



## RESEARCH ARTICLE - BEES

## Nesting Preference, Nesting Biology and Associated Natural Enemies of *Chalichadoma flavipes* Spinola, 1838 (Hymenoptera: Megachilidae)

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### Abstract

Bees of the genus *Chalichadoma* comprise a diverse group with numerous species mainly present in the Mediterranean region. Most of these species use mud to build their nests, while some species use cavity nesting using available holes in the nesting area. The present research was conducted between 2020 and 2022 after discovering natural nests of many solitary bee species in some villages around El Tal El Kebir and El Qassasin areas, 30.31N 31.48E (Eastern Egypt). However, the most important species were *Chalichadoma nigripes* Spinola, 1838, *Chalichadoma flavipes* Spinola, 1838; and several species of the genus *Megachile*. Meanwhile, the most abundant species were *M. minutissima* Radoszkowski, 1876, and *M. patellimana* Spinola, 1838. A few species of Osmiini were also recorded, especially *Osmia submicans* Morawitz, 1870. All nests were associated with several cleptoparasitic bees, parasitic wasps, and predators such as beetles of the genus *Trichodes*. Several materials were examined to choose the recommended material for artificial nesting. Wood was the most suitable material. The study has also highlighted some notes on the biology of *Chalichadoma flavipes* Spinola.

### Introduction

There are approximately 20,000 bee species known worldwide (Michener, 2007). Bees are the primary pollinators of most wild and crop plant species. The value and economic services of the pollinators were estimated in Europe to be 14 billion EUR (Leonhardt et al., 2013). The contribution of bee pollinators for some crops, such as alfalfa and broad beans, occurs in Egypt but with no detectable monetary contribution (Shebl et al., 2009; Mohamed et al., 2024).

The decline of some bee species is well documented globally due to several reasons, such as climate change, lack of natural foraging resources, pest and disease outbreaks, and elimination of nesting habitats (You et al., 2005; Goulson, 2003; Shebl et al., 2013). The urbanization and replacement of natural habitats that change the whole landscape considerably impact the bee species richness and abundance (Banaszak-Cibicka & Zmihorski, 2012). Urban areas might offer good

bee nesting sites (Osborne et al., 2008; Goulson et al., 2010; Hall et al., 2017; da Rocha-Filho et al., 2018). However, in some cases, the effect of urbanization in promoting bee abundance is high compared to natural habitat (Sirohi et al., 2015; Kaluza et al., 2016; Xie et al., 2020).

When compared with historical data, information about the current status of Egyptian bee fauna is still in the infancy stages. Nevertheless, there are several alarming signs implying that the bee species composition and abundance are endangered and threatened due to environmental change Okely et al. (2023). Some observations in Egypt during interval samplings throughout the last decades indicated lower bee species richness and abundance. Climate change, coupled with other anthropogenic changes, could be one of the causes of the decline of bees. The decline of pollinators and its effects on Egyptian agricultural production was estimated to be approximately 13.5 billion Egyptian pounds (LE). There was a 46% loss in alfalfa seed production due to pollinators'



decline in Egypt. In the long term, the decline in pollinators would affect the economy, with an annual loss equal to 1.9 billion Egyptian pounds (LE) (Brading et al., 2009). One of the most critical anthropogenic human-induced changes is habitat loss and fragmentation (Winfree et al., 2009). An apparent sharp decline was noticed in several cavity-nesting bees of the family Megachilidae (Shebl et al., 2018). Some species are considered endangered in this family, such as *Chalicodoma flavipes* Spinola, 1838, *Megachile minutissima* Radoszkowski, 1876, *Megachile submucida* Alfken, 1926 and *Osmia submicans* Morawitz, 1870 (Shebl et al., 2013; Shebl et al., 2021). Future decline scenarios were elucidated and confirmed the possible extinction of several cavity-nesting bee species (Okely & Shebl, 2023). The research aims to present current efforts to conserve the mason bees group of the genus *Chalicodoma* in Egypt. In addition, it was also meant to elucidate some notes on the nesting biology of *Chalicodoma flavipes* Spinola, 1838, under artificial nesting conditions. The utmost goal is to increase public awareness of how much cavity-nesting bees are threatened by anthropogenic activities, which might lead to bee decline.

