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RESEARCH ARTICLE

MOLECULAR CONFIRMATION OF TWO HONEYBEE SPECIES (*Apis mellifera* L. and *A. cerana* F.) IN APIARY AND THEIR FORAGING BEHAVIOR IN LITCHI ORCHARD

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ABSTRACT

The foraging of honeybees is one of the most well-organized and admirable behaviors that exists among social insects and being greatly influenced by nectarine sources and habitat adaptability. In Bangladesh, apiculture is mostly confined to rearing of European honeybee *Apis mellifera* L. despite of having the native *A. cerana* F. due to lack of information about comparative foraging efficiency and productibility of two species in Asian cropland ecosystem. The present study aimed to molecular characterization of two honeybee species in apiary and their foraging performance on litchi orchard. The genetic identity was revealed thorough phylogenetic analysis with >90% bootstrap value using mitochondrial cytochrome oxidase sub unit- 1 (CO1) gene and nucleotide sequence data deposited to NCBI GenBank with accession number ON680900-ON680902 for *A. mellifera* and ON703291-ON703293 of *A. cerana*. Upon placing the identified bee hives in litchi orchard, the foraging efficiency were studied based on egression and ingression rate, number of bees visited flowers per minutes, and nectar and pollen collection efficiency in varied time series of the day. Principal component analysis (PCA) for measuring the contribution of different foraging parameters and the species wise PCA biplot revealed the better foraging efficiency by *A. mellifera* L. compared to *A. cerana* F. in litchi blooms. However, foraging efficiency of other nectarine sources should be analyzed for suggesting best performing bee species in apiculture.

KEYWORDS

Foraging, European and Asian Honeybee, MtDNA, Honeybee Phylogeny

1. INTRODUCTION

Honeybees are an important part of the natural ecosystem since its contribution to biodiversity and agricultural output by providing vital pollination services, which are based on the ecological principle of mutual interactions between fertilized plants and pollinators (Vaziritabar et al., 2015). The role of bees as pollinators and honey production, in turn, depends on their foraging ecology, including foraging range, daily patterns of activity, and exploratory behavior. Their close relation to a lot of imperative crops and its foraging behavior makes them successful insect pollinators (Said et al., 2015). An understanding of different bee foraging biology is especially important in tropical ecosystems, where the vast majority of agricultural crops depend upon bee pollinators and where ecosystems are currently under threat from human actions such as land use change, pesticide use, and pollution. (Brown et al., 2016; Thimmegowda et al., 2020; Donaldson-Matasci and Dornhaus, 2012; Dainese et al., 2019). The knowledge on bee behavior and foraging activity and their interactions with different plant species are pre-requisite to frame on strategy for effective crop pollination and beehive productions for different agro-ecological regions (Pudasaini and Thapa, 2014).

Honeybees, (genus *Apis*), are important pollinators in both agricultural and natural ecosystems. Although there are more than 3000 pollinators other than honeybees, but among them honeybees are ranked first (Vaziritabar et al., 2015). In Bangladesh, Apiculture has huge impacts on agricultural, ecological and socio economical aspects (Rumman et al., 2021). Beekeeping in Bangladesh mostly meant by rearing of the European honeybee, *A. mellifera* for production of honey, with rare exceptions using *A. cerana*. About 25000 people involved with beekeeping that collect honey from mustard, coriander and black cumin fields apart from litchi garden and the Sundarbans in Bangladesh and the country produces nearly 10,000 tons of honey annually (Topu and Parvez, 2021). The European honeybee, *Apis mellifera* was introduced into Bangladesh on an experimental basis in 1992 (Sivaram, 2012). However, the two species, *Apis mellifera* and *Apis cerana* are mostly used by the beekeepers worldwide for production of honey including Bangladesh (Hung et al., 2018; Requier et al., 2019).

As a managed agricultural species worldwide, recent studies have highlighted only the role of the western honeybee *A. mellifera*, as a potential threat to wild pollinators that are in danger of extinction (Requier et al., 2019). Numerous studies have also demonstrated that, this introduced honeybee can decrease the survival, growth, reproduction, and dietary behaviors of native pollinators because of their aggressive dominance (Stanley et al., 2015; Liu, 2016). On the other hand, due to native origin of *Apis cerana* bee colonies, it is easy to find and cultivate (Islam, 2016). In many countries of the world, *A. cerana* is considered one of the desired bee species in beekeeping because of the nutritional quality and price of *A. cerana* honey. Like other Asian countries, in Bangladesh, the price of *A. cerana* honey is usually three to five times higher than that of *A.*

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mellifera honey due to limited productivity and local consumer preferences. *A. cerana* is originated from Asia and thus, it may have better survival capacity against insect pest and also can play more efficient role in foraging and honey production in Asian cropland ecosystem however, this species has been underrated.

