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A New Technique for Marking Queen Bees (*Apis mellifera*) for Better Visibility and Easier Spotting

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Abstract: While marking queens is an optional rather than mandatory technique, it is increasingly becoming a standard practice in modern beekeeping. Finding queens in strong colonies and large apiaries is a time-consuming process. The visible and durable marking of the queen enables it to be seen more quickly, directly improving productivity in apiary management. This study examined a new technique for marking queens using an oil-based marker, which involved marking not only the thorax (as a standard technique) but also the wings and abdomen. The durability of the marking was assessed by measuring color retention at the start of the experiment and after five months. Two groups of queens were formed: an experimental group, marked with the new technique on three body parts—Group O (n = 12) and a control group of unmarked queens—Group N (n = 12). The most durable color retention was observed on the thorax (54.4%) and abdomen (14.4%), while retention on the wings was weaker (2.4%), necessitating reapplication during the season. Considering the proportion of the total marked area, abdomen marking gave better results (9.5%) compared to the thorax (5.4%) and wings (0.6%) marking. The application of this marking technique showed no negative effects on queen acceptance, survival, or supersedure. Marking three body parts can increase the queen's visibility in a non-invasive way, improving work efficiency.

Keywords: queen marking; queen visibility; beekeeping practice

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1. Introduction

In modern beekeeping, queen marking is one of the standard beekeeping practices [1]. Worldwide, various methods and techniques are used to facilitate marking, and recently, significant efforts have been directed toward discovering new technologies for faster recognition and spotting of queens [2,3]. Regular monitoring of bee colonies often involves finding (locating) queens within the colonies. If the queens are unmarked, locating them becomes a time-consuming and laborious task, especially in large apiaries and strong colonies. Having unmarked queens increases labor costs and reduces productivity in the apiary. The first mentions of insect marking date back to the 1920s [4], while the oldest references to animal marking date back to 218 BCE [5].

Marking and identifying individual animals within a population are used not only for easier tracking but also for various biological and ethological studies. However, unlike

vertebrates and larger animals, which can be marked using techniques such as tattooing, tagging, or banding, marking insects has always presented a challenge for biologists. Today, certain insects are marked by attaching colored wires to different parts of their bodies [6,7]. Marking with fluorescent dyes, which are added to food or directly applied to larvae, leading to pigment accumulation in specific tissues, is also used [8]. Due to the small size of insects (e.g., spiders, mosquitoes), researchers used various techniques, some of which have been abandoned. These methods include marking with rare elements (rubidium, strontium, etc.) and radioactive isotopes [9–11]. Genetic marking, which involves gene transfer (transgenic markers) to label mass-produced insects, has shown stability of markers even after 15 generations [12,13].

Wineriter and Walker [14] tested 26 different types of paints on insects and found that the most durable ones are non-water-soluble. Additionally, the authors stated that the paint must be easy to apply, non-toxic, lightweight, resistant to peeling, and quick-drying. Researchers have marked insects with various paints to study toxicity, while the most common method of marking bees (family *Apidae*) involves using different paints and numbered tags [15–17]. In addition to standard paints (markers), other methods are used to monitor natural behavior, such as self-marking techniques for studying pollination activities. These techniques use fluorescent powders that are most visible under UV light. Besides fluorescent powders, protein-based powders (from egg albumin and milk casein) are used, with detection performed via analytical biochemical tests [5,16].

Advancements in technology offer new possibilities for researchers and entomologists to use electronic devices and sensors for tracking the movement, behavior, and longevity of bees [18,19]. One marking method involves using radio-frequency identification (RFID), which is used not only for marking honey bees [20] but also for bumblebees [21] and solitary bees [22,23]. RFID tags are used for biomonitoring, tracking foraging activities, and individual monitoring of bees [22].

In recent decades, marking bees with radar transponders has enabled harmonic tracking of individual bees (direction, route, speed, and distance). Using transponders, the exact flight paths and how a queen or drone reaches the congregation area for mating can be monitored [24]. Through digital technologies and artificial intelligence, it is possible to track bee behavior, with each bee individually marked with a unique barcode [25]. Barcode marking of queens can assist in monitoring their daily activities, egg-laying patterns, and other activities depending on the time of day [26].

