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Short Communication

Identification of Potential Medicinal Plant Species in Multiflora *Trigona* Species Honey from Riau Using a Metabarcoding Approach

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ABSTRACT

Stingless bees (*Trigona* sp.) naturally produce *Trigona* honey from the nectar of different plants. This honey is known to have various health benefits that are influenced by the composition of the nectar collected by the bees. The aim of this study was to use the DNA metabarcoding approach to identify the plant species that makeup *Trigona* sp. multiflora honey from Riau, Indonesia. DNA was extracted from the honey samples obtained from the Riau Forest following standard procedure. The extracted DNA was amplified by polymerase chain reaction (PCR) using universal primers for the target genes internal transcribed spacer 2 (*ITS2*). and sequenced using next generation sequencing (NGS) technique. The results of the analysis showed the presence of three plant genera, namely; *Syzygium*, *Amaranthus*, and *Capsicum*. *Syzygium aromaticum* was found as the dominant species with a relative abundance of 46%, followed by *Amaranthus dubius* (44%) and *Capsicum annuum* (10%). This metabarcoding method has proven effective in identifying plant species that make up *Trigona* multiflora honey and has the potential to identify herbal medicinal plants, such as *Syzygium aromaticum* which has antibacterial and anti-inflammatory properties. These findings did not only provide insight into the food sources of *Trigona* bees but also open up opportunities for further exploration into the use of plants as herbal medicines.

Keyword: Honey, ITS2, *Trigona* sp., Plant species, Metabarcoding.

Introduction

Honey is a natural sweet substance produced by bees from plant nectar or excretions of plant-sucking insects collected by bees. Honey contains substances such as water, carbohydrates, minerals, and amino acids that can be used to meet individual nutritional need. In addition, honey contains phytochemical compounds with various pharmacological properties including antibacterial, hepatoprotective, hypoglycemic, anti-hypertensive, gastroprotective, antifungal, anti-inflammatory, and antioxidant properties.[1]

Trigona bees are known as stingless bees and are a type of bee that are widely found in Riau, Indonesia. *Trigona* sp. honey is known to have a unique taste and many health benefits.[2] The antioxidant content of honey produced by *Trigona* sp. bees is 45% higher than that of *Apis mellifera*.[3] Based on the source of plant nectar, honey is divided into monofloral honey (derived from one plant flower) and multifloralhoney (derived from many plant flowers)[4] The origin of plant nectar is one of the determinants of honey quality. In addition, the difference in flower sources in honey affects the phenolic and flavonoid contents of honey. For example, honey that has antibacterial properties is usually derived from plants such as *Corymbia calophylla*, *Eucalyptus marginata*, and *Leptospermum polygalifolium*.[5]

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The phenolic content and phytochemical compounds of honey depend on several factors, such as the source of plant nectar, geographical location, season, and technology used to extract the honey. Therefore, techniques are needed to determine the source of plant nectar in honey and the content of biological substances that are important for human health. Several methods have been used to determine the source of plants and geographical origin of honey. For example, the traditional method of using a microscopic examination to determine the pollen contained in honey is called melissopalynology.[6] Observation of pollen morphology has limitations, such as difficulty in identifying plants at the family level, thus affecting the knowledge of the origin of the plant. Organoleptic identification and several chemical markers of honey are also used to determine the origin of honey, but these markers depend on the details of beekeeping techniques and honey processing. Therefore, this analysis is most often used to complement the analysis of traditional honey's origin.[7] Due to the cumbersome nature of the work and the limitations of traditional methods used to identify the origin of plant nectar in honey, it is necessary to develop a DNA-based method. The development of DNA-based molecular techniques is often chosen especially for the species identification process. This method is chosen because it is fast, precise, and reliable.[8, 9].

One of the molecular methods developed for species identification is DNA metabarcoding. The use of this technique can identify multispecies with high throughput from environmental or whole organism samples. Metabarcoding focuses on analyzing specific subsets of genes within a genome. So, the metabarcoding approach aims to document and characterize species diversity in an ecosystem and have better coverage in identifying rare taxa.[10, 11] Research has shown that the DNA metabarcoding method can identify family orders and plant species often infested by honey bees.[12] Research has also shown that using two metabarcoding markers, namely the *ITS2* gene and the *trnL* P6 loop, successfully identified 55 plant families, 67 genera, and 43 species in Australian honey.[13] The findings from the study of Khansaritoreh *et al*. (2020) showed that using DNA metabarcoding of

the *ITS2* and *rbcL* genes, 926 plant species in Iranian honey samples were successfully identified, where some of the identified species could not be identified using only the traditional pollen observation method.[14] Research on the metabarcoding method for identifying plant species in honey in Indonesia, especially in Riau, has not been carried out. This study aimed to identify the plants species contributing to *Trigona* sp honey and assess their relative abundance.