To reveal the role of the two bee species in their foraging perspective in Asian croplands, it is necessary to confirm their genetic identity in the hives placed in the apiary. There are few literatures describing about the foraging parameters of the honeybee, but a systematic analysis is lacking prior to those study. For the identification of bee species reared in the apiary, the most relevant instruments for species differentiating proof in animal products and processed food are DNA-based entomological authentication procedures (Amaral et al., 2016). Mitochondrial cytochrome oxidase subunit I (COI) gene region is one of the gene markers used to classify and identify honeybee species now a days (Zhao et al., 2014). DNA-based approaches are thought to be faster and more exact than other methods, with soundness and ubiquity in all cell types. The correlations between DNA characteristics and cytochrome oxidase subunit -1 amino acid content in *Apis* reflecting an opportunity to evaluate if there is any hint of such a relationship for this mitochondrial gene, at least on a general level (Crozier and Crozier, 1992). As well as there is no comparative study on foraging behaviors of *A. cerana* and *A. mellifera* is available.

Since the beekeepers of Bangladesh has mostly shifted to mono-floral honey production strategies, therefore, determining the best foraging bee species with its associated crops will boost up the honey production and pollination scenario. Despite of the importance of Asian honeybee species in Asian tropical ecosystems, their foraging ecology remains poorly studied as compared to the Western honeybees (*A. mellifera*) (Kohl et al., 2020). Although investigations have been made on species specific behavior, efforts comprising both the species in the same cropland ecosystem should certainly provide a valid information regarding the efficiency of foraging behavior of those species. Thus, the findings can be transfer to the beekeepers for choosing the best fitted and suited bee species for smart apiculture in Bangladesh. The molecular characterization of both the species prior to analyzing the foraging ability will provide authentic data for further study of ecosystem restoration using these bee species. Conceiving all these thoughts and ideas, the present study was undertaken with the objectives to reveal and confirm the genetic identity of the honeybee species in the hives using mitochondrial *cytochrome oxidase subunit- 1 (CO1)* gene for characterizing the bee colony in apiary and to determine the foraging behavior of *A. mellifera* and *A. cerana* in litchi orchard as swarmed from the identified bee colony

2. Materials and Methods

The present research included two experiments where the first part focused on molecular characterization for revealing the taxonomic identity of the honeybee species in the apiary while the 2nd part emphasized on determination of foraging performance of *A. cerana* F. and *A. mellifera* L. in litchi orchard. The experiment was conducted from February 2021 to March 2022. The detailed methodology of these two experiments were described under the following subheadings:

2.1 Sample Collection and Specimen Preservation

For taxonomic identification of the honeybees from apiary, honeybee samples were collected from the two distinct apiaries managed by the beekeepers. Both the apiary was placed in the litchi orchard of village Pazulia, Joydebpur, Gazipur district. The experimental area was belonging to latitude 24°02′57′′N and longitude 90°26′30′′E. From the litchi orchard, a total of 8 hives from each apiary were selected based on beekeeper's species-specific recommendation for molecular study for revealing their taxonomic identity. Three adult honeybee workers from each hive of a single apiary have been taken for molecular characterization. The collected specimens were preserved in 99.9% Ethanol prior to DNA extraction.

2.2 Characterization of The Beehives by Revealing its Taxonomic Identity

The molecular analyses were conducted in the Advanced Entomology Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh. The molecular identification was done by the characterization of honeybee samples by sequencing mtDNA. Genomic DNA was extracted from alcoholic preserved specimens by using *QAGEN DNeasy Blood and Tissue kit*, following manufacturer's instruction (Zhao et al., 2014). Amplification of DNA was done by polymerase chain reaction (PCR) *TaKaRa Ex Taq* PCR kit, according to the manufacturer's instructions. The target site was detected through mitochondrial DNA of Cytochrome b region. For, mitochondrial DNA analysis, primers for COI gene fragment, forward and reverse primers were used as COI 1–3 (5′ ATAATTTTTTTTATAGTTATACC'3) and COI 2–4 (5′ TCCTAAAAAATGTTGAGGAAA'3) as cited (Crozier and Crozier, 1993).

The thermal cycling parameters for COI basically followed the protocols established, including 95 0C for 5 min for initial denaturation, 35 cycles of dissociation (92 0C, 1 min), annealing (54 0C, 1min), and extension (70 0C, 2 min) (Sameshima et al., 1999). The purified PCR product was sequenced with the results derived from nucleotide sequencing company and the sequenced data of mtDNA derived from mitochondrial *cytochrome oxidase subunit 1* gene was submitted to GenBank of NCBI, for accession number. Upon receiving the accession numbers from NCBI of all the collected bee samples, the nucleotide sequence data had been processed for further analysis. The retrieved nucleotide sequences were aligned using the MEGA 11 software's Clustal X. For *A. cerana* F. and *A. mellifera* L., a total of 627 bp and 798 bp of nucleotide sequences were used in the analysis, respectively. The phylogenetic study revealed the nucleotide diversity of collected honeybee samples and thus, their taxonomic identity is retrieved through boots strap consensus. The hives with identified bee species were placed in the litchi orchard for studying the foraging performance.

