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Annual Population Dynamics and Daily Activity Rhythm of Adult Oriental Hornets (*Vespa orientalis* L.), an Important Pest of Iranian Honeybee in Ahvaz, Iran

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ABSTRACT

The Oriental hornet, *Vespa orientalis* L. (Hymenoptera: Vespidae), is one of the most important and serious enemies of honeybees. In this study, the annual population dynamics and daily activity rhythm of Oriental hornet were examined, based on the number of adults trapped in an apiary located in Ahvaz (southwest of Iran) during a period of two years (January 2021 to December 2022). Samplings were performed using the box traps available in the market. The bait used in the traps was fresh chicken liver, with the traps checked daily in four time periods. The results revealed that the first overwintering queens (gynes) emerged in March and two population peaks of newly emerged hornet workers occurred during the year, one in July and the other in October. In late November and early December, no adult hornet was trapped. The daily activity rhythm of adult hornets was observed mostly during 9–12 hours, almost twice as much as in the afternoon period (12–15 hours). The lowest activity was recorded in the evening period and night (6 pm-9 am). During both sampling years, the seasonal abundance of adult hornets displayed a significant positive correlation with air temperature and a significant negative correlation with relative humidity. By comparing the results of our findings with other studies performed in the same and different climate zones, it can be hoped to present effective methods to control the population of *V. orientalis*, especially in areas that have extensive beekeeping.

Keywords: *Apis mellifera* meda, Bait trap, Khuzestan Province, Population fluctuations, Vespidae.

INTRODUCTION

The Vespidae are a large (more than 5000 species) and cosmopolitan family (Pickett *et al*., 2004; Aguiar *et al.*, 2013), divided into the subfamilies Polistinae (wasps) and Vespinae. The Vespinae are split into four genera including *Dolichovespula*, *Provespa, Vespa* (hornets), and *Vespula* (yellow jackets) (Archer, 2012). Hornets of the genus *Vespa* have 22 species where five of the most important species of this genus include *V. mandarinia*, *V. tropica*, *V. velutina* and *V. orientalis,* and are distributed in Asia and Oceania regions, while *V. orientalis* is expanded to north of Africa, Mediterranean regions and across middle-east (Carpenter and Kojima, 1997; Perrard *et al*., 2013). *Vespa crabro* (European hornet) is naturally distributed in Europe, around Black sea (Carpenter *et al*., 2013; Perrard *et al*., 2013), and later imported to North American countries as a biological control agent to control different lepidopteran, coleopteran, dipteran immatures (Cowan, 1991). *Vespa velutina* is native of south Asian regions and migrated to other Asian and European countries

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lately. This Asian hornet was recorded at South Korea in 2003 (Kim *et al*., 2006; Choi *et al*., 2012), France in 2004 (Haxaire *et al*., 2006; Villemant *et al*., 2006) and 2012 at Japan (Sakai and Takahashi, 2014; Minoshima *et al*., 2015), and right now rapidly spreading across the region (Takeuchi *et al*., 2017). Two species of *V. orientalis* and *V. crabro* are present in Iran: the first species is distributed throughout the country except for Mazandaran, Golestan, and Guilan provinces (Caspian coast), and the second species is distributed only in the Caspian Coast (Ebrahimi and Carpenter, 2012).

Vespa orientalis is a medium-size hornet with the weight of about 250 mg. In the spring, the overwintering mated queens form the annual colonies, usually in underground cavities. During the summer, the queen reproduces rapidly, such that a queen develops a colony of up to 2000 individuals including drones and sterile workers, by the beginning of autumn (Ishay, 1976). The sterile workers function as food gathering, cleaning, enlarging nest, and defense (Cappa *et al*., 2021), while the males attend only to mate with the queens (Cowan, 1991). The young mated females (gynes) will enter diapause during the winter, and as the only overwintering individual of the community, they will establish the next year colony (Perez and Aron, 2020).

