

## ORIGINAL PAPER

# IoT-based analysis of temporal dynamics in beehive conditions under electrical and light stimulation

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## ABSTRACT

This study investigated the effect of light stimuli on the internal conditions (temperature, relative humidity, and atmospheric pressure) of honeybee colonies of varying strengths (5, 7, and 9 frames) during bee venom collection using electrical stimulation. This work uses IoT to monitor beehive conditions and environmental factors, studying the effects of light stimuli with a bee venom collector device (BVCD). Experiments were conducted during both day and nighttime, with two collection instances per colony. Results indicated that colony strength and the time of day exerted a more pronounced influence on internal temperature and humidity than the applied light stimuli. Stronger colonies exhibited higher internal temperatures during venom extraction, likely due to increased metabolic activity. Smaller colonies tended to have higher relative humidity during nighttime. Atmospheric pressure remained largely stable and unaffected by the experimental manipulations, except average pressure values were highest in the 9-frame beehives. These findings suggest that while visual cues have a limited immediate impact on the overall hive environment during venom collection, factors such as colony size and diurnal cycles are more significant. Further research is warranted to elucidate the specific mechanisms underlying the observed effects of colony strength, time of day, and green light on beehive conditions, aiming to optimize venom collection practices and minimize stress on bee colonies.

## 1. Introduction

Nowadays, the internet permeates every aspect of human life. The concept of the Internet of Things (IoT) emerged due to the widespread adoption of computing and Internet access. (Akkaş and Sokullu, 2017; Bhujel et al., 2020). The Internet of Things (IoT) is critical to achieving sustainable development goals, The IoT is an infrastructure that enables devices to communicate with each other over the Internet such as data collection, generation, processing, analysis, and application handling (Zeng et al., 2024). It enables machine-to-machine (M2M) communication by establishing a cloud connection between devices from different stations (Bhujel et al., 2020; Khanna and Kaur, 2019). The IoT architecture mainly consists of three layers. The perception layer is

the foundational level and includes sensors that collect data and transmit it to the network layer. The network layer handles the reception and transportation of data to the application layer. The application layer stores data in databases, and various tools are used for data analysis (Bellini et al., 2022; Kumar et al., 2019). Each sensor controller can send data directly to the cloud using this cutting-edge precision approach, enabling the central system to analyze real-time data effectively and dynamically. As a result, it is now possible to connect and manage every end device via a central system (Borero and Zabalo, 2020).

While sensors have existed for some time, their widespread use in various industries began with the development of wireless sensor networks (WSNs) and

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advances in semiconductor, networking, and material science technologies (Alahmad et al., 2023). Intelligent systems have become increasingly attractive due to their advantages, including enhanced agricultural output, sustainable environmental practices, and optimized resource management. For accurate and automated management, real-time monitoring, control, and data analysis are made possible by the integration of IoT technology (Ferentinos et al., 2017; Singh et al., 2021).

Karan et al. (2024) have developed a Smart Beehive Monitoring System for Sustainable Apiculture, which utilizes IoT sensors for real-time hive weight assessment, temperature/humidity insights, ambient light sensors, and a microphone for in-depth sound analysis. The data collected is easily transferred to the cloud for analysis using Node MCU (Microcontroller Unit) ESP32. This all-inclusive system sets a new beekeeping standard, optimizing hive management and promoting sustainable practices through a mobile or online application. It also offers real-time insights.

In other case, Andrijević et al. (2024) propose an autonomous smart beehive based on artificial intelligence, equipped with an advanced system for monitoring bee entry and exit, as well as collecting data on weather. The hive uses an array of sensors controlled by Espressif ESP32 and Arduino Mega microcontroller boards to continuously optimize ventilation system operation, monitor energy consumption, and adapt to changing conditions.

The ESP32's environmental impact varied with its assigned tasks. To reduce its carbon footprint, the team implemented a sleep cycle, switching the ESP32 into deep sleep mode to lower carbon emissions. This strategy helped the ESP32 meet the team's fourth criterion: cost-effectiveness, making it the top choice for the project. Its capacity for WiFi connectivity, minimal environmental impact, and strong programmability and security collectively made it the optimal solution for completing the assigned tasks (Espressif Systems, 2023; Ursillo et al., 2024).

However, there is a scarcity of research that deals with the condition of the hive under the use of a BVCD, to its development and modernization, which requires a lot of studies and development and the use of modern methods to study the condition of the hive in this case, in which the hive is exposed to a state of severe stress according to Ali et al., 2023; Younis and Ali, 2024). They added that A bee monitoring system (BMS) was set up, containing a BVCD. Data was collected before and after the device ran at three levels of colony strength (CS) during the autumn season. The results showed that the temperature returned to natural temperature within 5 minutes. The number of collection times and CS were significant factors affecting temperature, maximum

temperature, and venom productivity (VP). Regression models were used to express the relationship between temperature and time and between VP and maximum temperature. The study found that the BVCD increased temperature due to stress in the colony, and venom productivity was correlated with maximum temperature. Nightly productivity was higher than daytime productivity. Therefore, this work aims to use an IoT sensor in the beehive, monitor the environmental conditions (temperature, relative humidity, atmospheric pressure), and study the effects of light stimuli on beehive conditions through the use of a bee venom device collector. In addition, the sensor data can be monitored on web pages and Android app platforms through the integration of the Internet of Things (IoT).

## 2. Materials and methods

The study was carried out in the Agricultural Research Centre (ARC), Plant Protection Research Institute (PPRI), Bee Research Department, Giza (latitude 30.04635° N, longitude 31.207320° E and altitude 18 m above sea level), in April 2025.

### 2.1. Bees

The experiment was carried out on Craniolian hybrid (*Apis mellifera* L.) bees. The monitoring system was set up to continue on six beehives through the spring of 2025. Colonies were placed in Langstroth-type hives made of wood with external dimensions of 510, 410, and 270 mm and internal dimensions of 485, 385, and 250 mm, and a wall thickness of 25 mm.