2.3 Study of The Foraging Behavior of Two Types of Honeybees

After confirming the honeybee species, 4 hives from each apiary were used to analyze the foraging attributes in litchi field. The data based on foraging time, number of worker bees egressing from the colony per minute, number of worker bees ingressing into the colony per minute, number of flowers visited per minute and number of worker bees entering with pollen and nectar into the hives per minute were taken. All the data were taken three times with four days intervals (17 March'22-27 March'22) as the litchi flower blooming is varied. The details of data analysis are described below:

2.3.1 Foraging Time

Foraging time of both the species was assessed in terms of timings of commencement and cessation of flight activity by noting the time when first bee started its flight in the morning and the last bee ceased its flight in the evening (Joshi and Joshi, 2010).

2.3.2 Number of Worker Bees Egressing from and Ingressing into The Colony Per Minute

Total number of worker bees egressed from and ingressed into the colony per minute was counted by digital watch. The egressing record of worker bees was taken for 3 weeks. In each week each existing data was recorded on hourly basis started from 9.00 to 14.00 hrs. The average number of worker bees egressing in each week at each hour of the day was calculated.

2.3.3 Number of Flowers Visited by Bees Per Minutes

Foraging of bees started from morning and ended in the afternoon. Foraging behavior of bees in respect to number of litchi flowers visited per minute was determined by tracking 5 bees at an hourly interval from 9.00 to 14.00 hour of the day. Hourly intervals were 9.00-10.00 hrs, 10.00-11.00 hrs, 11.00-12.00 hrs, 12.00-13.00 hrs, 13.00-14.00 hrs. At each hourly interval a foraging worker bee (irrespective of pollen or nectar collector) was tracked and recorded the time (second) spent on a flower. The same bee was tracked when it flown to another flower. The bee was followed until it gone beyond the sight and recorded the total number of flowers visited. In this way 5 bees were tracked at each hourly interval from morning to afternoon. This tracking was done for 3 weeks beginning from flower initiation.

2.3.4 Number of Worker Bees Entering The Colony with Pollen and Nectar Per Minute

The number of worker bees entering the colony with pollen and nectar per minute were recorded separately on four-day interval (17 March'22-27 March'22) of the season. The number of bees carrying pollen and nectar during 9.00 to 14.00 hrs of each day of the week were recorded. Pollen collectors were identified by the presence of pollen load on their hind legs. Nectar collectors do not bear such load on their legs. Data was collected by observing workers bees carrying pollen or nectar from litchi field and landing on the entrance.

2.4 Data Analysis

The molecular nucleotide sequence was analyzed using MEGA 11

(Molecular Evolutionary Genetic Analysis) software and the reference data was retrieved from National Center for Biotechnology Information, NCBI to construct the phylogenetic tree. Principal component analysis (PCA) was done to determine the contribution of different parameters in foraging efficiency. All the statistical analysis to determine the foraging efficiency of the two types of honeybees were performed through statistical package "R".

3. RESULTS

The results of the current study were described in this chapter with two sections. The first part of the results focused on revealing the genetic identity of the honeybees in hive while the second section described the foraging efficiency of those identified beehives in the apiary.

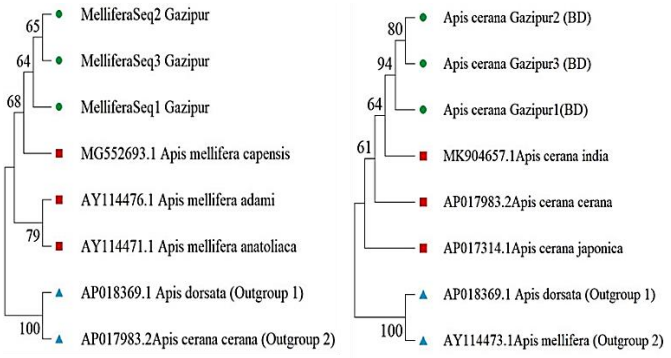
3.1 Molecular Identification of Honeybee Species from Beehives Placed in Apiary

The identification of honeybee species in apiary was done by mitochondrial DNA analysis through *cytochrome oxidase subunit-1* gene. The nucleotide sequences were submitted to NCBI GenBank and received the accession number (ON703291-ON703293 & ON680900-ON680902) respectively for *A. cerana* and *A. mellifera*.