## Material and Methods

### *Natural nesting habitat and species determination*

This research was conducted from 2020 to 2022. It was initiated after discovering several natural nests in some El Tal El Kebir and El Qassasin villages (30.31N 31.48E, Eastern Egypt). Several species of solitary bees were found nesting in old mud walls, such as *Chalichadoma nigripes* Spinola, 1838 and *Chalichadoma flavipes* Spinola, 1838, as well as several species of the genus *Megachile*. However, the most abundant species were *Megachile minutissima* Radoszkowski, 1876, and *M. patellimana* Spinola, 1838. A few species of *Osmia* were also found in the same habitat, specially *Osmia submicans* Morawitz, 1870 (Fig 1). Species were identified based on a reference collection deposited at the Department of Plant Protection, Faculty of Agriculture, Suez Canal University. Specimens were checked by using a stereoscopic microscope Optometron D-81829 Munchen-Germany.



**Fig 1.** Natural nesting sites on mud walls of *Chalichadoma nigripes* Spinola, 1838 and *Chalichadoma flavipes* Spinola, 1838.

## Artificial nesting materials

### Wooden nests:

Fourteen wooden blocks about 12 cm thick were used as nesting material ( $n = 14$ ), and holes were made mechanically. The length of the hole was 9 cm. These holes look like tunnels or tube openings naturally made by the female bees. The units were glued and gathered to form the shelter (Fig 2).

### Foam nests:

The shelter of artificial nests was made of wooden frames containing several polystyrene foam pieces stacked one above the other ( $n = 18$ ). Each foam piece was 50 cm in length, 12 cm in width, and 2 cm in thickness, containing 26 holes with 10 cm in depth and 6 mm in diameter. An appropriate number of paper tubes, each 10 cm long and 5.2 mm internal diameter, were used, with only one paper tube placed in each hole. The artificial nests were painted brown from the outside to look natural. The shelters with artificial foam nests were distributed and hung on the mud walls of the natural nests where the species were present (Fig 2).



**Fig 2.** The shelters with artificial foam and wooden nests hanging on the mud walls which containing the Megachilids natural nests.

The wooden trapping nests were collected by the end of the nesting activity seasons and transferred to the Faculty of Agriculture, Suez Canal University, in July 2020 and 2022. These nests were subjected to further observations to record the total number of available nests, the total number of occupied nests, and the number of emerged individuals.

### Laboratory studies:

After the successful completion of artificial nesting, by June/July every year, the artificial foam and wooden nests were collected from the sites and transferred to the laboratory. All nests were scrutinized, and all insect cells were extracted. Each type of artificial nest and constructed cells were counted individually. The most dominant nests in the foam were used

by *M. minutissima* Radoszkowski, 1876 which was studied previously. Nests of *C. flavipes* Spinola, 1838 were subjected to nest description and nesting behavior since they were not studied previously. The wooden nests were placed inside the laboratory of the Bee Research Center at the Faculty of Agriculture, Suez Canal University, covered with adhesive tape for counting the existing holes caused by several parasites and predators five times over five months, then covering the holes with adhesive tape again, to calculate the parasitism rate.

#### Statistical analysis

The chi-square test was performed on the nest unit data through a 2 x 2 contingency table for material type (wooden and foam) and nest occupancy (nested and unnested)

**Table 1.** Number of total, nested and unnested tubes of *C. nigripes* and *C. flavipes* in wooden traps.

Nests	Total Number of tubes	Number and percentage of nested tubes		Number and percentage of Unnested tubes	
		No.	%	No.	%
1	249	4	1.6	245	98.4
2	152	5	3.3	147	96.7
3	250	193	77.2	57	22.8
4	249	156	62.7	93	37.3
5	248	181	73.0	67	27.0
6	249	160	64.3	89	35.7
7	249	166	66.7	83	33.3
8	249	112	45.0	137	55.0
9	249	199	79.9	50	20.1
10	249	213	85.5	36	14.5
11	249	160	64.3	89	35.7
12	249	18	7.2	231	92.8
13	249	144	57.8	105	42.2
14	249	135	54.2	114	45.8

variables. We used GraphPad Prism 8 for Windows version 8.0.2 (236), GraphPad Software.

