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Unraveling the Nesting Architecture and Foraging Behavior of *Ceratina viridissima* in the Sub-Tropical Terrain of Jammu

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ABSTRACT

Ceratina viridissima, commonly referred to as the small carpenter bee, serves as a crucial pollinator in both agricultural and horticultural crops. In this study, we investigated its nesting habits through controlled laboratory observations. Our examination focused on its nesting behavior within trimmed pithy stems of *Rosa* spp., *Saccharum spontaneum* and *Saccharum bengalense*. These bees exhibit polylectic behavior, gathering pollen balls from diverse sources for their brood cells, and engaging in nest protection activities. Nest construction involves the excavation of pith from various plant stems, resulting in distinct chambers housing eggs, larvae, pupae, and adults. The life cycle of *C. viridissima* spans approximately 43.77±2.13 days. Notably, pupal stages display a range of eye pigmentation. Foraging endeavors are predominantly observed from early morning to late evening, closely linked to sunlight and temperature fluctuations. Detailed analysis of nest architecture revealed chambers partitioned by septa. A comprehensive understanding of the nesting behavior and relatively short life cycle of *C. viridissima* across different plant hosts contributes significantly to their conservation efforts and underscores their potential for enhancing agricultural crop pollination practices.

Key words: *Nesting behavior, Conservation, Polylectic and foraging activity*

Introduction

The global decline in honey bee populations, attributed to Colony Collapse Disorder (CCD) (Oldroyd, 2007; van Engeldorp *et al.,* 2008; Ratnieks and Carreck, 2010), extensive utilization of pesticides (Hopwood *et al.,* 2012), climate change (Bartomeus *et al.,* 2011), and the expansion of uniform agricultural environments, which diminish biodiversity and the food sources accessible to bees, underscores the importance of exploring wild or domesticated non-Apis bee species and deciphering their role as potential pollinators for agricultural crops, especially in regions where honey bee colonies are facing challenges or limitations (Chagnon *et al.,* 1993; Wilmer *et al.,* 1994; Javorek *et al.,* 2002; Hoehn *et al.,* 2008; Brittain *et al.,* 2013). So far, only a small selection of bee species other than Apis have been extensively utilized in agricultural activities such as *Bombus terrestris* (Linnaeus, 1758), *Megachile rotundata* (Fabricius, 1787), *Nomia melanderi* (Cockerell, 1906), *Osmia rufa* (Linnaeus, 1758), as well as certain stingless bee species (Westerkamp and Gottsberger, 2000; Hogendoorn *et al.,* 2006; Greenleaf and Kremen, 2006; Slaa *et al.,* 2006 and Hoehn *et al.,* 2008). These bees have demonstrated their effectiveness as pollinators, matching or even surpassing honey bees when it comes to specific crop plants (Greenleaf and Kremen, 2006).

Ceratina viridissima, also known as the small carpenter bee, represents a commonly found group of bee species categorized under the Xylocopinae subfamily. These bees are bright metallic blue to green in color and forage on a variety of crop plants (Michener, 2007). These bees make linear burrows in dead woods and pruned pithy stems to build their nests (Raju and Rao, 2006). Adult female bees chew the central pith of the pruned stem to build small cells and later female bee leaves nest for foraging to collect pollen and molds them into pollen balls and places them inside each cell of the nest, and these pollen balls act as a substrate for laying eggs (McIntosh, 1996). Few species of these small carpenter bees have also been reported to close the nest entrance with a membranous layer using the chewed pith and leave the nest. Small carpenter bees have the potential to act as efficient pollinating agents, especially for agricultural crops, and could serve as a counter-strategy in regions with declining honey bee populations. These bees can be easily conserved in-situ using pithy stems of different plants in different habitats (Kapil and Kumar, 1969; Batra, 1976). However, the decreasing abundance and species richness of native bees in agricultural landscapes have been well-documented due to habitat loss and a lack of foraging resources. Ceratina is a taxonomically diverse species but still are poorly known (Vanitha, 2023).