3.1.1 Molecular Identification of A. Mellifera L. and A. Cerana F. From Bee Hives

The molecular identification of *A. mellifera* L. and *A. cerana* F. from beehives was done by phylogenic analysis. The analysis was done using the 3-nucleotide sequence of *A. mellifera* L. samples collected from different hives, with 3 reference data of *A. mellifera* from GenBank and 2 outgroup of *A. cerana cerana* and *A. dorsata* in case of *A. melliferea*. While 3-nucleotide sequence of *A. cerana* F. samples collected from different hives, with 3 reference data from GenBank and 2 outgroup of *A. mellifera* and *A. dorsata* were used in case of *A. cerana*.

For the phylogenetic analysis of *A. mellifera* L. and *A. cerana* F from bee hives, the evolutionary history was inferred using Neighbor- joining tree generated by MEGA 11 software (Figure 1). Each of the analysis involved 8 nucleotide sequences. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Tamura et al., 2004). All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 798 positions and 627 positions in the final dataset for *A. mellifera* and *A. cerana* respectively.



(Green circles indicates *A. mellifera* L. species of beehives, red rectangle indicates standard *A. mellifera* for reference from GenBank and *A. cerana cerana* and *A. dorsata* used as outgroup (blue triangle)).

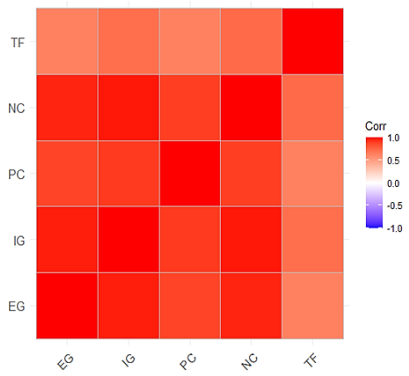
Figure 1: Neighbor-joining tree of A. mellifera and A. cerana

Result retrieved through the molecular study of collected honeybee samples confirmed the genetic identity of two honeybee species as of *A. mellifera* L. and *A. cerana* F. The foraging efficiency of the honeybee on litchi orchard are described under the following subheadings.

3.2 Foraging Pattern of *A. Mellifera* L. and *A. Cerana* F. on Litchi

To determine the foraging behavior of two types of honeybee species in litchi bloom, the rate of ingression and egression per minute were measured along with their spent time per flower with nectar and pollen collection efficiency. All the parameters taken in this analysis are strongly correlated with significant contribution towards determining the foraging efficiency. Performance of different variables for aforementioned purposed are showed in Figure 2.

The correlation among the variables was analyzed using the correlation matrixes, where the red to white colors indicated positive correlation and the white to blue color indicated negative correlation. Deeper the red color indicated the strong positive (Figure 2). In this figure, PC had positive correlation with TF (64%). EG had strong positive correlation with PC (88%) than TF (64%) but didn't have any correlation with the other variables. IG had strong positive correlation (97%) with EG than PC (91%) and had lowest positive correlation (72%) with TF but didn't have any correlation with the variable of NC. NC had strong positive correlation (98% & 96% respectively) with both IG and EG than PC (90%) and had lowest positive correlation (74%) with TF (Figure 2).



(PC- Pollen collectors entering into hive with pollen load per minute; EG- Egression of worker bees from hive per minute; IG- Ingression of worker bees into hive per minute; NC- Nectar collectors entering into hive per minute with nectar TF- Time spent per flower)

Figure 2: Correlation matrix of different parameters for determining foraging efficiency.

Heatmap dendrogram was used to visualize the result of a hierarchical clustering calculation of the variables. The result of a clustering was presented as the distance or the similarity between the clustered rows or columns depending on the selected distance measure (Figure 3). In this heat map, NC was closely associated with IG rate as they offered the short distance followed by the rate of EG. TF showed in distant clades with other variables and provided a significant importance in determining the foraging performance (Figure 3).

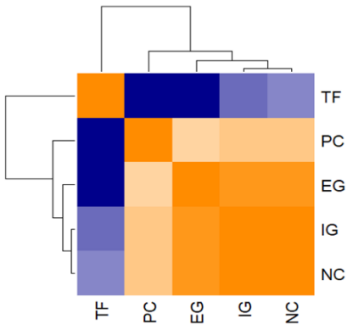


Figure 3: Heatmap dendrogram to visualize the result of a hierarchical clustering calculation of the analyzed parameters

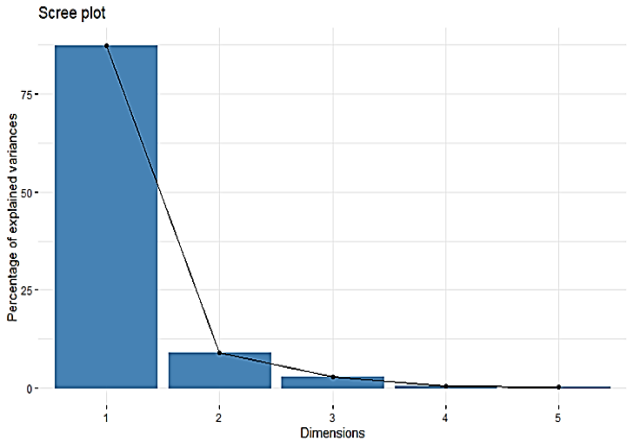


Figure 4: Principal Component analysis (PCA) among the foraging variables of two honeybees

Principal component analysis (PCA) was done with variables of data collections of two honeybee species and it was found that, the first two components could explain more than 96% of the variation presented in Figure 4. Hence, in the PCA biplot analysis, two dimensions were considered with refereeing to variance 1 and 2.

