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Editorial

Preface

With the publication of Volume 3, Issue 1, APIS has completed its first four issues as an international gold open-access journal dedicated to honeybee-related sciences.

During these issues, the journal published contributions from **68 authors affiliated with institutions in 19 countries**, demonstrating a steadily expanding international presence. Authors represented countries from **Europe, Asia, Africa, North America, and South America**, reflecting the journal's commitment to fostering global scientific dialogue across disciplines related to honeybee biology, bee health, pollination, apitherapy, and the history of apicultural sciences.

The broad geographic distribution of authors illustrates the growing visibility of APIS within the international scientific community. Particularly encouraging is the participation of researchers from both established scientific centres and emerging regions of apicultural and apitherapeutic research, creating opportunities for knowledge exchange across diverse cultural and scientific traditions.

The Editorial Board expresses its gratitude to all authors, reviewers, and readers who have contributed to the development of the journal. We hope that APIS will continue to serve as an accessible platform for high-quality scientific communication and international collaboration in the years ahead.

The Editorial Board would also like to inform readers of an important development concerning the journal's publishing model.

During the publication of Volumes 1 and 2, APIS operated without requesting or receiving publication fees from authors. To improve transparency and align the journal's terminology with widely recognised international publishing practices, the Editorial Board has revised the formal description of the journal's publication model.

Beginning with Volume 3, Issue 1, APIS is published as a Gold Open Access journal, and publication-related charges, where applicable, are described using the internationally recognised term Article Processing Charge (APC).

This change reflects a clarification of the journal's publishing framework and terminology and does not affect its commitment to accessible scientific communication, editorial independence, or the dissemination of high-quality research.

We look forward to continuing the development of APIS as an international forum for bee-related sciences and apitherapy and welcome future contributions from researchers around the world.

Editor-in-Chief

Dr. János Körmendy-Rácz

APIS

Original Research

Dietary supplementation with a molybdenum-based complex is associated with higher emergence weight in Apis mellifera queens under field conditions

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ABSTRACT

The quality of the queen is a key factor in the performance of honeybee colonies. Yet the role of trace-element supplementation in queen rearing remains insufficiently documented. This study investigated whether dietary supplementation with the molybdenum-based complex abbreviated Na-Mo_2O_4-EDTA affected queen-rearing traits in Apis mellifera under field conditions. Experiments were conducted in spring 2024 in two apiaries in western France. Supplemented colonies received sugar syrup containing Na-Mo_2O_4-EDTA, whereas control colonies received the same syrup without supplementation. Queen emergence weight was assessed at both sites, and additional queen-rearing traits were measured at one site.

Three replicates were conducted at both sites, although one replicate at site 1 was excluded because of high mortality. After accounting for colony identity, site, and week effects using mixed-effects models, dietary supplementation with Na-Mo_2O_4-EDTA was significantly associated with increased queen emergence weight. In contrast, no consistent treatment effect was detected for queen cell length, royal jelly production, or morphometric traits. These results provide preliminary evidence that molybdenum supplementation may influence queen developmental outcomes under field conditions and support further investigation of trace-element supplementation in honey bee queen rearing.

Keywords: Molybdenum, Honey bees, Queen rearing, Nutrition

1. INTRODUCTION

Honey bee colonies are exposed to multiple stressors, including pathogens, parasites, pesticides, environmental change, habitat alteration, and nutritional shortages, all of which can impair colony health and survival (Goulson et al., 2015; Vanbergen, 2014; Tsuruda et al., 2021; Lin et al., 2024; Steinhauer et al., 2018). In eusocial honey bees (*Apis mellifera*), caste differentiation depends strongly on larval rearing conditions, with nutrition playing a central role in queen development (Kamakura, 2011). Queen quality is a multifactorial concept that includes reproductive capacity, longevity, immune status, nutritional status, and interactions with workers (Margarita, 2020), and colony performance is closely linked to queen condition (Copeland et al., 2024; Holmes et al., 2023; Nelson & Gary, 1983; Rangel et al., 2013; Yu et al. 2023). In particular, larval diet, including the quantity and composition of royal jelly, is known to influence queen development (Slater et al., 2020; Pirk, 2018; Taha et al., 2025).

In this context, nutrition is an important determinant of colony resilience and queen production in apiculture (Lau et al., 2023; Tsuruda et al., 2021; Vaudo et al., 2015; De Souza et al., 2019; Fèvre, 2024). Honey bee nutritional requirements remain complex and incompletely understood, as they depend on a balance between macro- and micronutrients (Bonoan et al., 2018; Bonoan et al., 2017; Lau et al., 2023). Honey bees obtain essential nutrients from nectar and pollen, but the availability and diversity of these resources vary with season, weather, and floral environment (Vaudo et al., 2015; Tsuruda et al., 2021; Topal et al., 2022).

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To compensate for nutritional limitations, beekeepers commonly use supplemental feeding, most often in the form of sugar syrups or fondants, which can also serve as carriers for additional nutrients such as vitamins, proteins, sterols, or microbial supplements (Tsuruda et al., 2021; Jovanovic et al., 2021; Ricigliano et al., 2022; García-Vicente et al., 2023; García-Vicente et al., 2024; Pavlović et al., 2025; Bogaert et al., 2025).

By contrast, the role of minerals, and especially trace elements, in honey bee nutrition remains comparatively less documented, despite their essential physiological functions (Herbert, 1979; Zhang et al., 2015; Bonoan et al., 2018). Recent work has highlighted the potential interest of mineral supplementation for worker physiology, including hypopharyngeal gland development under laboratory conditions (Ghasemi et al., 2025). Among trace elements, molybdenum is of particular interest because it serves as a cofactor for several oxidoreductases involved in intermediary metabolism and redox regulation (Schwarz et al., 2009; Dow, 2017). Honey bees naturally contain molybdenum at low levels in the 0.2 to 0.5 ppm range (Benner et al., 2025; Fuior et al., 2025). In particular, Fuior et al. (2025) evidenced that Mo is found in the cuticle and in all tagma of the honey bees, especially in the head (brain, hypopharyngeal glands) and in the abdomen, but its role in honey bee health, its impact in honey bee nutrition and the benefits of molybdenum supplementation in apiculture and notably in queen rearing remain little or not explored

Previous studies have shown that supplementing bees with the complex $Na_2[Mo_2O_4(EDTA)]$ (denoted hereafter Na-Mo_2O_4-EDTA), both in the laboratory and under field conditions, leads to a significant increase in molybdenum levels in honeybees, associated with beneficial effects at the colony level, notably higher honey production and reduced winter mortality under specific experimental conditions (Fuior et al., 2025; Benito-Murcia et al., 2025). To our knowledge, the question of whether such supplementation can also influence characteristics related to queen rearing has not yet been investigated under real field conditions, and this aspect could provide further insights into the mode of action of this molecule.

The aim of the present study was therefore to assess whether dietary supplementation with Na-Mo_2O_4-EDTA, delivered in a commercial sugar syrup, was associated with variation in queen-rearing traits in Apis mellifera under field conditions in two apiaries in western France. We examined royal jelly production, queen cell length, queen emergence weight, and selected morphometric traits. Because queen emergence weight is commonly used as an informative indicator of their developmental conditions and has been associated with their later reproductive performances (Hatjina et al., 2014; Amiri et al., 2017; Kahya et al., 2008), this parameter was considered alongside the other measured traits, without being treated as a comprehensive proxy for overall queen quality.

2. MATERIALS AND METHODS

2.1. Study sites

The study took place in two independent apiaries in two neighboring departments in the Nouvelle-Aquitaine region, in the West part of France, during the spring 2024. The first site was located in Lagord (lat: 46.208, long: -1.034, Charente-Maritime, France) and was used for experiments on royal jelly characteristics, queen cells sizes, morphological characteristics (queen head width, thorax width, wings length) and queen weights at emergence. The second site was located in Saint-Laurent-de-la-Salle (lat: 46.593, long: -0.938, Vendée, France) and was used for experiments on royal jelly characteristics and queen weights at emergence.

2.2. Feeding syrups composition

The control diet was composed of plain sugar syrup ('Apistar', ICKO Apiculture®). This syrup was composed of 34% of sucrose, 33% of fructose and 33% of glucose. For the supplemented syrup, the molybdenum-based complex Na-Mo_2O_4-EDTA, was diluted in the syrup at a concentration of 4 mg/L (1.15 mgMo/L, 1.7 mgMo/kg with a density of the syrup equal to 1.46). The concentration of Mo was checked by ICP-MS method by laboratory LEAV, La Roche sur Yon (France). The complex was synthesized as previously described (Fuior et al 2022, 2025). Two liters of Na-Mo_2O_4-EDTA provided 8 mg of the complex per colony.

2.3. Experimental design and measurements

At site 1 (Lagord), the experiment followed a 12-day queen-rearing protocol. Three colonies received either syrup supplemented with Na-Mo_2O_4-EDTA while 3 colonies received the corresponding control syrup. Colonies were randomly assigned to the control or supplemented feeding condition. All grafted larvae originated from a single selected donor colony. For each feeding condition, three breeder colonies were prepared at the start of each replicate. The protocol was repeated three times, starting on 10, 17, and 24 June 2024, respectively, using the same breeder colonies across weeks. The third replicate at site 1 was excluded from analysis because high mortality in both groups resulted in an insufficient number of viable queens for meaningful comparison. The cause of this mortality could not be established.

Three days before grafting (D−3), colonies were pre-fed with 2 L of syrup. On day 0 (D0), each breeder colony received 30 grafted larvae, resulting in 90 queen cells per feeding condition, and colonies were then fed again with 2 L of the corresponding syrup. At D+3.5, approximately 10 queen cells per hive and per feeding condition were sampled to quantify royal jelly production per cell, and a small amount of royal jelly was retained for 10-HDA analysis. At D+7, queen cell length was measured on the remaining cells using a caliper, after which the cells were placed in an incubator. At D+12, newly emerged queens were weighed using a precision balance (1 mg resolution), and morphometric traits (head width, thorax width, and wing length) were measured from scaled photographs using ImageJ. Morpho-

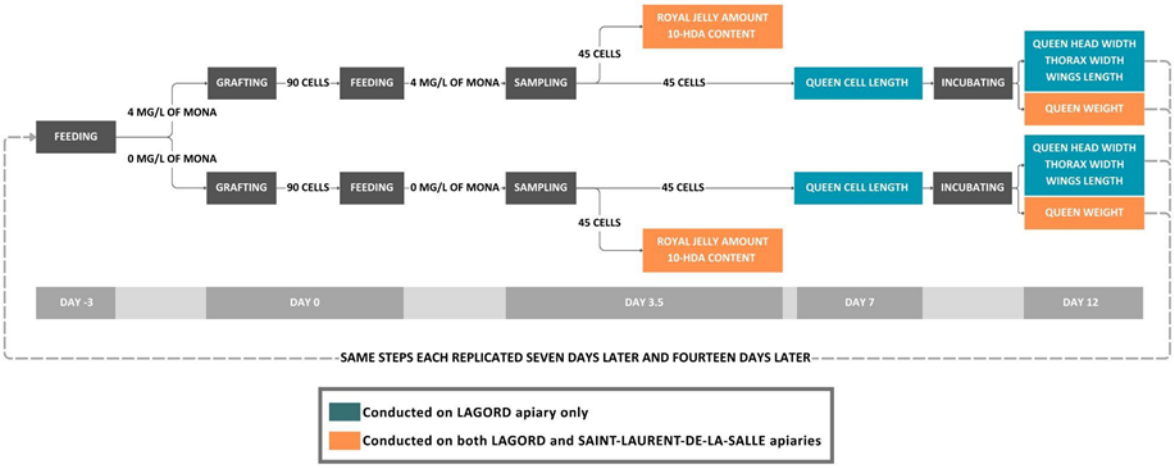

Figure 1: Experimental protocol scheme. Days are colored in gray above and below the principal scheme. Actions in the process are colored in black. Measurement types are colored in orange if conducted on both Lagord and Saint-Laurent-de-la-Salle apiaries and in blue if conducted only on Lagord apiary.

metric measurements were performed by a single operator blinded to feeding conditions.

At site 2 (Saint-Laurent-de-la-Salle), the same protocol was applied with 6 colonies, randomly divided into two groups of 3 for each modality, to assess royal jelly production and queen emergence weight. The experiment was conducted in three replicates starting on 14, 21, and 28 June 2024. All raw data are provided in Tables S1–S4 in the Supporting Information.

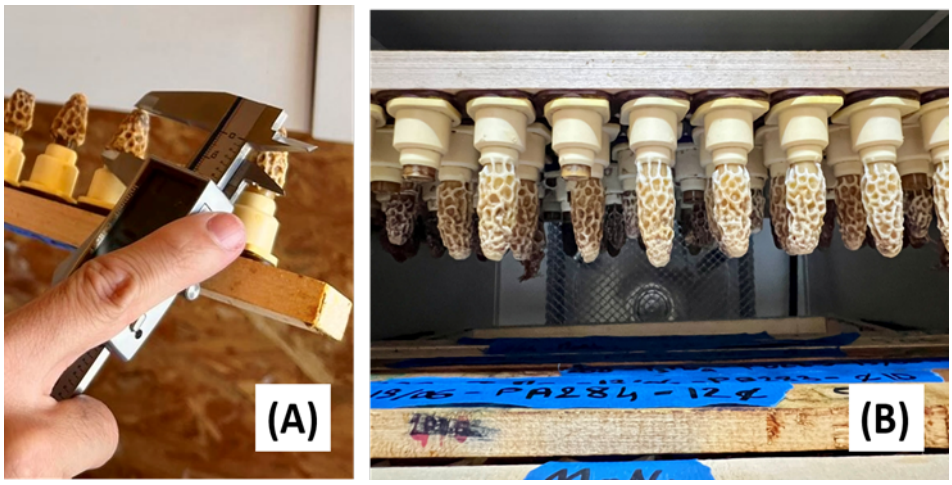

Figure 2. (A) Queen cells for the measurements of the royal cell length; (B) Queen cells in incubation.