The present investigation was carried out with the objective of providing insights into nest architecture, nesting preference, life cycle, foraging behaviour, as well as delineating the effect of weather parameters on the foraging activity of *Ceratina viridissima*.

Materials and Methods

Study area

The study was conducted at the Research Farm and laboratory of the Division of Entomology, Faculty of Agriculture, SKUAST-Jammu, it is located between 32.6529° N geographical longitutude and 74.8071°geographical latitude and its maximum altitude from sea level is 1850 meters.

Study method

The nest architecture and life cycle of *Ceratina viridissima* were investigated by thoroughly monitoring its activity on selected host plants, namely *Rosa spp*., *Saccharum spontaneum*, and *Saccharum bengalense*. Bee nest construction was identified by holes at the cut ends of pruned stems, with bees seen entering these holes. We collected thirty nests from each host plant, ensuring adult bees had returned from foraging by evening before closing the nest entrance with cotton and a rubber band to prevent escape. Using a sharp knife, we opened each nest from the entrance hole, examining parameters like nest length, brood cell dimensions, presence of pollen provisions, and number of immature stages. After examination, we rejoined split stems to provide a suitable micro-climate for brood development. Immature stages were reared in the lab inside rearing boxes covered with cotton cloth until adult emergence. Regular inspections allowed monitoring of the bee's life cycle stages.

Foraging behavior was observed over 10 days, focusing on five marked nests during early morning hours. We recorded departure and return times, as well as time spent within the nest. To understand weather's effect on foraging, observations were made in natural field conditions, recording the number of flowers visited per minute and time spent on

Fig. 1. Exploring foraging tactics and nesting architecture of small carpenter bee

each flower, and correlated with weather parameters.

The data underwent statistical analysis using SPSS version 16.0. Standard errors and means were computed, and a one-way analysis of variance (ANOVA) was conducted at a significance level of P ≤ 0.05. All graphs were generated using Microsoft Excel.

Results

Thirty nests were collected from selected host plants, including *S. spontaneum*, *S. bengalense* and *Rosa* spp. Among these, ten nests were found on *S. bengalense*, one of which was an active brood nest housing a solitary mother and developing brood. Ten nests discovered on *Rosa spp*. contained no pollen mass, with only adult bees inhabiting the cells. The remaining nests on *S. spontaneum* were observed to be full brood nests, exhibiting varying sizes of pollen masses and stages of larvae within the cells (Fig. 2).

Life cycle of *C. viridissima* comprises egg, larvae, pupae, and adult stages, as depicted in Table 1. Adult females laid single eggs in individual cells atop the pollen provision. These provisions, measuring between 0.6 to 0.8 cm in length (n=30), appeared yellowish-brown, slightly viscous, and soft. The spindle-shaped eggs were translucent white and strategically positioned dorsally over the pollen ball to ensure immediate access to food upon hatching. The apodous larvae actively fed on the pollen mass within each cell. Notably, the size of the pollen mass was observed to vary, being larger in cells housing