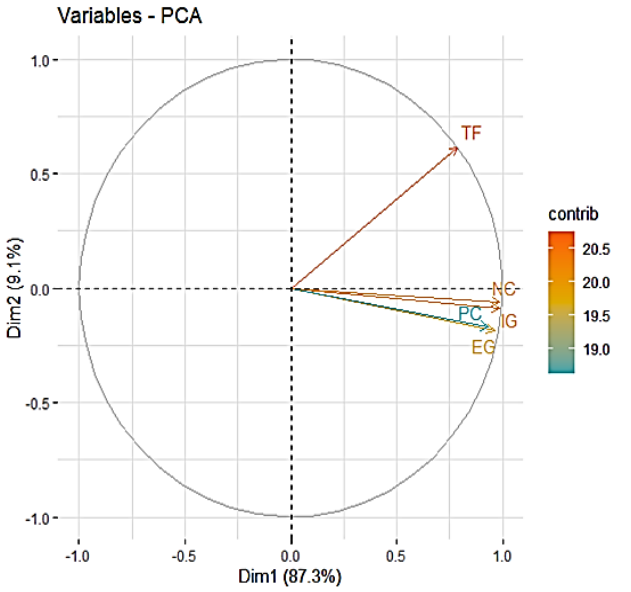
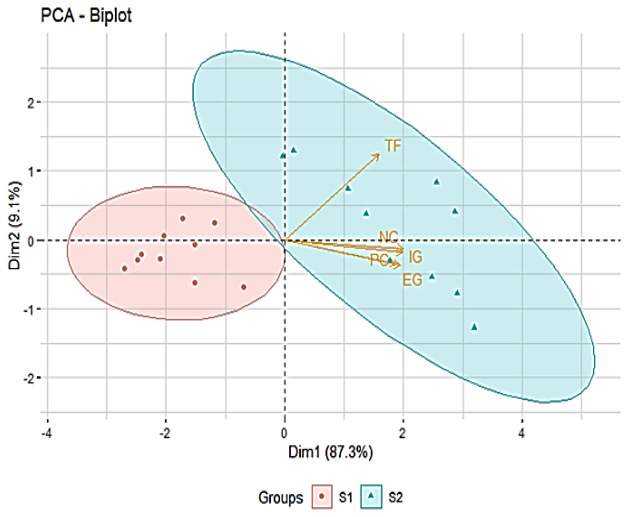


Figure 5: Principle component analysis representing different variables for both *A. cerana* F. and *A. mellifera* L. honeybees.

Among the 5 parameters of which the data have been collected to evaluate the comparative behavior of both species (Figure 5), TF exposed strong positive correlation within other variables by contributing 14.21% in dimension 1 and 83.50% in dimension 2; while PC exposed weak contribution of 19.90% in dimension 1 and 6.41% in dimension 2 among all other variables. These correlations among the variables may differ due to change in the presence of flower, colony needs, day temperature, humidity, wind speed and other situation.

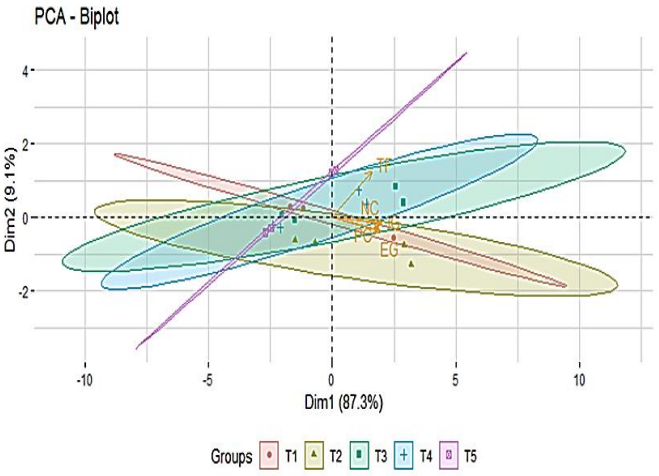
The contribution of different variables in PCA with its corresponding honeybee species is presented in Figure 6. TF played the most positive correlation for determining the signature pattern of the bee species. In S2, NC and IG had high correlation between them as they almost overlapped each other; as well PC and EG also had high correlation between them as they also overlapped each other. On the other hand, TF had lower correlation with other variables as it was distant from the others (Figure 6).