## Results

### Artificial nesting of *C. nigripes* and *C. flavipes*

The present results indicated that wooden nests were the most preferred material used by both species. A significant relationship exists between nesting and nest types,  $p < 0.01$ . There was also a variation in nesting percentage, but overall, the average nesting percentage was 54.5, which is considered quite a good indicator for both species' future conservation. In some cases, the nesting percentage was 85.5%, which is relatively high and promising for protecting both species from expected decline due to habitat loss (Tables 1-3, Figs 3 and 4A-B). The female bees preferred using wooden material over foam material.

### Nest construction and cell provisioning of *Chalicodoma flavipes*

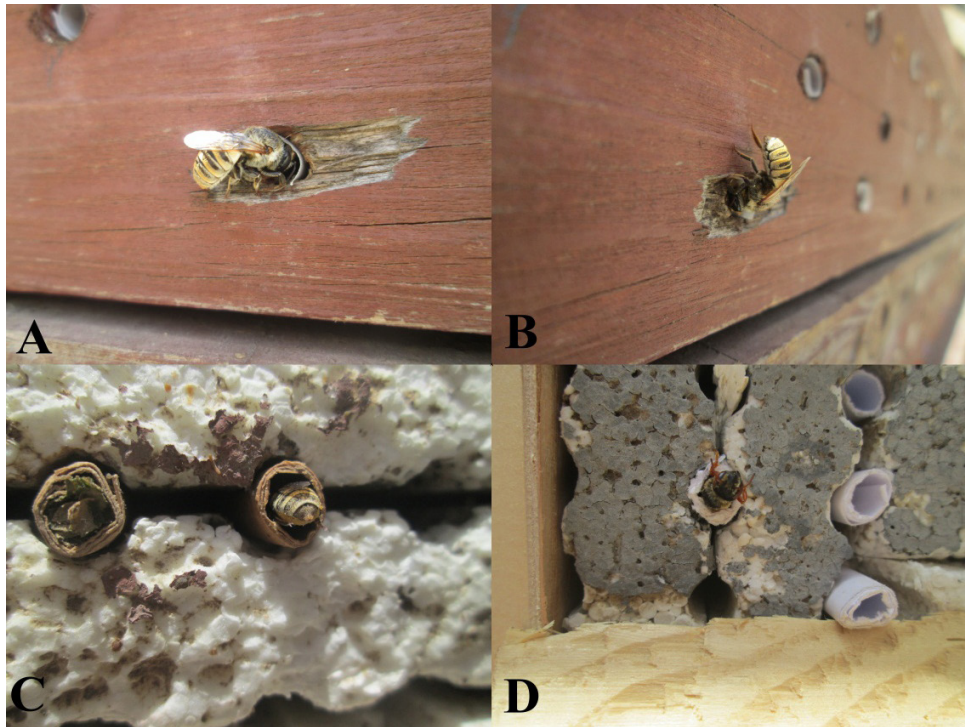
The present study showed that female bees had built straight mud tunnels, where the tunnel length ranged between 1 to 3.7 cm ( $n = 34$  nests) with 1 to 3 cells for each tunnel ( $n = 34$  nests). The average cell length ranged between 0.7 cm and 1.6cm, and the cell diameter ranged between 0.4 cm and 0.5 cm (Fig 3). The mating behavior was noticed around the nesting area above the ground, lasting a few minutes in early May. In Megachilids, males use their legs, mandibles, and head capsules to grasp females during mating. By then, females started building their nests using many loads of mud, followed by cell provisioning and laying eggs. It was rather complicated to check the development of immature stages of this species because of the small number of nests, besides the hardness of the cells. Moreover, some larval stage development observations and notices were taken (Fig 4A-E). The whole nesting area was attractive to several cleptoparasitic bee species, such as *Coelioxys decipiens* Spinola, 1838, *Coelioxys coturnix* Pérez, 1884 and *Radoszkowskiana rufiventris* (Spinola, 1838), in addition to the predatory beetles of the genus *Trichodes*.



**Fig 3.** Completed constructed tunnels inside artificial wooden nests.

The results obtained from covering wooden nests with adhesive taps revealed that studied parasites and predators have destroyed all the young generations inside the wooden nests. The most common predators and parasites subjected to the study were *Polistes gallica*, *Trichodes* sp., the larva of *Trichodes* sp., *Tribolium* sp., and *Melittobia* sp., (Figure 6).

