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作品名稱	BeeMind AI: Development of an AI-Based System to Assess Honeybee Health, Behavior, and Nutrient Effects on Learning and Memory
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關鍵詞	<u>Artificial Intelligence, Beehive, Honeybee System, C60 Nanoparticles, Neonicotinoids, Free-Flying, T-Maze</u>
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BeeMind AI: Development of an Artificial Intelligence-Based System to Assess Honeybee Health, Behavior, and Nutrient Effects

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Abstract—This research proposed and built the first integrated AI-based honeybee health assessment system called BeeMind AI. The BeeMind AI system had eight sensors including a microphone, temperature and humidity, carbon dioxide, atmospheric pressure, and camera, which enabled BeeMind AI to monitor both in-hive and external conditions. BeeMind AI has several diverse applications due to its ability to analyze honeybee movement and behavioral patterns to determine honeybee health, and it was used to evaluate the effects of four nutrients on honeybee health through video analysis in two experimental settings, one in a newly designed tri-chambered maze based on a Delayed Matching-to-Sample procedure, and another in a free-flying homing paradigm. The free-flying experiment was conducted to study the effect of nutrients on return rates of honeybees at distances of 300 m, 500 m, and 800 m, and it was found that the base return rates of the control group even at 800 m was close to 75%. It was observed for the first time that C60 nanoparticles had significant positive effects on learning, memory, and flying capabilities, improving return rates by around 9% at 300 m, 16% at 500 m, and 20% at 800 m, while neonicotinoid pesticides had negative effects on return rates, reducing them significantly by up to 30%. The developed BeeMind AI system has a significant impact on honeybee-related research, especially in the evaluation of honeybee learning and memory.

Keywords—Artificial Intelligence, Beehive, Honeybee System, C60 Nanoparticles, Neonicotinoids, Free-Flying, T-Maze

I. INTRODUCTION

Due to their pollination services, honeybees are one of the most ecologically vital animals, being singlehandedly responsible for nearly 80% of global agricultural pollination [1]. However, in recent years, they have experienced large declines in populations, and as a survey reported roughly 50% of beekeepers in the US lost their honeybee colonies [2]. These losses are experienced globally due to a combination of many factors, including but not limited to habitat loss, pesticides, climate change, and other invasive species [3, 4].

One of the biggest factors attributed to the decline of honeybee colonies is the usage of pesticides, specifically neonicotinoids [3-6]. Neonicotinoid compounds have been used globally since their introduction in the early 1990s [4]. Studies have shown that neonicotinoids can have both sublethal and lethal effects on honeybees, depending on the dosages that they are exposed to, as neonicotinoids bind to nervous system receptors of honeybees [7]. These effects can range from behavior changes to altered motor functions [7-9].

Among the reported effects, one of the more significant ones is the effect of neonicotinoids on honeybee learning and memory [10, 11]. Additionally, there is a lack of availability for methods of monitoring of honeybee hives, essentially meaning that the only methods to track honeybee health are through obtrusive physical methods of inspection.

This paper aims to develop a novel AI-based honeybee health assessment system, able to monitor beehives using the following functions: continuous temperature and humidity monitoring both inside and outside the hive, as well as video and audio recording to assess honeybee health as well as population. In addition, this system can be used for honeybee-related studies such as nutrition effects and evaluation on health, learning, and memory. To do this, four types of nutrition have been studied and their effects have been analyzed by a deep learning approach.

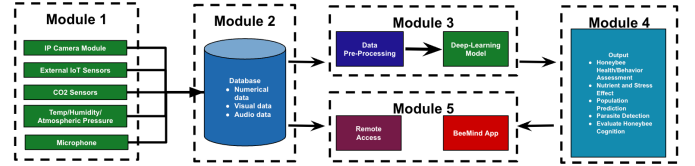


Fig. 1. Diagram of BeeMind AI system design consisting of 5 modules.