### 2.2. Bee Venom Collector Monitoring System "BVCMS"

This section describes the design and implementation of a Bee Venom Collector Monitoring System (BVCMS), focusing on the hardware architecture, sensing mechanisms, and integration with Internet of Things (IoT) platforms. The development encompasses material selection, hardware implementation, circuit design, and the creation of a sensing subsystem for real-time monitoring of a bee venom collector. A smart system design, leveraging HTML and the Kodular platform for IoT application development, was employed to create a functional prototype at an actual scale. The system's modular architecture, comprising monitoring and sensing modules, a MCU, and a Power Unit, facilitates data acquisition, processing, and wireless communication over WI-FI.

### 2.3. System Architecture

As illustrated in Fig. 1, the BVCMS adopts a modular design consisting of three primary units: the Monitoring and Sensing Modules, the MCU, and the Power Unit. The system facilitates three distinct pathways: data transmission from the sensors to the MCU and subsequently to the cloud, power distribution to the various components, and wireless communication via WiFi for remote monitoring and control.

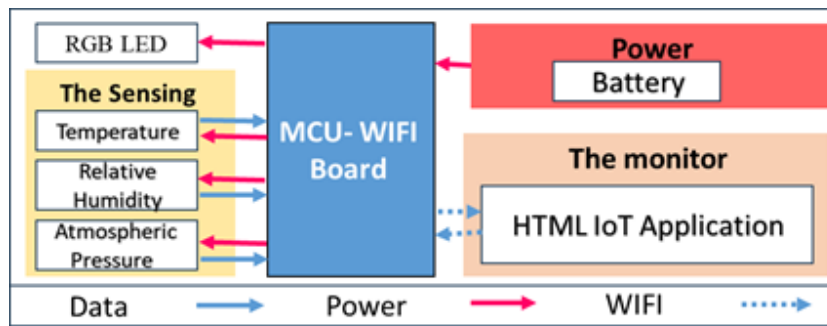


Fig.1. Schematic diagram of the BVCMS.

#### 2.4. Hardware Implementation

**Base Unit:** The mechanical foundation of the monitoring unit was constructed from wood, incorporating a dual-frame assembly as previously described by Ali et al. (2023). This configuration, depicted in Fig. 2, was strategically positioned beneath the beehive cover to mitigate direct solar exposure and external environmental influences.

**Electronic Monitoring System:** The electronic monitoring framework builds upon the methodology proposed by Ali et al. (2023), employing an MCU (specifically the ESP32-s) and a distributed sensor network. This network is partitioned into two distinct groups; each mounted on a separate frame within the assembly (Fig. 2).

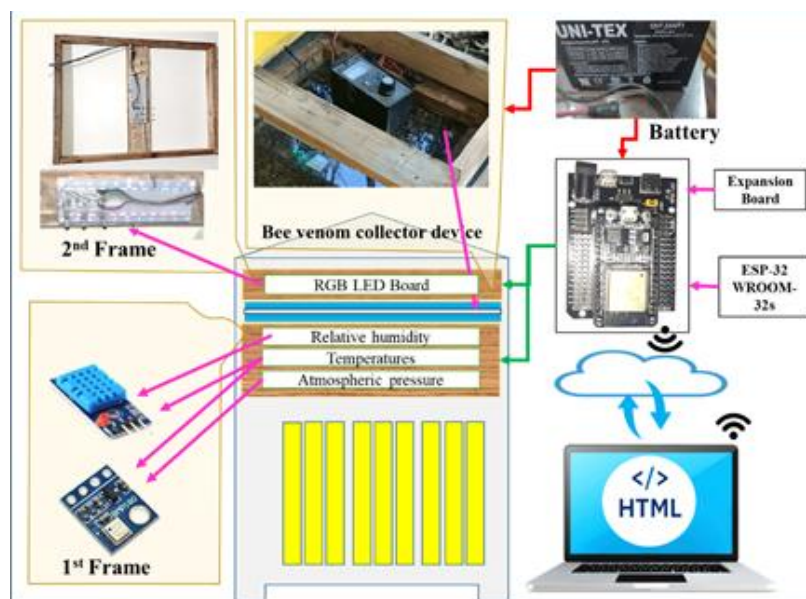


Fig. 2. Schematic diagram of BVCMS consists (Hardware design based)

**MCU-Wi-Fi Module:** The ESP32 microcontroller was selected as the core processing unit due to its inherent security features (secure boot), robust open-source library support facilitating high and low-level programming, and energy efficiency achieved by implementing deep sleep cycles, thereby minimizing its environmental impact. Furthermore, its integrated WiFi connectivity and cost-effectiveness rendered it an optimal choice for this project (Espressif Systems, 2023).

The system uses the ESP-WROOM-32 module from Espressif Systems, featuring a dual-core ESP32 microcontroller, 2.4 GHz WiFi, and Bluetooth at an affordable price. It is built on the ESP32-12E WiFi module for easy

internet connectivity. The development platform includes a 30-pin Type-C Micro USB GPIO Breakout Board and an ESP32 Shield 30P, both known for low power consumption. The module offers internal RAM, 4MB SPI Flash memory, an integrated antenna, 16 digital I/O pins, 18 analog inputs, 2 DACs, and 8 PWM channels.

**Sensing and Stimulation Module:** The Sensing Module comprises two sensors strategically positioned on opposing edges of the bottom frame's interior. A DHT11 sensor is employed to measure ambient temperature and humidity, while a BMP180 sensor monitors atmospheric pressure and temperature. The primary

function of this module is to trigger the BVCMS and control an RGB LED array based on commands received from the HTML platform, driven by both human user input and sensor readings. The stimulation component, consisting of three RGB LED units (comprising white, red, green, and blue light-emitting diodes), is centrally mounted on the top frame, facing the Bee Venom Collecting Device (BVCD). The activation sequence and timing of the RGB LEDs are programmable.