The larva of *Trichodes* sp. devours everything inside the cell, whether it is larva or food (pollen and nectar). Observation also confirmed the presence of unknown parasites and predators found during this study. These unknown species were photographed, but they could not be identified. The number of existing holes made by the parasite and predators in five



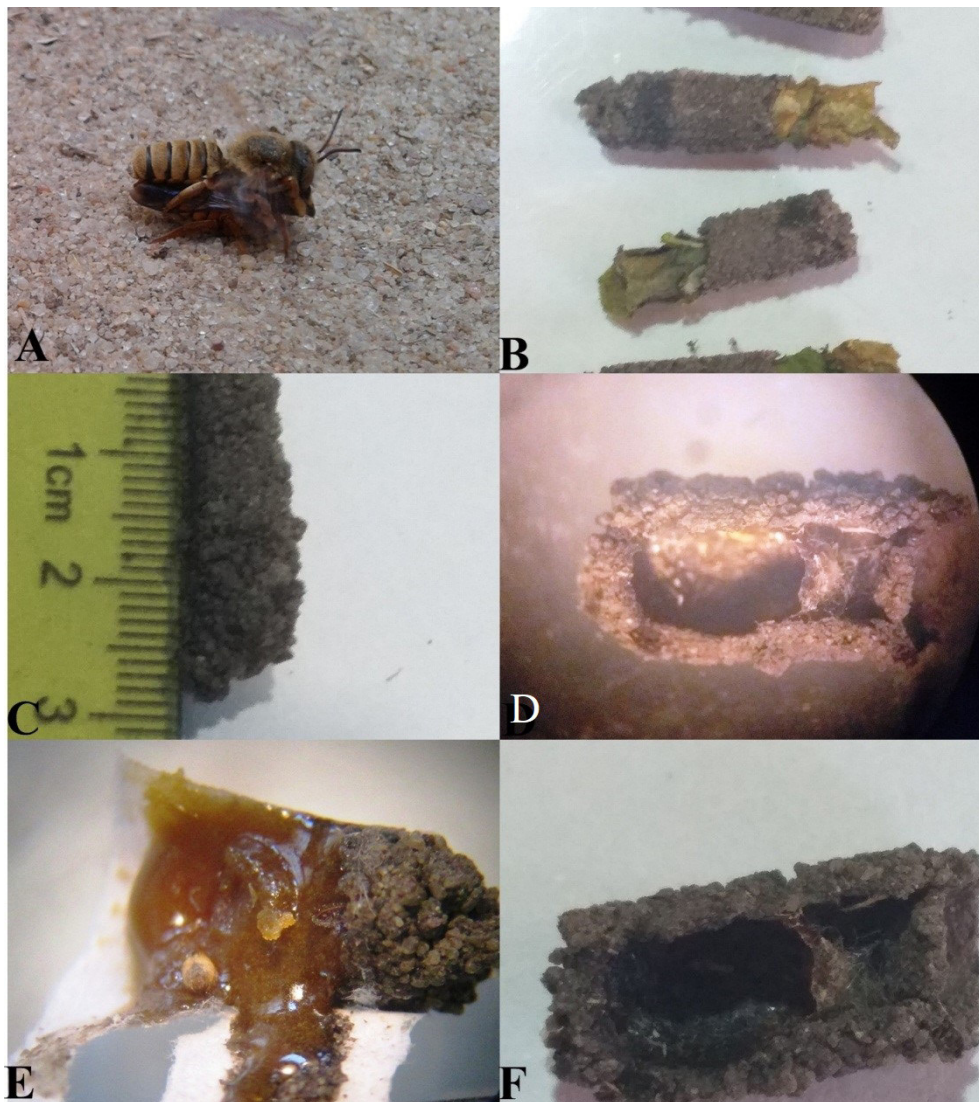
**Fig 4.** Artificial nesting material used for *C. nigripes* and *C. flavipes*.