(S1= *Apis cerana* F.; S2= *Apis mellifera* L.)

Figure 6: Biplot generated through principal component analysis corresponding with honeybee species cluster.

Time series ranging from 9:00 AM to 3:00 PM provided marked impact on the foraging efficiency as it had a strong correlation in different foraging activities of the bees. The contribution of different variables in PCA with time is presented in this figure 7. T5 showed distinct relation among others crossing both dimension 1 and dimension 2 with completely positive relation with dimension 1 and completely negative relation with dimension 2. T3 and T4 overlapped each other having positive relation with dimension 1 and negative relation with dimension 2. On the other hand, T1 and T2 overlapped each other having both positive relation in dimension 1 and dimension 2.



(T1= 9:00-10:00; T2= 10:00-11:00; T3=11:00-12:00; T4= 13:00-14:00; T5= 14:00-15:00)

Figure 7: Biplot generated through principal component analysis corresponding with different time

3.3 Comparative Foraging Efficiency of *Apis Mellifera* L. and *Apis Cerana* F. in Litchi Orchard

The foraging behavior of both the species were influenced with several factors namely, total foraging time, time spent per flower, number of flowers visited per minute, ingression and egression rate. The comparative foraging efficiency of two honeybee in several parameters are shown in table 1. Between the two species, *A. mellifera* L. and *A. cerana* F. the European bees, *A. mellifera* L. showed significant values of egression and ingression of worker bees per minute, number of pollen and nectar collectors entering into hive per minute and time spent per flower on a foraging trip than *A. cerana* F.

Table 1: Comparative Foraging Behaviors of Two Honeybee Species in Different Parameters

Foraging Parameters	*A. Mellifera* L.	*A. Cerana* F.
Egression of Worker Bees Per Minute	76.02 a	34.92 b
Ingression of Worker Bees Per Minute	85.18 a	35.04 b
Pollen Collectors Entering into Hive with Pollen Load Per Minute	9.60 a	0.66 b
Nectar Collectors Entering into Hive Per Minute with Nectar	76.06 a	30.18 b
Time Spent Per Flower	8.040 a	4.703 b

Foraging time was significantly higher in case of *A. cerana* F. than *A. mellifera* L. and *A. cerana* F. as mentioned in Table 1. *A. cerana* F. had started foraging early in the morning (6:10±0.5hrs) than *A. mellifera* L. (6:30±0.5hrs). Similarly in the evening *A. cerana* F. ceased its flight (18:45±0.10hrs) later than *A. mellifera* L. (18:30±0.10hrs). The flight activity of *A. cerana* F. lasted for 12:35±0.10 hrs while in *A. mellifera* L. it lasted for 12:00±0.10 hrs. Therefore, these results suggested that *A. mellifera* visit considerably a less amount time span than that of *A. cerana* (Table 1).

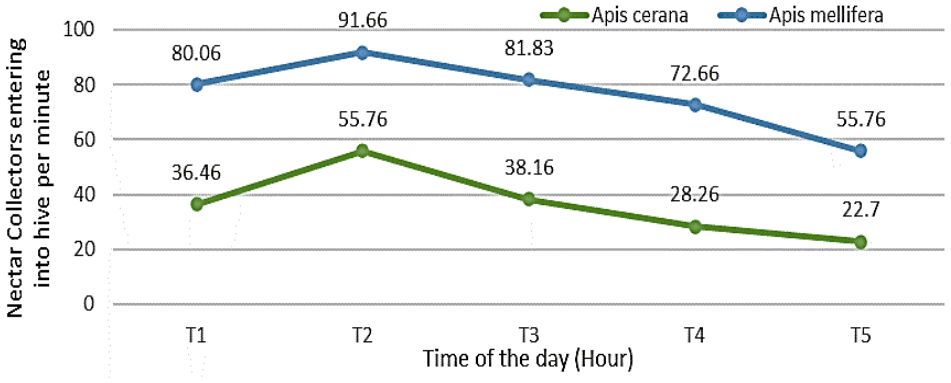
Egression and ingression of worker bees per minute was statistically significant during full blooming of litchi flower for *A. mellifera* L. and *A. cerana* F. at different time of the day (Table. 2). Egression of worker bees was maximum for both of the species from 10:00am-11:00 am. In case of ingression, *A. mellifera* L. showed non-significant value from 9:00 am to 12am and *A. cerana* F. showed maximum ingression from 10:00am- 11:00 am.