2.4. Analyses of the royal jelly

As an exploratory analysis, the 10-HDA content of royal jelly was assessed because this compound is a characteristic bioactive component of royal jelly (Genç & Aslan, 1999; Wang et al., 2016; Howe et al., 1985). To obtain sufficient material for HPLC analysis, royal jelly samples were pooled by feeding condition, following the analytical approach described by Kim and Lee (2010). One pooled sample per feeding condition was analysed by Intertek (Germany). Because no replicated analytical measurements were available at the treatment level, 10-HDA results were considered descriptive only and were not subjected to statistical comparison.

2.5. Statistical analysis

Because the dietary treatment was applied at the colony level, colonies were considered the primary experimental units. To account for the hierarchical structure of the dataset and avoid pseudo-replication, the effects of treatment were analyzed using mixed-effects models.

For queen emergence weight, the following model was fitted:

Weight ~ Treatment + Site + Week + (1|Colony)

where Treatment, Site and Week were included as fixed effects and Colony identity as a random effect (to account for repeated measurements performed on the same breeder colonies across experimental weeks).

For queen cell length, a mixed-effects model including Treatment and Week as fixed effects and Colony as a random

effect was fitted.

Morphometric traits (head width, thorax width and wing length) were analysed using similar mixed-effects models with Treatment and Week as fixed effects and Colony as a random effect.

Royal jelly production was considered exploratory because of the limited number of independent observations available at the colony level. Therefore, no inferential statistical analysis was performed and results are presented descriptively.

All analyses were performed in R (R Core Team, 2024) using mixed-effects modelling approaches (Mixed-effects models were fitted using the lme4 package and p-values were obtained using lmerTest).

3. RESULTS

At site 1, results are reported for the first two experimental weeks only, as the third replicate was excluded because of high mortality and an insufficient number of viable queens for analysis (n = 6 or less).

3.1. Royal jelly production

Royal jelly production varied among weeks and sites, but no consistent pattern associated with dietary supplementation was observed (Figure 3).

Given the limited number of independent colony-level observations available for this parameter, royal jelly production was considered exploratory and no inferential statistical analyses were performed. Consequently, the results are presented descriptively only.

The pooled royal jelly sample obtained from supplemented colonies showed a slightly higher 10-HDA content (3.80%) than the pooled control sample (3.59%). Because these measurements were performed on pooled samples

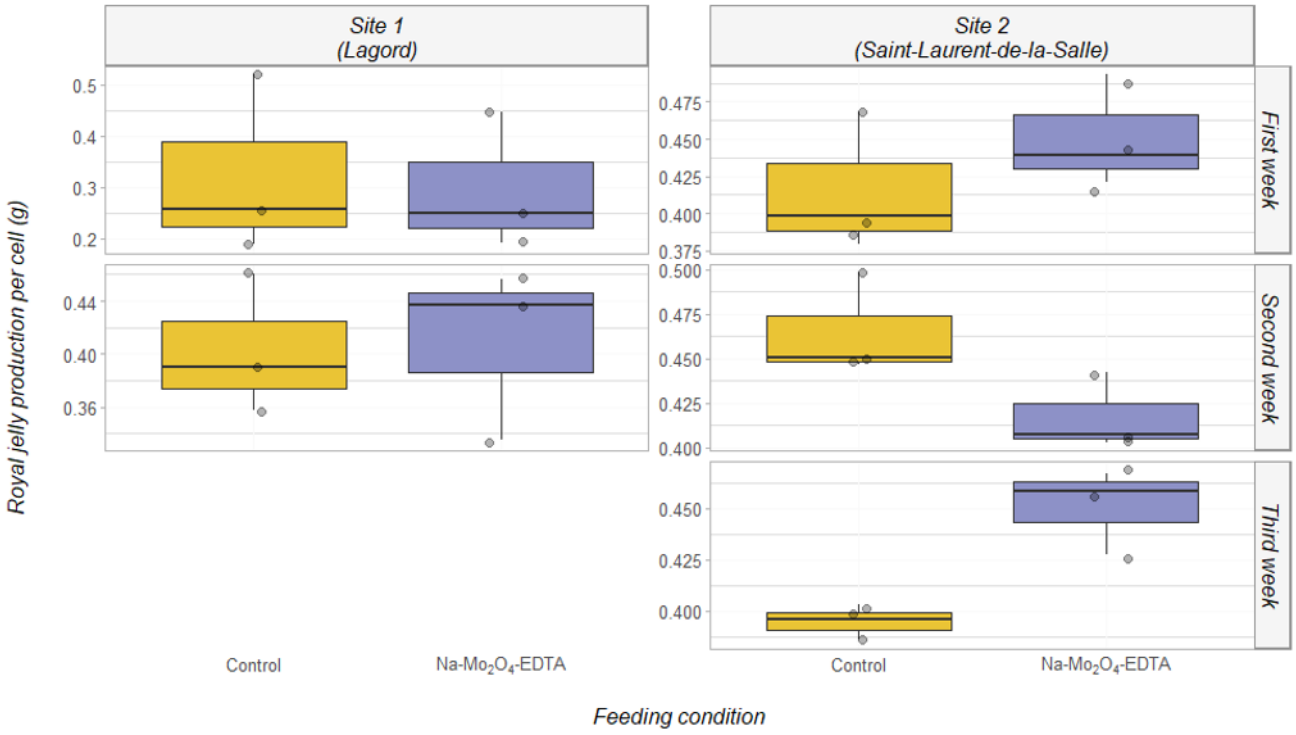

Figure 3. Royal jelly production per cell (g) according to feeding condition ("Na-Mo_2O_4-EDTA" or "Control"), study site, and experimental week. Boxplots show the median, first and third quartiles, and whiskers extending to 1.5 × the interquartile range. Individual points represent colony-level observations. Given the limited number of independent observations available, these data are presented descriptively and were not subjected to inferential statistical testing.

without analytical replication, they should be considered descriptive only.

3.2. Queen cell length

At site 1, Figure 4 shows queen cell length according to feeding condition, with raw data provided in Table S2 (SI).

The mixed-effects model revealed a significant effect of week on queen cell length (p = 0.0009), whereas no significant effect of dietary supplementation was detected (p = 0.405).

Although queens reared in supplemented colonies tended to develop in slightly longer queen cells during some experimental replicates, this pattern was not consistent enough to support an overall treatment effect after accounting

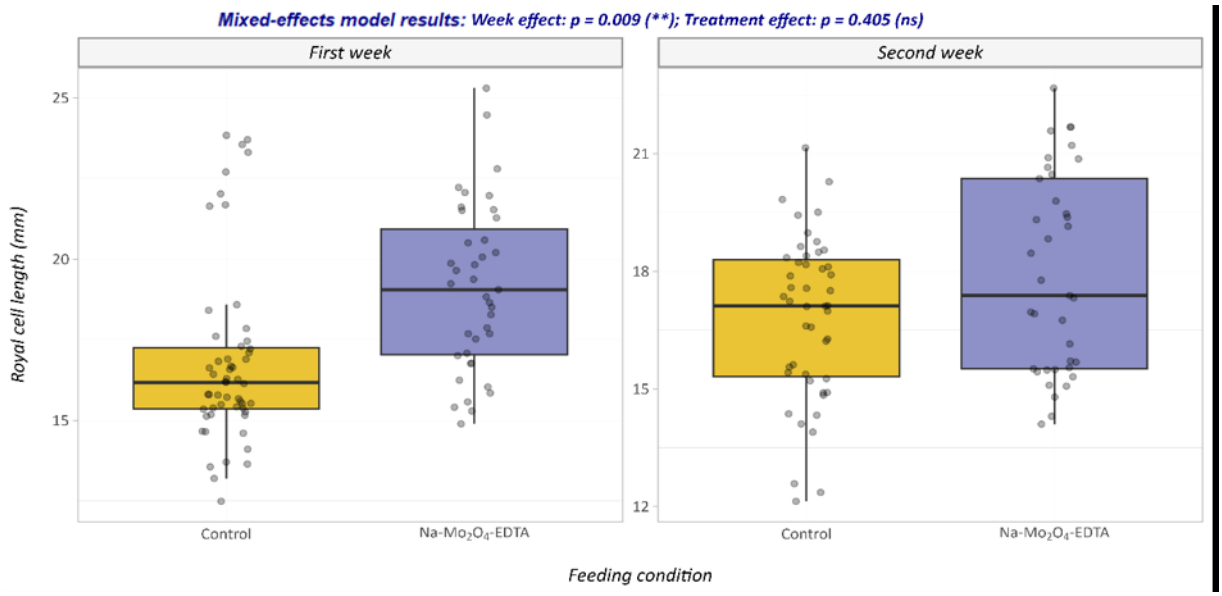

Figure 4: Queen cell length (mm) according to feeding condition ("Na-Mo_2O_4-EDTA" or "Control") and experimental week. Boxplots show the median, first and third quartiles, and whiskers extending to 1.5 × the interquartile range. Individual points represent measured queen cells. Results from the mixed-effects model are shown above the figure (Week effect: p = 0.0009; Treatment effect: p = 0.405).

for colony-level variability.

3.3. Queen weight at emergence

The mixed-effects analysis revealed a significant effect of dietary supplementation on queen emergence weight (Treatment effect: p = 0.009; Figure 5). Queens originating from colonies receiving Na-Mo_2O_4-EDTA supplementation were consistently heavier than queens originating from control colonies across most experimental replicates. A strong site effect was also detected (p = 0.0001), indicating substantial differences between the two apiaries. In contrast, the effect of experimental week was not significant (p = 0.172). These results indicate that the positive association between Na-Mo_2O_4-EDTA supplementation and queen emergence weight remained significant after accounting

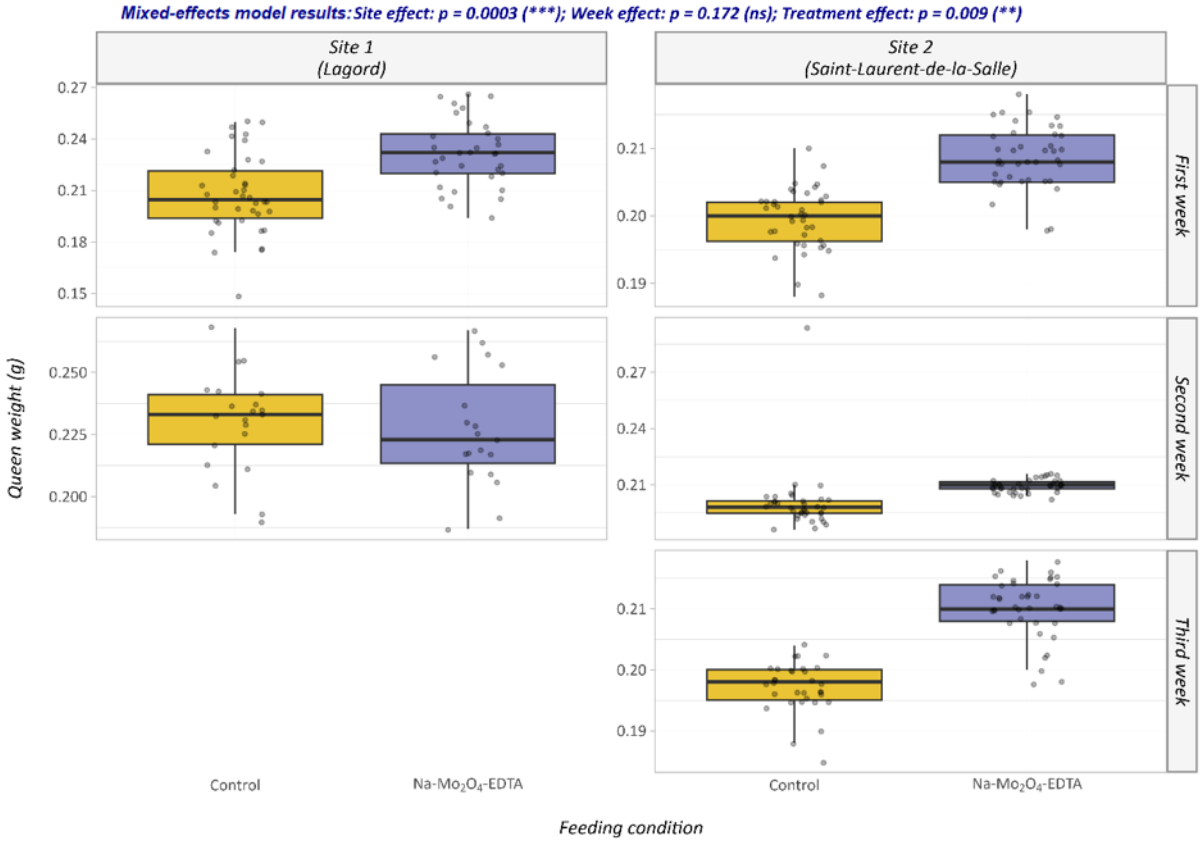

Figure 5: Queen emergence weight (g) according to feeding condition ("Na-Mo_2O_4-EDTA" or "Control"), study site, and experimental week. Boxplots show the median, first and third quartiles, and whiskers extending to 1.5 × the interquartile range. Individual points represent emerging queens. Results from the mixed-effects model are shown above the figure (Site effect: p = 0.0003; Week effect: p = 0.172; Treatment effect: p = 0.009).

for colony-level variability and the hierarchical structure of the experiment.

3.4. Morphometric parameters

Mixed-effects analyses did not detect any significant effect of dietary supplementation on head width ($p = 0.333$), thorax width ($p = 0.249$) or wing length ($p = 0.789$) (Figure 6).

A significant week effect was detected only for thorax width ($p < 0.0001$), whereas no significant week effect was observed for head width ($p = 0.497$) or wing length ($p = 0.176$).

Overall, the morphometric data do not support a consistent effect of Na-Mo2O4-EDTA supplementation on structural queen morphology.