Materials and Methods

Collection of Trigona sp honey sample

The honey samples used in this study were multifloral *Trigona* sp. honey type with red colour obtained from forests in Riau, Indonesia. Honeybee farmers assisted in the honey extraction process using the drain method. In this method, honeycomb containing ripe honey was collected and cut into small pieces. The pieces were filtered through a muslin cloth, and the separated honey was stored in an air-tight container.

DNA extraction

The total DNA from 10 grams of honey was extracted according to the method previously described by Hawkins *et al*. (2015).[12] Total DNA from each honey sample was extracted four times using a total of 40 grams per sample. The honey sample was placed into a 50 mL centrifuge tube, and 30 mL of ultrapure water was added. The sample was incubated at 65°C for 30 minutes and then centrifuged at 15,000g for 30 minutes. The supernatant was discarded, and 400 µL of AP1 buffer from the DNeasy Plant Mini Kit (Qiagen) was added to the pellet, followed by the addition of 80 µL of proteinase K (1 mg/mL). The sample was homogenized by vortexing and incubated for 10 minutes in a water bath at 65°C. The subsequent steps were performed according to the manufacturer's instructions. The extracted DNA was stored at -20°C until further analysis. The quality and quantity of DNA was analyzed using nano spectrophotometer (Implen N80) by measuring absorbance value at wavelength A_{260} and A_{280} to determine the purity and DNA concentration.

Amplification of the ITS2 gene

DNA was amplified using universal primers for the target genes *ITS2*. The primers used were based on previous research by Urumarudappa *et al*. (2020).[15] The primer sequences are presented in Table 1. Polymerase chain reaction (PCR) was performed with a final reaction volume of 25 µL. A total of 2.5 µL of DNA template was mixed with 12.5 µL of HotStart Taq polymerase master mix (Qiagen), 0.5 µL of each primer (5 µM), 1.0 µL of BSA (10 µM) and 8 µL of nuclease-free water. The PCR cycle was carried out according to the following program: predenaturation at 95°C for 5 minutes, followed by denaturation at 95°C for 40 seconds, annealing at 48°C for 60 seconds, extension at 72°C for 30 seconds for 35 cycles, and a final extension at 72°C for 5 minutes. Visualization of PCR results was conducted using electrophoresis. This process was performed using 1.5% agarose gel in 1X TAE buffer at 70 volts for 70 minutes. Afterward, the gel was stained with Gel Red and visualized under a UV transilluminator to observed the amplified DNA bands. The Thermo Scientific GeneRuler 100 bp DNA Ladder was used to determine the size of the DNA bands. The electrophoresis results were processed using the Gel Analyzer 2010 program.[16]

Next Generation Sequencing (NGS)

The sequencing process was done in Novogene Beijing, China. DNA samples were amplified with target specific primer presented in Tabel 1. Library preparation was performed using the final PCR products. The final library was sequenced on Illumina platform to generate paired-end raw reads. After the DNA Sequencing results were obtained from the honey samples, the sequencing data were analyzed using bioinformatics software.

Data Analysis

The sequence data obtained from the NGS results were analyzed bioinformatically. The analysis started by removing other nucleotide sequences in the sample, such as index tags, adapter tags, and primers, and then proceeded by separating all samples for subsequent analysis (demultiplexing) using Cutadapt.[17] DADA2 was used to correct sequencing errors, remove low quality sequences and chimera error.[18] The resulting ASVs data was used for taxonomic classification against Blast-NCBI. Downstream analysis and visualizations were performed using packages in RStudio (R version 4.2.3) (https://www.R-project.org/), Krona Tools (https://github.com/marbl/Krona).