Fig. 3 outlines a flowchart for the automated system (BVCMS) that controls various colored lights based on

elapsed time. The process begins with activating the "WIFI-ESP32" after an initial five-minute check. The system then sequentially turns on green, red, blue, and white lights at specific time intervals of 15, 17, 19, and 21 minutes, respectively, before turning all lights off at the 25-minute mark. Simultaneously, the system collects various time-related data, including T1, T2, AP, and RH, to calculate parameters such as air pressure, temperature, and humidity. These parameters are then output as generated mass and visualized on a graph.

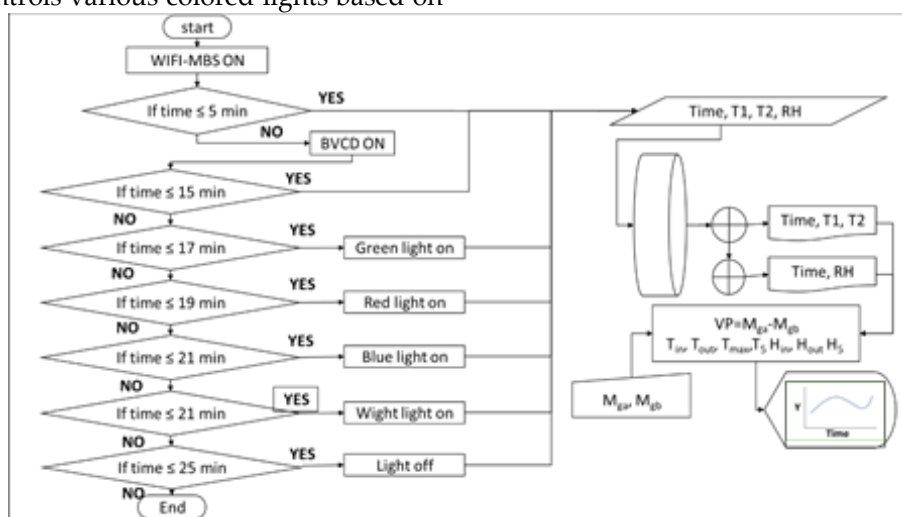


Fig. 3. Flowchart flowchart for the automated system (BVCMS).

**Monitor Module (BVCMS):** This module facilitates the IoT functionality of the BVCD system. It utilizes the WiFi capabilities of the NodeMCU ESP32 development board to transmit the status of the beehive structure to an HTML-based IoT web application. The selection of the WiFi protocol was driven by its capacity to handle substantial data volumes over the internet, crucial for comprehensive monitoring. The IoT web page, designed using HTML to enable monitoring via a PC screen, was hosted on a free Weebly domain. Subsequently, the Kodular platform was employed to convert this web page into an Android application (Fig. 4), providing a rapid and user-friendly method for remote monitoring of the hardware project from any Android-based device.

## 2.5. Software Implementation

**Code Upload to NodeMCU:** The NodeMCU ESP32-s was programmed using the Arduino Integrated Development Environment (IDE) with code designed to establish communication with the Blynk application, enabling remote control functionalities.

**WiFi Connection Establishment:** The NodeMCU is configured to connect to the local WiFi network using user-provided credentials, facilitating communication with the HTML web page and the corresponding Android application on the user's smartphone.

**Display and Indication Unit:** For continuous monitoring of sensor values, a PC screen was interfaced with the MCU-Wi-Fi module via a USB-Wi-Fi module. This allowed users to observe and record real-time data on temperature, humidity, atmospheric pressure, and light stimuli. Data logging was implemented using a Google Sheets file as a cloud-based storage solution, chosen for its simplicity and cost-effectiveness. Furthermore, the internal controller of the MCU-Wi-Fi module offloads the continuous data transmission burden to the display unit.

## 2.6. BVCD

Bee venom was collected from colonies by the Bee Venom Collector Device (BVCD). The technical specifications of the BVCD components (Electric shock device, VC-6F, Apitronic, Canada) are shown in Table 1.

The bee venom collection was conducted by the device. The collector frame was connected with wires to the collector device and the collection time is 30 minutes at every collection treatment. During this period, the device works automatically and supplies preset impulses to the wire grids. The collector frame was removed from the bee colony. Then, the deposit of bee venom on the glass plate was scrapped using a scraping knife.



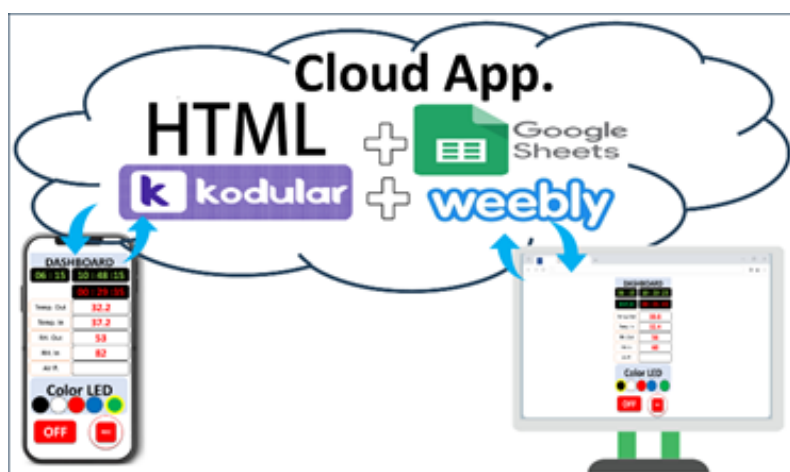


Fig. 4. Cloud Software design based on the free app.

Table 1.