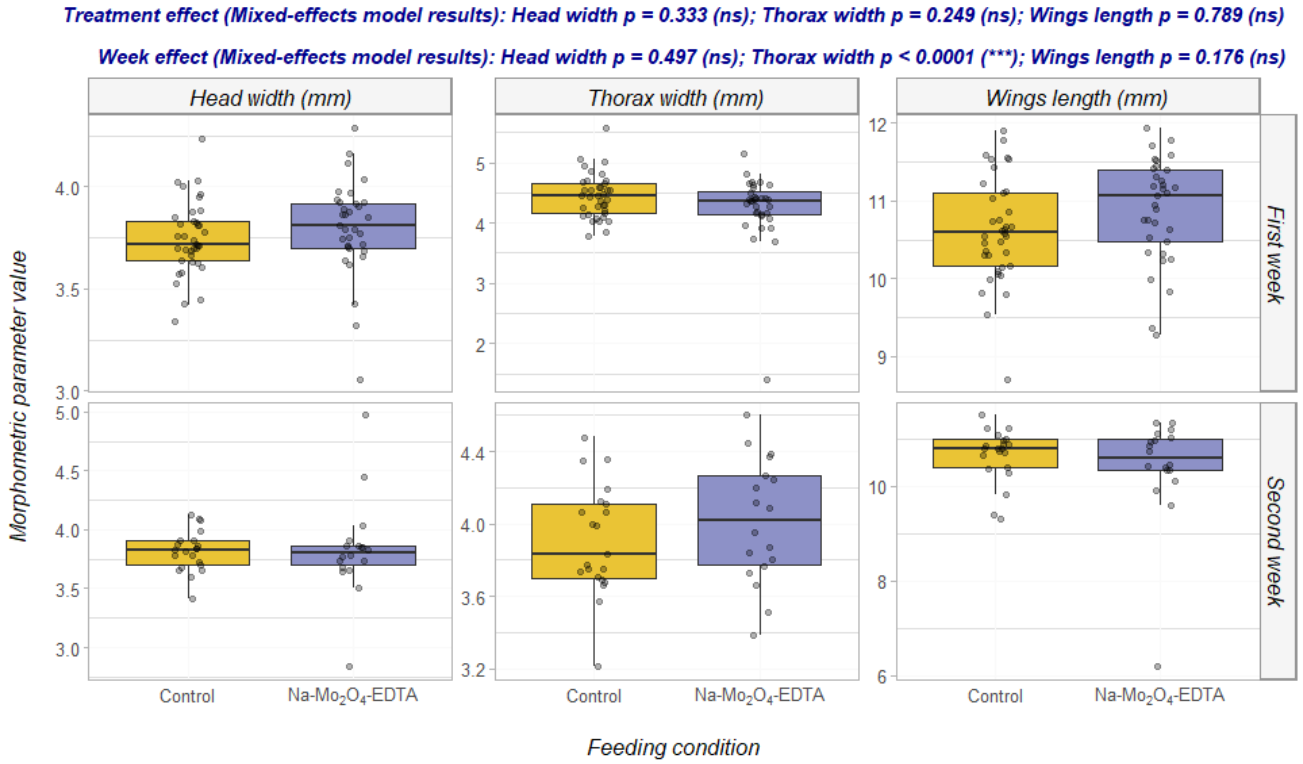

Figure 6: Head width (mm), thorax width (mm), and wing length (mm) of queens according to feeding condition ("Na-Mo_2O_4-EDTA" or "Control") and experimental week. Boxplots show the median, first and third quartiles, and whiskers extending to 1.5 × the interquartile range. Individual points represent measured queens. Results from the mixed-effects models are shown above each panel. No significant treatment effect was detected for head width ($p = 0.333$), thorax width ($p = 0.249$), or wing length ($p = 0.789$). A significant week effect was detected only for thorax width ($p < 0.0001$).

4. DISCUSSION

The present study investigated whether dietary supplementation of honey bee colonies with the molybdenum-based complex Na-Mo_2O_4-EDTA was associated with variation in queen-rearing traits under field conditions. After accounting for the hierarchical structure of the experiment through mixed-effects modelling, the principal finding was that queens originating from supplemented colonies exhibited a significantly higher emergence weight than queens originating from control colonies. In contrast, no overall treatment effect was detected for queen cell length, royal jelly production, or the morphometric traits measured.

The increase in queen emergence weight is of particular interest because emergence weight is commonly considered an informative indicator of queen developmental conditions and has been associated with subsequent reproductive performance, sperm storage capacity, and longevity (Amiri et al., 2017; Hatjina et al., 2014; Kahya et al., 2008). Under the conditions tested here, queens produced by colonies receiving Na-Mo_2O_4-EDTA supplementation were consistently heavier across sites and experimental replicates. Importantly, this association remained significant after accounting for colony identity, site effects, and temporal variation, suggesting that the observed difference was not solely attributable to colony-specific characteristics or environmental heterogeneity. Nevertheless, emergence weight alone cannot be considered a comprehensive measure of queen quality, and the present results should not be interpreted as evidence that all aspects of queen performance are improved by supplementation.

A major contribution of the present work is the use of a statistical framework that explicitly accounts for the experimental design. Because supplementation was applied at the colony level rather than at the level of individual queens, colonies represent the true experimental units. The mixed-effects models used in the analyses account for this structure and reduce the risk of pseudo-replication. Using this model, the treatment effect on queen emergence weight appears significant. This outcome strengthens confidence that the observed pattern reflects a genuine biological signal rather than an artefact arising from non-independent observations.

The mechanisms through which molybdenum supplementation may influence queen development remain unclear. Molybdenum is an essential trace element involved in several oxidoreductase systems and metabolic pathways (Schwarz et al., 2009; Dow, 2017; Marelja et al., 2018). Previous studies have shown that supplementation with Na-Mo_2O_4-EDTA can increase molybdenum concentrations in honey bee tissues and may improve physiological parameters related to oxidative balance and colony performance (Fuior et al., 2025; Benito-Murcia et al., 2025). It is therefore conceivable that enhanced nutritional or physiological status of nurse bees could indirectly affect larval development and queen growth. However, the present study was not designed to investigate physiological mechanisms, and any causal link between molybdenum supplementation, nurse-bee physiology, royal jelly composition, and queen development remains hypothetical.

In contrast to queen emergence weight, no overall treatment effect was detected for queen cell length. Although some individual experimental replicates suggested longer queen cells in supplemented colonies, this pattern was not consistent across the study. Queen cell size has previously been associated with developmental and morphometric characteristics of queens (Mattiello et al., 2022; Wu et al., 2018), but the present results do not provide evidence that Na-Mo_2O_4-EDTA supplementation systematically influences this parameter. The significant week effect observed for queen cell length indicates that temporal factors may have contributed more strongly to variation than dietary supplementation.

Similarly, no significant treatment effect was detected for head width, thorax width, or wing length. Morphometric traits are known to exhibit substantial biological variability and may be sensitive to measurement error despite careful standardization (Fox et al., 2020; Fruciano, 2016). The absence of detectable differences in these parameters suggests that any influence of supplementation on structural morphology is either limited or smaller than the effect observed for emergence weight. Additional studies involving larger numbers of colonies and complementary measurements of reproductive anatomy would be required to determine whether molybdenum supplementation influences other dimensions of queen quality.

Royal jelly production and 10-HDA content were evaluated as exploratory variables. No consistent pattern associated with supplementation was observed for royal jelly production across sites and weeks. Furthermore, 10-HDA measurements were performed on pooled samples and therefore do not allow statistical inference. Although previous work has suggested that molybdenum supplementation may influence hypopharyngeal gland physiology (Fuior et al., 2025), the present data are insufficient to determine whether changes in royal jelly characteristics contributed to the differences observed in queen emergence weight.

A significant site effect was detected for queen emergence weight, highlighting the importance of environmental context in queen-rearing experiments. Differences in floral resource availability, weather conditions, colony strength, brood dynamics, and overall nutritional status are known to influence queen development and colony performance (Vaudo et al., 2015; Lau et al., 2023; Tsuruda et al., 2021). Such factors likely contributed to the variability observed between apiaries and may partly explain why the magnitude of the treatment effect differed among replicates. Consequently, caution is warranted when extrapolating these findings beyond the specific conditions investigated here.

Overall, the present results provide preliminary field evidence that dietary supplementation with Na-Mo_2O_4-EDTA may positively influence queen emergence weight in honey bees. However, because the study involved a limited number of colonies and was conducted during a single season, further experiments across multiple environmental conditions and years will be necessary to assess the reproducibility of this effect and to determine whether the increase in emergence weight translates into measurable improvements in queen performance, colony productivity, or colony survival.

5. CONCLUSION

Under the conditions tested in two field apiaries, dietary supplementation with the molybdenum-based complex Na-Mo_2O_4-EDTA remained significantly associated with higher queen emergence weight in Apis mellifera, after accounting for colony-level variability whereas effects on royal jelly production, queen cell length, and morphometric traits were limited or inconsistent across sites and experimental weeks. These results suggest that molybdenum supplementation may influence some queen-rearing outcomes under field conditions, but they do not support a general improvement across all measured traits.

The present study therefore provides preliminary field evidence that trace-element supplementation deserves further investigation in honey bee queen rearing. Additional studies are needed to assess the reproducibility of the effect across colonies, seasons, and environmental conditions, to deeply investigate the impact on the quality of royal jelly (notably biochemical or nutritional profiling), and to clarify whether the observed association with queen emergence weight translates into measurable differences in subsequent queen performance and colony development.

AUTHOR CONTRIBUTIONS

Conceptualization, P.C., B.P. and S.F.; Methodology, A.M., P.C., B.H., R.A., B.P. and S.F.; Formal Analysis, P.C.; Writing-Original Draft Preparation, A.M., P.C. and S.F.; Writing-Review and Editing, P.C., A.M., B.P. and S.F. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY

Raw data can be found online as supplementary data from the present publication.

CONFLICT OF INTEREST

SF is linked to a patent about the technology used in this study (European Patent EP4185594B1 delivered on 4th December 2024) and consultant for Oligofeed SAS (as SFConsulting), which cofunded this work and aims to commercialize the complex for the beekeeping industry. The authors declare these interests in the interest of full transparency and affirm that the reported findings are presented objectively and without bias.

FUNDING

We would like to thank SATT Paris-Saclay (Project APIMONA) for funding the test on Site 2, the preparation of syrup for both sites and the analyses of royal jelly. The complementary studies in site 1 were funded by Oligofeed SAS.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

Statement: The authors did not use generative AI technologies for preparation of this work. The author takes full responsibility for the publication's content.

SUPPORTING INFORMATION

Table S1 contains raw data concerning the royal jelly production on sites 1 and 2; Table S2 contains raw data about the cell length measured on site 1; Table S3 contains raw data about queen weight, head width, thorax width and wings length measured on site 1 for weeks 1 and 2; Table S4 contains raw data about queen weight measured on site 2 for weeks 1-3 (Cochard et al., 2026).

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APIS

Original Research

Use of BEE BREAD and Drone Larvae Homogenate in the Treatment of Chronic Bacterial Prostatitis: Results of an Early-Phase Clinical Study

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ABSTRACT

The development of apitherapy began many centuries ago. However, currently, it is still not a widespread method for the treatment of various diseases. To strengthen the evidence base for apitherapy, a series of clinical studies of bee products was organized.

In 2021, at the Kharkiv National Medical University (Ukraine), at the clinical sites of the Department of Urology, Nephrology and Andrology, an early-phase clinical study was conducted on the use of bee products in the complex treatment of chronic bacterial prostatitis.

The study included 60 patients with chronic prostatitis who had previously used standard treatment methods. In the complex treatment of these patients, the following combination of bee products was used: 1) lyophilized drone larvae homogenate, 2) suppositories with drone homogenate, 3) bee bread (perga). An important objective of the study was to investigate the tolerability and safety of these bee products, as well as to assess the potential for further in-depth research on this topic.

As a result of the complex treatment, high and moderate effectiveness was achieved for 93.3% of patients, and low - only for 6.7%. A significant improvement in clinical and laboratory parameters was achieved: 1) pain on palpation disappeared, 2) the size of the prostate gland decreased and the heterogeneity of its structure disappeared, 3) the number of leukocytes decreased, 4) the number of erythrocytes decreased, 5) testosterone levels increased, etc.

It is important to note that this study was conducted as an early-phase study using a «patient-as-own-control» methodology, which was reflected in both the study design and the interpretation of its results.

An early-phase clinical study of the use of homogenate of drone larvae and bee bread for patients with chronic bacterial prostatitis showed their high effectiveness and tolerability. Therefore, these natural and safe products are very promising for further research and practical application for the prevention and treatment of various diseases.

Keywords: apitherapy, prostatitis, drone homogenate, apilarnil, bee bread.

INTRODUCTION

Prostatitis is currently one of the most common urological diseases in men. This condition significantly impairs men's quality of life, so the task of improving the effectiveness of its prevention and treatment is highly relevant. At the same time, the use of apitherapy in solving men's health problems has a long and successful history.

Unfortunately, this successful experience has not been sufficiently researched and described, which significantly complicates its study, dissemination and practical implementation. In order to strengthen the evidence base for the effectiveness of apitherapy and to encourage the use of natural and safe methods of improving men's health, an early-phase clinical study was initiated and conducted.

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This study was conducted in 2021 at the Department of Urology, Nephrology, and Andrology at Kharkiv National Medical University (Kharkiv National Medical University, 2022). The study focused on the efficacy and safety of bee products in combination therapy for chronic bacterial prostatitis.

MATERIALS AND METHODS

To conduct this study, two bee products were used: bee bread (perga) and drone larvae homogenate.

Bee bread (perga) is a product made from bee pollen processed by bees and stored in honeycomb cells (State Standard of Ukraine, 2010). Bee bread is a mixture of pollen granules, honey, and lactic acid bacteria (Ministry of Agriculture, Agri-food and Food Sovereignty, n.d.). Active production and distribution of bee bread on the European continent began about 20 years ago. Therefore, in many countries, this bee product may currently be completely unavailable.

However, bee bread is already authorized as an ingredient for dietary supplements in the European Union. And in Ukraine, for example, where the production and use of bee products are widespread and objectively ahead of those of many other countries, a national standard for these bee products has been in effect since 2009 (State Standard of Ukraine, 2010).