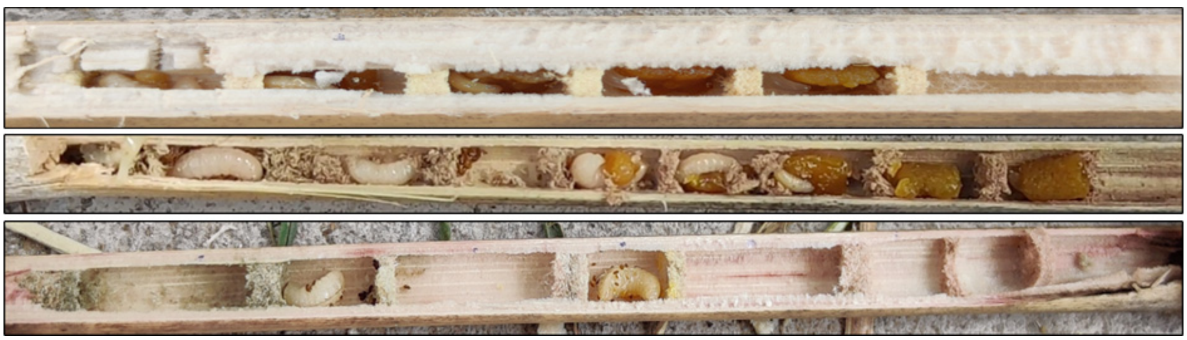

Fig. 2. Nesting Chambers and Pollen Stores of *Ceratina viridissima* in *Saccharum spontaneum* Hollow Stems

Table 1. Life cycle of *Ceratina viridissima*

Stage	Life stage description Duration	(days) (N=30)
Egg	-	5.83±0.85
	A fifth of the size of a pollen ball	4.00±0.79
	A pollen particle twice the size of a pollen ball	4.77±0.42
Larvae	Fully developed larvae Total larval period	4.23±0.62
	A fifth of the size of a pollen ball	4.27±0.88
	A pollen particle twice the size of a pollen ball	15.48±1.48
Prepupae	-	4.50±0.44
	White eyed	3.77±0.71
Pupae	Pale brown eyed	2.87±0.42
	Dark brown eyed	3.28±0.50
	Black eyed	4.57±0.71
	Total pupal period	12.50±1.07
	1/2 body pigmented	2.28±0.31
	1/4 body pigmented	2.60±0.45
Adult	3/4 body pigmented	2.43±0.62
	Full body pigmented	4.60±1.41
	Total adult period	8.77±1.70
Eggto Adult	-	43.77±2.13

*N-Number of nests

early instar larvae and smaller in cells containing mature larvae, suggesting differences in nutrient intake during larval development. The larval stage lasted for an average of 15.48 ± 1.45 days (n = 30), while the pupal stage persisted for approximately 12.5 ± 1.07 days (n = 30). Upon complete pigmentation, pupae molted into winged adults, which were subsequently observed chewing on the partition layer of cells to facilitate emergence from the nest. The entire life cycle of *C. viridissima* was completed in an average of 43.77 ± 2.13 days.

Ceratina viridissima constructs elongated nests within the cut tips of pithy plant stems. Upon dissection, adult female bees were found in a protective posture, positioned either with their heads or abdomens oriented upwards, safeguarding the developing brood cells. Each fully constructed nest was guarded by adult female bees positioned at the entrance. In some nests, a few adult females were observed near the septa of the second or third cells. The nests were unbranched, with an average length of 20.77 ± 0.78 mm (n = 10) in S. spontaneum, 14.38 ± 0.73 mm (n = 10) in *S. bengalense*, and 5.79 ± 1.38 mm in Rosa spp., respectively. Each nest had a single entrance point, with multiple chambers separated by chewed piths. The entrance widths of the selected host plants were nearly identical, recorded at 2.62 ± 0.07 mm with minor variations. Nests of *S. spontaneum* typically contained 6-8 chambers, with average lengths of 16.82 ± 1.00 mm and breadths of 3.06 ± 0.22 mm. In *S. bengalense*, 3-4 chambers were observed, with average lengths of 8.17 ± 0.86 mm and breadths of 2.12 ± 0.50 mm. No chambers were observed in *Rosa* spp. Nests of *S. spontaneum* exhibited the maximum number of larvae (3), pupae (2), and adult bees (3), followed by *S. bengalense*, which had no larvae or pupae but reported 1-2 adult bees. In contrast, *Rosa* spp. nests had 2 adult bees with no larvae or pupal stages (Fig. 3).