The technical specifications of BVCD

Input DC Voltage & Current, V & A	Timer, s		Collector Frames, m	Operation Mode	Temp., °C	Humidity (max). % (at 40 °C)	Max operating time, h
	ON	OFF					
11.5-12.75 & 0.15	0.5 - 2	3 - 5	0.5 × 0.4	Semi- automatic	-5: 40	95	8

## 2.7. Methodology

The automated BVCMS operation involved a sequence of procedures for data acquisition, environmental control, and remote monitoring. The CPU generated control signals for the RGB LED stimuli to regulate the internal beehive environment. The integration of the hardware components and the software system culminated in a comprehensive IoT-based smart BVCMS. It provides the beekeeper with continuous remote access to beehive conditions via a PC or smartphone-based system. This microcontroller-based circuit continuously monitors and records temperature and humidity values within the BVCD's operational zone, maintaining an updated log to facilitate optimization for both maximum safety and productivity of the beehive.

This study investigated the influence of light stimuli on beehive conditions during bee venom collection. Six honeybee colonies with varying strengths (5, 7, and 9 frames) were selected for the experiment. Three colonies underwent venom collection during daylight hours, and the remaining three were subjected to the process at night. Each colony participated in two venom collection sessions, separated by a two-week interval, following a factorial experimental design that considered colony strength, time of collection (day/night), and the sequential collection number (first or second session).

Four distinct light stimuli (red, blue, green, and white) were applied uniformly across all treatment groups during each venom collection session. Throughout the experimental duration, internal beehive temperature ( $T$ , °C), relative humidity (RH, %), and atmospheric pressure (AP, N/cm<sup>2</sup>) were continuously monitored using the integrated sensors described previously.

The experimental design ensured a balanced representation across all combinations of daytime collections (5 frames - session 1 & 2; 7 frames - session 1 & 2; and 9 frames - session 1 & 2) and nighttime collections (5 frames - session 1 & 2; 7 frames - session 1 & 2; 9 frames - session 1 & 2). This structured approach facilitated a robust statistical analysis to determine the individual and interactive effects of colony strength, time of collection, and the application of specific light stimuli on the internal beehive environment during the venom extraction process.

## 3. Results and discussions

### 3.1. The visual data overview

The visual data overview of the collected data (Fig. 5) illustrates temporal dynamics of temperature ( $T$ , °C), relative humidity (Rh, %), and atmospheric pressure (AP, N/cm<sup>2</sup>) under the specified experimental conditions. The x-axis represents the experimental treatments, while the y-axis depicts the temporal

progression of measurements within each trial. The left-most panel indicates the applied light stimulation, including potentially a control condition alongside the red, blue, green, and white light stimuli. Data are

further stratified by colony strength (5, 7, and 9 frames) and the time of collection (Day and Night), aligning with the established experimental design.

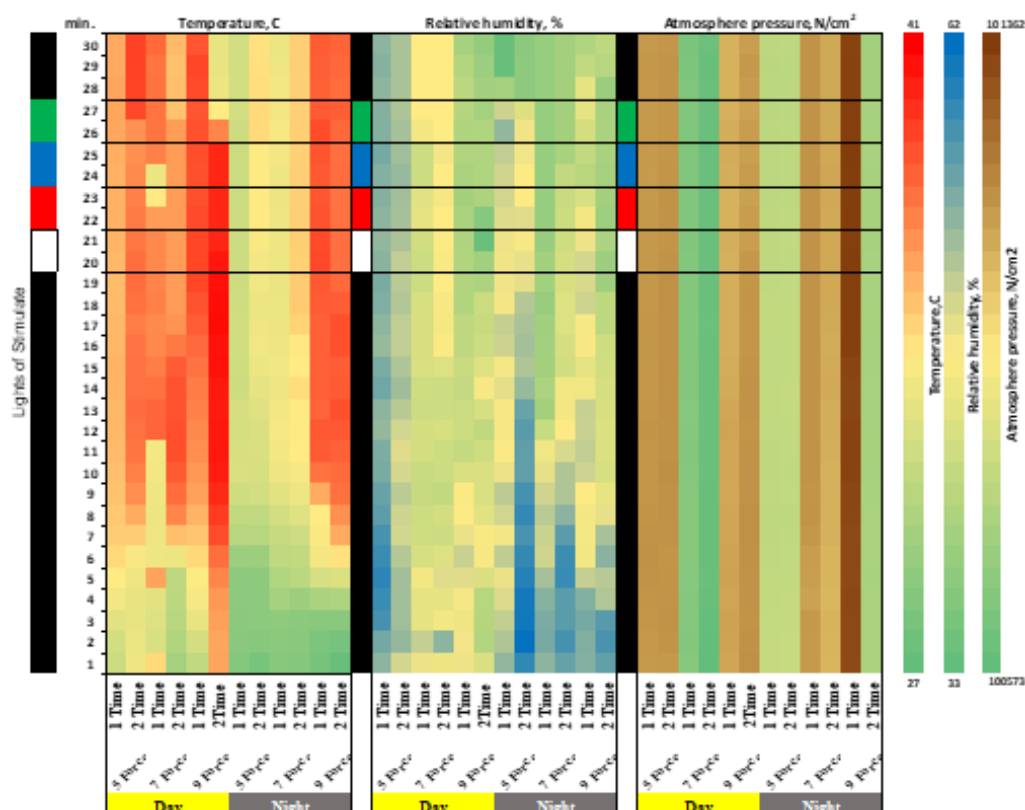


Fig. 5. The visual data overview of the Experiments

**Temperature:** Analysis of the temperature profiles revealed no discernible variations attributable to the different light stimuli. However, distinct temperature differences were observed across colony strengths and between day and night durations. As anticipated, daytime temperatures within the hives were generally higher than nighttime temperatures, likely due to solar radiation. Furthermore, a trend of increasing internal hive temperature over time during the operation of the bee venom collection device was noted, particularly in colonies with the largest frame number (9 frames) during both day and night collections. This suggests that the venom collection process itself, potentially coupled with the larger bee population in stronger hives, contributes to increased internal temperature. The influence of colony strength on temperature regulation was also apparent, with stronger colonies exhibiting a tendency towards higher overall temperatures under the conditions of venom extraction.