II. EXPERIMENTAL CONDITIONS AND SYSTEM SETUP

A. BeeMind AI System Design and Build Up

An artificial intelligence-based system (BeeMind AI) was designed to assess honeybee health and behaviors when implemented directly into a beehive. The beehives used in this study were three 8 frame deep box hives with approximately 20,000 honeybees (*Apis mellifera*) each. As shown in Figure 1, BeeMind AI's system design consists of 5 separate modules. Module 1 is data collection of both in-hive and external conditions, and contains 8 integrated sensors, capable of collecting visual data, audio data, and numerical data. Different sensors were placed both in-hive, mounted externally, or placed inside the BeeMind AI terminal, such as sound (MAONO USB Lavalier Microphone) and CO₂, temperature, and humidity (Adafruit SCD-41) placed inside of the hive, as well as a camera (Raspberry Pi Camera Module 3) contained in the terminal to track the entering and exiting of honeybees, and the ShillehTek

Inside Hive I2C temp, humidity, CO2 Sensor

I2C Bus 0

Raspberry Pi 4B

Outside Hive I2C temp, humidity, pressure sensor

I2C Bus 4

CSI Camera Port

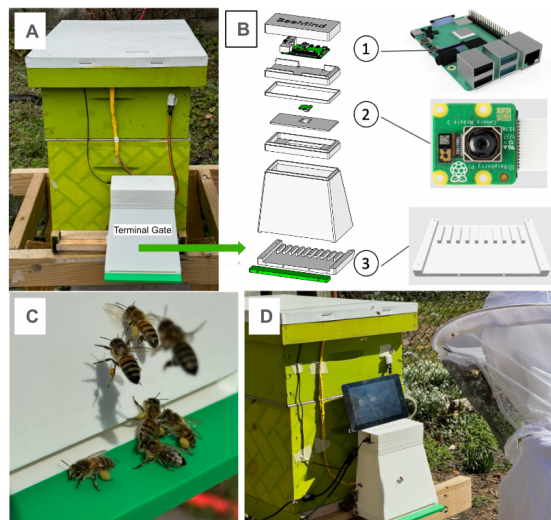
Live Stream

USB Audio Sensor

1D Entrance Divider

HDMI 7" Display

The BeeMind AI terminal gate also consisted of multiple different components and was where the Raspberry Pi Board as well as the camera module were located as shown in Figure 3.



B. BeeMind AI Deep Learning Architecture

The deep learning model used in this research was YOLOv8 as shown in Figure 4. The selection was based on YOLOv8's higher accuracy and speed as well as fewer parameters, making it efficient and suitable for hardware such as the Raspberry Pi 4B model used in BeeMind AI. The parameters used in the AI model were epochs= 100, image size (imgsz)= 640, and learning rate (lr)= 0.01, with all other parameters being default settings. This approach allowed BeeMind AI to monitor inflow and outflow of honeybees at the entrance divider as well as track honeybee flight paths.

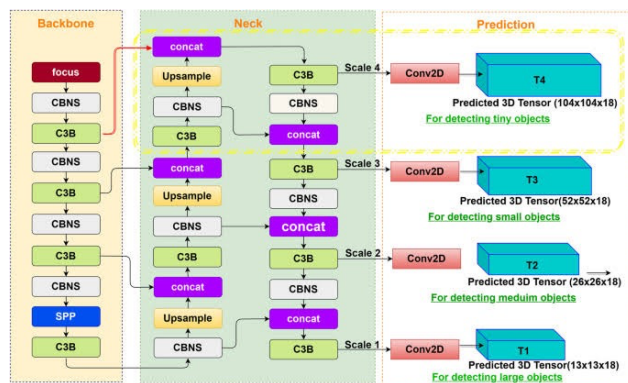


Fig. 4. YOLOv8 architecture diagram of AI-based video analysis for the BeeMind AI system.

C. Two-Level Tri-Chamber Maze Design

To test honeybee learning and memory, two experiments were designed and conducted based on a previously proposed procedure known as Delayed Matching-to-Sample (DMTS) [12]. The first design to test honeybee learning and memory was a maze consisting of two levels and three chambers, as shown in Figure 5. Each level of the maze was 32 x 32 x 34 inches and was made from cardboard and acrylic. Every level had 3 exits with a unique pattern disc above each exit that was 2 inches in diameter. Figure 5A shows the pattern disc hierarchy used in the tri-chamber maze. The first level consisted of 3 patterns that were clearly distinct from each other, meaning that the bees would have an easier time making the correct selection. If the bees made the incorrect choice in the first level, they would fly into the next chamber where they would be faced with the exact same 3 patterns to see if they would make the correct selection if given a chance to retake the test. If the bees successfully passed the first level, they were faced with a second level with 3 much more similar patterns. Additionally, a scoring system was implemented to compare the performances of different honeybees. Honeybees that selected the correct pattern at both Level 1 and Level 2 received 3 points, while the ones that selected the correct pattern at Level 1 but not Level 2 received 2 points. The honeybees that selected the incorrect pattern at Level 1 but selected the correct pattern at the retake received 1 point, and the rest of the honeybees that made all incorrect selections received 0 points.