There are numerous scientific studies confirming the high antimicrobial potential of bee bread, for example (Pełka et al., 2021). This is why this bee product was included in this clinical study.

The second and most important bee product used in the study is drone larvae homogenate. This product is sometimes called drone milk or Apilarnil. The name Apilarnil was introduced at the end of the last century by Romanian researcher Nicolae Iliesiu. To avoid conflicts of interest, we do not use this name and instead characterize the product as drone larvae homogenate, basing our definition on the national standard adopted in Ukraine in 2013. Drone larvae homogenate is obtained by grinding 7-day-old drone larvae (State Standard of Ukraine, 2013).

Scientists from around the world have actively studied this bee product for several decades. Drone larvae homogenate contains proteins, amino acids, vitamins, minerals, fatty acids, and hormone-like substances and has been traditionally used in apitherapy because of its biological activity (Bogdanov, 2017; Isidorov et al., 2019). The unique composition of drone larvae homogenate determined the choice of this bee product for this clinical study.

Generally, the study included 60 patients aged 28 to 55 years with chronic prostatitis, defined as symptoms persisting for more than three months (Ministry of Health of Ukraine, 2017). These patients had previously used standard treatments, but their effectiveness was quite low. Therefore, the decision was made to use a combination therapy using bee products.

A «patient-as-own-control» methodology was used in the design of this early-phase study and in the interpretation of its results.

In the complex treatment of these patients, the following combination of bee products was used: 1) lyophilized drone larvae homogenate, 1 capsule 3 times a day (per os), 2) suppositories with drone homogenate and wheat germ oil 1 time a day (per rectum), 3) bee bread (perga), 5-7 granules per day 30 minutes before meal (per os). Patients took the complex of bee products for 30 days.

All bee products for the clinical study were produced and provided by MEDOK Company LLC (Ukraine). The product “Drones larvae homogenate lyophilized” was patented in Ukraine in 2015. One capsule of this product includes 250 mg lyophilized drones larvae homogenate. Suppositories containing drone homogenate and wheat germ oil were patented in Ukraine in 2018. Supporitories with drone larvael homogenate were used as an auxiliary/supportive remedy, patients received the main part of the drone homogenate in capsules.

All patients also received the standard basic therapy, including antibiotics, immunomodulators, enzymes, and anti-inflammatory products, as well as physiotherapy procedures.

A complete clinical and laboratory examination was performed pre- and post-treatment, including the following: recording of medical history, evaluation of symptoms and previous treatment; digital rectal examination; prostate and bladder ultrasound examination with determination of residual urine volume (Toshiba Aplio 500 apparatus); laboratory tests: complete blood count, urinalysis, blood biochemistry, sex hormone test, microscopy of prostatic secretion; bacterioscopic examination with Gram, Romanowsky–Giemsa, and methylene blue staining (Olimpus BH2 microscope); bacteriological examination of the prostatic secretion with pathogen identification by the morphological, tinctorial, and culture-based methods (by J. Holt at al., 1997).

The colony-forming unit (CFU) counts were determined by the serial dilution method followed by the inoculation of media. The sensitivity of the isolated cultures to the antimicrobial medicinal products was determined by the Bauer–Kirby disk diffusion method using the commercial disks according to Order of the Ministry of Health of Ukraine No. 167.

Hormone tests. The testosterone, luteinizing hormone (LH), prolactin, and estradiol serum levels were determined by enzyme-linked immunosorbent assay (ELISA) using CIS Bio International (France) test kits.

Evaluation of clinical symptoms and quality of life. The International Prostate Symptom Score (IPSS) with quality of life index (QoL) was used to evaluate the severity of the lower urinary tract symptoms. The questionnaire was completed pre- and post-treatment.

Evaluation of efficacy and safety. The therapy efficacy was evaluated using the Composite Clinical Score Scale (Table 1) involving the changes of symptoms, laboratory tests, and investigation results over time.

The side effects were classified using the five-score scale: 5 scores — no side effects; 4 scores — mild, not requiring product discontinuation; 3 scores — moderate, affecting the patient's condition but not requiring discontinuation; 2 scores — marked, requiring therapy discontinuation; 1 score — severe, requiring discontinuation of product and medical intervention.

High efficacy	CP index decreased by ≥ 14 scores by NIH-CPSI questionnaire, 1999. Statistically significant reduction of WBC count in prostatic secretion. Decrease in tenderness and density of the prostate gland according to digital rectal examination findings.
Moderate efficacy	Two of the above conditions are met
Low efficacy	One of the above conditions is met
Lack of efficacy	None of the above conditions is met

Table 1: Evaluation of efficacy

RESULTS

This early-phase clinical study concluded with a set of important results. The main ones will be described below.

Changes in Symptom Severity and Quality of Life

As a result of the treatment received, the patient-reported IPSS total score was decreased by 93.9%, quality of life was improved by 29.3% and performance status from severe to mild by total score (S+L) (Table 2).

Measure	Pre-treatment	Post-treatment
IPSS	24.5 ± 0.3	2.3 ± 0.1
L	4.1 ± 0.1	1.2 ± 0.1
S+L	28.6 ± 0.3	3.5 ± 0.1

Table 2: Results of symptom evaluation during the treatment

IPSS – International Prostate Symptom Score (0–35 points), a validated questionnaire used to assess the severity of lower urinary tract symptoms.

L – Quality of Life Index (0–6 points), assessed by the patient.

S + L – Composite score calculated as the sum of the IPSS symptom score and the Quality of Life Index. Overall condition was classified as mild (≤7 points), moderate (8–19 points), severe (20–35 points), or very severe (>35 points).

Digital Rectal Examination Findings

During the pre-treatment digital rectal examination, the prostate tenderness was observed in all patients and swelling - in 36 (60%); post-treatment, the tenderness was observed only in 8 (13.3%) patients. In addition, the well-defined prostate contours were detected pre-treatment only in 44 (73.3%) patients, and post-treatment in all patients (Table 3).

Findings of prostate digital rectal examination	Pre-treatment, n (%)	Post-treatment, n (%)
Tenderness on palpation	60 (100)	8 (13.3)
Well-defined contours	44 (73.3)	60 (100)
Swelling	36 (60)	0

Table 3: Findings of clinical examination of patients with chronic prostatitis

Laboratory and Hormonal Findings

The laboratory tests of patients did not show any significant differences in the complete blood count, blood biochemistry, and urinalysis results (Table 4). At the same time, the pre- and post-treatment prostate secretion test demonstrated significant differences ($p < 0.001$). Thus, the pre-treatment WBC count per field of view (for treatment in general) was 21.8 ± 23.2, and post-treatment it was 7.3 ± 3.2, i.e. it was decreased by 66.5%; the pre-treatment RBC count was 0.4 ± 0.6, and post-treatment it was 0.1 ± 0.2, i.e. it was decreased by 75%; the pre-treatment lecithin count was 112.6 ± 71.6, and post-treatment it was 164.3 ± 79.6, i.e. it was increased by 68.5%.

The significant changes ($p < 0.001$) were also demonstrated in the hormone blood tests. The testosterone level was increased from 6.4 ± 2.4 to 8.2 ± 3.1 ng/ml, i.e. by 22%, and the estradiol level, on the contrary, was decreased by 6.7% — from 28.3 ± 9.3 to 26.4 ± 7.6 pg/ml.

Laboratory test results	Pre-treatment	Post-treatment
Complete blood count:		
Hemoglobin, g/l	150.6 ± 16.9	151.4 ± 15.7
WBC, 109/l	6.3 ± 1.7	6.1 ± 1.2
Lymphocytes, %	29.9 ± 8.7	29.4 ± 8.9
Monocytes, %	6.9 ± 2.2	6.7 ± 2.3
ESR, mm/g	6.3 ± 5.9	5.7 ± 4.8
Blood biochemistry		
Glucose, mmol/l	4.4 ± 0.6	4.3 ± 0.7
Total protein, g/l	73.9 ± 5.1	74.2 ± 5.3
Urea, mmol/l	5.6 ± 1.4	5.6 ± 1.3
Total bilirubin, μmol/l	13.2 ± 3.4	13.3 ± 3.5
Direct bilirubin, μmol/l	3.6 ± 0.9	3.7 ± 0.8
ASAT, mmol/g·l	29.0 ± 8.4	28.7 ± 7.9
ALAT, mmol/g·l	29.1 ± 13.8	28.9 ± 12.4
Urinalysis		
Specific gravity	1016.3 ± 5.6	1016.5 ± 6.4
pH	5.2 ± 0.3	5.3 ± 0.4
Protein, g/l	0.03 ± 0.09	0.03 ± 0.08
WBC, units per field of view	4.9 ± 7.3	4.3 ± 2.8
RBC, units per field of view	1.0 ± 1.3	1.0 ± 0.6
Epithelium, units per field of view	1.5 ± 0.1	1.5 ± 0.1
Prostatic secretion:		
WBC, units per field of view	21.8 ± 23.2	7.3 ± 3.2
RBC, units per field of view	0.4 ± 0.6	0.1 ± 0.2
Lecithin granules, units per field of view	112.6 ± 71.6	164.3 ± 79.6
Blood hormone test		
Testosterone (ng/ml)	6.4 ± 2.4	8.2 ± 3.1
LH (IU/l)	5.0 ± 2.0	4.9 ± 2.1
Prolactin (IU/l)	7.2 ± 1.5	7.1 ± 1.4
Estradiol (pg/ml)	28.3 ± 9.3	26.4 ± 7.6

Table 4: Laboratory test findings

Ultrasound Findings

The pre-treatment ultrasound examination showed the inhomogeneous prostate structure in 52 (86.7%) patients, and its inhomogeneity was detected post-treatment only in 14 (23.3%) patients. During the treatment, a significant decrease ($p < 0.05$) in the prostate dimensions was also demonstrated. The pre-treatment cranio-caudal prostate dimension was 37.8 ± 3.2 mm, and post-treatment — 32.6 ± 2.9 mm; the pre-treatment lateral prostate dimension was 30.6 ± 3.4 mm, and post-treatment —28.4 ± 3.2 mm. The pre-treatment residual urine with the maximum volume up to 30 cm3 was detected in 6 patients. None of the patients demonstrated the post-treatment residual urine (Table 5).

Prostate ultrasound examination	Pre-treatment	Post-treatment
Cranio-caudal dimension, mm	37.8 ± 3.2	32.6 ± 2.9
Lateral dimension, mm	30.6 ± 3.4	28.4 ± 3.2
Structure homogeneity	inhomogeneous in 52 (86.7%)	inhomogeneous in 14 (23.3%)
Residual urine, cm3	2.5 ± 6.8	0

Table 5: Investigation findings.

The pre-treatment ultrasound examination detected the local changes of prostate structure in 8 (13.3%) patients, and post-treatment — in 6 (10.0%) patients. At the same time, the pre-treatment diffuse changes were observed in 52 (86.7%) patients, and post-treatment — only in 14 (23.3%) patients (Table 6).

Symptoms	Values	
	Pre-treatment, n (%)	Post-treatment, n (%)
Tenderness on palpation	60 (100)	8 (13.3)
Focal changes in ultrasound examination	8 (13.3)	6 (10.0)
Diffuse changes in ultrasound examination	52 (86.7)	14 (23.3)

Table 6: Objective prostate changes over time

Overall Clinical Efficacy

The high and moderate efficacy was achieved in 93.3% of patients, and low efficacy only in 6.7% of patients with chronic bacterial prostatitis who received 30-day combination therapy (Table 7).

Level	Number of patients	%
High	36	60
Moderate	20	33.3
Low	4	6.7
Lack of effect	0	0

Table 7: Evaluation of efficacy of combination therapy used: suppositories with drone homogenate and wheat germ oil, lyophilized drone homogenate in capsules, and bee bread in granules.

Safety and Tolerability

Only 2 (3.3%) patients reported mild skin itching during treatment suppositories with drone homogenate and wheat germ oil, lyophilized drone homogenate capsules, and bee bread, no side effects were observed in other patients (Table 8).

Scores	Patients	%
5	58	96.7
4	2	3.3
3	0	0
2	0	0
1	0	0

Table 8: Evaluation of side effects when using the combination therapy.

DISCUSSION

The results of this early-phase clinical study suggest that the combination of bee bread (perga) and drone larvae homogenate may provide beneficial adjunctive effects in patients with chronic bacterial prostatitis. Improvements were observed in symptom scores, quality of life, laboratory findings, hormonal parameters, digital rectal examination findings, and ultrasound characteristics of the prostate. The high or moderate efficacy observed in 93.3% of patients supports their potential use in urological practice.

The observed effects may be related to the complex biochemical composition of the bee products used in the study. Bee bread contains proteins, amino acids, vitamins, minerals, and biologically active compounds and has demonstrated antimicrobial activity in previous studies (Pełka et al., 2021). Drone larvae homogenate is also characterized by a rich composition of biologically active substances, including proteins, amino acids, vitamins, minerals, and hormone-like compounds, which may contribute to its physiological effects.

Recent studies published after completion of the present clinical study have identified drone larvae homogenate as a rich natural source of spermidine (Tausendfreund & Gloger, 2025). Spermidine has attracted increasing scientific interest because of its potential effects on cellular metabolism, inflammatory processes, tissue regeneration, and healthy aging. Although these findings were not available when the study was designed and conducted in 2021, they may provide a possible biological explanation for some of the beneficial effects observed in the present study.

The present study was designed as an early-phase exploratory clinical investigation. A patient-as-own-control methodology was used. Although this approach was formally discussed in the apitherapy literature only after completion of the present investigation (Körmendy-Rácz, 2025), the underlying methodological concept has long been

recognized in clinical research through within-subject and self-controlled study designs. All patients continued to receive standard medical treatment, while bee products were introduced as adjunctive interventions. Therefore, each patient served as his own reference point for evaluating changes over time. The primary objective of the study was to evaluate the tolerability and potential clinical value of bee products in patients with chronic bacterial prostatitis.

Within this framework, clinically meaningful improvements were observed in symptoms, laboratory findings, hormonal parameters, digital rectal examination findings, and ultrasound characteristics of the prostate. The high proportion of patients achieving high or moderate efficacy, together with the excellent tolerability profile, supports the potential value of bee bread and drone larvae homogenate as adjunctive components of complex therapy in patients with chronic bacterial prostatitis.