**Relative Humidity:** Temporal fluctuations and variations in relative humidity were evident across different treatments. Higher relative humidity levels appeared to correlate with nighttime collections and lower colony strength (5 frames), suggesting that smaller colonies might experience increased humidity, especially

during periods of reduced ventilation associated with nighttime bee inactivity. Conversely, larger colonies (9 frames) tended to maintain lower relative humidity levels.

**Atmospheric Pressure:** As an external environmental variable, atmospheric pressure exhibited less variation within each treatment group than temperature and humidity. Minor temporal changes were observed, likely reflecting broader meteorological shifts during the experimental period. The applied light stimuli and colony strength did not appear to directly influence atmospheric pressure within the hives. However, subtle, indirect correlations might exist, potentially linked to alterations in bee activity and hive ventilation that could marginally affect the internal microenvironment.

These data suggested that colony strength and time of day (day vs. night) had a greater effect on the internal temperature and humidity of honeybee colonies during venom collection than the light stimuli tested. The increase in temperature observed in stronger hives during venom extraction requires further investigation into the metabolic heat produced by the larger bee population under these conditions. Similarly, the higher humidity in smaller hives during nighttime collections

suggests differences in ventilation and moisture regulation capabilities related to colony size. The lack of a clear effect from the different visual spectra on the measured parameters indicates that under the conditions of this experiment, visual cues might not be a primary driver of changes in the hive microclimate. However, further quantitative statistical analysis of the data is crucial to confirm these observed trends' significance and elucidate any potential subtle interactions between the experimental factors. This will involve extracting the numerical data from the visual representations and employing appropriate statistical methods to assess the main and interactive effects of light stimulation, colony strength, and collection time on the measured environmental variables.

**Table 2**

Beehive temperature through BVCD work

Treatment			Tin when BVCD ON						
Time	Frames	Times	T <sub>out</sub>	T <sub>ON</sub>	T <sub>Max</sub>	t <sub>max</sub>	T <sub>diff</sub>	CV <sub>T</sub>	SD <sub>T</sub>
Daytime	5	1st	32.7	36.2	37.3	18.85	1.1	0.7	0.26
		2nd	35	35.4	38.8	13.08	3.4	2	0.76
	7	1st	33.8	35.4	39.5	15.43	4.1	4.16	1.54
		2nd	30.5	34.7	39.4	10.66	4.7	2.69	1.02
	9	1st	32	36.1	39.6	14.03	3.5	2.33	0.9
		2nd	37.6	36.5	40.8	11.2	4.3	5.03	1.98
Nighttime	5	1st	28.7	29.5	34.1	4.63	4.6	2.16	0.72
		2nd	27.5	29.5	36.1	16.93	6.6	4.33	1.51
	7	1st	28.5	31.4	35.1	19.72	3.7	2.29	0.8
		2nd	28.6	32.3	36.8	12.03	4.5	3	1.08
	9	1st	28.1	35.1	39.3	5.87	4.2	2.97	1.15
		2nd	27	35.3	39.4	7.37	4.1	1.69	0.65

**Temperature Response to Venom Collection and Colony Strength:** Consistent with the initial analysis, the data confirms a clear increase in internal hive temperature (T<sub>ON</sub> and T<sub>Max</sub>) during BVCD operation across all colony strengths and collection times, indicating a metabolic response to the electrical stimulation. Notably, T<sub>Max</sub> consistently reached its highest values in the 9-frame beehives during both daytime (40.8 °C) and nighttime (39.4 °C) collections, across both the first and second collection instances. This reinforces the earlier finding that stronger colonies exhibit a more pronounced temperature increase, likely due to the larger population's collective metabolic activity in response to the stimuli. Furthermore, the text highlights that this increase in T<sub>Max</sub> was proportionally greater during the daytime, potentially attributable to the higher ambient temperatures (T<sub>out</sub>) amplifying the bees' thermoregulatory efforts and metabolic heat generation. A similar trend of elevated T<sub>Max</sub> was observed in the 7-frame

### 3.2. Temperature

Table 2 presents a detailed overview of temperature dynamics within honeybee colonies of varying strengths (5, 7, and 9 frames) during bee venom collection under both daytime and nighttime conditions, across two collection instances. The parameters recorded include the ambient temperature (T<sub>out</sub>), the temperature inside the hive after opening the Bee Venom Collection Device (T<sub>ON</sub>), the maximum temperature reached inside the hive during BVCD operation (T<sub>Max</sub>), the temperature difference between T<sub>Max</sub> and T<sub>out</sub> (T<sub>diff</sub>), the coefficient of variation of the temperature inside the hive (CV<sub>T</sub>), and the standard deviation of the temperature inside the hive (SD<sub>T</sub>).

beehives during the daytime, further supporting the influence of higher external temperatures on the internal thermal response during venom collection.

**Temporal Dynamics of Maximum Temperature:** The time taken to reach the maximum temperature (t<sub>max</sub>) was significantly longer during the daytime (average 13.87 min) compared to the nighttime (average 11.08 min). This observation may be due to the bees' spatial distribution within the hive. During the night, with all bees likely congregated inside, their response to the BVCD might be faster and more synchronized, leading to a quicker attainment of T<sub>Max</sub>. Similarly, t<sub>max</sub> was higher during the first collection (average 13.08 min) compared to the second (average 11.87 min), potentially indicating a faster reaction of the bees upon subsequent exposure to the venom collection process.

**Temperature Difference and Stability:** The values of T<sub>diff</sub>, CV<sub>T</sub>, and SD<sub>T</sub> generally showed an increasing trend

from 1.1°C, 0.7%, and 0.26 to 4.7°C, 5.03%, and 1.98, respectively, with rising beehive CS during the daytime. This suggests that stronger colonies not only experienced a larger overall temperature increase but also exhibited greater variability in  $T_{in}$  during the venom collection process under daytime conditions. Conversely, the supporting text indicates an "inverse trend" for these parameters during the nighttime. The contrasting trend at night might be due to the densely populated hive, potentially leading to a more uniform and rapid thermal response within the confined space.