Attaching tags to queens is achieved using various commercially available adhesives. The technique is simple, involving the application of adhesive to the thorax, between the wings, with a minimal amount sufficient to hold the attached tag. However, little data are available on the chemical impact of adhesives on honey bees. According to Splitt et al. [27], shellac adhesive yielded better results compared to nitrocellulose adhesive, and shellac, as a natural resinous substance made from the secretions of the insect *Laccifer lacca*, is more suitable for use. Synthetic cyanoacrylic (CA) glue showed the worst results in *Osmia bicornis* bees, causing high mortality (17% survival). The results indicated that cyanoacrylate adhesives were not toxic to the Colorado potato beetle (*Leptinotarsa decemlineata*) and plum curculio (*Conotrachelus nenuphar*) but were highly toxic to corn rootworms (*Diabrotica* sp.), where mortality exceeded 40% after just 4 h [28].

In queen marking, attention must also be given to the weight of the tag. In general, insects are known for their ability to carry loads exceeding their body weight multiple times over [29]. While data on load effects on queen longevity are lacking, some data exist for worker bees. Loads exceeding 20 mg reduced worker bee lifespan [30]. Unlike queens, workers are often burdened with loads they bring to the hive during activities outside. Average pollen loads account for 27% of a bee's body weight, while nectar loads

average 40%. Pollen loads averaging 18% of body weight had no effect on wing fanning frequency or body posture during flight [31]. Queens are generally twice as heavy as workers, meaning the weight of a tag or microchip may not significantly impact normal functioning (19). The average chip weight (e.g., UHF Hitachi chip) is about 5 mg, which represents ~5% of an average worker bee's body weight of 98 mg [32] or <3% of an unmated queen's weight, which averages 198.8 mg, with variations from 168 mg to 219 mg [33–35]. According to Alburaki et al. [19], the optimal size of microchips for tracking queen nuptial flights is 2.5 × 2.5 × 0.4 mm.

Considering the above, it is evident that no current method of queen marking meets all beekeepers' requirements. The marking technique should be simple and efficient, ensuring quick queen spotting while being safe for the queen and the colony.

The aim of this study was to examine a new queen-marking technique, involving oil-based marker coloring on the thorax, wings, and abdomen, with an assessment of color retention at the beginning of the trial and after five months. Additionally, the study monitored the acceptance of queens marked using this technique, along with the queen supersedure and survival rates.

2. Materials and Methods

The experiment was conducted in the vicinity of Belgrade (44°44′58″ N, 20°20′22″ E) at the apiary of the company "Golden Bee doo, Belgrade, Serbia.", which specializes in the professional production of queen bees. In the study, we used the honey bee subspecies *Apis mellifera carnica* [36,37]. At the beginning of May (1 May 2024), 48 queen cells were introduced into four-way mating nucs (Figure 1a) to produce a minimum of 24 mated queens. Sixteen days after introducing queen cells, mating success was checked, and 24 successfully mated queens were transferred to a beekeeping trailer that contains production colonies (queenless for one day). The usage of insulated beekeeping trailer (Figure 1b) eliminates variability in factors like hive material, roof type, and position (in the shade or in the sun), ensuring equalization of all factors inside the trailer (humidity, temperature, etc.). The beekeeping trailer is located at one site, so nectar source enviroment is the same for all colonies. The mated queens were introduced into these queenless colonies using cages, along with the belonging frames and bees from the origin mating nucs (their own brood frames and bees). Seven days after the introduction of queens, the acceptance of queens in production colonies was assessed.

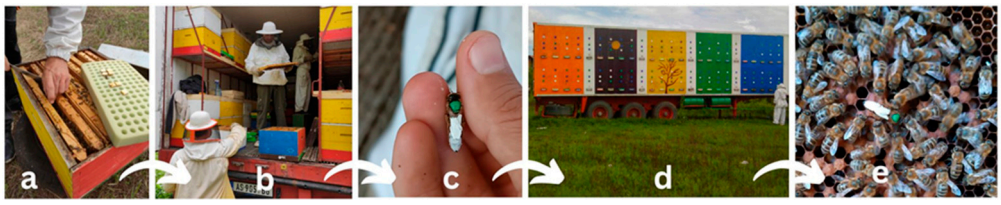

Figure 1. Protocol during the conduct of the experiment ((**a**) adding queen cells and obtaining mated queens; (**b**) bringing mating nucs with mated queens and frames in the trailer; (**c**) experimental marking of queens; (**d**) beekeeping trailer where the 24 colonies that are in the trial are located; (**e**) checking for queen acceptance).