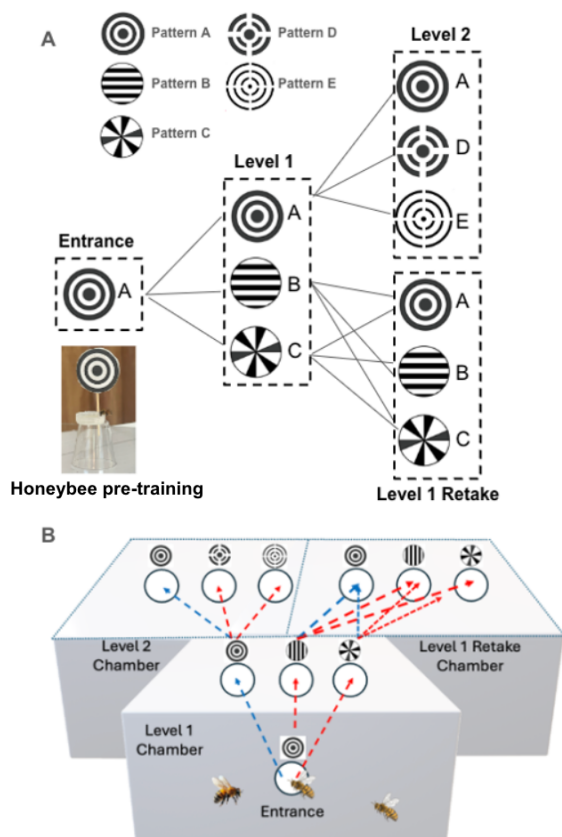


Fig. 5. Maze design: (A) shows different pattern disc hierarchy. The left-most column disc was displayed outside chamber 1 at the entrance, the middle column discs (Level 1) were mounted inside chamber 1 on the way into chamber 2 and 3, and the right-most column discs (Level 2 and Level 1 Retake) were placed inside chamber 2 and 3 while (B) shows 3D design of two-level tri-chamber maze.

D. Nutrition and Concentration Selection

To ensure a comprehensive study of nutrients with a wide range of effects, two positive and two negative nutrients were selected and are listed in Table 1. The two negative nutrients were neonicotinoid pesticides, one being thiamethoxam and another being acetamiprid, and they were both purchased from Sigma-Aldrich, USA. Dosages for neonicotinoids were based on previous literature for calibration purposes. The two positive nutrients chitosan and water soluble C60 were both nanoparticles. C60 was purchased from Sigma-Aldrich, USA, while chitosan was purchased from Nanochemazone, Canada. C60 is a powerful antioxidant that has shown anti-aging effects [13]. Additionally, Baati et al. discovered that C60 could in fact nearly double the lifespan of rats when dissolved into olive oil [14]. Chitosan also has similar reported health benefits [15, 16]. All nutrients were diluted in series in a 1:1 sucrose solution of sugar and water. To prepare chitosan, 5 grams of chitosan were diluted in the sucrose solution into a final solution of 1.2 ng. C60 was diluted so that each bee received 5 ug. Thiamethoxam was diluted to a final 1 ng per bee, and acetamiprid was diluted to a final 1 ug per

bee. All bees were fed 10 uL of sugar water with the added nutrient, and the control group received pure sugar water.