The data strongly indicates that collecting bee venom leads to a measurable increase in  $T_{in}$ . The extent and timing of this increase are significantly influenced by CS and the time of day. Stronger colonies showed higher  $T_{max}$  and greater temperature fluctuations during venom collection conducted in the daytime. The quicker attainment of  $T_{max}$  of 39.4°C, reached in  $t_{max}$  of 7.37 seconds during nighttime and in subsequent collections, suggests that the bees may learn or acclimatize in response to the bee venom collection dynamics (BVCD). The contrasting trends in temperature difference and stability between day and night in relation to colony strength warrant further investigation,

potentially linked to the spatial distribution and collective behavior of the bee population within the hive under different light conditions. These findings have practical implications for optimizing venom collection protocols to minimize stress on bee colonies by considering factors such as colony size and the timing of collection. The reference to Ali *et al.*, 2023 for establishing a baseline temperature after hive opening provides methodological context for ensuring the measured temperature changes are attributable to the BVCD operation rather than hive disturbance.

### 3.3. Relative Humidity

Table 3 presents the humidity dynamics within honeybee colonies of varying strengths (5, 7, and 9 frames) during bee venom collection under both daytime and nighttime conditions, across two collection instances. The parameters recorded include the ambient humidity ( $H_{out}$ ), the humidity inside the hive when the Bee Venom Collection Device (BVCD) was ON (Hum. When BVCD ON), the maximum humidity reached inside the hive ( $H_{max}$ ), the minimum humidity recorded inside the hive ( $H_{min}$ ), the coefficient of variation of the humidity inside the hive ( $CV_H$ ), and the standard deviation of the humidity inside the hive ( $SD_H$ ).

**Table 3**

Beehive relative humidity through BVCD work

Treatment			$H_{out}$	Hum. When BVCD ON				
Time	Frames	Times		$H_{avg}$	$H_{max}$	$H_{min}$	$CV_H$	$SD_H$
Daytime	5	1st	48	53.18	58	50	2.88	1.53
		2nd	40	49.49	52	45	2.76	1.36
	7	1st	41	42.21	45	40	3.6	1.52
		2nd	42	43.55	46	40	4.24	1.84
	9	1st	45	40.64	50	38	6.66	2.71
		2nd	43	40.69	47	33	7.45	3.03
Nighttime	5	1st	53	45.72	54	33.4	9.81	4.48
		2nd	63	48.76	59	35.8	13.45	6.56
	7	1st	54	39.92	51	37	11.02	4.4
		2nd	62	43.57	59	37	12.5	5.44
	9	1st	54	44.38	50	38	6.89	3.06
		2nd	63	41	53	36	9.43	3.86

Influence of Colony Strength and Diurnal Variations on Humidity: The note corroborates that during daytime, the average humidity ( $H_{AVA}$ , When BVCD ON) exhibited a decreasing trend with increasing colony strength from 50 to 33% and the same trend in  $H_{max}$ , and  $H_{min}$ . As suggested, this inverse relationship between colony size and internal humidity during the day might be linked to the increased thermal conditions

within larger hives (as discussed in the temperature results) leading to a reduction in relative humidity despite potentially higher moisture input from the larger bee population. This aligns with the note's explanation of lower atmospheric relative humidity to the internal hive environment during the day.

Conversely, the note highlights a key difference at nighttime.  $H_{out}$  was generally higher than  $H_{max}$  during



the night, which contrasts with the daytime, where  $H_{\max}$  tended to be higher than  $H_{\text{out}}$ . This observation, supported by Ali *et al.* (2023) regarding higher nighttime  $T_{\max}$  (which our temperature data also partially supports in stronger colonies), suggests that the concentrated bee population within the hive at night, coupled with potentially elevated temperatures due to their collective metabolism, contributes to a higher internal humidity compared to the outside environment.

**Humidity Stability and Colony Strength:** The trends in humidity stability, as indicated by  $CV_H$  and  $SD_H$ , are further clarified by the note. Consistent with the previous analysis, both  $CV_H$  and  $SD_H$  showed an increasing trend with rising colony strength during the daytime, indicating greater humidity fluctuations in larger hives under daylight conditions. This suggests that stronger colonies maintain a more stable internal humidity environment during the night, potentially due to a more effective and coordinated effort in humidity regulation by the larger, clustered bee population. The note proposes a direct relationship between humidity (H) and temperature (T), implying that the thermal stability in larger nighttime colonies might contribute to their more stable humidity levels.

In conclusion, it provides additional valuable support and further explanation for the observed humidity dynamics during bee venom collection. The inverse relationship between colony strength and internal humidity during the day is likely influenced by the increased thermal conditions in larger hives. The contrasting humidity relationship with the external environment

**Table 4**

Beehive Atmospheric pressure through BVCD work

Treatment			Atmospheric Preusser under 30 min						
Time	Frames	Times	$AP_{ON}$ N/cm <sup>2</sup>	$AP_{Avg}$ N/cm <sup>2</sup>	$AP_{Min}$ N/cm <sup>2</sup>	$AP_{Max}$ N/cm <sup>2</sup>	$DIFF_P$ N/cm <sup>2</sup>	$CV_P$	$SD_P$
Daytime	5	1st	10.116	10.117	10.116	10.119	0.0031	0.0061	0.0006
		2nd	10.117	10.118	10.116	10.12	0.0034	0.006	0.0006
	7	1st	10.063	10.066	10.063	10.068	0.0049	0.0096	0.001
		2nd	10.058	10.059	10.058	10.061	0.0029	0.0041	0.0004
	9	1st	10.114	10.113	10.111	10.116	0.0016	0.0102	0.001
		2nd	10.119	10.117	10.101	10.12	0.0013	0.0144	0.0015
Nighttime	5	1st	10.085	10.082	10.08	10.085	0.0002	0.0084	0.0009
		2nd	10.085	10.083	10.08	10.085	0.0002	0.0084	0.0009
	7	1st	10.115	10.118	10.115	10.12	0.005	0.0051	0.0005
		2nd	10.111	10.113	10.111	10.115	0.0044	0.008	0.0008
	9	1st	10.133	10.135	10.133	10.137	0.0045	0.0088	0.0009
		2nd	10.074	10.076	10.074	10.078	0.0039	0.0063	0.0006