The tested queens were placed in the beekeeping trailer (Figure 1d) in standardized European Langstroth (LR) hives [38]. Two groups of queens were formed: one consisting of 12 queens marked on the thorax, wings, and abdomen (Figure 1c), labeled as Group O, and the other, labeled as Group N (control) that included 12 unmarked queens. On queens belonging to Group O, the colored areas were measured, while in both groups, the acceptance rate of added queens, queen supersedure, and queen survival were monitored.

The marking of queens was performed in mid-May (17 May 2024), and on the same day, they were introduced into queenless colonies. One week later, the acceptance (presence) of queens in both groups was evaluated (Figure 1e). The evaluation of color retention was conducted at the end of the season, after a five-month period (22 October 2024). During this time, the colonies containing the tested queens were used for regular production purposes.

In the experiment, we used green and white oil-based markers of the same brand: uniPaint Marker (Uni Mitsubishi Pencil Co., Ltd., Ho Chi Minh City, Vietnam). The marking of queens in the experimental group (Group O) was performed in the following way: the thorax was marked with green paint, while the wings and abdomen were marked with white paint (Figure 2). The green color represents the year of queen production (2024), while the white color was used to improve visibility due to its high contrast.

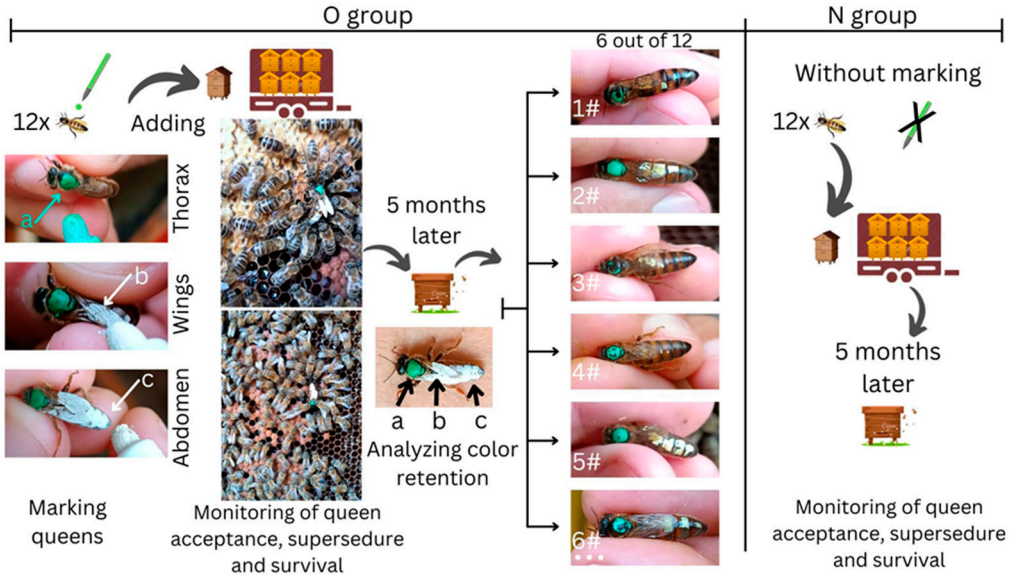

Figure 2. Experimental design, using the first six queens as examples (a—thorax marking; b—wing marking; c—abdomen marking).

Determination of partial color retention and total colored area—The colored area was measured at the beginning and after five months of trial. The partial color retention was determined based on the difference between the marked surface area (for each body part) at the beginning of the experiment and after five months. The marking of individual body parts (thorax, wings, and abdomen) was expressed as partial color area, while the multiplication of partial color retention (expressed as percentages) and the surface area of the body parts was expressed as total colored area. By graphic analysis of the queens, we calculated the percentage of color retention on the marked parts of the queen's body (area of partial retention).