TABLE I. NUTRIENTS AND CHEMICAL FORMULAS

Nutrient	Formula	Structure	Molecular Weight (g/mol)
Acetamiprid	$C_{10}H_{11}ClN_4$		222.67
Thiamethoxam	$C_8H_{10}ClN_5O_3S$		291.71
Chitosan Nanoparticles	$(C_6H_{11}NO_4)_n$		161n
Water Soluble C ₆₀	$C_{60}(OH)_n$		720.66

E. Method Development of Honeybee Feeding

There are two methods of feeding honeybees: group feeding and individual feeding. Group feeding simplifies feeding large groups of bees but does not allow for controlling the amount of nutrients that bees individually consume. Individual feeding allows for flexible control but makes mass feeding more challenging. In this research, to feed honeybees the desired nutrients, a multi-step process was developed to individually feed honeybees, as shown in Figure 6. First, honeybees were attracted near the hive entrance using a coffee filter soaked in a sucrose solution (Figure 6A). They were then individually moved to a feeding station in plastic cups, where the solutions of the specified nutrients had been dropped. The cups were kept above them to ensure that the honeybees would fully consume the prepared solutions (Figure 6B), and when the solution had been visually fully consumed, the bees were moved into a freezer, where they were stored for a few minutes (Figure 6C). When they had stopped moving significantly, they were taken out of the freezer and marked with the color corresponding to the solution they were given. They were then ready for experimentation, as seen in Figure 6D. This method ensured that all bees individually received the same amounts of a certain nutrient.

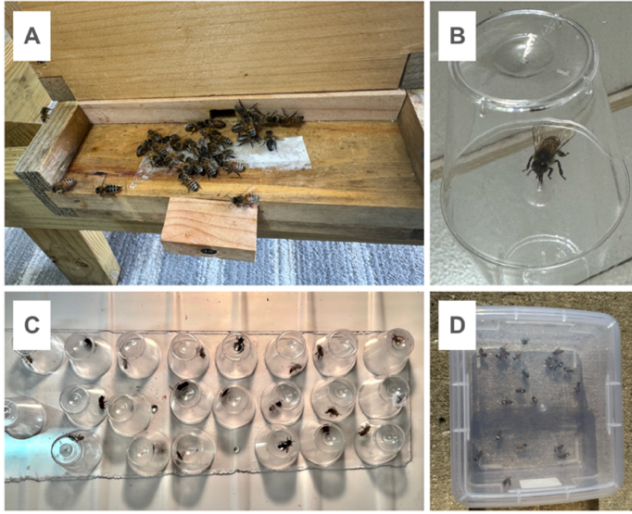


Fig. 6. Development of individual honeybee feeding method: (A) Bees were attracted outside of the hive using a coffee filter soaked in sucrose solution, then (B) placed under plastic cups to feed and to prevent physical contact. (C) Bees were then moved into a freezer, color-marked, and (D) gathered for experimentation.

F. Free Flying Method

In order to evaluate the effects of nutrients on honeybees in terms of memory and navigation in field-realistic situations, the second design was a free-flying paradigm where bees were released at varying distances (300 m, 500 m, and 800 m) from their hive to see their return rates (Figure 7A). Multiple groups of thirty bees each were released between 10:00 AM - 1:30 PM in Deltona, Florida, when the weather conditions were clear, sunny days between 60-65 °F, with around 9-16 MPH winds and 15 mile visibility (Figure 7B and 7C). The number of bees that had returned were counted by a combination of video analysis through AI as well as manually checking the hives for the marked bees (Figure 7D-7G). In this way, the memory of honeybees could be tested by the performances in these designs after their treatment with a specified nutrient.

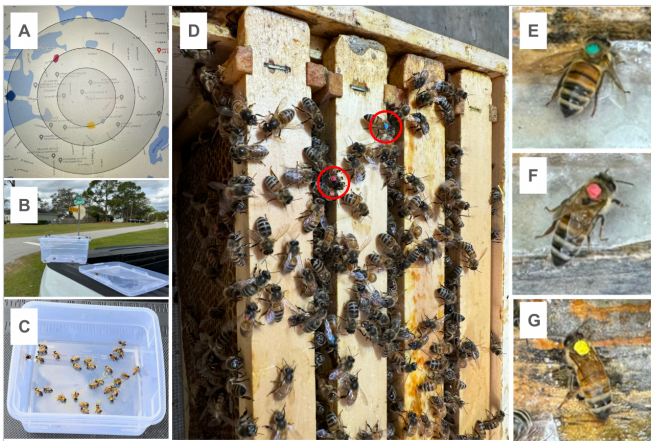


Fig. 7. Free-flying method: (A) Map showing release distances of 300 m, 500 m, and 800 m. (B) Bees warm from freezer temperature before release. (C) shows the release station, with dimensions 15 cm (Length) x 18.7 cm (Width) x 9.2 cm (Height). (D) Tallying of marked bees gives return rate. (E), (F), and (G) show examples of color marks indicating different nutrient solutions consumed.