**Temporal Variations in Atmospheric Pressure:** During the daytime, the 7-frame configuration often demonstrated comparatively lower variability, with an  $SD_P$  of 0.0004 and a  $CV_P$  of 0.0041 during the second

between day and night is attributed to the bees' concentrated presence and potentially higher internal temperatures at night. Furthermore, the opposing trends in humidity stability with colony strength between day and night underscore the complex interplay between colony size, diurnal patterns, and the bees' collective thermoregulatory and hygroscopic behaviors during the venom collection process. These findings emphasize the need to consider both thermal and humidity conditions within the hive when optimizing venom collection techniques and assessing potential stress on bee colonies. The references Ali *et al.* (2023) and Younis and Ali (2024) provided a link to existing research supporting the observed temperature patterns and their potential influence on humidity.

### 3.4. Atmospheric pressure

Table 4 presents the atmospheric pressure dynamics within honeybee colonies of varying strengths (5, 7, and 9 frames) during bee venom collection under both daytime and nighttime conditions across two collection instances. The parameters recorded include the atmospheric pressure when the Bee Venom Collection Device (BVCD) was ON ( $AP_{ON}$ ), the average atmospheric pressure recorded over 30 minutes during the BVCD operation ( $AP_{AVA}$ ), the minimum atmospheric pressure ( $AP_{Min}$ ), the maximum atmospheric pressure ( $AP_{Max}$ ), the difference between  $AP_{Max}$  and  $AP_{Min}$  ( $DIFF_P$ ), the coefficient of variation of the atmospheric pressure ( $CV_P$ ), and the standard deviation of the atmospheric pressure ( $SD_P$ ). All pressure values are reported in N/cm<sup>2</sup>.

collection, similarly, at nighttime. This suggests an optimal balance in internal dynamics under these specific conditions and exhibits the highest pressure stability. Interestingly, the 9-frame configuration at night

showed a marked increase in variability, with an  $SD_P$  of 0.0009 and a  $CV_P$  of 0.0088 in the 1st, while daytime showed a greater increase, with an  $SD_P$  of 0.0015 and a  $CV_P$  of 0.0144 in the 2nd. This suggests that larger internal volumes at night might allow for greater, rather than reduced, pressure fluctuations. This could be attributed to increased internal air currents or subtle thermal gradients within a larger, less occupied space.

**Pressure Difference ( $DIFF_P$ ) and Colony Strength/Diurnal Cycle:** Although overall pressure changes  $DIFF_P$  were small, 9-frame beehives showed a notable change during daytime collections. These results suggest this might be due to the 'closed room' effect within the larger hive, potentially leading to slightly more pressure fluctuations during the day when bee activity and thermoregulation are higher. Interestingly, this effect was reportedly absent at nighttime, which the text attributes to a "mass escape of bees from the beehive in all treatments spatially in high beehive power." This suggests that at night, despite the larger colony size, increased ventilation or bee dispersal might mitigate any pressure build-up observed during the day in stronger hives. Although a slightly higher  $DIFF_P$  is observed for the 9-frame (2nd collection) daytime treatment.

The results suggest subtle influences of colony strength and diurnal cycles. There is a potential trend for slightly higher average internal pressure in stronger colonies. Furthermore, the increased pressure variation ( $DIFF_P$ ) in larger hives during daytime collections, potentially due to metabolic activity and hive structure, and its absence at night (possibly due to bee dispersal) warrant further investigation. However, the minimal magnitude of these pressure differences suggests that atmospheric pressure is likely a less critical factor compared to temperature and humidity in assessing the impact of venom collection on the hive environment (Younis and Ali, 2024). Future research could focus on more precise measurements and the correlation between bee activity levels and minute pressure changes within the hive during different stages of venom collection and under varying environmental conditions.

### 3.5. Effect of light stimuli on Beehive conditions under BVCD work

Tables 5, 6 and 7 present the internal temperature " $T$ " ( $^{\circ}C$ ), relative humidity " $Rh$ " (%), and atmospheric pressure " $AP$ " ( $N/cm^2$ ) within honeybee colonies under different light stimuli (white, red, blue, green, and off) during bee venom collection using a BVCD. Measurements were conducted across colonies of varying strengths (5, 7, and 9 frames) during both daytime and nighttime, with two collection instances. Baseline conditions for temperature, humidity, and atmospheric

pressure were recorded prior to the application of light stimuli.

**Baseline temperatures** exhibited expected diurnal variations, with higher values from 37.06 to 40.66  $^{\circ}C$  during the day and a general increase with colony strength, particularly during the daytime. Baseline relative humidity showed diurnal variability, with higher levels at night, and no consistent correlation with colony strength. Baseline atmospheric pressure remained remarkably stable across all experimental conditions, indicating a consistent internal environment.

**Temperature:** White, red, and blue light stimuli generally resulted in minimal and inconsistent changes in internal beehive temperature compared to baseline across all treatments. Greenlight also showed no significant uniform impact, although a specific decrease was observed in the 9-frame colony during the second daytime collection. The "off" condition did not significantly alter hive temperature. Overall, light stimuli did not substantially or consistently influence internal temperature, primarily driven by diurnal cycles and colony strength.

**Relative Humidity:** White, red, and blue light stimuli did not consistently affect internal relative humidity. However, green light induced a notable decrease in humidity during some nighttime instances across all tested colony strengths. The "off" condition showed humidity levels similar to other treatments. This suggests a potential specific behavioral response to green light under low light conditions affecting hive humidity regulation.