Care was taken to ensure precise coloration. When wings were marked, the paint was applied exclusively to the distal parts, avoiding contact with the wing bases. Similarly, when thorax and abdomen were marked (dorsal side), the paint did not come into contact with the head or antennae. After the paint was applied, time was allowed for it to dry on the queens.

The measurement of the colored areas was conducted digitally using the software ImageJ v1.54k. To ensure consistency in the distance between the camera and the queen bee, we simply placed a plastic ruler between the camera and the queen bee before each photograph was taken. Before taking the picture, the ruler was removed, so the ruler served as a physical spacer, ensuring a fixed distance. Additionally, all measurements were conducted in pixel units, where the total area of the body parts was calculated

proportionally. This ensures comparable results regardless of possible slight variations in camera distance. So, we did not compare absolute physical dimensions (e.g., mm^2), and the camera distance did not affect the proportional results (the proportional relationship remained unchanged). For measurements, appropriate polygon selection tools were used to outline the colored areas of the queens, followed by the calculation of marked body areas. Statistical analysis of the obtained values for the marked areas of the queens was performed using a two-tailed *t*-test ($p < 0.05$) for the tested queens. The data were analyzed using the software GraphPad Prism version 9.0.0.

3. Results

The queen acceptance rate was 100% in both groups ($\sum = 24$), where young open brood in the egg stage was observed in all colonies. Throughout the five-month duration of the experiment, all queens remained alive, and the colonies functioned normally during the active beekeeping season. No supersedure cells were observed in the colonies of either group, which would have indicated queen dysfunction (defect) due to this marking technique.

After five months, the color retention of the marking on different body parts was measured. Using graphical representation and digital analysis, the data obtained are presented in a graph (Figure 3).

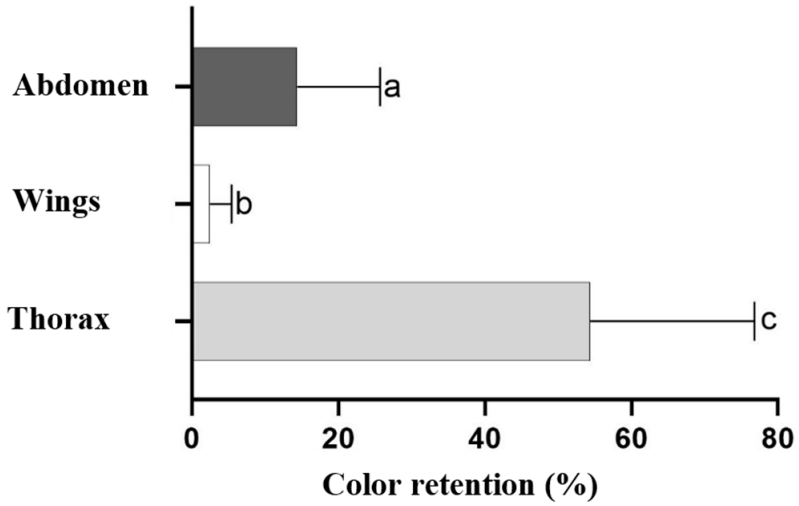

Figure 3. Retention of color (%) on the dorsal area of the abdomen, wings, and thorax of queens after a five-month period. Color retention was calculated based on the painted surface area at the beginning of the experiment and the remaining colored area at the end. The results are presented as the mean value of 12 independent measurements ± SD (standard deviation). The values marked with different letters differ significantly, where the two-tailed *t*-test reveals significant differences in color retention between the following groups: wings and thorax ($p < 0.0001$, t = 7.938, df = 22), abdomen and thorax ($p < 0.0001$, t = 5.507, df = 22), and abdomen and wings ($p = 0.0019$, t = 3.525, df = 22).

A graphical representation of the queens (using the first three queens as examples) during the process of determining the color retention on different body parts is shown in Figure 4.

A representation of partial color retention of queens from the O group that were experimentally marked is presented (Table 1).