Table 1: List of primer sequences used for amplification and metabarcoding

Target Gene	Name of Primer	Primer Sequence 5'-3'
ITS2	SF	5'-ATGCGATACTTGGTGTGAAT-3'
	SR	5'-GACGCTTCTCCAGACTACAAT-3

Results and Discussion

DNA from Trigona sp. multiflora honey

The DNA extraction process led to the acquisition of both quantitative and qualitative data. The concentration of the extracted DNA was expressed in quantitative data, while qualitative data was used to indicate the purity of the extracted DNA. The concentration of the extracted DNA from Trigona honey was 351 ng/µL, and the purity was 1.43.

According to Sophian *et al*., 2021,[19] a purity value below 1.8 indicates protein, phenol, or other contamination during the DNA extraction process. The purity value of good DNA extraction ranges from 1.8 to 2.0.[20]

PCR amplification of ITS2 gene

The use of primers for genetic species identification is essential to distinguish between species accurately. Primer selection is an important step in PCR amplification. In this study, nuclear gene ITS2 were selected because this gene has been used and validated as DNA barcode genes for plant identification. In addition, the ITS2 genes have conservative but quite variable characters, so they are very informative for species identification, even in very closely related species.21 The results of amplification of the ITS2 genes are presented in the form of electropherogram image (Figure 1).

Based on the electrophoregram results, the amplification of the target gene in the DNA sample from Trigona sp Honey was good. This was observed from the DNA bands produced, which were clear, firm, and there was no smear. The absence of a smear in the PCR product amplification results indicates an optimal reaction in the PCR.[22] The size of the PCR product produced was around 450bp (base pairs). The results of the PCR amplification was used for metabarcoding sequencing using the NGS (Next Generation Sequencing) method.

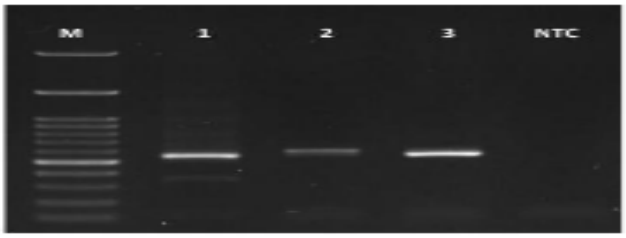

Figure 1: Electrophoregram of *ITS2* gene amplification results in DNA samples from *Trigona* sp honey

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Metabarcoding results

ITS2 gene sequencing was conducted on the DNA extracted from the honey to identify the plant species comprising the Trigona sp. multiflora honey. The obtained sequencing data included 75,462,933 base pairs read. Following trimming, filtering, and denoising, 70,392,484 base pairs were obtained. The mean length of the sequence read was 454 base pairs.

The ASV data analysis identified three plant genera representing the taxonomy of *Trigona* sp. multiflora honey. Figure 2 illustrates the relative abundance of the species successfully identified from the pollen in the honey sample and their taxonomic classifications at the phylum, class, family, and genus levels. The figure reveals that the botanical composition of *Trigona* sp. multiflora honey includes a limited number of plant species, suggesting that *Trigona* sp. bees have restricted foraging diversity.

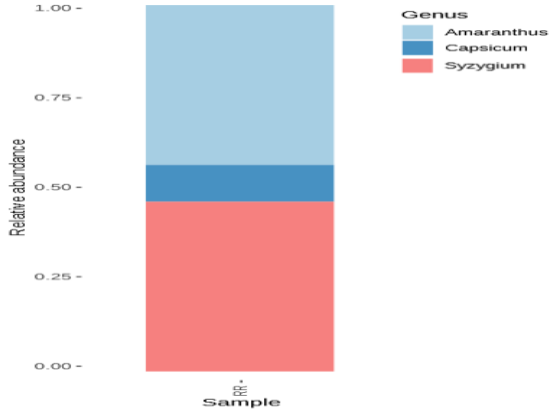

Figure 2: Relative abundance (%) of pollen identified in Multiflora *Trigona* sp. honey samples

Analysis of *Trigona* sp multiflora honey samples revealed a diversity of pollen from three distinct plant species, all included under the phylum Streptophyta. Of all the genera identified, *Syzygium aromaticum* was the most dominant taxon, comprising 46% of the relative abundance, followed by *Amaranthus dubis* at 44%, while *Capsicum annum* represented the least at around 10%. The taxonomic information of the plant pollen constituting *Trigona* sp multiflora honey is shown in Table 2.