The observed effects are considered to be associated with the biological properties of bee bread and drone larvae homogenate rather than with a specific commercial product. Comparable effects may potentially be achieved using products of equivalent quality and composition.

In the absence of officially approved and generally accepted methods for conducting clinical trials of bee products in Ukraine, the design of such studies is largely determined by their authors and may be subject to debate among specialists. To address this issue, leading apitherapy scientists are working to develop methodological standards for such research (Kurek-Górecka et al., 2024). Strengthening the methodology of bee product research is essential for improving the scientific quality, comparability, and practical value of future studies

CONCLUSION

To our knowledge, this is the first study to examine the use of drone larvae homogenate and bee bread in the treatment of chronic bacterial prostatitis. The study was conducted as an early-phase investigation using a patient-as-own-control methodology; therefore, these methodological characteristics should be taken into account when interpreting the findings.

High or moderate efficacy was achieved in 93.3% of patients, while tolerability was excellent.

The findings of this early-phase clinical study support the potential value of bee products as adjunctive components of complex therapy in patients with chronic bacterial prostatitis. The observed effects may be related to the health-promoting, anti-inflammatory, antibacterial, immunomodulatory, regenerative, and endocrine-regulating properties of bee products and to their complex biochemical composition.

The results support further investigation of bee products, particularly drone larvae homogenate, in larger and more rigorously controlled clinical studies. Of particular interest are the biological effects of drone larvae homogenate as a relatively new and still insufficiently studied bee product, as well as the potential effects of combining different bee products in therapeutic applications.

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CONFLICT OF INTEREST

MEDOK Company LLC is a producer of lyophilized drone larvae homogenate, suppositories with drone homogenate and wheat germ oil, bee bread (perga).

This study was partly financed by MEDOK Company LLC (Ukraine).

INFORMED CONSENT

Written informed consent for the processing of clinical data and their further use was obtained from patients participating in the study.

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APIS

Original Research

Experimental Investigation of the Oncobiological Effects of Apitoxin in a Sarcoma-45 Model

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ABSTRACT

Intensive research is currently being conducted worldwide into novel biologically active compounds and integrative therapeutic approaches for the treatment of malignant diseases. Bee venom (apitoxin) is a natural substance with a complex biological composition containing peptides, enzymes, and other bioactive components. Over recent decades, preclinical studies have increasingly focused on the oncobiological activity of melittin, phospholipase A2, and other peptide components of bee venom.

The aim of the present study was to describe the antitumour effects of bee venom observed in an experimental Sarcoma-45 tumour model and to provide a brief overview of the relevant preclinical literature. Native dried apitoxin obtained by electrical stimulation was administered to rats implanted with Sarcoma-45 tumours using different solvent systems. The control group received no treatment.

Marked tumour regression was observed in the apitoxin-treated groups by days 3–4 of treatment. By day 10, tumour size had decreased to approximately half of the initial volume. The treated animals demonstrated improvement in general condition, increased activity, and improved appetite, whereas the control group exhibited progressive tumour growth and significant mortality.

These findings suggest that the bioactive components of bee venom may influence several biological mechanisms involved in tumour progression and may also affect immunological and inflammatory regulatory pathways. Although further controlled preclinical and clinical studies are required to assess clinical applicability in humans, the available molecular, cellular, and experimental evidence supports further investigation of apitoxin within the field of integrative oncology.

Keywords: apitoxin; bee venom; melittin; integrative oncology; Sarcoma-45; tumour biology; preclinical research; apitherapy; tumour regression; immunomodulation

INTRODUCTION

Intensive research is being conducted worldwide into the development of novel antitumour therapeutic strategies and biologically active compounds. Malignant diseases continue to represent a major global public health burden, with both incidence and mortality rates increasing in many countries (Xia et al., 2022). Large-scale epidemiological studies indicate that the incidence of several malignancies is also increasing among younger age groups (Bleyer & Barr, 2009; André et al., 2025; Sung et al., 2021). Tumour development is a complex multifactorial process resulting from interactions between genetic, epigenetic, environmental, and lifestyle-related factors. Although numerous molecular mechanisms involved in carcinogenesis have been identified, the complete process of tumour formation and the determinants of individual susceptibility remain incompletely understood (Hanahan, 2022; Vineis & Wild, 2014).

Cytotoxic and other systemic oncological therapies used in cancer treatment frequently possess a narrow therapeutic window, and their clinical use may therefore be limited by significant toxicity. Although chemotherapy has improved survival and therapeutic outcomes in many malignant diseases, its administration is

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commonly associated with immunosuppression, myelosuppression, hepatotoxicity, and impairment of haematopoietic and other physiological regulatory processes (Niraula et al., 2012; Wang et al., 2019).

Although surgery, radiotherapy, and systemic oncological therapies have significantly improved survival outcomes in numerous tumour types, substantial therapeutic challenges remain in advanced and metastatic disease. During antitumour treatment, particular importance is placed on reducing treatment-associated toxicity, preserving quality of life, and supporting and restoring physiological functions throughout the course of therapy (Miller et al., 2022; Sung et al., 2021).

Increasing attention is currently being directed towards integrative oncology approaches, which incorporate various supportive and immunological strategies alongside standard antitumour therapies. These may include selected naturally derived bioactive substances, immunomodulators, and adaptogens intended to support physiological and immunological functions and improve treatment tolerability. Integrative approaches may fulfil different supportive roles during various stages of oncological treatment (WHO, 2013).

One area investigated within integrative oncology is apitherapy, which examines the potential physiological and immunological effects of biologically active substances derived from bee products. Numerous preclinical and experimental studies have investigated the potential antitumour, immunomodulatory, and anti-inflammatory properties of royal jelly, propolis, pollen, and the biologically active components of bee venom. Published findings suggest that these substances may exert both specific and non-specific biological effects; however, their clinical applicability requires further investigation (Cui et al., 2024; Pandey et al., 2023; Rady et al., 2017).

Townsend et al. first identified one of the principal bioactive fatty acids of royal jelly, 10-hydroxy-2-decenoic acid (10-HDA), which demonstrated antitumour activity in experimental leukaemia and ascitic tumour models (Townsend et al., 1959). In subsequent investigations, the authors further analysed the in vitro effects of 10-HDA on tumour cells and compared its biological activity with that of other fatty acids (Townsend et al., 1960).

Later experimental studies evaluated the effects of royal jelly in various tumour models, including Ehrlich and Sarcoma systems. Some studies reported inhibition of tumour progression and effects on survival in experimental cancer models (Tamura et al., 1987).

In our own clinical observations, we investigated the potential effects of royal jelly administration in patients receiving palliative oncological care. Preliminary clinical experience suggested that, in some cases, improvements in quality of life, reduction of pain, enhancement of general condition and functional status, and clinical signs suggestive of tumour regression could be observed. According to our observations, the efficacy of royal jelly appeared more limited in hormone-dependent tumours. However, these observations were not obtained from controlled clinical trials. The primary objective of the present article is therefore not the analysis of human clinical outcomes, but rather the presentation of the effects of apitoxin observed in experimental tumour models.

Investigation of the biologically active components of bee venom has become an area of growing interest in integrative and experimental oncology research. Bee venom (apitoxin) is a secretory product of the venom glands of honeybees and possesses a complex biological composition. It contains proteins, enzymes, peptides, amino acids, biogenic amines (histamine, dopamine, noradrenaline), acetylcholine, lipids, minerals, and other bioactive molecules. Peptides, enzymes, and biogenic amines are considered particularly important contributors to the biological activity of bee venom, and their potential immunological and cellular effects have been examined in numerous preclinical investigations.

Current evidence indicates that melittin, the principal peptide component of bee venom, demonstrates significant cytotoxic activity against several tumour cell types in vitro and in experimental animal models. Reported mechanisms include disruption of tumour cell membranes, induction of apoptosis, and inhibition of certain signalling and proliferation pathways. Nevertheless, the authors emphasise that further studies are required before clinical application in humans can be considered (Pandey et al., 2023).

In the study by Duffy et al. (2020), bee venom and melittin demonstrated rapid and selective cytotoxic effects against highly aggressive breast cancer cell lines, including triple-negative and HER2-positive subtypes. According to the authors, melittin was capable of rapidly disrupting tumour cell membranes and reducing the activity of specific growth factor receptors (Duffy et al., 2020).

According to the review by Cui et al. (2024), melittin and phospholipase A2 are among the most important potential antitumour components of bee venom. The review summarised experimental findings indicating that these substances may induce apoptosis, modulate tumour signalling pathways, and be utilised in various targeted drug delivery systems (Cui et al., 2024).

Rady et al. (2017) identified melittin as one of the most important biologically active components of bee venom, demonstrating antitumour activity in numerous preclinical tumour models. The authors emphasised that one of the principal limitations of therapeutic melittin application is its non-specific cytotoxicity and haemolytic activity, necessitating the development of conjugates and targeted delivery systems (Rady et al., 2017).

Apamin and MCD peptide have primarily been investigated because of their neuroimmunological and membrane-physiological effects, whereas adolapin is mainly known for its anti-inflammatory and analgesic properties. At pres-

ent, only limited preclinical and mechanistic data are available regarding the potential oncobiological relevance of these components. In vitro studies demonstrated that apamin reduced proliferation, migration, and invasive properties of K562 leukaemia cells through inhibition of SK-type calcium-activated potassium channels. These observed effects may be associated with modulation of calcium-dependent signalling and membrane-physiological processes in tumour cells (Vasileva et al., 2023). MCD peptide (mast cell degranulating peptide) is primarily recognised for its mast cell-activating and immunomodulatory properties. Experimental investigations demonstrated enhancement of mast cell degranulation and histamine release, as well as alterations in membrane permeability and inflammatory mediator activity. Its potential oncobiological relevance is mainly being investigated in relation to modulation of immunological and inflammatory processes within the tumour microenvironment (Moreno & Giralt, 2015). Adolapin became known primarily because of its anti-inflammatory and cyclooxygenase-inhibitory activity, which may be relevant to inflammatory regulation within the tumour microenvironment. Melittin remains the most extensively studied component, and its cytotoxic and tumour cell-biological effects have been analysed in greater detail in both in vitro and animal models (Son et al., 2007; Rady et al., 2017; Cui et al., 2024).

Various bioactive components of bee venom may additionally influence immunological, neuroendocrine, and inflammatory regulatory pathways. Experimental studies suggest that bee venom administration may affect the hypothalamic–pituitary–adrenal axis as well as the production of inflammatory mediators and corticosteroid hormones. The immunomodulatory activity of phospholipase A2 and other peptide components has been examined in several preclinical models, particularly with regard to inflammatory and intercellular regulatory processes (Son et al., 2007; Moreno & Giralt, 2015). The precise mechanisms by which bee venom may affect hormone-dependent tumours remain incompletely understood, and further investigations are therefore required.

The present article reports previously unpublished experimental observations performed in 1997 in a Sarcoma-45 animal model and discusses them in the context of current research on the oncobiological effects of bee venom.

MATERIALS AND METHODS

In 1997, laboratory investigations were conducted to study the effects of bee venom on tumour growth in rats implanted with Sarcoma-45 tumours. Native dried bee venom (apitoxin) obtained by electrical stimulation from Carpathian honeybees was used in the experiments. The bee venom was stored in dark glass containers at 4–5 °C. Physiological saline solution and 0.5% novocaine solution were used as solvents.

The study was performed on rats weighing 190–220 g that had been implanted with Sarcoma-45 tumours. Tumour implantation had originally been carried out within an educational experimental programme at the Sverdlovsk Medical Institute. The experimental animals were subsequently made available for additional investigations. A total of 45 animals participated in the present study, with 15 animals in each group. Animals were selected so that tumour size was approximately equivalent between the groups.

The animals were divided into three groups:

1 In the first group, 0.5% novocaine solution was used as the solvent for apitoxin.
2 In the second group, physiological saline solution was used as the solvent.
3 The third control group received no bee venom treatment.

Apitoxin was administered using a gradual dose-escalation protocol over a 10-day period. Treatment was initiated with lower doses, and the administered amount was progressively increased up to a dose equivalent to 5 bee stings. No treatment-related acute toxicity or mortality was observed during dose administration. Tumour regression was observed during the gradual dose-escalation period.

Tumour size was monitored at regular intervals using linear measurements. The study was conducted in accordance with the available experimental animal regulations and institutional practices applicable at that time.

RESULTS

By days 3–4 of treatment, marked tumour regression was observed in both apitoxin-treated groups. No substantial difference in the degree of tumour regression was observed between the solvent systems used (0.5% novocaine and physiological saline solution).

The general condition of the apitoxin-treated animals improved: activity increased, mobility improved, and appetite also showed improvement. By day 10 of treatment, tumour size had decreased to approximately half of the initial volume.

Progressive tumour growth was observed in the control group, and significant mortality occurred during the observation period.

No acute toxicity or immediate severe adverse effects associated with treatment were observed.

Tumour size assessment was performed using linear measurements.

DISCUSSION

Investigation of biologically active natural compounds in tumour biology generally proceeds through sequential stages of research. The initial phase typically involves analysis of cellular and biochemical effects of individual molecules, including their activity on cell membranes, signalling pathways, gene expression, and immunological regulatory mechanisms. These investigations are followed by in vitro tumour models and subsequently by animal studies, which permit analysis of more complex biological effects and organism-level responses.

The molecular and cellular effects of various bioactive components of bee venom — particularly melittin, apamin, and phospholipase A2 — have been examined in numerous preclinical studies over recent decades. The present experimental animal observations demonstrated that natural apitoxin retained its biological activity when administered as a complex natural preparation, and no loss of antitumour activity was observed despite the simultaneous presence of multiple components.

According to the principles of integrative medicine, the primary objective of patient care is improvement of health status, quality of life, and survival outcomes. Accordingly, scientific investigation of any natural or synthetic bioactive substance demonstrating verifiable biological activity and an acceptable safety profile may be considered justified.