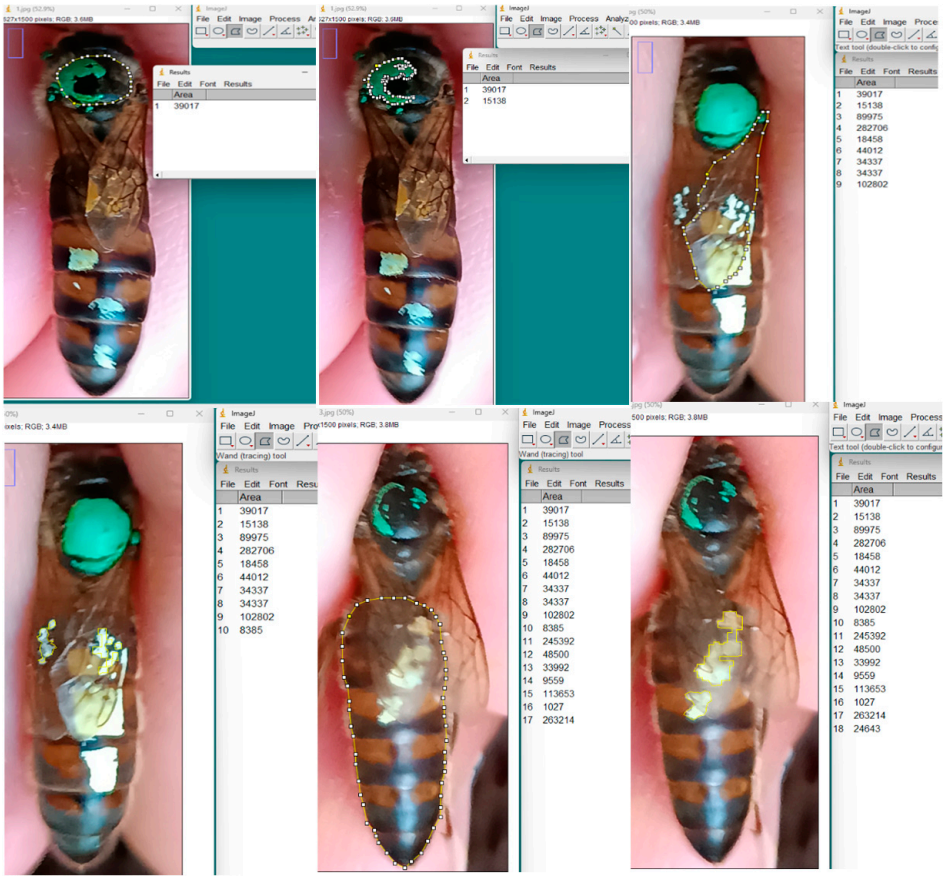

Figure 4. Digitized representation of the calculation of the color retention of the thorax of queen #1, the wings of queen #2, the abdomen of queen #3 after 5 months.

Table 1. Representation of the area of partial color retention in percentages at the beginning of the test (Initial) and at the end of the 5-month test period (After). The measurement unit of area is expressed in pixels (their total number).

Queen	Thorax		%	Wings		%	Abdomen		%
No#	Initial	After		Initial	After		Initial	After	
1	39,017	15,138	38.80	89,975	0	0.00	282,706	18,458	6.53
2	44,012	34,337	78.02	102,802	8385	8.16	245,392	48,500	19.76
3	33,992	9559	28.12	113,653	1027	0.90	263,214	24,643	9.36
4	36,819	22,198	60.29	90,246	0	0.00	273,228	3074	1.13
5	44,740	37,172	83.08	107,827	3310	3.07	277,002	97,648	35.25
6	53,389	16,817	31.50	91,813	1101	1.20	282,967	41,196	14.56
7	44,513	20,483	46.02	92,389	5047	5.46	275,486	19,572	7.10
8	39,001	35,713	91.57	99,036	1353	1.37	281,459	52,178	18.54
9	37,705	18,471	48.99	110,075	0	0.00	275,416	4012	1.46
10	42,788	10,548	24.65	97,305	7512	7.72	268,451	89,571	33.37
11	49,065	24,517	49.97	95,822	1118	1.17	274,538	52,483	19.12
12	38,507	27,473	71.35	101,024	317	0.31	245,127	15,487	6.32
$\bar{x}$	41,962	22,702	54.4	99,331	2431	2.4	270,416	38,902	14.4

The average color retention ($\bar{x}$) of the thorax after 5 months was 54.4% (min 24.65%–max 91.57%). Thorax color retention showed higher values compared to wing and abdomen color retention. The average color retention of the wings showed lower values, where some values represented the complete absence of color (0%). The average wing color retention values were 2.4% (min 0%–max 8.16%). The average color retention of the abdomen after 5 months was 14.4% (min 1.13%–max 35.25%) (Table 1).