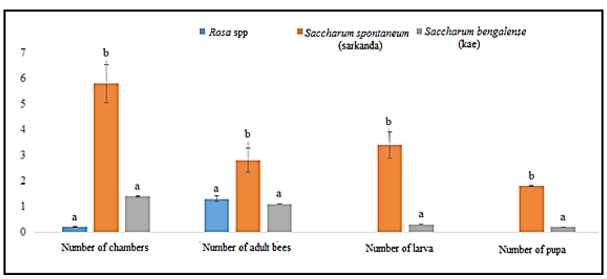

Fig. 3. Nesting architecture of *Ceratina viridissima* in different host plants All the data are presented as mean ± standard error and different letters above each bar indicate a significant difference between treatments ((p< 0.05, one way ANOVA).

Small carpenter bees are known for their polylectic foraging behaviour, visiting numerous species of flowering plants. In our investigation, these bees were observed foraging on flowers of *Ocimum sanctum* and *Trifolium alexandrinum*. On average, they spent 55.26 ± 20.77 seconds on each flower, collecting pollen rewards to take back to their nests. Foraging typically commenced in the morning around 9:00 am and concluded in the late evening between 4:50 and 5:15 pm. Our findings revealed that maximum temperature and sunshine had a notable positive impact on foraging activity, as illustrated in Fig. 4 and Fig. 5. Conversely, foraging activity showed a negative correlation with relative humidity and rainfall. Small carpenter bees demonstrated a preference for higher temperatures and ample sunshine while foraging, avoiding cloudy weather and lower temperatures. Interestingly, relative humidity did not significantly affect the foraging activity of these bees.

Discussion

Study revealed a clear preference of *Ceratina*

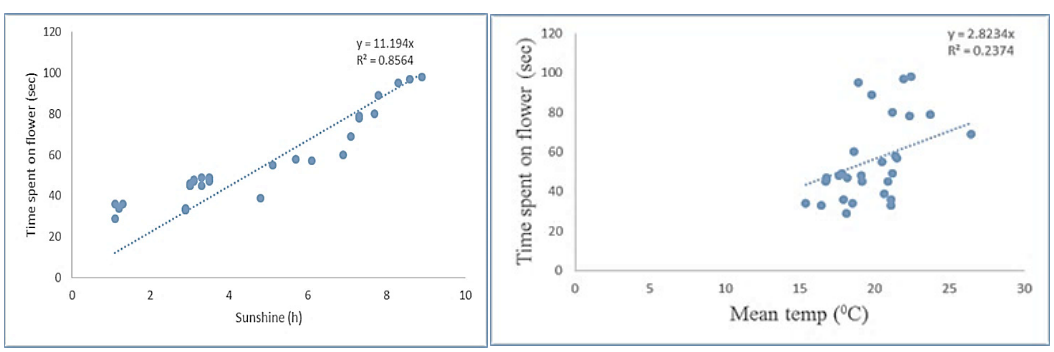

Fig. 4. Sunshine and temperature influence on foraging duration of *Ceratina viridissima* on *Ocimum sanctum* flowers.

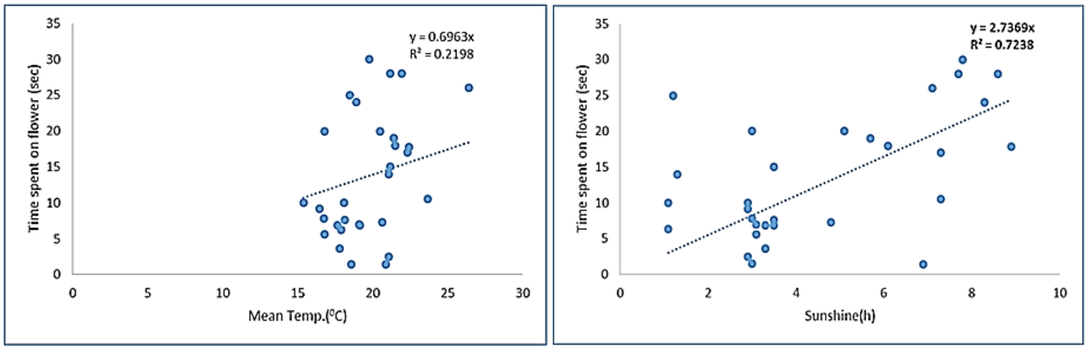

Fig. 5. Sunshine and temperature influence on foraging duration of *Ceratina viridissima* on *Trifolium alexandrinum* flowers.