Assessment of the clinical applicability of naturally derived bioactive substances is particularly complex. In the case of substances with which humanity has coexisted over long evolutionary periods, and which possess no known severe toxic adverse effects when properly administered — with the exception of allergic reactions — preclinical evidence of safety and efficacy may justify investigation of human clinical application.

In Russia, apitherapy has a longstanding tradition within medical practice and research.

The institutional scientific foundations of Soviet medical apitherapy had already been established by the 1940s. Artemov's monograph on the physiological and therapeutic effects of bee venom (Artemov, 1941) is considered one of the earliest scientific works in this field. During the 1950s, N. P. Ioyrish published several studies concerning the medical application of bee products and the clinical significance of apitherapy. The institutional integration of apitherapy into the Soviet healthcare system is further demonstrated by the fact that, in 1959, the Scientific Medical Council of the USSR Ministry of Health officially approved the clinical instruction for apitherapy using bee venom.

Following completion of medical education and clinical specialisation, physicians may obtain apitherapy qualifications through specialised postgraduate training programmes. Such training is available at several medical institutions, including Ryazan State Medical University, and includes instruction in the physiological, pharmacological, and clinical application of bioactive substances derived from bee products.

Beginning in the 1990s, the author applied the integrative medical use of bee venom in the palliative care of patients with advanced and inoperable malignancies, based upon professional knowledge supported by preclinical and experimental observations, including the author's own animal experiments performed in 1997 and described in the present article. In all cases, treatment was conducted following detailed patient information, voluntary informed consent, and consideration of the ethical principles of the Declaration of Helsinki.

According to the author's clinical experience, apitoxin administration favourably influenced the general condition, quality of life, and symptoms of patients in numerous cases. Nevertheless, systematic clinical investigation and standardised data collection regarding these observations remain important future objectives.

It should also be noted that the review by Li Wanyao (2016) summarised the results of five clinical studies in which bee venom demonstrated oncobiological activity.

CONCLUSION

Based on the present experimental observations, administration of apitoxin in the Sarcoma-45 tumour model was associated with tumour regression, improvement in general condition, and increased activity of treated animals. The findings suggest that the bioactive components of bee venom influenced certain biological mechanisms involved in tumour progression and exerted effects on immunological and inflammatory regulatory processes.

According to the author's clinical experience, bee venom administration in the integrative palliative care of patients with advanced or inoperable malignancies may have been associated with favourable symptomatic and quality-of-life effects. The applied therapeutic approach was based upon the longstanding clinical and postgraduate educational traditions of medical apitherapy in Russia.

Although these observations were not derived from controlled clinical trials, the available molecular, cellular, and experimental findings justify further preclinical and clinical investigation of the bioactive components of bee venom.

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APIS

Case studies

Effects of Bee-Derived Products, Yoga, and Dietary Intervention on Metabolic Parameters in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Background

Type 2 Diabetes Mellitus (T2DM) is a rapidly increasing global health concern, closely associated with lifestyle-related factors such as obesity, dietary habits, and physical inactivity. Integrative approaches combining nutritional and lifestyle interventions may offer complementary strategies for improving metabolic outcomes under real-world clinical conditions.

Objective

This study aimed to evaluate the effects of a combined intervention including bee-derived products (propolis and bee bread), dietary modification, and structured physical activity on glycaemic control and metabolic parameters in patients with T2DM.

Methods

This pilot, practice-based observational study applied a within-subject design, in which each participant served as their own control. A total of 130 patients initiated the intervention, and 10 participants who completed the full protocol were included in the final analysis. The intervention consisted of daily supplementation with propolis (15 drops) and bee bread (10 g), a structured yoga programme (three sessions per week), and a progressive dietary intervention including time-restricted feeding over a three-month period. Primary outcome measures included fasting blood glucose (FBG), glycated haemoglobin (HbA1c), and liver function parameters (SGPT and SGOT).

Results

Significant improvements were observed in glycaemic and metabolic parameters. Mean fasting blood glucose decreased from 234.1 ± 71.5 mg/dL to 110.0 ± 23.7 mg/dL ($p = .001$), while HbA1c levels decreased from 9.5 ± 2.1% to 5.8 ± 0.6% ($p = .001$). Liver function parameters also improved, with reductions in SGPT and SGOT levels. Postprandial physical activity was associated with additional reductions in blood glucose levels. HPLC-DAD analysis of propolis identified bioactive compounds including caffeic acid phenethyl ester (CAPE) and chlorogenic acid.

Conclusion

The findings suggest that a combined intervention including bee-derived products, dietary modification, and structured physical activity may be associated with substantial improvements in metabolic parameters in patients with T2DM. These results support the potential relevance of integrative, multimodal approaches in real-world clinical settings. Further studies with larger sample sizes and optimised intervention protocols are required to confirm these findings.

Keywords: Type 2 diabetes mellitus; propolis; bee bread; dietary intervention; yoga; glycaemic control

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INTRODUCTION

The global prevalence of Type 2 Diabetes Mellitus (T2DM) has increased markedly over recent decades and is expected to continue rising. According to the International Diabetes Federation, the number of adults aged 20–79 years living with diabetes is projected to increase from 537 million in 2021 to approximately 783 million by 2045, corresponding to an increase from around 10% to over 12% of the global adult population (Sun et al., 2022).

Regional trends further highlight the growing burden of the disease. In Europe, the number of individuals affected by diabetes is expected to rise from 61.4 million in 2021 to 69.0 million by 2045, while in North America and the Caribbean, cases are projected to increase from 51.7 million to 63.0 million. Substantial increases are also anticipated in Central and South America (32.5 million to 48.9 million), the Middle East and North Africa (73.0 million to 135.7 million), South-East Asia (90.2 million to 152.8 million), and the Western Pacific region (206.0 million to 260.2 million). Africa is expected to experience one of the most pronounced relative increases, rising from 24.0 million to 55.0 million (Sun et al., 2022).

These trends demonstrate that diabetes represents a rapidly escalating global public health challenge. The increase is closely linked to lifestyle-related factors, particularly the rising prevalence of overweight and obesity. According to the World Health Organization (WHO, 2021), approximately 39% of adults worldwide are overweight, while a further 13% are classified as obese. Epidemiological evidence indicates that approximately 70–90% of individuals with Type 2 Diabetes Mellitus are overweight or obese, underscoring the central role of excess body weight in the development and progression of the disease (Colberg et al., 2010; Meigs, 2010; Ginter & Simko, 2012; Jaacks et al., 2016; Kahn et al., 2014).

From a clinical and epidemiological perspective, T2DM is increasingly recognised not merely as a disorder of glycaemic control, but as a complex metabolic condition involving multiple organ systems. It is characterised by insulin resistance, progressive β-cell dysfunction, and a close relationship with hepatic metabolism (Petersmann et al., 2019). This evolving understanding supports the need for comprehensive therapeutic strategies that extend beyond glycaemic control and target the broader metabolic context of the disease.

Although diabetes is widely studied at the population level, data on specific professional subgroups remain limited. In a cross-sectional study of 138 healthcare workers, diabetes mellitus was reported in 6% of physicians and 8% of nurses, while substantially higher rates of obesity were observed in both groups (47% and 48%, respectively), suggesting the presence of metabolic risk factors even within healthcare populations (Shah, 2023).

The intervention protocol was designed as a multimodal therapeutic approach integrating several lifestyle and nutritional components previously associated with beneficial metabolic effects in patients with Type 2 Diabetes Mellitus.

The aim of the present study was to evaluate the effects of a combined intervention consisting of bee-derived products (propolis and bee bread), dietary modification, and structured physical activity on glycaemic control and metabolic parameters in patients with Type 2 Diabetes Mellitus under real-world clinical conditions.

MATERIALS AND METHODS

This was a pilot, practice-based observational study with longitudinal evaluation of metabolic parameters under real-world clinical conditions. The study investigated the effects of bee-derived products in combination with lifestyle interventions on metabolic parameters in patients with Type 2 Diabetes Mellitus (T2DM). A high attrition rate was observed during the study.

Study Design and Participants

A total of 130 patients with Type 2 Diabetes Mellitus (T2DM) initiated the intervention. Participation and adherence to the prescribed dietary and lifestyle protocol were monitored throughout the three-month study period. Only participants who completed the full intervention and adhered to the protocol were included in the final analysis (n = 10). Participants who did not complete the intervention or did not meet adherence criteria were not included in the final analysis. A high attrition rate was observed during the study.

Participants were monitored over a period of three months, during which metabolic parameters were recorded and evaluated. Blood glucose levels were measured daily using a finger-prick method, both before and after meals, to assess short-term glycaemic responses.

Inclusion Criteria

Patients diagnosed with T2DM, aged 40–60 years, without major comorbidities, and willing to follow the prescribed dietary and lifestyle intervention.

Adherence

Adherence was defined as compliance with the prescribed dietary and postprandial physical activity protocol.

Standard Medical Treatment

Participants continued their standard medical treatment as prescribed by their physicians, and no modifications were introduced as part of the study protocol during the intervention period.

Ethical Considerations

This study represents a retrospective analysis of data collected during routine clinical practice. All participants provided informed consent for the use of their anonymised data. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Materials

Propolis, a resinous substance collected by honeybees, is rich in phenolic compounds and has been associated with anti-inflammatory and metabolic regulatory properties. Bee bread, a fermented pollen product, contains a wide range of nutrients, including proteins, vitamins, and bioactive compounds that may support metabolic health. In addition to nutritional supplementation, a structured yoga programme and a controlled dietary regimen were implemented as part of the intervention.

Bee bread, a fermented pollen product, contains proteins, amino acids, vitamins, minerals, fatty acids, and phenolic compounds, and has been described as a functional food with potential nutritional and metabolic benefits (Mattila et al., 2012).

The propolis and bee bread samples were provided from the apiary of Honey Bee Science Center, Gagalphedi (N: 27° 46' 5" ; E: 85° 26' 56"), Nepal. The propolis samples collected from Gagalphedi at elevation of 1,950 m, Kathmandu, Nepal were surrounded by *Alnus nepalensis* (Utis) and *Pinus roxburghii* (Pine) trees.

The comparison of the botanical occurrence profile of CAPE with the local vegetation surrounding the apiary provides strong evidence that the investigated propolis can be classified as poplar-type propolis (Bankova et al., 2018).

The ethanolic extract of propolis was obtained by maceration using a 1:3 propolis-to-ethanol ratio (with 70 % of ethanol), with intermittent shaking during the 7 days extraction period, followed by filtration of the extract (López-Patiño et al., 2021).

Biochemical Analysis

Chemical analysis of raw propolis samples collected from Kathmandu was performed to determine their composition using high-performance liquid chromatography with diode-array detection (HPLC-DAD). The raw propolis was crushed into small pieces, accurately weighed, and extracted in 70% ethanol (60:100, w/v). The mixture was continuously shaken at room temperature for 72 hours and subsequently filtered using Whatman No. 4 filter paper.

The identification and quantification of chemical compounds in the propolis extracts were carried out using HPLC-DAD, following previously described methods (Pellati et al., 2011; 2013) with slight modifications. Chromatographic separation was performed on a C18 column (250 × 4.6 mm i.d.). The mobile phase consisted of (A) 0.1% formic acid in water and (B) acetonitrile.

Gradient elution was applied as follows: 0 min (10% B); 0–3 min (20% B); 3–10 min (30% B); 10–40 min (40% B); 40–50 min (60% B); 50–60 min (80% B); and 60–65 min (90% B). The flow rate was set at 1 mL/min, with a post-run time of 10 minutes. The column temperature was maintained at 30 °C, and the injection volume was 5 µL.

Detection was performed over a wavelength range of 190–450 nm. Gallic acid, caffeic acid, and quercetin were used as external standards, while salicylic acid served as the internal standard.

Figure 1. The Yoga sessions are based on five asanas. (Photo by Sarmila Moktan)

INTERVENTION PROTOCOL

Nutritional Supplementation

Participants received a daily oral dose of:

- 15 drops of propolis
- 10 g of bee bread

Supplementation was administered continuously for 12 weeks.

Physical Activity (Yoga Intervention)

Participants were instructed to engage in a structured yoga programme consisting of three sessions per week, with a duration of 30 minutes per session. The intervention was implemented throughout the study period and was designed to support metabolic regulation through moderate, regular physical activity (see figure 1.).

Dietary Intervention

Participants followed a structured dietary plan developed by Api and Natural Therapy. The intervention was progressive and included both a defined macronutrient composition and time-restricted feeding patterns.

The recommended diet consisted of approximately (see figure 2.):

- 50% vegetables
- 25% protein-rich foods (including meat, fish, and eggs)
- 25% complex carbohydrates (primarily rice)

Meal frequency was gradually reduced over the study period:

- Month 1: four meals per day (breakfast, lunch, snack, and dinner), all completed before 19:00
- Month 2: two meals per day (lunch and dinner), completed before 19:00
- Month 3: one meal per day (lunch)

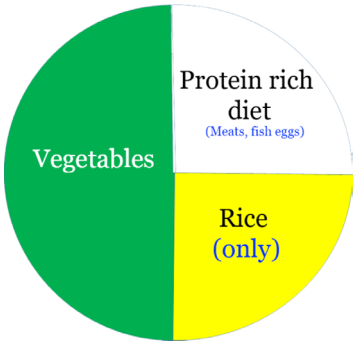

Figure 2. Api and Natural Therapy Diet Plan

This combined approach to dietary composition and meal timing was intended to support metabolic adaptation and glycaemic control.

Safety and Tolerability

The dietary intervention, including the reduction to one meal per day, was implemented under routine clinical supervision. Participants were monitored throughout the study period for tolerability.

Some participants reported transient symptoms of weakness during the initial adaptation phase (approximately 1–2 weeks), which resolved without further intervention. No severe adverse events were reported.

According to Ayurvedic dietary principles, reduced meal frequency is traditionally considered beneficial for metabolic balance. In addition, bee bread contains a range of vitamins, minerals, and bioactive compounds and may have contributed to maintaining nutritional status during the intervention.

Blood Glucose Measurement Protocol

Blood glucose levels were measured using a finger-prick method. During the first month, measurements were performed before meals and two hours after meals. During the second and third months, measurements were performed twice daily: before the first meal of the day and before going to bed.