In addition to partial color retention, the size of individual body parts (total coloration) should also be considered (Table 2).

Table 2. Representation of the area of total coloring (T × P) in queens after five months in relation to the size of body parts. The symbol $\bar{x}$ indicates average values.

	$\bar{x}$	Total Colored Area Initial (T)		Color Retention (Partial) After Five Months (P)	Total Colored Area After Five Months (T × P)
Thorax (t)	41,962	(t/Σ)	10%	54.4%	5.4%
Wing (w)	99,331	(w/Σ)	24%	2.4%	0.6%
Abdomen (a)	270,416	(a/Σ)	66%	14.4%	9.5%
Σ	411,709	100%			

When the queen bee is marked using the examined technique, its total coloration represents 100%, with the thorax area accounting for 10%, the wing area for 24%, and the abdominal area for 66% (Table 2). Although the color retention on the abdomen is weaker (compared to the retention on the thorax), the coloration of the abdominal part is greater than that of the thorax after a period of five months. The best color retention was observed on the thorax (54.4%), but comparing the thorax and the abdomen shows that the abdomen gives better results due to its larger size. This confirms that abdomen marking provides greater visibility (9.5%) compared to thorax marking (5.4%) and wing marking (0.6%), which contributes to easier queen spotting.

4. Discussion

The purpose of this study was to examine an innovative queen marking technique, focusing on the color retention of the marking on different body parts of queens, as well as the impact of this technique on queen acceptance, queen supersedure, and the survival of experimentally marked queens.

Our findings demonstrate that marking queens on the thorax, wings, and abdomen does not negatively affect their acceptance, survival over a five-month period, or lead to the queen supersedure.

Based on the obtained results, we can conclude that marking the abdominal part, in combination with thorax marking, is an effective method for marking queen bees. Given that the abdomen is the largest area marked using this technique, its coloration contributes the most to the total colored area of queen bees. This technique can significantly aid and simplify the process of finding queen bees for both professional beekeepers and hobbyists. Regarding wing marking, the procedure must be repeated during the season due to weaker color retention over a period of five months.

To our knowledge, there are no data on the color retention in honey bee queens over a certain period. There are older references on the color retention in other insect species where the color retention was nearly 50% after 6 weeks of testing [14], between 1 and

13 weeks depending on the type of paint and insect species [14], while for certain insect species, the retention was only 48.2 ± 9.2% after two weeks [39].

Thorax marking is a standard technique used worldwide [1]. However, current beekeeping practices do not involve marking other body parts of the queen. The presented marking technique is a completely non-invasive method. In the study, no negative effects of the presented marking method were recorded on the queens themselves (acceptance, supersedure, survival). When the thorax, wings, and abdomen are marked, care must be taken not to mark other body parts. This marking technique should avoid applying paint to the head and the lateral sides of the abdomen, as this may close the queen's spiracles, leading to respiratory tract dysfunction.

Thorax marking—Thorax marking is a common practice in queen marking. In our study, the best color retention results were observed on the thorax with an average of 54.4%, likely due to the high density of hair covering [40]. The thorax width in queens is 4.55 ± 0.016 mm, and the length is 4.74 ± 0.018 mm [41], which is significantly smaller than the surface area of the abdomen and wings. Additionally, irrespective of thorax marking, wings and abdomen marking should be considered a regular practice in modern beekeeping. The durability of the marking on the thorax (54%) was remarkable, which means that it could remain visible for several more months. Visibility depends on several other factors, such as the detectability of different colors and the placement of the mark on the body. These aspects could be explored further in future research. It would be valuable to understand the effect of different colors and marking locations on the speed of queen detection.