Table 2: Number of Worker Bees Exiting from (EG) and Entering Into (IG) Hive Per Minute of Two Species at Different Time of The Day					
Egression of Honeybee/ Minute			Ingression of Honeybee/ Minute		
A. mellifera L.	9:00-10:00	81.6 b	A. mellifera L.	9:00-10:00	92.3 a
A. mellifera L.	10:00-11:00	100.8 a	A. mellifera L.	10:00-11:00	105.7 a
A. mellifera L.	11:00-12:00	82.7 b	A. mellifera L.	11:00-12:00	94.6 a
A. mellifera L.	12:00-13:00	70.1 bc	A. mellifera L.	12:00-13:00	77.9 ab
A. mellifera L.	13:00-14:00	44.9 de	A. mellifera L.	13:00-14:00	55.4 bc
A. cerana F.	9:00-10:00	37.3 ef	A. cerana F.	9:00-10:00	39.7 c
A. cerana F.	10:00-11:00	55.1 cd	A. cerana F.	10:00-11:00	51.4 bc
A. cerana F.	11:00-12:00	33.6 ef	A. cerana F.	11:00-12:00	NS
A. cerana F.	12:00-13:00	24.9 f	A. cerana F.	12:00-13:00	NS
A. cerana F.	13:00-14:00	NS	A. cerana F.	13:00-14:00	NS

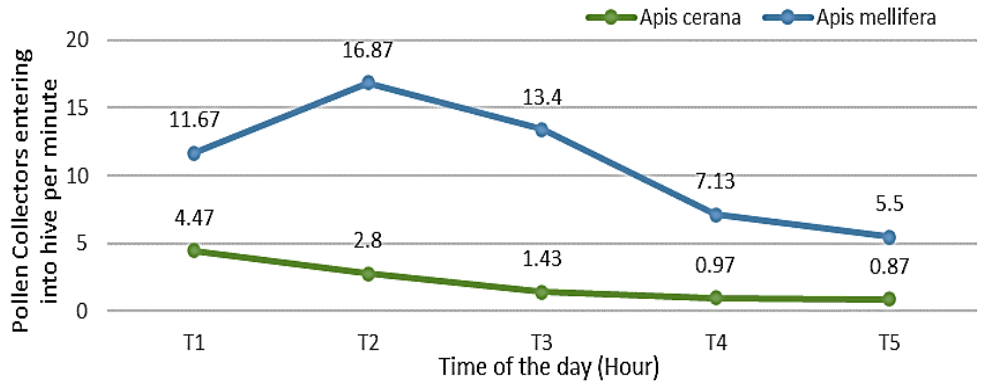
(*NS= non-significant)

The nectar and pollen collection efficiency for both the species are presented in Figure 8. The number of nectar and pollen collectors entered into hive per minute was significantly different in case of *A. cerana* F. and *A. mellifera* L while it was also different at different time of the day (hour).

The highest number of nectar collectors' entry for both the species was at T2 and it was lowest for both at T5. That indicated similar trend of nectar collection efficiency in case of both the species.



a) Number of nectar collectors entering into hive per minute at different time of the day (hour)



b) Number of pollen collectors entering into hive per minute at different time of the day (hour)

(T1= 9:00-10:00am; T2= 10:00- 11:00am; T3=11:00-12:00am; T4=12:00-13:00pm; T5= 13:00-14:00pm)

Figure 8: Relationship between numbers of nectar collectors (a) and pollen collectors (b) entering into hive with nectar and pollen per minute at different time of the day (hour)

On the other hand, maximum number of pollen collectors were seen entering into the hive with pollen load at T1 in case of *A. cerana* F. which was lowest at T5; while, maximum number of pollen collectors were seen entered into hive at T2 in case of *A. mellifera* L. which was lowest at T5 (Figure 8).

4. Discussion

Molecular characterization of different honeybee species using mitochondrial DNA is one of the most reliable methods for measuring the parental inheritance. Apart, of using *mt*DNA, some approaches based on nuclear DNA has been also made however, using *mt*DNA is found more congenial as it contains the maternal traits where, *nDNA* is used to detect the heterozygosity in the bee colony (Chalapathy, 2014). The results toward molecular detection of two bee species reflected a comprehensive phylogenetic tree based on *mt*DNA of *COI* gene where the analyzed honeybee species were being supported with higher rate of bootstrap value and that identical with the findings of Genchi (García et al., 2018).

Behavior of *A. mellifera* L. and *A. cerana* F. was studied in this research using number of worker bees exiting from and entering into the hive per minute, pollen and nectar collectors entering into hive per minute with pollen load and nectar and time spent per flower by the bees. This study revealed that, foraging activities including ingression of worker bees into hive and egression of workers from hive per minute were higher at the morning time. Bee forging activity varies every hour depending on the availability of floral supplies, the needs of their colony, and the weather conditions (Hemalatha et al., 2018). Foraging activity was found to be at

its peak in the morning due to ideal air temperature, relative humidity, wind speed, and floral availability; on the other hand, foraging activity decreases with higher temperature and higher wind speed (Young et al., 2021; Hemalatha et al., 2018). The activities of the workers were greatly influenced by time (Chen, 2016).