Oriental hornets are general predators (Richards, 1962). In some areas, they are considered a significant agricultural pest, damaging summer fleshy fruits, such as grapes, peaches, dates, figs, pomegranates, and some vegetables (Dvorak, 2006; Glaiim, 2009; Taha, 2014), which may reduce the marketability of agricultural products (Glaiim, 2009; Al-Mahdawi and Al-Kinani, 2011; Abdelaal *et al*., 2014). Oriental hornets also cause direct damage to the trees by chewing the bark of citrus trees and ornamental trees, to use them in the construction of nest walls (Havron and Margalith, 1995). They are very aggressive and their venoms usually cause painful reactions and anaphylaxis in people sensitive to stings (Landolt, 1998; Landolt and Wash, 2000). In some areas, the activity of these hornets may make sensitive people unwilling to leave their house during the summer season (Sackmann *et al*., 2001; Bacandritsos *et al*., 2006; Sackmann and Corley, 2007).

The relation of native honeybee species (*Apis laboriosa, A. dorsata, A. cerana, A. florea*) and hornet are very old, as these organisms live together since ancient times and still living together at oriental regions. These native honeybee species possess perfect defensive behavior (as, shimmering, bee-carpet, heat balling, changed flying strategy, etc,) against these hornets and economic damage is few (Cappa *et al*., 2021). The problem was aggravated with the introduction of *A. mellifera* in these regions, where this species lack those defensive behaviors against native hornets, and these hornets have become the major problem for *A. mellifera* beekeeping (Chantawannakul *et al*., 2016).

In tropical or subtropical regions, as the ambient temperature rises, the number of various available insects and spiders usually drops, causing increased hunting and more damage to honeybee colonies by these invasive hornets. Hornets usually hunt alone by approaching the hive entrance to catch the foraging workers and drones, or by hunting the honeybee individuals around or away from the hives (Baracchi *et al*., 2010). In some cases, it has been observed that Oriental hornets, in addition to hunting all adult honeybees, enter the hive to feed on all larvae and pupae (Papachristoforou *et al*., 2007; Ebadi and Ahmadi, 2010). Although the hornets are usually considered the secondary pest of honeybees, in some areas, especially tropical and subtropical regions, the damage caused by these invasive hornets is so serious that they have recently threaten the beekeeping industry in these regions (Khodairy and Awad, 2013).

Living organisms with a wide geographical range are exposed to various weather conditions (Bridle and Hoffmann, 2022), which affects their phenotypic,

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physiological, biological, and genetic characteristics (Jackson *et al*., 2020). Oriental hornet has a wide geographical distribution and is the only vespid species found in desert environments (Cohen *et al*., 2022). This hornet shows appropriate adaptation in extreme climatic regions (Spradbery, 1973; Harris, 1991), and even recent changes in the climate have led to further spread of this species into central Asia, Europe, and the Americas (Werenkraut *et al*., 2021).

This study aimed to investigate the annual population dynamics and daily activity rhythm of adult Oriental hornets (*V. orientalis*) during two years in the southwest of Iran (Ahvaz City, the capital of Khuzestan Province) with very extreme weather conditions (38.4°C and 25.5% R.H., average summer season).

MATERIALS AND METHODS

In order to determine the population fluctuations of adult Oriental hornets, *V. orientalis*, daily samplings were conducted using bait traps during a period of two years (from March 2021 to February 2023), at the Unit of Bee Research of Shahid Chamran University of Ahvaz, Iran (southwest of Iran; 31° 20' N, 48° 38' E). The monthly average of temperature and relative humidity is mentioned in Figure 1. The Iranian honeybee *A. mellifera meda* was comprised of nine colonies, kept in standard Langstroth hives containing 10 frames. The hives were placed at least three meters apart in each direction.