**Table 2.** Numbers of total tubes and number nested tubes of *C. nigripes* and *C. flavipes* in foam traps.

Nests No.	Total Number of tubes	Number and percentage of nested tubes		Number and percentage of Unnested tubes	
		No.	%	No.	%
1	572	7	1.2	565	98.8
2	598	11	1.8	587	98.2
3	572	0	0.0	572	100.0
4	572	4	0.7	568	99.3
5	598	3	0.5	595	99.5
6	1040	52	5.0	988	95.0
7	1551	52	3.4	1499	96.6
8	1014	44	4.3	970	95.7
9	1014	8	0.8	1006	99.2
10	1040	42	4.0	998	96.0
11	1066	39	3.7	1027	96.3
12	1014	59	5.8	955	94.2
13	520	1	0.2	519	99.8
14	1530	17	1.1	1513	98.9
15	546	0	0.0	546	100.0
16	1014	108	10.7	906	89.3
17	572	4	0.7	568	99.3
18	800	7	0.9	793	99.1

intervals in wooden nests is shown in Figure 7. Based on the obtained number of holes, it was indicated that the highest number of parasites and predators was recorded in August, followed by July. Numbers decreased gradually in September, October, and November, respectively.

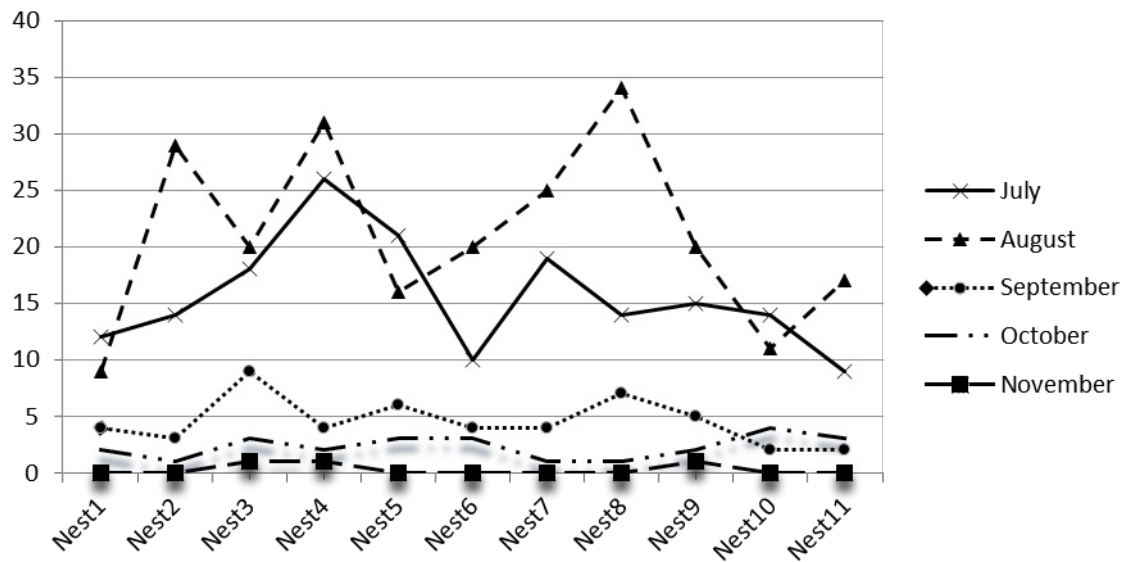
Megachilidae species cells collected from artificial nests and placed in the refrigerator were randomly counted and divided into live, empty, and dead cells. Numbers were recorded five times during the season (Figure 8). Observed parasites and predators were eliminated, and their spread was limited in the second season.