viridissima for nesting in the pithy stems of *Saccharum spontaneum*, although nests were also found in *S. bengalense* and *Rosa* spp. The selection of nest sites, nest architecture, and life cycle of *C. viridissima varied* significantly based on species, climate, and plant availability. According to Ali *et al.* (2016), *Ceratinas maragdula* in Pakistan constructed nests in the wooden stalks of Ravenna grass (*Saccharum ravennae* L., Poaceae) and completed its life cycle within 28 to 32 days under laboratory conditions. Our observations align with this, showing similar nest construction behaviour. Adult female bees were frequently found guarding the nest entrance, either with their heads or abdomens oriented upwards. This behaviour varied but generally involved the female bee in a protective posture over the developing brood cells. During nest dissections, adult bees were observed crawling over the brood cells, which may indicate inspection of the provisioned pollen. This behavior was also noted by Maeta *et al.* (1997), who reported sub-social behavior in female bees, involving crawling through cell partitions to examine offspring.

Nest guarding by adult females is a critical behaviour for the survival of the offspring. It provides protection against natural enemies and increases the likelihood of offspring reaching maturity. Our study found that 100% of the adult bees emerged without signs of parasitism, suggesting that the guarding behaviour significantly contributed to the high survival rate. Mikat *et al.* (2016) also reported increased offspring survival in *C. cucurbitina* due to the guarding strategy of adult females. *Ceratina calcarata,* a sub-social species, exhibits similar behaviour. Females construct nests solitarily but remain in the nest to care for their offspring until they reach adulthood, as observed by Durant *et al.* (2015). Rehan and Richards (2010) documented a similar nesting biology for *C. calcarata*, noting a 46-day period to complete a single generation. Our study also showed that all examined nests contained bees at different life stages. Some nests had empty cells, indicating that certain broods had completed their development and emerged, leaving behind chewed cell partitions and molted exuviae. The orientation of pupae near the nest entrance was consistent with findings by Yogi (2014) for *C. simillima*, which nested in *Caesalpinia* stems. Hongjamrassilp and Warrit (2014) reported similar linear unbranched nest architecture for *Xylocopa nasalis*, another bee in the subfamily *Xylocopinae*, with nests constructed by the founding female using pithy partitions.

In our research, we also examined the influence of weather factors on the foraging habits of *C. viridissima* on *Ocimum sanctum* and *Trifolium alexandrinum*. Our observations showed that *C. viridissima* made significantly more visits to flowers compared to other pollinators, spending an average of 55.26 ± 20.77 seconds per flower. This behaviour aligns with findings from Lunau *et al.* (2020) and Rodney and Purdy (2020), who also reported similar foraging times. Our data indicated a positive relationship between foraging activity and both maximum temperature and sunshine. These weather factors significantly impacted the bees' foraging behaviour, with higher temperatures and increased sunshine correlating with increased foraging activity. This is consistent with studies by Bajiya and Abrol (2017) and Sharma and Abrol (2017) Painkra *et al.* (2017), who also found that temperature and sunshine positively influenced bee foraging behaviour. Conversely, our findings showed a negative correlation between foraging activity and relative humidity and rainfall. Small carpenter bees preferred elevated temperatures and ample sunshine for foraging, avoiding cloudy weather and lower temperatures. Relative humidity did not have a significant effect on the foraging activity of *C. viridissima*, highlighting the bees' adaptability to varying environmental conditions.

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Conflict of interest

The authors declare that the research work was carried out without any financial or commercial dealings that could be raised as a probable conflict of interest.

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