Samplings were performed using the box traps available in the market. Upon encountering the first adult Oriental hornets in the environment, 10 traps were installed at a height of one meter from the ground and near the hives. For this purpose, box traps (30×50 cm in size and 30 cm in height) made of wood and metal mesh were used. The bait used in these traps consisted of pieces of fresh chicken liver, which are very attractive for Oriental hornets according to previous studies (Al-Heyari *et al*, 2016; Karam Kiani, unpublished data). The baits were refreshed every two days, along with the test. During the sampling period, the

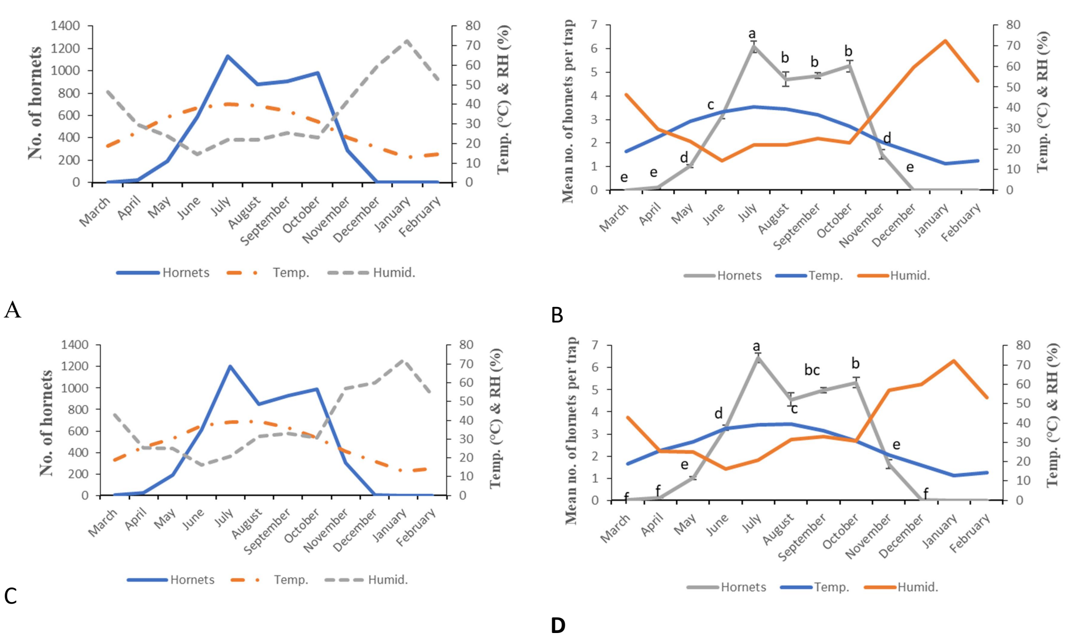

Figure 1. The total numbers of the captured adult Oriental hornets, *Vespa orientalis* by 10 baits traps, in different months (A and B) and the mean daily number of captured hornets by each trap (C and D), under the natural conditions of Ahvaz, Iran, during 2021-2023.

number of hornets caught in all traps was counted and recorded daily in four periods (including 9 - 12, 12 - 15, 15 - 18, and from 6 pm until 9 am the next day). After counting, the captured hornets were removed from the traps and used in other studies. The ambient temperature and relative humidity were recorded by a digital data logger on a daily basis.

Data Analysis

One-way repeated measures analysis of variance was used to determine any differences between the numbers of captured adult hornets within all sampling dates. Factorial two-way analysis of variance was employed to analyze the number of trapped adult hornets in different sampling dates (months of the year), and different daily sampling periods (four different periods), as independent fixed factors. The correlation coefficient test was also utilized to assess a possible linear association between temperature and relative humidity with the annual population fluctuations (SPSS, 1998). The Excel software was used to draw the graphs of population changes.

RESULTS

The oriental hornet, V. *orientalis* was the only species of hornet encountered during the study period.

The total numbers of captured adult Oriental hornets by 10 traps, during each month (Figures 1-A and -B) and the mean daily numbers of the captured adult Oriental hornets by each trap (Figures 1-C and -D) are presented. The analyses of data revealed that the abundance of the Oriental hornet changed significantly during the activity period (from March to December) (in 2021: $P< 0.001$, $F_{9, 1.620}= 66.730$; in 2022: $P< 0.001$, $F_{9, 1.541}= 68.645$).