Among the two honeybee species, *A. cerana* F. foraged for longer time than *A. mellfiera* L., and *A. cerana* F. started foraging early in the morning (6:10±0.5hrs) and ceased later than *A. mellifera* L. According to a study, *Apis cerana* bees began foraging early in the morning (06.14 ± 0.004) and ended late night (Singh, 2008). Similar results of foraging time were also seen in the observations of many authors where they mentioned *A. cerana* started foraging earlier and ceased later than A. *mellifera* and foraged for longer time (Joshi and Joshi, 2010; Said et al., 2015; Vaziritabar et al., 2015; Aryal et al., 2016; Mattu and Bhagat, 2016).

Egression was maximum for both the species at 10:00-11:00 am and ingression was maximum at 9:00-12:00 am for *A. mellifera* L. and at 10:00-11:00 am for *A. cerana* F. According to a study, maximum number of *A. mellifera* and *A. cerana* entering into hive as well as leaving the hive per five minute was highest at noon and lowest at 5pm (Aryal et al., 2016). In case of time spent per flower, similar result was found by other authors also. *A. cerana* visited more flowers per minute and took much longer to complete a single forging trip than *A. mellifera* (Joshi and Joshi, 2010; Pudasaini and Thapa 2014; Mattu and Bhagat 2016; Ahmad et al., 2017).

In this study, nectar collectors of both *A. cerana* F. and *A. mellifeara* L. foraged maximum at 10:00-11:00 am at the morning and foraging was lowest at 13:00-14:00 pm. According to the greatest nectar collection activity of the Indian bee was seen in the morning between 1000 and 1200 hours and nectar harvesting process came to an end around 1400 hours (Anandhabhairavi et al., 2020). Among the species, nectar collection was higher in *A. mellifera* L. than *A. cerana* F. Since *A. mellifera* has a larger body size, a greater flying range, and greater defensiveness when compared to *A. cerana*, it is the greatest advantage of *A. mellifera* when thieving nectar (Ernesto and Vergara, 2011). As well as *A. cerana* honeybees cover less ground in search of nectar and collects less nectar than *A. mellifera* (Mamatha et al., 2018).

Pollen collection is seen to be maximum at early morning due to ambient temperature for both the species. *A. cerana* F. collected maximum pollen at 9:00-10:00 am and lowest was seen at 13:00-14:00 pm and in case of *A. mellifera* L. it was highest at 10:00-11:00 am and lowest at 13:00-14:00pm. According to foraging activities of *A. cerana* were slowly turned down to its minimum level during late hours of the day (Said et al., 2015). A much higher number of flowers are visited around 0800-1000h than during the other time intervals (Anandhabhairavi et al., 2020). Other authors mentioned similar result in case of *A. cerana* pollen collection, where the maximum pollen load was found early in the morning which is to be between 0900 and 1000 hours (Partap et al., 2000; Singh, 2008; Bhagawati et al., 2016).

A study conducted in India revealed that, the greatest pollen collection activity of the Indian bee was found between 0800 and 1000 hours of the day (Anandhabhairavi et al., 2020). According to bees caried highest pollen load during 1000–1100 hours (Rajkhowa and Deka, 2013). Between 9.00 and 10:00 am, there was more activity and pollen gathering were higher for *A. mellifera* (Layek et al., 2020). Also in comparison to *A. cerana*, *A. mellifera* transports much higher pollen loads, a higher number of pollen grains (Joshi & Joshi 2010; Said et al., 2015; Ahmad et al., 2017) because *A.cerana* honeybees cover less ground in search of pollen due to their smaller size and smaller foraging area (Mamatah et al., 2018). Foragers activity for pollen collections varied due to season, resource availability, time of the day and weather conditions.

5. Conclusion

In this study, the presence of both the honeybees; *A. mellifera* L and *Apis cerana* F in the hives were revealed by analyzing the mitochondrial DNA of COI gene with accession number (ON680900- ON680902 & ON703291-ON703293). *A. mellifera* found to be superior over *A. cerana* in nectar and pollen collection, egression and ingression efficiency despite of spending more foraging time by *A. cerana*. However, in some parameters, *A. cerana* showed almost similar trend like *A. mellifera*. The time series data revealed that, in the morning, between 9:00 AM to 11:00 AM was found effective for the foraging activities for both the bee species while it decreased with the increasing temperature and light intensity. The foraging behavior were studied in litchi orchard where the litchi bloom was the only source of nectar and pollen. However, the foraging efficiency can be influenced by other corresponding factors like types of nectarine sources, flower orientation, floral types and weather condition. Therefore, before recommending the most efficient bee species, similar experiment with diversified flowering sources is suggested.

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