The Simpson and Shannon diversity index values were used to assess the species diversity generated by *Trigona* sp multiflora honey pollen. The findings indicate that the species diversity level in this honey sample is classified as moderate, with a Simpson index value of 0.6 and a Shannon index value of 1.1. The rarefaction plot is illustrated in Figure 3.

DNA study in honey samples is becoming more crucial for identifying the origin or species that may serve as herbal medicine. The *ITS2* primer used in this work effectively amplified the isolated DNA, enabling the analysis of the botanical composition of the *Trigona* sp multiflora honey sample. In metabarcoding studies, *ITS2* is often used because of its high efficacy in species identification within the plant kingdom. The findings from this study corroborated that of Urumarudappa *et al*. (2020), where it was found that the use of *ITS2* gene can ascertain the content of herbal medicine.[15] The study of Huda *et al*. (2023) showed that the *ITS2* gene effectively identified plant species in *A. dorsata* and *H. itama* honey from Malaysia.

Table 2: Plant pollen classifications (Phylum, Class, Order, Family, and Genus) in multiflora honey produced by *Trigona* sp.

Phylum	Class	Order	Family	Genus	Species
Streptophyta	Magnoliopsida	Caryophyllales	Amaranthaceae	*Amaranthus*	*Amaranthus dubius*
Streptophyta	Magnoliopsida	Myrtales	Myrtaceae	*Syzygium*	*Syzygium aromaticum*
Streptophyta	Magnoliopsida	Solanales	Solanaceae	*Capsicum*	*Capsicum annum*

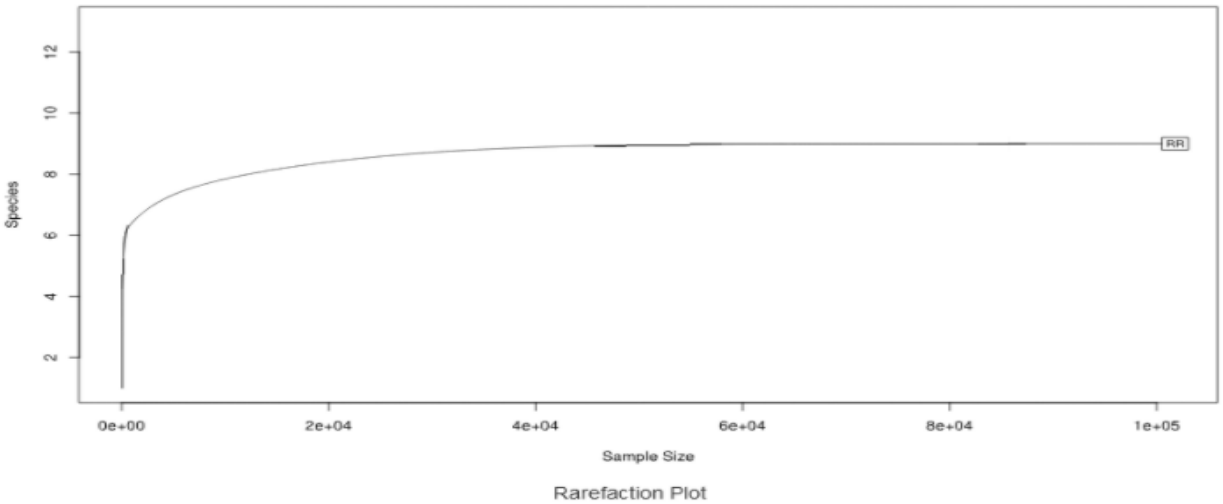

Figure 3: Rarefaction curve of the number of annotated species for Multiflora *Trigona* sp honey samples

This study identified three plant species used as food by *Trigona* bees in Riau. These include *Syzygium aromaticum, Amaranthus dubis*, and *Capsicum annuum*. *Trigona* sp bees are recognized for their capacity to use diverse pollen sources based on local availability for sustenance. Identifying these three species indicates that Riau *Trigona* sp bees depend on a combination of cultivated plants, weeds, and wild flora for sustenance.