G. Data Analysis Using a Statistical Method

To identify the difference between two sets of data, statistical t-tests were employed. The obtained p-values were then compared to an alpha value of 0.05 to determine whether differences between two sets of data were statistically significant or not. The statistical tests were used to compare the different groups in the free-flying paradigm.

III. RESULTS AND DISCUSSION

A. BeeMind AI Functions

1) Monitoring In-hive Conditions

By integrating 8 different sensors, different conditions that were both in-hive and external could be tracked in real time, with data being updated every 5 minutes as shown in Figure 8. The temperature and humidity levels inside the hive were maintained steadily over time. The CO₂ levels were also shown to be between the levels of 1000 and 3000 ppm. The external pressure levels were also consistently between 1010 and 1011 Pa. These levels are consistent with previously reported values [17].

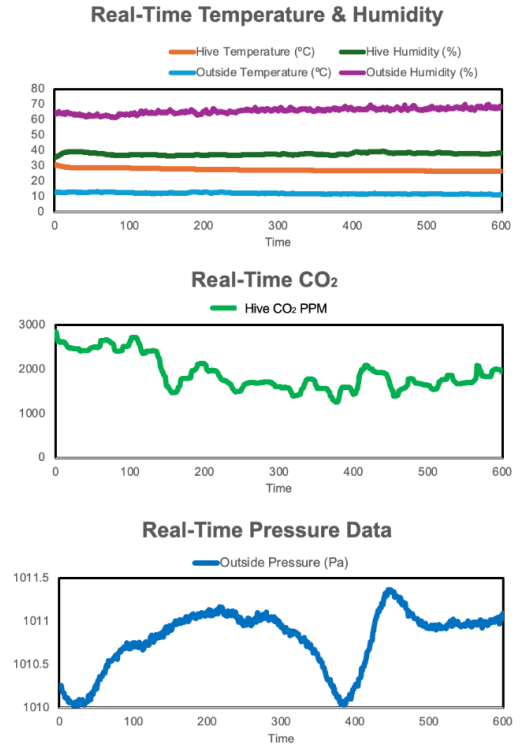


Fig. 8. Real-time monitoring of both in-hive and external conditions such as (A) temperature and humidity, (B) CO₂, and (C) atmospheric pressure. One unit on the x-axis corresponds to 5 seconds in real time.

2) Tracking Honeybee Inflow and Outflow

At the entrance of the hive, the camera module of BeeMind AI was capable of monitoring the entrance divider, and thus could count the number of honeybees entering and exiting the hive over a period of time, allowing for tracking of marked bees in the free-flying experiment. As shown in Figure 9A, the blue rectangle was the area of counting, with honeybees passing the top blue line adding to the upward count and honeybees passing

the bottom blue line adding to the downward count. As shown in Figure 9B, in the afternoon, the honeybees entering the hive exceeded those exiting the hive, which indicated normal circumstances.

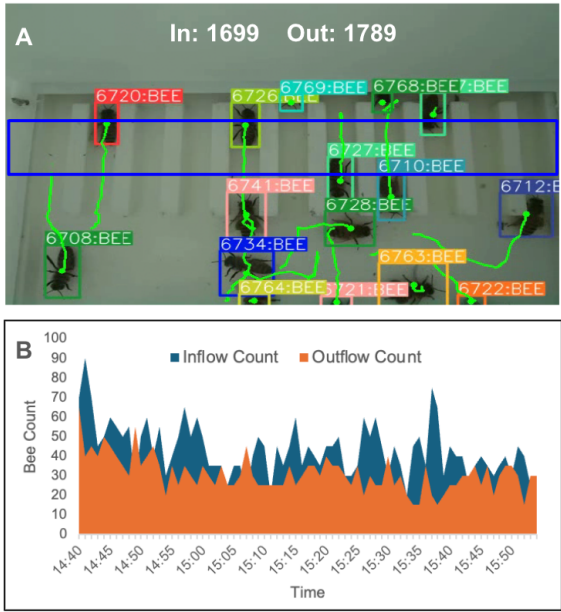


Fig. 9. (A) BeeMind AI camera module monitoring entrance divider of hive and tracking inflow and outflow of honeybees. Blue rectangle represents area of counting with top line registering upward count and bottom line registering downward count. (B) Real-time data of inflow and outflow of honeybees taken every 5 minutes.