During both sampling years, the first overwintering queens (gynes) appeared in the second half of March (on 16th and 18th March, for the two years, respectively). The gynes' population continued increasingly until the middle of June.

The sterile workers produced by emerged gynes gradually emerged and we found two population peaks during the year, one in mid July (6.09±0.25 in Jul. 15, 2021; 6.45±0.21 in Jul. 22, 2022) and the other in late September/early October (5.16±0.24 in Sep. 26, 2021; 5.33±0.24 in Oct. 01, 2022) (Figures 1-C and -D).

With the increase in air temperature in early August (39.4°C, monthly average), the average density of the hornet population was reduced to a minimum. Then, it again gradually rose and the second peak occurred in late September/early October. The hornet population declined considerably from the second half of November, so that no hornet was captured after late November (Nov. 22, 2021 and Nov. 26, 2022).

The seasonal population changes of adult hornets displayed a significant positive correlation with air temperature ($r= 0.468$, $P< 0.001$; $r= 0.454$, $P< 0.001$) and a significant negative correlation with relative humidity ($r= -0.548$, $P< 0.001$; $r= -0.598$, $P< 0.001$), respectively, for the two years (Figure 1).

In both sampling years, the main effects of sampling dates (active months of the year), daily sampling periods (four different periods), and their interaction were significant on the number of trapped Oriental hornets (Table 1).

In all active months (from April to November) of both years, the presence of foraging workers of *V. orientalis* was significantly higher at the beginning of the days (the period 9-12) than other periods ($P< 0.01$ for all months). Thereafter, higher activity was observed in the afternoons (between midday and 15 hours). The least number of hornets were captured in the evenings (15–18 hours) and thereafter (from 6 pm until 9 am) (Tables 2 and 3).

DISCUSSION

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Table 1. Two-way ANOVA of the effects of different sampling dates (months of the year; from March to December) and different daily sampling periods (four different periods) on the number of trapped adult Oriental hornet (*Vespa orientalis*), under the natural conditions of Ahvaz, Iran, during 2021-2022.

Variables	Sampling year					
	2021			2022		
	F	*df*	*P*	*F*	*df*	*P*
Sampling months	291.732	9	< 0.001	330.025	9	< 0.001
Daily sampling periods	1494.601	3	< 0.001	1698.936	3	< 0.001
Sampling months×Daily sampling periods	135.611	27	< 0.001	157.338	27	< 0.001
df residue		1200			1200	

Table 2. Mean (±SE) number of captured adult Oriental hornets, *Vespa orientalis* by bait traps, on different sampling dates and different daily sampling periods, under the natural conditions of Ahvaz, Iran during 2021.

Sampling months	Daily sampling periods				F	df	P
	9-12	12-15	15-18	18-9			
March	0.03±0.03 Fa	0.03±0.03 Da	0.00±0.00 Ea	0.00±0.00 Ca	0.667	3, 120	0.574
April	0.38±0.12 Fa	0.22 ± 0.08 Dab	0.03 ± 0.03 Eb	0.06±0.04 Cb	3.879	3, 120	0.011
May	3.29±0.20 Fa	1.93 ± 0.14 Cb	0.77 ± 0.13 Dc	0.22±0.08 BCc	82.558	3, 120	< 0.001
Jun	11.48±0.62 Da	4.70 ± 0.20 Bb	2.35 ± 0.12 ABc	0.32±0.08 BCd	206.710	3, 120	< 0.001
July	27.09±1.32 Aa	5.93 ± 0.18 Ab	2.64 ± 0.13 Ac	0.87±0.15 Ac	324.253	3, 120	< 0.001
August	20.25±1.39 Ca	5.70 ± 0.17Ab	1.74 ± 0.27 BCc	0.54±0.12 ABc	158.833	3, 120	< 0.001
September	21.93±0.02 BCa	5.09 ± 0.13 ABb	1.77±0.15 BCc	0.38±0.08 BCd	914.193	3, 120	< 0.001
October	24.00±1.07 Aba	5.83 ± 0.26 Ab	1.38±0.18 CDc	0.38±0.08 BCc	381.098	3, 120	< 0.001
November	7.16±0.93 Ea	1.93 ± 0.34 Cb	0.03±0.03 Ec	0.03±0.03 Cc	45.498	3, 120	< 0.001
December	0.03±0.03 Fa	0.03 ± 0.03 Da	0.00±0.00 Ea	0.00±0.00 Ca	0.667	3, 120	0.574
F	179.070	188.011	58.253	10.699			
Df	9, 300	9, 300	9, 300	9, 300			
P	< 0.001	< 0.001	< 0.001	< 0.001			

