optimal vegetation coverage is essential for mitigating soil erosion, providing habitats for diverse organisms, facilitating carbon sequestration, and contributing to nutrient cycling.

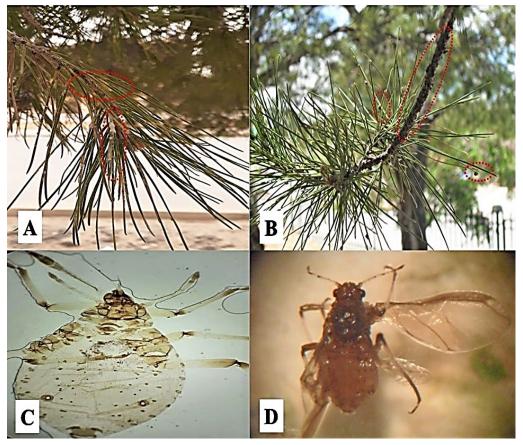
## Performance of P. silana as a Parasitoid

The parasitism rate observed in the Kais region was 3.98% in 2019, increasing slightly to 4.41% in 2020. This modest rise suggests a limited impact of *P. silana* on the substantial *C. maghrebica* populations. Parasitized aphids were first detected on March 21, 2019, and parasitism gradually escalated to 56 specimens by April. However, a significant reduction in parasitoid activity was observed by late May and June, with nearly no parasitism occurring (Fig. 4).

II: Numerical importance of C. maghrebica in the three localities

Habitas	Abundance of aphids per sampling year			
	2019	2020	2021	
Kais	4771	1 952	180	
El Hamma	Absent	Absent	Absent	
Khenchela	Absent	Absent	Absent	

Absent: No data was recorded or aphids were not present in these locations during the sampling period

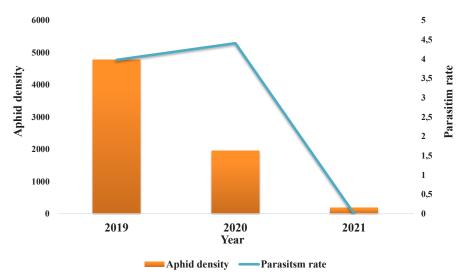


3: Cinara damage to the Aleppo pine
A - Effects of Aphids honeydew on Aleppo pine needles, B - Branch of Aleppo pine infested with several mummies, C - C. magherbica, apterae form, D - C. magherbica, winged form

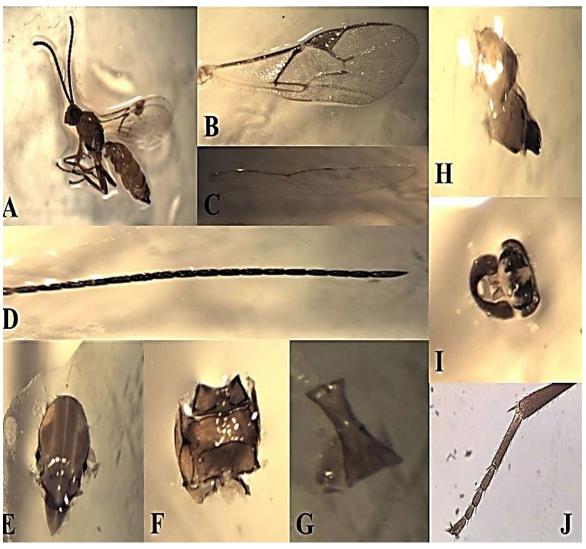
III: Overall coverage rate (%) of the plant transects of each study site

Species	Kais	Khenchela	El Hamma
Pinus halepensis (Mill., 1768)	84.9	65.08	51.92
Cupressus sempervirens (L., 1753)	-	0.1	18.4
Quercus ilex (L., 1753)	-	13.3	8.07
Juniperus oxycedrus (L., 1753)	-	-	7.49
Olea europaea (L., 1753)	-	5.32	3.9
Phillyrea angustifolia (L., 1753)	-	2.5	-
Globularia alypum (L., 1753)	-	-	2.29
Stipa tenacissima (L., 1753)		-	1.2
Stipa nitens (Ball, 1878)	15.05	1.8	-
Ampelodesmos mauritanicus (Durand & Schinz, 1894)	-	-	0.33
Artemisia herba-alba (Asso, 1779)	-	2.9	2.6
Rosmarinus officinalis (L., 1753)	-	-	3.8
Acanthorrhinum ramosissimum (Coss. & Durieu, 1855) Rothm., 1943	-	3.9	-
Cistus villosus (L., 1753)	-	3.9	-
Chenopodium murale (L., 1753)	-	0.9	-
Plantago albicans (L., 1753)	-	0.3	-
Global Recouverment (%)	99.9%	86.7%	100%

<sup>-:</sup> Absence of plant in the region



 $4:\ Variation\ in\ parasitism\ rate\ depending\ on\ the\ quality\ of\ the\ aphid\ population\ in\ the\ Khenchela\ region\ between\ 2019\ and\ 2021$ 