Wing marking—The wing area in queens represents a relatively large surface area compared to the entire body. The length of the forewing in queens varies between 9.50 and 9.70 mm, while the width of the forewing varies between 2.90 and 3.05 mm, measured using a scanner [42]. This means that wing marking in queens significantly improves their visibility, making it easier for beekeepers to find them. After mating, the queen does not actively use its wings, except during swarming. In practice, to prevent swarming, some beekeepers cut their wings. Previous research has shown that shortening 7.5% of the forewing area, representing an area of ~1.3 mm^2, does not impair the queen's flight or affect the mating success rate [43]. Moreover, shortening the wings to half their length did not have negative consequences on the normal functioning of the queen or colony development [1]. Compared to these radical practices of wing clipping, our technique, which involves surface application of paint on the wings, cannot pose a serious threat to the life and functioning of the queen. In our study, wing marking did not have negative consequences on the queens' functioning or colony development (queen acceptance, queen supersedure, and queen survival) compared to the control group where queens were unmarked.

However, the study results showed that the paint is not durable after 5 months. Therefore, to achieve maximum results (quick spotting), the painting procedure should be repeated as needed during the season during regularly scheduled apiary tasks. This ensures durability throughout the season. Otherwise, wing marking will give excellent results in the first part of the season, but the colored area is expected to decrease over time. The reason for this is likely the high mobility of the wings (compared to the thorax and abdomen), as well as mutual grooming and self-grooming by the queens [44,45], which leads to paint removal over time.

Certain beekeeping production processes (e.g., royal jelly production, queen cell production, etc.) require frequent finding of queens. In such beekeeping practices, wing marking is very useful as it significantly facilitates the process of spotting queens. During these frequent colony manipulations, repainting the wings can ensure visibility throughout the active production process. It is certain that color retention also directly depends on the

quality of markers. Further research should be conducted to investigate paint retention concerning the type (quality) of markers.

Abdomen marking—Given the size of the queen's abdomen, abdomen marking will greatly contribute to easier queen finding. The queen is the member of the colony with the longest abdomen, and it is worth noting its significant width, where the width of the first abdominal tergite in queens averages 4.8 ± 0.21 mm [46]. Compared to wings, marking the abdominal part is significantly more durable, probably due to its lower mobility compared to the wings and the higher density of hair, which contributes to color retention [40]. Color retention on the abdomen, even after a longer period (5 months), gives excellent results (values reaching up to 35.25%). The thickness of the dorsal abdominal membranes is twice that of the ventral side, with the maximum thickness of the exoskeletal cuticle of the first tergite being 26.8 ± 2.1 μm [47,48]. Given the strength and thickness of the exoskeleton, surface application of paint on the dorsal thick cuticle of the abdomen cannot endanger the queen's functioning.

Overall, the presented technique for marking queen bees has advantages over other available techniques. This method is simple, widely applicable, and can be carried out by a beekeeper who does not have specialized experience. Other marking methods require more complex handling of queens and using specialized tags, sensors, and glues. On the other hand, the use of fluorescent powder, rare elements, genetic marking, and other techniques requires professional experience. In addition to the above, marking the queen bee with numbers and tags involves the use of glue, which can lead to excessive glue spillage, potentially causing cause damage to the queens or loss of the tag after some time. In terms of price, usage of oil-based markers is one of the most affordable methods of queen marking, which is also an important aspect of modern beekeeping.

5. Conclusions

So far, no ideal queen-marking method has been found. The results of this study indicate that marking larger parts of the queen is a simple technique that can be helpful in modern beekeeping because it can significantly increase queens' visibility in a non-invasive way.

Five months after queen marking, our results show the following:

- Thorax marking gives the best results in terms of partial color retention (54.4%).
- Abdomen marking show weaker partial color retention (14.4%), but, on the other hand, it gives the best results in terms of total coloration (5.4%) and contributes the most to the visibility of marked queens due to its size.
- Wing marking contributes to the quick spotting of queens; however, reduced color retention (2.4%) has been observed, requiring the marking process to be repeated during the season.
- The presented marking technique did not negatively affect queen acceptance, queen supersedure, or the survival of marked queens.

Marking queens by painting their wings and abdomens represents a better solution compared to the traditional thorax marking, as it ensures better visibility. This technique is simple and has no negative effects on the queens' functioning or their regular activities.

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