3) Flight Path Tracking and Analyzation

In order to study the effects of different nutrients on honeybees, an AI-based video analysis method was developed to track honeybee flight patterns and behaviors in the tri-chamber maze design. By utilizing cameras mounted above each chamber, the flight paths of honeybees throughout every chamber could be tracked and analyzed. As shown in Figure 10, different honeybee flight patterns can be broken down into four time zones based on their flight times: T1, T2, T3, and T4. More detailed work on this method will be published elsewhere.

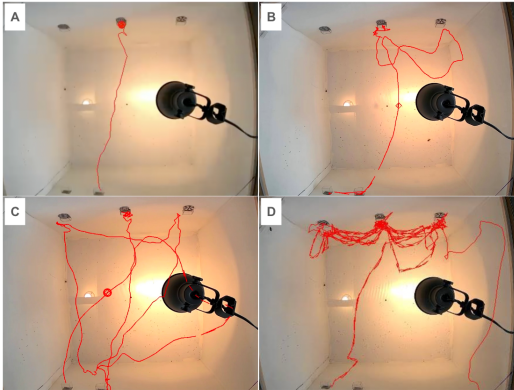


Fig. 10. BeeMind AI camera flight path tracking capabilities used to evaluate the effect of different nutrients on honeybee flight times in maze with different flight time zones shown (A) T1 (flight time ≤ 10 seconds) (B) T2 (flight time ≤ 20 seconds) (C) T3 (flight time ≤ 30 seconds) and (D) T4 (flight time > 30 seconds).

B. Tri-Chamber Maze

Experimentation with the tri-chamber maze design was conducted to test honeybee memory and yielded some insights on honeybee behavior. The first finding is how the memory of honeybees is very strong, being able to recognize the correct target sign among many other different signs after training. It revealed how honeybees rely on their visual memory to make decisions, as honeybees took around 15% more time at Level 2 where the targets were more similar than at Level 1 where the targets were more distinct. Another insight is that honeybees can become careless, as most honeybees that did not pass level 1 spent longer on the retake and successfully passed the retake. 75% of honeybees selected the correct symbol at Level 1, while 48% of honeybees were able to get both Level 1 and Level 2 correct, with the average score of all honeybees being 2.1.

C. Free Flying Design

In the free flying experiment where bees were captured, marked, and released, several key findings were obtained. The first key finding was the ability of nanoparticles to significantly improve the return rates of foraging honeybees. As shown in Table 2, honeybees which received C60 had the highest return rates, followed by the chitosan group, control group, and then the neonicotinoids. The second key finding was the effect that different nutrients had on the time it took for honeybees to leave the release station. As shown in Figure 11, the neonicotinoid pesticide groups took a shorter amount of time to depart compared to the control and nanoparticle groups. This experiment confirmed the positive benefits of nanoparticles under field-realistic conditions as well as the detrimental effects of neonicotinoids.

TABLE II. FREE FLYING RETURN RATE BY NUTRIENT AND DISTANCE

	Control	Acetamiprid 1ug/bee	TXM 1 ng/bee	Chitosan 1.2 ng/bee	C60 5ug/bee
300 m	88%	73%	50%	90%	95%
500 m	80%	68%	43%	85%	93%
800 m	75%	55%	38%	75%	90%

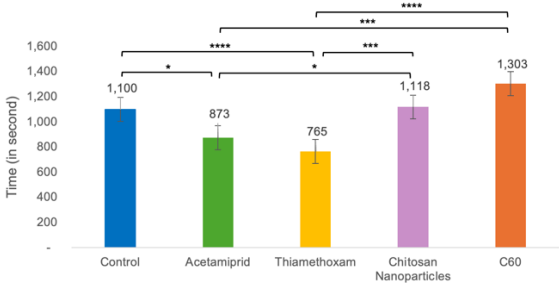


Fig. 11. Bar graph of the average times taken for bee departure from the release station after nutrient feeding. Significant p-values indicated: *p≤0.05, **p≤0.01, ***p≤0.001, and ****p≤0.0001.