5: Pauesia silana morphological details: A. - Female, B. - Forewing, C. - Hinwing, D. - Antenna, E. - Mesonotum, F. - Propodeum, G. - Ptiol, H. - Female genitalia, I. - Male genitalia, J. - Tibia

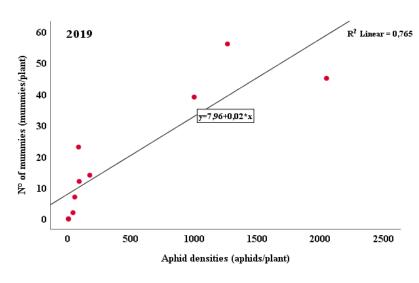
This trend is crucial for understanding the effectiveness of *P. silana* in controlling aphid populations. The data outline the proportion of parasitized aphids relative to the total aphid count, offering a quantifiable measure of the parasitoid's impact on aphid population dynamics over the observed period. The slight increase in parasitism rate from 2019 to 2020 suggests a possible adaptive response of *P. silana* to the environmental conditions.

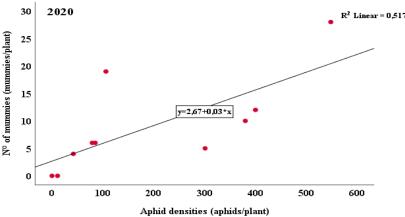
This study highlights the temporal dynamics of *P. silana* parasitoids and their interaction with *C. maghrebica* populations (Fig. 4), revealing a marked reduction in *P. silana* mummies over three years. A peak of 198 mummies in 2019 was followed by a reduction to 90 in 2020, with none observed in 2021, suggesting intricate ecological dynamics. The adult emergence rate crucial for evaluating parasitoid efficacy, exhibited significant annual variability between 2019 (47.92%) and 2020 (24.65%). These fluctuations indicate that changes in host-parasitoid dynamics and environmental conditions likely modulate parasitoid success.

# Mummy Formation and Emergence Rate Correlation with Aphid Densities

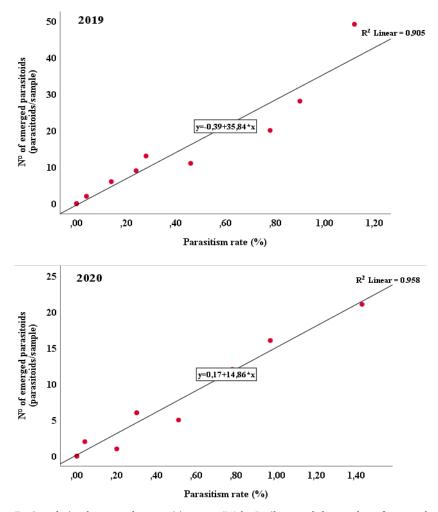
The correlation between aphid density and the number of mummies demonstrates the intricate relationship between these variables. The density of aphids on Aleppo pine showed a strong positive correlation with the number of mummies (p < 0.001) (Fig. 6). The relationship between aphid densities and other factors, including parasitism rates and the number of emerged parasitoids, was also analysed for 2019 and 2020. This analysis provided insight into the interdependencies within this ecological framework.

The emergence rate of *P. silana* reflects the parasitoid's success in developing and surviving within its host. This rate is influenced by the density of aphid populations, particularly those that emerge from mummies during the spring. The relationship between aphid densities and parasitism rates showed a strong correlation, demonstrating that fluctuations in aphid populations directly impact parasitoid emergence rates (Fig. 7).





6: Correlation between the density of C. magherbica and the number of mummies formed due to P. silana in Khenchela (2019 / 2020)



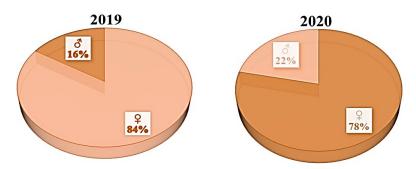
7: Correlation between the parasitism rate (%) by P. silana and the number of emerged parasitoids from C. magherbica in Khenchela (2019/2020)

## Sex Ratio and Reproductive Dynamics of P. silana

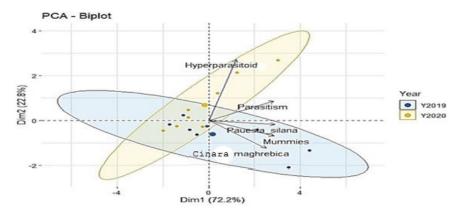
Another aspect of the research focused on the sex ratio of *Pauesia silana*, representing the relative proportion of males to females among the emerged parasitoids. The sex ratio provides insights into the reproductive dynamics and selective pressures influencing this distribution.

The sex ratio, quantifies the percentage of males within the total emergent progeny. In 2019, the

emergence rate was composed of 84% females and 16% males, while in 2020, the composition shifted slightly to 78% females and 22% males. The predominance of females highlights a common reproductive strategy among many parasitoid species, where females, due to their critical role in parasitizing hosts, tend to outnumber males. This female dominance is important for maintaining the parasitoid population's ability to control aphid populations effectively (Fig. 8).