Among natural enemies of *Apis mellifera*, hornets appear to be the most dangerous predators for honeybee colony, which can even lead to complete destruction of hives (Matsuura and Yamane, 1990; Power *et al*., 2022). Our sampling indicated that *V. orientalis* was the only *Vespa* species distributed in the studied area. This result is consistent with the previous reports where *V. orientalis* proved to be more adapted to arid climates, while *V. crabro* appeared to be more adapted to cold and dry conditions (Lioy *et al*., 2023). Similarly, Ebrahimi and Carpenter (2012) emphasized that the distribution of both species was completely separated from each other in Iran, so that *V. orientalis* occurred from the southern slopes of the Alburz Mountains to Persian Gulf, while the distribution of *V. crabro* was limited between the northern slopes of the Alburz Mountains and the Caspian Coasts. Our results, similar to those of Ebrahimi and Carpenter (2012) question the results obtained by Abd-Rabou *et al*. (2005)

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Table 3. Mean (±SE) number of captured adult Oriental hornets, *Vespa orientalis* by bait traps, on different sampling dates and different daily sampling periods, under the natural conditions of Ahvaz, Iran, during 2022.[a]

Sampling months	Daily sampling periods				F	df	P
	9-12	12-15	15-18	18-9			
March	0.09±0.05 Fa	0.06±0.04 Da	0.00±0.00 Da	0.00±0.00 Da	1.901	3, 120	0.133
April	0.41±0.12 Fa	0.25±0.09 Dab	0.03±0.03 Db	0.06±0.04 Db	4.008	3, 120	< 0.004
May	3.16±0.22 Fa	1.93±0.14 Cb	0.77±0.13 Cc	0.22±0.08 BCDc	68.038	3, 120	< 0.001
Jun	11.96±0.61 Da	4.87±0.17 Bb	2.54±0.12 Ac	0.38±0.08 BCDd	231.932	3, 120	< 0.001
July	29.29±1.09 Aa	5.58±0.20 ABb	2.77±0.15 Ac	1.09±0.15 Ac	543.751	3, 120	< 0.001
August	19.70±1.36 Ca	5.45±0.18 ABb	1.70±0.27 Bc	0.54±0.11 Bc	156.301	3, 120	< 0.001
September	22.45±0.63 BCa	5.09±0.14 Bb	1.83±0.14 Bc	0.51±0.10 BCd	902.097	3, 120	< 0.001
October	23.74±1.04 Ba	6.00±0.26 Ab	1.64±0.17 Bc	0.61±0.11 Bc	383.098	3, 120	< 0.001
November	7.38±0.94 Ea	2.12±0.35 Cb	0.19±0.07 CDc	0.12±0.06 CDc	45.097	3, 120	< 0.001
December	0.09±0.05 Fa	0.03±0.03 Da	0.00±0.00 Da	0.00±0.00 Da	2.105	3, 120	0.103
F	210.672	176.222	61.400	14.831			
df	9, 300	9, 300	9, 300	9, 300			
P	< 0.001	< 0.001	< 0.001	< 0.001			

[a] Means in each row bears the same lower case letter, and means in each column bears the same upper case letter were not significantly different (One-way ANOVA with post-hoc Tukey HSD; P> 0.05).