An interesting behavior was noticed during the free-flying experiment. Honeybees that had received sublethal doses of neonicotinoids were crawling on the edges before taking off, while the honeybees from the control group and the nanoparticle groups were found to take off directly from the bottom of release station as shown in Figure 12. This behavior is still under ongoing evaluation through AI-based video analysis.

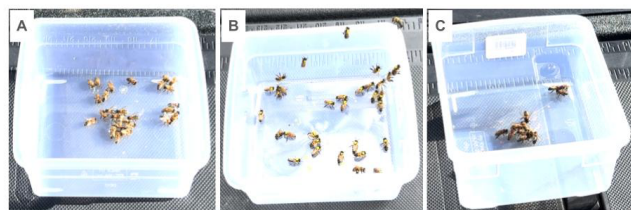


Fig. 12. Different groups of honeybees with different degrees of crawling and flying behaviors displayed with (A) Control Group, (B) Acetamiprid, and (C) C60.

IV. CONCLUSION

In this research, an AI-based system was successfully developed to monitor honeybee behaviors, and the impact of different nutrients on honeybees was studied. The first design for bee learning and memory evaluation of a tri-chamber maze was shown to be a successful test of honeybee memory and was the first free-flying maze to incorporate multiple levels with symbols of different complexities. In the field-realistic free-flying homing design, it was observed that for the time it took for bees to depart from the release station, both neonicotinoid pesticide groups took a statistically significantly shorter amount of time to depart compared to the control group, while the nanoparticle groups took a statistically significantly longer amount of time compared to the control group as shown in Figure 8. Additionally, C60 nanoparticles showed a significant positive effect on return rates of foraging honeybees, with a 9% increase at 300 m, 16% increase at 500 m, and 20% increase at 800 m compared to the control group. The future work of this research will be focused on continued optimization of AI with larger sample size, and different nutrients and concentrations in the free-flying experiment.

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Strengths :

1. Innovative Data Collection :

BeeMind AI integrates multiple sensors and employs the YOLOv8 deep learning model, offering a non-invasive and precise method for tracking honeybee behavior and environmental conditions.

2. Comprehensive Experimental Design :

The study combines indoor memory evaluations with outdoor navigation tests, providing a multi-dimensional perspective on honeybee learning, memory, and field performance. The use of t-tests validates the statistical significance of the findings.

3. Impactful Results :

Detailed data demonstrate the beneficial effects of water-soluble C60 and chitosan on honeybee navigation and the detrimental impact of neonicotinoids, offering actionable insights for pollinator health management.

Weaknesses and Recommendations

1. Limited AI Applications :

The AI component focuses primarily on motion tracking and counting, missing opportunities for advanced behavioral pattern recognition, anomaly detection, or predictive modeling. Future studies could implement machine learning algorithms to analyze complex behavioral data and predict colony health under different conditions.

2. Narrow Nutrient Selection :

The study tested only two positive (C60 and chitosan) and two negative (Acetamiprid and Thiamethoxam) substances, excluding other potentially impactful nutrients such as natural plant extracts. Expanding the range of tested substances would improve the generalizability of findings.

3. Small Sample Size and Short-Term Observations :

The experiments used a limited sample size and focused on short-term effects, which may not fully represent long-term impacts or variability across different honeybee populations and environmental conditions. Increasing the sample size, including diverse honeybee species, and conducting longitudinal studies would strengthen the findings.

4. Data Reliability in High-Density Activity :

The accuracy and reliability of the BeeMind AI system in scenarios of high-density bee activity need further validation. Enhancements to the system's hardware and

software should be explored to maintain data quality under such conditions.

5. Scalability and Practical Applications :

While the system has demonstrated success in research settings, its scalability and adaptability for widespread use remain untested. Future developments should focus on refining the system for practical applications in diverse environments.

Conclusion

BeeMind AI represents a significant advancement in honeybee monitoring technology, offering a robust tool for understanding the effects of nutrients and pesticides on honeybee health and behavior. Despite its limitations, the system provides a foundation for future research, with potential applications in pollinator conservation and agricultural sustainability. Implementing the recommended improvements could maximize the impact and utility of this innovative AI system.