8: The sex ratio of P. silana emerging from mummified aphids during the 2019–2020 study period



9: Visualization of variables projected onto the PCA axes 1 and 2 for the years (2019/2020)

Principal component analysis (PCA) was used to explore the relationships among different variables in the 2019 and 2020 datasets. The first two dimensions of the PCA explained 95% of the total variance. The 2020 dataset was characterized by the prevalence of hyperparsitoids, while the 2019 dataset showed a stronger association with *C. maghrebica* mummies and *P. silana*. These variables demonstrated a positive correlation, suggesting that the dynamics of mummies, parasitoid emergence, and aphid populations are interconnected (Fig. 9).

#### **DISCUSSION**

The period from March 21, 2019, to March 07, 2020, and extending to May 29, 2021, revealed subdued aphid activity, peaking at 6% parasitism. From the beginning of April, a gradual increase in aphid numbers was observed, reaching a peak of 2050 individuals on May 2, 2019, and 585 individuals on May 16, 2020. This surge coincided with an average temperature of 14 °C, which is favorable for adult aphid flight. These observations align with Robert's (1987) findings, which indicated that the flight initiation threshold for *Rhopalosiphum padi* is around 11 °C daytime temperature. However, in 2021, a shift in the number of affected individuals corresponded with an average temperature increase to 33 °C.

Temperature plays a vital role in aphid longevity, with accelerated aging at higher temperatures. Aphids are especially vulnerable to survival fluctuations beyond 25 °C and typically die at 30 °C (Dean, 1974). This temperature sensitivity underscores the complex influence of environmental factors on aphid populations and, by extension, parasitoid effectiveness.

Aphid flight activity is also contingent upon temperature and humidity. Aphids are most active between 20 °C and 30 °C at 75% humidity, while flight is rare below 13 °C and in conditions exceeding 75% humidity (Bonnemaison, 1950). This study found that *P. silana* ability to control *C. maghrebica* was limited, potentially due to the aphid's rapid reproduction and colonization capabilities or the parasitoid's reliance on alternative hosts.

Interestingly, a reduction in parasitism from late May to June suggests a multifaceted interaction among parasitoid lifecycle, environmental factors, and aphid dynamics. This decline may be influenced by factors such as the synchrony between *P. silana* emergence and aphid prevalence, host availability, or other environmental variables impacting parasitoid activity.

The relationship between host aphid density and *P. silana* performance highlights the complexity of host-parasitoid interactions. While higher aphid densities may provide more parasitoid hosts, potentially reducing parasitism rates. Previous studies by He *et al.* (2006) and Mdellel *et al.* (2015) have shown that parasitism end emergence rates often decline when aphid population densities are too high.

Three hyperparasitoid species were identified, belonging to two taxonomic families: Pteromalidae and Encyrtidae. Specifically, Asaphes vulgaris (Walker, 1834), Pachyneuron aphidis (Bouché, 1834), and Syrphophagus aphidivorus (Mayr, 1876) (Hymenoptera: Chalcidoidea: Pteromalidae), were documented, each constituting 21% of the total. Similar families were previously reported in Algeria by Chehma and Laamari (2014), and in Tunisia by Ben Halima et al. (2022).

Observations indicated that hyperparasitism rates increased towards the end of the season, particulary duriing warmer periods. Zuparko and Dahlsten (1993) obeserved that, within the primary parasitoid complex, hyperparasitoids accounted for significant mortality, with just over 50% of all mummies yielding viable adults. Consequently, a pronouced presence of hyperparasites could undermine aphid biological control efforts, as noted by Gómez-Marco et al. (2015). Hyperparasitoids are thought to impact biological control through two principal mechanisms: prompting the premature departure of adult primary parasitoids from aphid colonies and leveraging hyperparasitism to affect the succeeding generation of primary parsitoids (Brodeur and Rosenheim, 2000; Ghelamallah et al., 2020).

## CONCLUSION

Understanding the intricate dynamics that underlie Aleppo pine's resistance to aphids is crucial not only for tree survival but also for maintaining the broader ecological balance of its native environment. The absence of *C. maghrebica* in natural habitats provides insights into predator-prey dynamics, plant health, and habitat conditions. Such observations emphasize the interconnectedness of species and the ecological implications of their presence or presence.

This study offers significant insights into the interactions between *C. maghrebica*, its parasitoid *P. silana*, and various ecological factors. The emergence rate of adult parasitoids is a critical indicator for gauging parasitic interaction effectiveness, and the findings here underscore the importance of *P. silana* in aphid population control. The concurrent evolution of emergence and parasitism rates relative to aphid and parasitoid densities highlights the mutual dependence of these entities, revealing delicate ecological balances.

The marked reduction in *P. silana* by 65.21% between 2019 and 2020 suggests that environmental shifts or interspecies interactions are influencing its population. Conversely, the increase in hyperparasitoid activity (up by 26.09%) may reflect changes in predator-prey dynamics or responses to the shifting population of primary parasitoids. The parasitoid emergence rate, calculated at 32.32%, reflects the success of the parasitic interactions, reinforcing the significant role of parasitoids in the ecological control of aphid populations.

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