(quoted by Bagjacik and Samin, 2011) who previously reported *V. crabro* in Khuzestan Province.

This study found that, unlike the climate conditions of the Tel Aviv region, where hornets regulate their flight activity to provide maximum temperature from the environment (Volynchik *et al*., 2008), in the Ahvaz region, foraging workers of *V. orientalis* employ some strategies to reduce the negative effects of high ambient temperature, including dedication of the activity rhythm of foragers to the early hours of the day (9-12 hours) during active season. Generally, it seems that the flight activity rhythm is season dependent and a function of the climatic conditions of the region, so that in cold months of Ahvaz (March, April and December), and in Egypt (Khater *et al*., 2001; El-Boulok *et al*., 2019) the activity rhythm of foragers continued until the afternoons (between midday until 15 hours), or even to the evenings (6 pm), in the Minya region of Egypt (Fouad *et al*., 2021). Despite all these explanations, Volynchik *et al*. reported that the flight activity of Oriental hornets was more related to the ultraviolet B radiation level than to ambient temperature (Volynchik *et al*., 2008).

The lower density of foraging hornets in September is not expected to be due to the population decline of the colonies, and it seems workers spend most time inside the nest, during this period. Indeed, Oriental hornets, *V. orientalis* use some methods so as not to face unfavorable environmental conditions, such as maintenance of colony temperature at around 35°C (not to exceed 37°C) in hot region and Dead Sea's environment (Volov *et al*., 2021). This arises

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from cooling down the colony, carried out actively by adult hornets by means of evaporating water drops and ventilation (Jones and Oldroyd, 2006). This method of lowering the temperature is so effective that the thermal microclimate of the nest, in two different extreme climates, changed only within a narrow range of temperatures (Volov *et al*., 2021). Under extreme climatic conditions, *V. orientalis* has also the physiological ability to maintain lipid composition with minimal changes (Volov *et al*., 2021).

Vespa orientalis is a thermophilic species (Taha, 2014; Thakur and Bagga, 2000), and in accordance with our finding, there was a significant positive relationship between seasonal population changes of adult *V. orientalis* and ambient temperature. Indeed, *V. orientalis* is a thermophilic species and its high adaptability to extreme conditions (desert) results in decline in interspecific competition, especially with other species belonging to this genus.

Heat adaptation of living organisms consists of two types: adaptation to humid heat and to dry heat. In the studied area (Ahvaz), both types of weather conditions, including dry heat and humid heat, occur on different days of summer. Our observations revealed that the number of catches in traps reached minimal values (close to zero) on days with high humidity. It seems that the foraging hornets prefer to stay in the nest during these unfavorable days.

Most studies show that overwintering queens (gynes) appear at the beginning of the year when the weather warms up (Ishay *et al*., 1974; Chhuneja *et al*., 2008; Volynchik *et al*., 2008). During both years of sampling in the studied area, the emergence of overwintering females (gyns) started from the beginning of the second half of the March (18.9 °C, monthly average) and it was continued until the middle June (37.7 °C, monthly average). These results were consistent with both studies conducted in Egypt (Khater *et al*., 2001; Taha, 2014). Also, in another study in Egypt, Shoreit (1998) reported that queens were visible from January to May and reached their highest density in March. The sterile workers produced by gynes were gradually increased and formed two population peaks during a year. These results are in agreement with the data obtained by some researchers on the same hornet species in Egypt (Sharkawi, 1964; Shoreit, 1998; Gomaa and Abd El-Wahab, 2006; Ibrahim, 2009; Omran *et al.*, 2011) and in India (Sharma and Raj, 1988; Sihag, 1992).