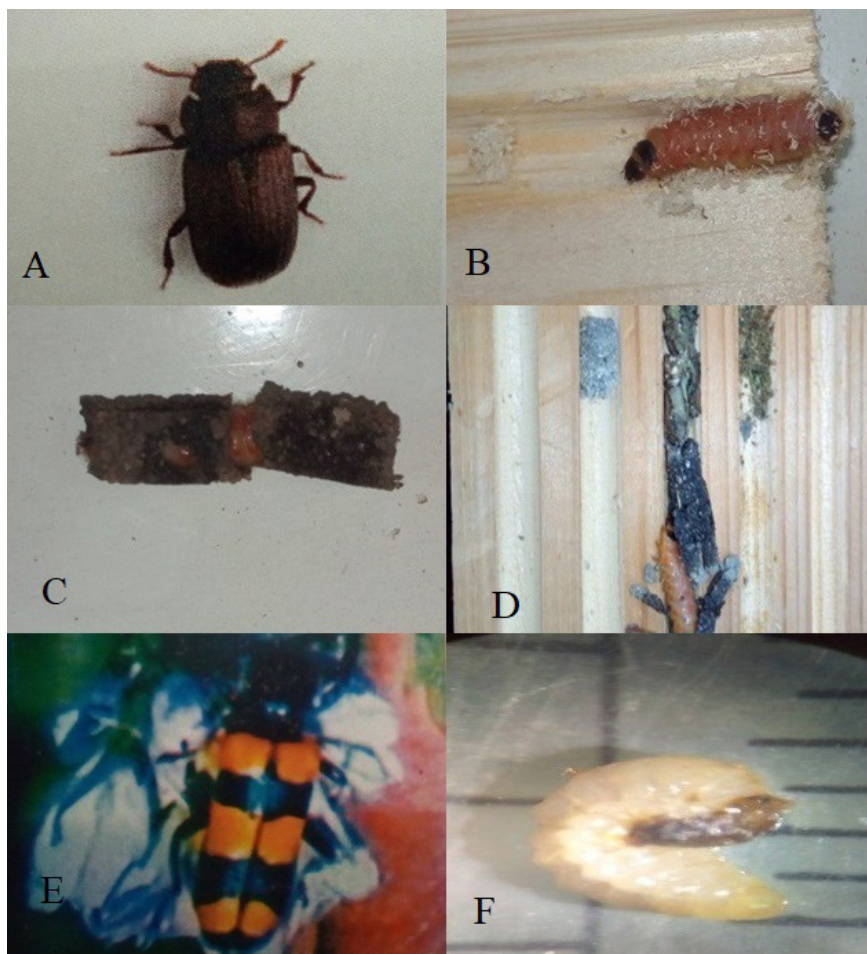
**Fig 5.** Nesting Biology of *C. flavipes* **A.** mating, **B.** Several built tunnels, **C.** Tunnel length (1cm to 3 cm), **D.** Empty cell (diameter 0.4 to 0.5 cm), **E.** Cell with provisioning for egg and larvae, **F.** Final stage with spinning cocoon.

**Table 3.** Comparison of nesting ratio of *C. nigripes* and *C. flavipes* in wooden and foam traps using Chi square test.

Nests	Total number of tubes		Nested tubes		Un-nested tubes	
	N		Number	%	Number	%
Wooden traps	3389		1846	54.47	1543	45.53
Foam traps	15633		458	2.93	15175	97.07
Chi square				6950.29		
P. value				0.00**		



**Fig 6.** Number of existing holes made by the parasites and predators in the Megachilidae bees in wooden nests.



**Fig 7.** Natural enemies found in *Megachile* bee cell, A: Adult of flour beetles; B, C and D: The larva of *Trichodes* sp.; E: Adult of *Trichodes* sp.; F: Unknown larva.

## Discussion

Information about the Egyptian bee faunal diversity is relatively meager. However, several alarming signs

referred to the composition and abundance of bee species and endangered and threatened species. Some observations were made in Egypt during interval samplings over the last decades, reflecting lower bee species richness and abundance.