Outcome Measures

The primary outcome measures included fasting blood glucose (FBS), glycated haemoglobin (HbA1c), and liver function parameters (SGPT and SGOT).

Additional Observations

Participants were also evaluated for the effects of postprandial physical activity: walking or squatting exercises after meals.

Control considerations

The study applied a within-subject design, in which each participant served as their own control. This approach is consistent with practice-based, real-world clinical research methodologies, particularly in the evaluation of complex, multimodal interventions where individual-level longitudinal changes are of primary interest (Körmendy-Rácz, 2025).

In this study, all interventions were implemented within routine clinical practice, and outcomes were assessed longitudinally within the same individuals. Given the chronic nature of Type 2 Diabetes Mellitus and the need for ongoing therapeutic management, the use of a traditional control group without access to potentially beneficial lifestyle components may present practical and ethical challenges in this context.

Therefore, a within-subject design was considered appropriate for the early-phase evaluation of this integrated, low-risk intervention, allowing for the assessment of real-world effectiveness while maintaining continuity of care.

Statistical Analysis

All results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, USA). A paired Student's t-test was used to compare fasting blood glucose, HbA1c, and liver function parameters before and after the three-month intervention. A p-value of < 0.05 was considered statistically significant.

RESULTS

The combined intervention including propolis, bee bread, yoga, and dietary modification was associated with significant improvements in multiple metabolic parameters in patients with Type 2 Diabetes Mellitus.

Chemical Composition of Propolis

HPLC-DAD analysis of Kathmandu propolis identified several bioactive compounds, including *caffeic acid phenethyl ester (CAPE)* and *chlorogenic acid*.

Baseline Blood Glucose Distribution

Baseline blood glucose values showed considerable variability among participants, ranging from 150 mg/dL to 380 mg/dL, indicating heterogeneous glycaemic status at study entry.

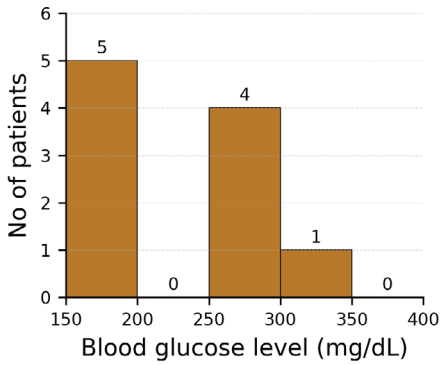

Figure 3. Distribution of baseline blood glucose values among patients with Type 2 Diabetes Mellitus.

Glycaemic Outcomes

Fasting blood glucose (FBG) levels decreased from 234.1 ± 71.5 mg/dL at baseline to 110.0 ± 23.7 mg/dL after the intervention. This reduction was statistically significant ($t(9) = 5.113$, $p = .001$). (see figure 4.)

Glycated haemoglobin (HbA1c) decreased from 9.5 ± 2.1% to 5.8 ± 0.6%, representing a statistically significant improvement ($t(9) = 5.019$, $p = .001$). (see figure 5.)

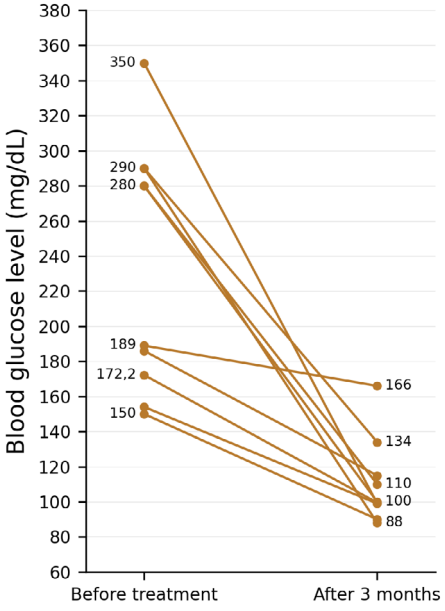

Figure 4. Fasting Blood Sugar before and after treatment

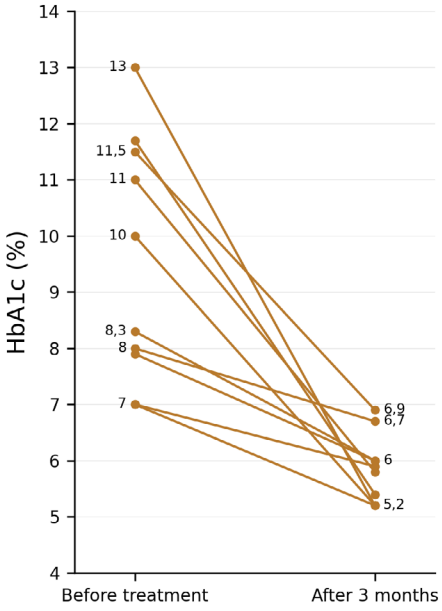

Figure 5. Glycated haemoglobin (HbA1c) before and after treatment

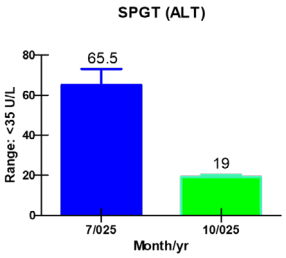

Figure 6. SGPT level before and after treatment

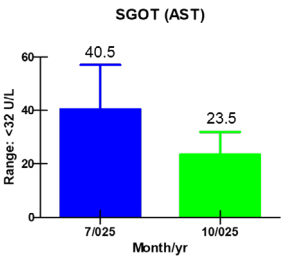

Figure 7. SGOT level before and after treatment

Liver Function Parameters

SGPT levels decreased from 65.5 ± 50 U/L to 19.0 ± 15 U/L ($p = .002$), (see figure 6.), while SGOT levels decreased from 40.5 ± 26 U/L to 23.5 ± 51 U/L ($p = .002$). (see figure 7.)

DISCUSSION

Overview of Findings

This pilot, practice-based observational study evaluated the effects of a combined intervention including bee-derived products, dietary modification, and physical activity in patients with long-standing Type 2 Diabetes Mellitus

(T2DM). The findings suggest that this integrated approach may be associated with improvements in glycaemic control and metabolic parameters under real-world clinical conditions.

Interpretation of Glycaemic Outcomes

The most prominent finding of the study was the significant reduction in fasting blood glucose (FBS) and HbA1c levels. The decrease in FBS from 234.1 mg/dL to 110.0 mg/dL, together with the reduction in HbA1c from 9.5% to 5.8%, indicates substantial improvement in both short-term and long-term glycaemic control.

Given the baseline variability in blood glucose values (150–380 mg/dL), the observed improvements suggest that the intervention may be effective across a heterogeneous patient population with differing levels of metabolic dysregulation.

Role of Propolis and Bee Bread

The identification of bioactive compounds in the propolis sample, including caffeic acid phenethyl ester (CAPE) and chlorogenic acid, provides a possible mechanistic explanation for the observed metabolic effects. Chlorogenic acid has been associated with regulation of glucose metabolism, reduction of hepatic glucose production, and modulation of gut microbiota.

These compounds have been associated with antioxidant activity (Sulaiman et al., 2011; Kuropatnicki et al., 2013; Diniz et al., 2020) and anti-inflammatory effects (Silva et al., 2012; Franchin et al., 2018), which may contribute to improved metabolic outcomes. Previous studies have also reported that propolis exhibits hypoglycaemic activity and may positively influence diabetic complications. It has been shown to modulate lipid metabolism, reduce lipid peroxidation, and scavenge free radicals (Matsui et al., 2004; Fuliang et al., 2005; Li et al., 2012).

Furthermore, propolis may influence glucose homeostasis through stimulation of insulin secretion or sensitivity and inhibition of intestinal α-glucosidase activity, thereby slowing carbohydrate digestion (Mujica et al., 2017; Wei et al., 2018), although these mechanisms were not directly assessed in the present study.

Bee bread, a functional food, may have contributed to the observed outcomes through its nutritional composition and enhanced bioavailability of nutrients resulting from fermentation. Its content of proteins, vitamins, and bioactive compounds may support overall metabolic function (Didaras et al., 2020). It is known that variations in flavonoid composition may be influenced by geographic origin, as reported in bee bread from Georgia (Tavdidishvili et al., 2014), Portugal (Sobral et al., 2017), and Romania (Dranca et al., 2020).

Contribution of Dietary and Lifestyle Interventions

The structured dietary intervention, including defined macronutrient composition and progressive changes in meal frequency, may have played a central role in the observed improvements. In particular, the reduction in meal frequency and the emphasis on vegetables and protein-rich foods may have contributed to improved insulin sensitivity and reduced glycaemic load.

Similarly, the incorporation of regular physical activity, including yoga and postprandial movement, is likely to have supported glucose utilisation and metabolic regulation (Yuniartika et al., 2021). A daily habit of yogic practice has been reported to reduce mental and oxidative stress and may be beneficial for glycaemic control (Hedge et al., 2011; Raveendran et al., 2018). Lifestyle interventions, including dietary modification and exercise, are widely recognised as first-line therapeutic strategies in T2DM management (Braga et al., 2019).

Hegde et al. (2011) reported that a 3-month Yoga intervention in addition to standard diabetes care resulted in significant reductions in HbA1c and fasting blood glucose levels. Furthermore, the review by Raveendran et al. (2018) summarised that Yoga-based interventions were associated with an average reduction in fasting blood glucose of approximately 23.7–25.7 mg/dL and HbA1c reductions of approximately 0.47%. In the present study, fasting blood glucose decreased from 234.1 ± 71.5 mg/dL to 110.0 ± 23.7 mg/dL, corresponding to a reduction of 124.1 mg/dL, while HbA1c levels decreased from 9.5 ± 2.1% to 5.8 ± 0.6%, corresponding to a reduction of 3.7%. These findings may suggest that the addition of progressive meal-frequency reduction, bee-derived products, and postprandial physical activity could further enhance the metabolic effects previously described for Yoga-based interventions alone.

The dietary composition applied in the present study is consistent with nutritional approaches previously associated with improved metabolic outcomes in patients with Type 2 Diabetes Mellitus, particularly diets characterised by high vegetable intake, moderate protein content, and controlled carbohydrate consumption (Evert et al., 2019; Esposito et al., 2009).

Previous dietary intervention studies in patients with Type 2 Diabetes Mellitus have demonstrated that nutritional approaches characterised by high vegetable intake and controlled carbohydrate consumption are associated with HbA1c reductions of approximately 0.5–1.2% and improvements in fasting blood glucose levels (Esposito et al., 2009). Similarly, studies investigating reduced meal frequency and time-restricted eating have reported HbA1c reductions of approximately 1–2% together with significant improvements in fasting blood glucose and insulin sensitivity (Carter et al., 2018). In the present study, fasting blood glucose decreased from 234.1 ± 71.5 mg/dL to 110.0 ± 23.7 mg/dL, while HbA1c levels decreased from 9.5 ± 2.1% to 5.8 ± 0.6%, corresponding to a reduction of 3.7%. These findings may suggest additive or synergistic metabolic effects resulting from the combined application of dietary modification, progressive meal-frequency reduction, Yoga, bee-derived products, and postprandial physical activity.

Postprandial Physical Activity

The observed reductions in blood glucose levels following walking (approximately 17%) and squatting (approximately 21%, data not shown) suggest that postprandial physical activity may have an immediate beneficial effect on glycaemic control. Although these findings are exploratory, they are consistent with previous observations that even moderate physical activity after meals can improve glucose metabolism (Colberg, 2012; Najafipour et al., 2017).

Liver Function and Metabolic Implications

The improvements observed in liver function parameters (SGPT and SGOT) suggest that the intervention may have broader metabolic effects beyond glycaemic control. The observed improvements in liver function parameters are consistent with the known role of hepatic metabolism in T2DM.

The hepatoprotective effects of propolis have been demonstrated in various experimental studies (Paulino et al., 2014; Wali, 2015; Omar et al., 2016). In addition, a clinical study reported that supplementation with Brazilian green propolis (830 mg/day) in elderly individuals living at high altitude was associated with reductions in liver enzyme levels over a two-year period (Zhu et al., 2018).

The magnitude of HbA1c reduction observed in the present study may reflect the combined metabolic effects of several intervention components that have individually been associated with improved glycaemic control in previous studies, including Yoga-based interventions, Mediterranean-style dietary modification, and reduced meal-frequency or intermittent fasting protocols. Since these interventions likely act through partially overlapping metabolic pathways, their effects cannot be assumed to be directly additive. Nevertheless, the observed 3.7% reduction in HbA1c is consistent with the possibility of additive or synergistic effects resulting from the multimodal intervention strategy.

Study Design Considerations

This study was conducted using a within-subject design, where each participant served as their own control under real-world clinical conditions. Such an approach is particularly relevant in the evaluation of complex, multimodal interventions, where isolating individual components may not fully reflect routine clinical practice.

Limitations

The intervention included multiple components, including Yoga, dietary modification, reduced meal frequency, bee-derived products, and postprandial physical activity. Several of these intervention elements have previously been associated with improvements in glycaemic control in patients with Type 2 Diabetes Mellitus. However, due to the multimodal nature of the present intervention, the relative contribution and interaction of the individual components (including postprandial physical activity) could not be determined within the current study design.

Future Directions

Further studies with larger sample sizes, extended follow-up periods, and optimised intervention protocols are required to confirm these findings. In particular, future research should aim to develop more feasible and sustainable intervention strategies in order to improve adherence and reduce attrition.

Future investigations may also focus on refining the implementation of combined lifestyle and nutritional interventions in real-world clinical settings.

CONCLUSION

The findings of this pilot, practice-based study suggest that a combined intervention including bee-derived products (propolis and bee bread), dietary modification, and structured physical activity may be associated with substantial improvements in metabolic parameters in patients with Type 2 Diabetes Mellitus. Significant reductions were observed in fasting blood glucose, HbA1c, and liver function markers over the course of the intervention.

These results highlight the potential relevance of integrated, multimodal approaches in the management of T2DM under real-world clinical conditions. The observed improvements within individuals support the applicability of within-subject evaluation frameworks in early-phase studies of complex, low-risk interventions.