**Atmospheric Pressure:** The application of all tested light stimuli (white, red, blue, and green) and the "off" condition had no discernible or consistent impact on internal beehive atmospheric pressure. Atmospheric pressure remained stable and consistent across all experimental conditions, primarily determined by external environmental factors.

The tested light stimuli had a limited and inconsistent effect on honeybee colonies' internal temperature and relative humidity during BVCD operation. Atmospheric pressure remained unaffected. While temperature was primarily influenced by diurnal cycles and colony strength, green light appeared to induce a decrease in relative humidity between 52.45 to 33.48 % during the day and nighttime.

The bees' thermoregulation is likely more responsive to ambient temperature and metabolic heat. Humidity regulation is a complex process influenced by ventilation and water balance. The lack of a consistent

response to most light stimuli suggests that under the current experimental parameters, these cues are not primary drivers of immediate changes in these microclimatic parameters. The specific response to green light at night regarding humidity warrants further investigation into potential behavioral mechanisms. Atmospheric pressure, being a predominantly external physical parameter, is not expected to be influenced by the biological activities or the applied light stimuli within the hive.

The application of white, red, and blue lights did not significantly affect internal beehive temperature or

relative humidity during bee venom collection. The green light showed a specific effect of decreasing relative humidity during nighttime. Atmospheric pressure remained stable and unaffected by the light stimuli. The primary factors influencing hive temperature were diurnal cycles and colony strength. Further research should focus on the specific mechanisms underlying the observed response to green light and exploring potential interactions between light spectra and bee behavior during venom collection to optimize beekeeping practices and minimize potential stress on colonies. The stability of atmospheric pressure provides a consistent baseline for future studies.

**Table 5**

Effect of visual stimuli on Beehive temperature under BVCD work.

Treatment	Daytime						Nighttime					
	5		7		9		5		7		9	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Before	37.06	38.57	38.16	37.88	39.22	40.66	33.26	35.14	34.94	36.51	39.11	39.01
White	37.19	38.43	37.89	37.7	39.45	40.2	33.13	35.75	35	36.6	39.34	38.62
Red	37.23	38.18	35.73	37.7	39.3	40.2	33.05	36.01	35.05	36.6	39.16	38.64
Blue	37.37	37.93	35.88	37.7	39.32	39.28	33.09	36.13	35.42	36.6	39.3	38.71
Green	37.41	37.55	35.67	36.97	39.37	34.71	33.4	36.24	35	36.6	39.02	38.83
Off	37.49	37.46	35.34	36.94	39.3	35.56	33.45	36.34	35.3	36.6	39.04	38.88

**Table 6**

Effect of visual stimuli on Beehive relative humidity under BVCD work.

Treatments	Daytime						Nighttime					
	5		7		9		5		7		9	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Before	52.48	49.22	41.61	44.81	39.46	39.84	46.93	48.79	37.72	42.09	46.42	39.28
White	52.05	50.41	42.25	45.18	38.7	34.63	46.63	46.25	37	41.18	45.14	38.1
Red	52.75	51.14	41.96	44.6	38.24	38.89	47.93	44.84	37	40.63	40.79	38.04
Blue	53.26	50.78	42.41	45.36	38.96	39	50.36	45.91	37	38.27	40.99	38.49
Green	52.45	50.22	45	45	38.78	33.98	33.48	36.24	37	38	40	39.53
Off	52.39	50.2	45	45	39.6	34.16	43.63	39.58	37	38	43	37.87

**Table 7**

Effect of visual stimuli on Beehive atmospheric pressure under BVCD work.

Treatments	Daytime						Nighttime					
	5		7		9		5		7		9	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Before	10.117	10.119	10.066	10.059	10.113	10.118	10.083	10.084	10.118	10.113	10.134	10.076
White	10.117	10.119	10.066	10.059	10.113	10.118	10.083	10.084	10.118	10.113	10.135	10.076
Red	10.117	10.119	10.066	10.059	10.113	10.118	10.083	10.083	10.118	10.114	10.136	10.076
Blue	10.117	10.118	10.066	10.059	10.112	10.117	10.083	10.083	10.118	10.114	10.136	10.076
Green	10.117	10.118	10.065	10.059	10.112	10.116	10.082	10.082	10.118	10.114	10.136	10.076
Off	10.117	10.118	10.065	10.059	10.113	10.116	10.082	10.082	10.118	10.114	10.136	10.076



#### 4. Conclusions and recommendations

The findings of this study indicate that while the tested light stimuli did not elicit significant and consistent changes in the monitored internal environmental parameters (temperature, relative humidity, and atmospheric pressure) of honeybee colonies during electrical stimulation, colony strength and the time of day (day vs. night) exerted a more pronounced influence, particularly on temperature and humidity. Atmospheric pressure remained largely stable and unaffected by the experimental manipulations, except average pressure values were highest in the 9-frame beehives. The lack of a clear and consistent effect of the different light spectra on the measured microclimatic parameters suggests that, under the conditions of this experiment, visual cues might not be primary drivers of immediate changes within the hive environment during venom collection. Further investigation with more controlled conditions and a focus on the behavioral responses of bees to specific light wavelengths in conjunction with venom collection. The stable atmospheric pressure readings reinforce that this parameter is primarily determined by external environmental conditions and is not significantly influenced by the tested light stimuli or the immediate biological responses of the bees during venom collection.

Future research should explore the link between colony size, bee activity during venom extraction, and metabolic heat generation to understand temperature increases in stronger hives. Studies are recommended to examine ventilation dynamics and moisture regulation in honeybee colonies of varying strengths, especially at night, to explain higher humidity in smaller hives. Investigate the long-term impacts of repeated venom collection, possibly combined with light stimuli, on colony health and productivity. When developing bee venom collection protocols, consider colony strength and collection timing (day vs. night) to minimize stress on the colonies.

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