The variety of food accessible to *Trigona* bees in the Riau Forest, including several blooming plant species, offers considerable ecological advantages. Similar to *S. aromaticum* and *A. dubius*, these plants may provide bee sustenance year-round, particularly during seasons when

other blooming plants are unproductive. Moreover, invading species like *A. dubis* demonstrated that *Trigona* sp bees adapt to resources in degraded environments or more exposed agricultural areas. Research undertaken by [24, 25] supports the notion that weeds and herbs might provide essential nutrients for bees, hence sustaining the life and health of bee colonies. The reliance of these bees on cultivated flora and weeds underscores the necessity of sustainable environmental management; deforestation and alterations in land use in Riau may jeopardize the availability of natural food sources for bees, potentially compromising the health of bee colonies and the continuity of pollination within the ecosystem.

The metabarcoding technique for identifying honey-producing plant species may also identify certain plant species suitable for use as herbal medicines. This approach may provide novel insights that contribute to identifying herbal medicines by examining the ecological connection between *Trigona* sp bees and the plant species they consume. Based on literature data of the species that were found in this study, *S. aromaticum* species has been found to possess several health advantages. This herb has antibacterial, anti-inflammatory, and analgesic properties. Furthermore, the *A. dubis* plant has been extensively used by local populations as a traditional remedy, notably for reducing blood pressure and enhancing stamina. *Capsicum annum* may also alleviate pain and enhance digestive function. The metabarcoding technology serves to identify plant species in honey and facilitates the discovery and preservation of medicinal plants beneficial to human health.

Conclusion

The metabarcoding technique using the ITS2 gene was employed to identify plant species present in multiflora honey produced by *Trigona* sp. in Riau. This approach enabled precise taxonomic classification of the plant-derived DNA within the honey samples, providing insight into the botanical composition and foraging behaviour of *Trigona* sp. bees in the region. Three plant species identified were *Syzygium aromaticum, Amaranthus dubius,* and *Capsicum annuum*. The species diversity indicates that *Trigona* sp bees rely on various food sources, including cultivated plants and weeds, which provide essential nutrients throughout the year. The findings of this study also show the potential of metabarcoding in identifying herbal medicinal plants, such as *S. aromaticum*, which has antibacterial and anti-inflammatory properties. These findings contribute to understanding the ecological relationship between bees and plants and open up opportunities for further exploration in the field of herbal medicine and plant conservation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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References