The hornet population in the apiary was significantly reduced in August due to the high daily temperature (39.4°C, 26.6% RH monthly average). Thereafter, with a relative decline in the ambient temperature in late summer, the population of workers was again increased in late September (36.3°C, 29.15% RH monthly average), and reached the peak in middle October (30.8°C, 26.75% RH monthly average). In the fall, with the weather cooling down (Late November: 23.35°C, 49.25% RH), a significant decline in the population was observed and, finally, the hornet workers disappeared. Other studies suggested that, while the weather cools down in the fall, the queen starts laying eggs that will develop into drones and new queens. The drones will die after mating, and the young fertilized queens go to overwintering (Chhuneja *et al*., 2008; Volynchik *et al*., 2008).

CONCLUSIONS

The results of the present study revealed that the seasonal dynamics of the population and the daily activity of the *V. orientalis* outside the nest are completely coordinated with the environmental conditions. In this study, the flight of queens was firstly observed in middle March and continued until the beginning of June. The peak population of emerged workers occurred during July and October. The daily activity rhythm of adult hornets was observed mostly during 9 am-12 noon. Since the present study was carried out in very extreme weather conditions, comparing the

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result of our findings with other studies performed in the same and different weather conditions (Sharkawi, 1964; Sharma and Raj, 1988; Sihag, 1992; Shoreit, 1998; Gomaa and Abd El-Wahab, 2006; Chhuneja *et al*., 2008; Omran *et al*., 2011) can increase our knowledge about the annual population dynamics and daily activity rhythm of adult Oriental hornets. This information, especially the exact time of overwintering queens' appearance and the population peaks of the sterile workers, may be used to better control the population of *V. orientalis*, in areas that have extensive beekeeping.

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پویایی جمعیت سالانه و ریتم فعالیت روزانه هورنت های شرقی بالغ (*Vespa orientalis* L.)، آفت مهم زنبور عسل ایرانی (*Apis mellifera* meda) در اهواز، ایران

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چکیده

زنبور هورنت شرقی، *Vespa orientalis* L. (Hymenoptera: Vespidae) یکی از مهم ترین و جدی ترین دشمنان زنبورهای عسل است. در این مطالعه، پویایی جمعیت سالانه و ریتم فعالیت روزانه هورنت شرقی بر اساس تعداد بالغین گرفتار شده در زنبورستان واقع در اهواز (جنوب غربی ایران) طی یک دوره دو ساله (ژانویه ۲۰۲۱ تا دسامبر ۲۰۲۲) مورد بررسی قرار گرفت. نمونه برداری با استفاده از تله های جعبه ای موجود در بازار انجام شد. طعمه مورد استفاده در تله ها جگر مرغ تازه بود که تله ها روزانه در چهار دوره زمانی بررسی می شدند. نتایج نشان داد که اولین ملکه های زمستان گذران (ژین ها) در ماه مارس ظهور کردند و سالانه دو

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پیک جمعیتی کارگران تازه ظاهر شده در طول سال، یکی در ماه جولای و دیگری در اکتبر رخ داد. در اواخر نوامبر و اوایل دسامبر، هیچ هورنت بالغی به دام نیفتاد. ریتم فعالیت روزانه هورنت های بالغ بیشتردر ساعت ۹ تا ۱۲، تقریباً دو برابر بیشتر از دوره بعد از ظهر (۱۵–۱۲) مشاهده شد. کمترین میزان فعالیت در ساعات عصر و شب (٦ عصر تا ۹ صبح) به ثبت رسید. در طول هر دو سال نمونه‌برداری، فراوانی فصلی هورنت‌های بالغ همبستگی مثبت و معنی‌داری با دمای هوا و همبستگی منفی معنی‌دار با رطوبت نسبی نشان داد. با مقایسه نتیجه یافته های ما با سایر مطالعات انجام شده در مناطق آب و هوایی مشابه و متفاوت، می توان امیدوار بود که روش های موثری برای کنترل جمعیت *V. orientalis* به ویژه در مناطقی که زنبورداری گسترده ای دارند ارائه شود.

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