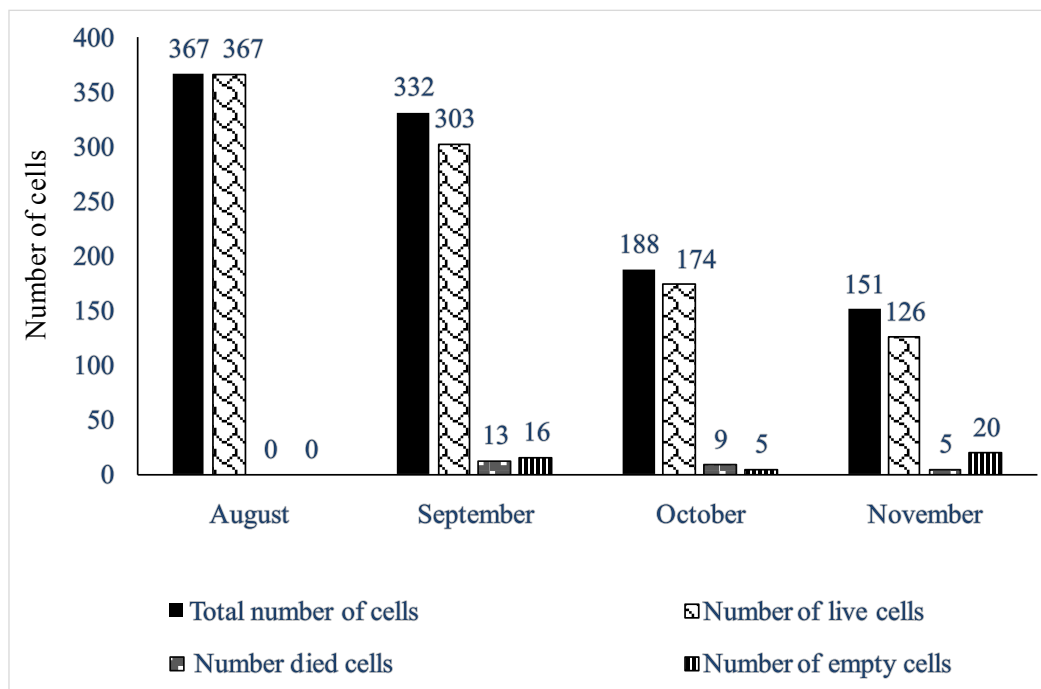


Fig 8. Number of expected emerged larvae of Megachilidae sp. cells preserved in cooling condition.

Hence, comparing historical data with the current situation is mandatory. In this respect, old data records from 1933 indicated that the *Chalicodoma* is well-abundant across the country, with 16 recorded species. However, the current species numbers and abundance are probably lower Okely et al. (2023). Conservation of domestic and solitary bees is crucial, particularly for Megachilids, to ensure good pollination services for several important crops, as most species are affected by climate change and habitat loss.

The genus *Chalicodoma* was first investigated one century ago in Egypt (Alfken, 1932) in addition to some subspecies of *C. (Chalicodoma) desertorum* and *C. (Chalicodoma) sicula*. About 16 species are currently known in Egypt (Shebl et al., 2013; Kuhlmann et al., 2024; Ascher & Pickering, 2024). The taxonomic position of the genus *Chalicodoma* is still debatable. It can be considered either a genus or a subgenus of *Megachile* (Gonzalez et al., 2019). Here, we consider *Chalicodoma* a genus based on Dufour's gland's nesting biology and structure (Mahmaoud & Shebl, 2024). Although much work was done on this genus, still more should be carried out on their conservation and their role in crop pollination since they are considered primary potential pollinators of some essential crops such as broad bean, alfalfa, and Egyptian clover (Mohamed et al., 2024; Shebl et al., 2013). Hence, the conservation of all of the species is crucial for assuring good pollination services. The conservation of *C. nigripes* was initiated in the eighteens of the last century, and several materials were used with large diameters of around 6 to 7 mm (Rashad & Ewis, 1985). The present results showed that the *C. nigripes* and *C. flavipes* preferred to nest in wood rather than foam. Most megachilids used to nest in wood as the best material for building their nests (Shebl et al., 2018).

The predominant nesting materials used for alfalfa leafcutting bees were wooden and foam nests, one of the present study recommendations. Similarly, the present study has revealed that the same genera and species, such as *Osmia rufa*, had a notable adaptation capability to artificial nests (Fairy & Lieveise, 1986). *Chalicodoma* species females used mud to line the inner surface of the cell and close it. The same behavior was also recorded in both species in our study. In this case, once the cell is shaped, nectar and pollen are supplied, and laying eggs is performed, then closing the cell would occur. (Ibrahim, 1979; Willmer, 1986). Meanwhile, *Chalicodoma sicula* built new nests close to old tunnels and cells (Ibrahim, 1979; Shoukry et al., 1997).

The bees could manipulate the parasitism rate and predation in natural conditions based on their high populations. Artificially, the rate was higher in some cases, especially with tiny parasites. Laboratory observation showed that observed parasites and predators were eliminated, and their spread was limited. Similarly, when samples resulting from the second season were placed in the refrigerator, their frequent examinations indicated that the degree of cooling works to preserve and save these samples and the cells from parasites and predators (Akram et al., 2022; Alvarez et al., 2022; Sabino & Antonini, 2017).

In this respect, it should also be noted that there are several worries about biodiversity loss worldwide. In this respect, bee faunal diversity is threatened by different causes, but habitat loss is one of the main drivers. Several endeavors should be considered to mitigate habitat loss. Offering natural materials for bees to build their nests and increase their population would be the best and easiest way to conserve megachilids.

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