1. Soares S, Amaral JS, Oliveira MBPP, Mafra I. Improving DNA Isolation From Honey for the Botanical Origin Identification. Food Contr. 2015; 48:130-136.
2. Yanti EN and Kustiawan PM. Study of Indonesian Stingless Bee (*Trigona* sp.) Propolis Potential as Antioxidant: A Review. J Food Sci Prod. 2023; 9(3):261-269..
3. Ávila S, Hornung PS, Teixeira GL, Malunga LN, Apea-Bah FB, Beux MR, Beta T, Ribani RH. Bioactive Compounds and Biological Properties of Brazilian Stingless Bee Honey Have a Strong Relationship With the Pollen Floral Origin. Food Res Int. 2019; 123:1-10.
4. Jain S, Jesus F, Marchioro G, Araújo E. Extraction of DNA From Honey and its Amplification by PCR for Botanical Identification. Food Sci Technol. 2013; 33(4):753-756.
5. Irish J, Blair S, Carter DA. The Antibacterial Activity of Honey Derived from Australian Flora. Plos One. 2011; 6(3):1-9.
6. Bodor Z, Kovacs Z, Benedek C, Hitka G, Behling H. Origin Identification of Hungarian Honey Using Melissopalynology, Physicochemical Analysis, and Near Infrared Spectroscopy. Molecules. 2021; 26(23):1-15.
7. Zhao H, Cheng N, He L, Peng G, Xue X, Wu L, Cao W. Antioxidant and Hepatoprotective Effects of *A. cerana* Honey Against Acute Alcohol-Induced Liver Damage in Mice. Food Res Int. 2017; 101:35-44.
8. Gultom ES, Hasruddin H, Wasni NZ. Exploration of Endophytic Bacteria in Figs (Ficus carica L.) with Antibacterial Agent Potential. Trop J Nat Prod Res. 2023; 7(7):3342-3350.
9. Hafzari R, Annisa A, Muchamad Nur C, Puspa KL, Huda PN, Nurbaity S, Kusuma MDRA. Precision and Reliability of Nanoplate Digital PCR System for Pork DNA Identification and Quantification. J Microbiol Biotechnol Food Sci. 2024; 14(1):1-3.
10. Mena JL, Yagui H, Tejeda V, Bonifaz E, Bellemain E, Valentini A, Tobler MW, Sánchez-Vendizú P, Lyet A. Environmental DNA Metabarcoding as a Useful Tool for Evaluating Terrestrial Mammal Diversity in Tropical Forests. Ecol Appl. 2021; 31(5):1-13.
11. Kumar P, Babu G, Lakra. DNA Metabarcoding: a new approach for rapid biodiversity assessment. J Mol Cell Biol. 2015; 2(1):1-9.
12. Hawkins J, Vere Nd, Griffith A, Ford CR, Allainguillaume J, Hegarty MJ, Baillie L, Adams-Groom B. Using DNA Metabarcoding to Identify the Floral Composition of Honey: A New Tool for Investigating Honey Bee Foraging Preferences. PloS One. 2015; 10(8):1-20.
13. Milla L, Sniderman K, Lines R, Mousavi-Derazmahalleh M, Encinas-Viso F. Pollen DNA Metabarcoding Identifies Regional Provenance and High Plant Diversity in Australian Honey. Ecol Evol. 2021; 11(13):8683-8698.
14. Khansaritoreh E, Salmaki Y, Ramezani E, Akbari Azirani T, Keller A, Neumann K, Alizadeh K, Zarre S, Beckh G, Behling H. Employing DNA Metabarcoding to Determine the Geographical Origin of Honey. Heliyon. 2020; 6(11):1-6.
15. Urumarudappa SKJ, Tungphatthong C, Prombutara P, Sukrong S. DNA Metabarcoding to Unravel Plant Species Composition in Selected Herbal Medicines on the National List of Essential Medicines (NLEM) of Thailand. Sci Rep. 2020; 10(1):1-11.
16. Balkanska R, Stefanova K, Stoikova-Grigorova R, Ignatova M. A Preliminary Assessment Of TRNH-PSBA as DNA Barcode for Botanical Identification of Polyfloral Honey Samples and Comparison with *Rbcl* Marker. Bulg J Agric Sci. 2020; 26(1):238-242
17. Bellemain E, Carlsen T, Brochmann C, Coissac E, Taberlet P, Kauserud H. *ITS* as an Environmental DNA Barcode for Fungi: An *In Silico* Approach Reveals Potential PCR Biases. BMC Microbiol. 2010; 10(1):1-9.
18. Martin M. CUTADAPT Removes Adapter Sequences From High-Throughput Sequencing Reads. EMBnet J. 2011; 17:10-12.
19. Sophian A, Purwaningsih R, Muindar M, Igirisa EPJ, Amirullah ML. Short Communication: Analysis of Purity and Concentration of DNA Extracted From Intron Patho Gene-Spin Extraction on Crab Processed Food Product Samples. Asian J Trop Biotechnol. 2021; 18(1):28-31.

20. Boesenberg-Smith K, Pessarakli M, Wolk D. Assessment of DNA Yield and Purity: An Overlooked Detail of PCR Troubleshooting. Clin Microbiol Newsl. 2012; 34 (1):1-6.
21. Ralte L, Ralte L, Singh YT. Use of rbcL and ITS2 for DNA Barcoding and Identification of Solanaceae Plants in Hilly State of Mizoram, India. Res J Chem Environ. 2021; 22(3):616-623.
22. Zrimec J, Kopinč R, Rijavec T, Zrimec T, Lapanje A. Band Smearing of PCR Amplified Bacterial 16S rRNA Genes: Dependence on Initial PCR Target Diversity. J Microbiol Methods. 2013; 95(2):186-194.
23. Huda N, Ullah S, Wahab RA, Lani MN, Daud NHA, Shariff AHM, Ismail NI, Hamid AAA, Mohamad MAN, Huyop F. The first ITS2 sequence data set of eDNA from honey of Malaysian giant honeybees (*Apis dorsata*) and stingless bees (*Heterotrigona itama*) reveals plant species diversity. BMC Res Notes. 2023; 16(1):211.
24. Ansaloni LS, Kristl J, Domingues CEC, Gregorc A. An Overview of the Nutritional Requirements of Honey Bees (*Apis mellifera* Linnaeus, 1758). Insects. 2025; 16(1):1-12.
25. Thakur M. Bees as pollinators – Biodiversity and Conservation. Int Res J Agric Sci Soil Sci. 2012; 2(1):1-7