The high level of attrition observed in this study underscores the importance of developing more feasible and sustainable intervention protocols in future research. Further studies with larger sample sizes and extended follow-up are required to confirm these findings and to optimise the implementation of such combined therapeutic strategies.

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APIS

Review

Propolis: Research, Apitherapy and Clinical Applications

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ABSTRACT

Background

The literature shows that propolis has been widely used in the treatment of inflammatory diseases and wound healing for centuries.

Objective

The aim of this paper is to present the key findings from in vitro and in vivo assays, as well as clinical trials, involving propolis.

Methods

Publications related to research conducted in vitro using different cell cultures, and in vivo using mice and other experimental animals, were examined. Clinical trials involving propolis were also analysed.

Results

Literature revealed the significant antimicrobial, anti-inflammatory and antitumor effects of propolis, either on its own or when combined with medicines, indicating its efficiency and usefulness. The pharmacological activities of propolis demonstrate its potential as an adjuvant or alternative to conventional drugs, either on its own or in combination with drugs commonly used to treat various diseases.

Conclusion

The in vitro and in vivo assays allowed for the conducting of clinical trials, which confirmed the benefits of propolis for human health.

Keywords: propolis; drug development; natural products

1. INTRODUCTION

Propolis has been used empirically for centuries due to its biological properties.

Ancient civilizations such as the Greeks and Romans used it to treat wounds due to its healing properties. The Incas used propolis as an antipyretic agent, the Persians used it to treat eczema, myalgia and rheumatism, and the ancient Egyptians used it for embalming the dead. During World War II, propolis was used to heal wounds and treat tuberculosis (Weis et al., 2022). These observations enabled us to confirm these pharmacological properties and explore its potential for developing new drugs, as well as its applications in the food and cosmetics industries (Berretta et al., 2020).

Propolis is produced from various parts of plants, such as leaf buds and tree bark. The bees mix these substances with their own secretions and beeswax. The word 'propolis' originates from the Greek for "defense of the city", which is fitting given that bees use it to seal holes and protect the hive from water and intruders.

The chemical composition and biological activities of propolis samples may vary depending on their geographical origin, due to local flora and climatic conditions (Burdock, 1998, Bankova et al., 2016). Propolis can be sourced from various plant species around the world, including alder, birch, palm, pine, poplar, willow, and others (Toreti et al., 2013). It may contain phenolic acids, aromatic aldehydes, terpenes, lignans, amino acids, esters, alcohols, fatty acids, vitamins and minerals (Braakhuis, 2019). Research into the biological effects of bee products

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has increased in recent years, revealing numerous pharmacological activities. In vitro and in vivo studies, as well as clinical trials, have demonstrated the efficacy of bee products in treating various diseases and maintaining health. (Weis et al., 2022) Propolis is currently used in cosmetics, health foods, beverages and extracts, as well as in pharmaceutical products such as capsules and mouthwashes (Conte et al., 2021, Santiago et al., 2018).

In light of the growing interest in propolis research, this paper aims to present in vitro and in vivo assays, as well as clinical trials and their main outcomes, which confirm on a scientific basis the effects of propolis.

2. RESEARCH ON PROPOLIS FALLS INTO DIFFERENT CATEGORIES, EACH WITH ITS OWN SPECIFIC GOALS

2.1. In vitro evidence

In vitro assays are essential for toxicity studies, allowing the control of variables, lower cost, and reduced animal use. In vitro studies have the limitation of being an isolated model, in which cells come into direct contact with propolis, allowing one aspect of its action to be observed without the influence of other factors. On the other hand, these studies provide insights into the activities of propolis, which can be further explored in other in vitro assays or even lead to the initiation of pre-clinical in vivo studies.

Several in vitro assays have been conducted to verify the pharmacological properties of propolis, shedding light on how it could promote human health. The assays revealed its anti-inflammatory, immunomodulatory, antioxidant, antibacterial, antifungal, antiviral, antitumor and anti-inflammatory properties, among others (Sforcin, 2016).

2.2. In vivo studies

In vivo assays are useful for evaluating the safety, efficacy, and toxicity of new drugs and treatments in laboratory animals. These assays expand our understanding of the effects of propolis and enable us to investigate its mechanisms of action. In vivo, the action of propolis can be observed in a broader context, involving other cells, tissues and systems, which is not the case in vitro.

In vivo studies have revealed a variety of propolis activities, including immunomodulatory, antioxidant, antitumor, antidiabetic, hypolipidemic, antidepressant, anxiolytic, analgesic, antihypertensive, antinephrotoxic, antipsoriatic, antiurolithiatic, hepatoprotective, neuroprotective, photoprotective, and wound and burn healing properties, among others.

2.3. Clinical applications

Gathering meaningful data in vitro and in vivo enables the design of clinical trials to determine whether propolis does indeed have preventive or therapeutic potential in relation to a range of conditions affecting human health.

Clinical trials have disclosed the efficacy of propolis in dentistry (Piekarz et al., 2017, Santiago et al., 2018, Askari et al., 2019, Dileep, 2019, Neto et al., 2020) and in subjects with diabetes (Henshaw et al., 2014, Oryan et al., 2018, Afsharpour et al., 2019). The efficacy of propolis as an anti-inflammatory agent has also been verified (Khayyal et al., 2003), as has its effectiveness in treating recurrent vaginal infections in women (Imhof et al., 2005). The antifungal action of propolis in patients with toenail onychomycosis has also been reported (Veiga et al., 2018). The effects of propolis on asymptomatic people infected with HIV who were being treated with antiretroviral therapy were investigated, revealing that propolis intake was safe and improved the immune response and exerted antiinflammatory effects on the patients (Conte et al., 2021, Tasca et al., 2024).

Possible interactions were investigated between a Brazilian propolis extract and commonly used drugs (fexofenadine, losartan, metoprolol, midazolam and omeprazole). It was found that propolis did not alter the activity of enzymes involved in drug metabolism, and that the magnitude of changes in the area under the plasma concentration-time curve was less than 20% for all drugs, which is considered safe with respect to possible interactions involving such enzymes (Cusinato et al., 2019).

The potential of propolis was reported to treat patients with SARS-CoV-2 infection, highlighting several mechanisms and perspectives (Ripari et al. 2021). The benefits of propolis were demonstrated as an adjunct treatment for adults hospitalized with SARS-CoV-2 infection. The propolis-treated groups had a shorter duration of hospitalization postintervention compared to the control group (Silveira et al., 2021).

Propolis has demonstrated therapeutic effects for patients with various diseases and has also been shown to benefit healthy individuals. Diniz et al. conducted a clinical trial in which participants received two different doses of propolis for seven days. Both doses decreased levels of a biomarker for lipid peroxidation and increased antioxidant enzyme activity. Propolis was also found to decrease a biomarker for DNA oxidation, indicating its potential to attenuate oxidative stress (Diniz et al. 2020). The positive effects of propolis supplementation were reported in the context of hypertension (Qu et al.).

In general, propolis is safe and non-toxic, and can be consumed both by healthy and unwell individuals, with few adverse effects (Braakhuis, 2019). Importantly, propolis is considered safer than many synthetic medicines (Toreti et al., 2013). However, cases of hypersensitivity after its topical application have already been reported mainly among beekeepers, so it is important to seek medical advice before using it (Braakhuis, 2019).

Based on clinical trials, propolis shows enormous potential for the development of new drugs in the treatment of several diseases. It is also a low-cost, easily obtainable treatment. However, its effectiveness as an adjuvant treatment for certain clinical conditions requires further investigation. It is worth highlighting that its activity depends on its botanical origin and that different samples should be compared in terms of their efficacy. Our group has been investigating so-called 'green propolis' (Sartori et al. 2024a), as well as red propolis (Ripari et al. 2025), and, more recently, a sample produced in the Brazilian caatinga biome (Sartori et al. 2024b). However, there is no consensus on how to prepare propolis extracts, what concentrations and doses to use, how long to take them for, or what other conditions are needed to obtain the same effects using propolis samples from different geographic regions. Thus, standardized extraction methods and large-scale clinical trials are required to validate its efficacy.

3. CONCLUSION

Traditional knowledge has provided insight into the effects of propolis in the treatment of various diseases. Despite the limitations of these models, in vitro and in vivo assays have revealed important findings related to its biological properties and mechanisms of action. Clinical trials assessing propolis have demonstrated its efficacy in treating various diseases, both internally and externally. Overall, these findings highlight the potential of propolis in the development of new medicines for dentistry, diabetes, immunity, tumors and other conditions.

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APIS

Practice report

Erasmus+ Supported Citizen Science on Bee-Relevant Flora: A Hungarian Field Study Informing Estonian Beekeeping

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ABSTRACT

This study presents a citizen science–based field survey of bee-relevant plant species conducted during a professional visit to the National Botanical Garden and Institute of Ecology and Botany. The aim was to document plant taxa of apicultural importance and to support cross-border knowledge transfer in beekeeping education between Hungary and Estonia within the framework of the Erasmus+ programme.

Beekeeping educators were divided into four working groups responsible for recording scientific (Latin), English, and Estonian plant names, photographic documentation, and key ecological and apicultural characteristics. The collected data were later integrated into a unified dataset. The recorded species were documented in a structured format, including multilingual nomenclature, resource type (nectar and/or pollen), and flowering periods in Estonia and Hungary.

The results highlight differences in flowering phenology between the two regions and demonstrate the value of collaborative, multilingual data collection in documenting bee forage plants. The approach supports applied learning and enhances the transferability of ecological knowledge across regions.

However, the study is limited by the botanical garden context, where curated plant collections may not represent typical regional flora. As a result, the observed species composition, flowering periods, and apicultural relevance may differ from those found in natural habitats. Further studies in more representative environments are recommended.

Overall, the study demonstrates the potential of citizen science and international collaboration in strengthening beekeeping education and improving the understanding of pollinator-supporting plant resources.

Keywords: citizen science, beekeeping education, bee forage plants, apicultural relevance, botanical garden survey, collaborative data collection, Erasmus+ programme, cross-border knowledge transfer, Estonia, Hungary

INTRODUCTION

Pollinator-supporting plant resources play a key role in sustainable beekeeping and ecosystem functioning. Understanding the availability and phenology of bee forage plants across different regions is particularly important in the context of environmental variability and changing climatic conditions.

This study applies a citizen science approach within an Erasmus+ framework to document bee-relevant flora and to support knowledge transfer between Hungary and Estonia. By combining field observations with multilingual plant identification, the study aims to contribute to applied beekeeping education and cross-regional ecological understanding.

MATERIALS AND METHODS

A group of beekeeping educators from Estonia participated in a professional field visit to the National Botanical Garden and Institute of Ecology and Botany (Hungarian: Nemzeti Botanikus Kert, Ökológiai Kutatóközpont), located in Vácrátót. The visit took place at the end of April, a period characterized by the active flowering of several early-season, bee-relevant plant species.

The guided tour was led by Mr. Gergely Lunk, head gardener and curator of the National Botanical Garden, who introduced plant species of particular importance for apiculture. The presentation focused on plant taxa observable during the late April phenological stage.

Participants were divided into four working groups, each assigned a specific data collection task:

- Group 1 recorded the Latin (scientific) names of the presented plant species and matched them with their Estonian equivalents.
- Group 2 documented the corresponding English names of the same plant species.
- Group 3 was responsible for photographic documentation, capturing visual records of the plants and their phenological state.
- Group 4 compiled key ecological and apicultural information shared during the guided tour, recording these in Estonian and comparing them with existing Estonian reference data.

Following the fieldwork, the four datasets were integrated through collaborative synthesis among the groups, resulting in a consolidated multilingual and multidisciplinary dataset on bee-relevant flora observed during the visit.

RESULTS

The recorded plant taxa are presented below in a structured format, using their scientific (Latin), English, and Estonian names. For each species, the main apicultural characteristics are provided, including the type of resource available for pollinators (nectar and/or pollen) and the flowering periods in Estonia (EE) and Hungary (HU).

This format reflects the collaborative data collection approach of the study, where multilingual terminology and ecological observations were integrated. The inclusion of Estonian and English plant names alongside the scientific nomenclature facilitates knowledge transfer between regions and supports the application of the findings in beekeeping education. The comparison of flowering periods highlights temporal differences between the two countries, which may be relevant for understanding the availability of bee forage resources under different climatic conditions.

In cases where a species is indicated as absent from Estonia, this refers specifically to the recorded taxon and does not exclude the presence of related taxa within the same genus or family.

Camassia sp.
English name: Camas lily
Estonian name: Preeriaküünal
Family: Asparagaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): May–June / April–May

Camassia sp.

Lavandula angustifolia
English name: Lavender
Estonian name: Tähklavendel
Family: Lamiaceae
Natural distribution: Estonia and Hungary – rare
Use: herb and ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): July–August / June–July

Salvia rosmarinus
English name: Rosemary
Estonian name: Harilik rosmariin
Family: Lamiaceae
Natural distribution: Estonia – annual or in a pot ; Hungary – rare
Use: herb
Bee resource: nectar
Flowering (EE / HU): April–May / March–April

Symphytum officinale
English name: Common comfrey
Estonian name: Harilik varemerohi
Family: Boraginaceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: medicinal plant
Bee resource: nectar, small amount of pollen
Flowering (EE / HU): May–July / April–June


Symphytum officinale

Symphytum tuberosum
English name: Tuberous comfrey
Estonian name: Mugul-varemerohi
Family: Boraginaceae
Natural distribution: Estonia – not present; Hungary – moderate
Use: medicinal plant
Bee resource: nectar, small amount of pollen
Flowering (EE / HU): May–June / April–May


Symphytum tuberosum

Euonymus alatus
English name: Winged spindle
Estonian name: Tiivuline kikkapuu
Family: Celastraceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: small amount of nectar and pollen
Flowering (EE / HU): May–June / April–May


Euonymus alatus

Cornus mas
English name: Cornelian cherry
Estonian name: Kirss-kontpuu
Family: Cornaceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: nectar
Flowering (EE / HU): March–April / February–March

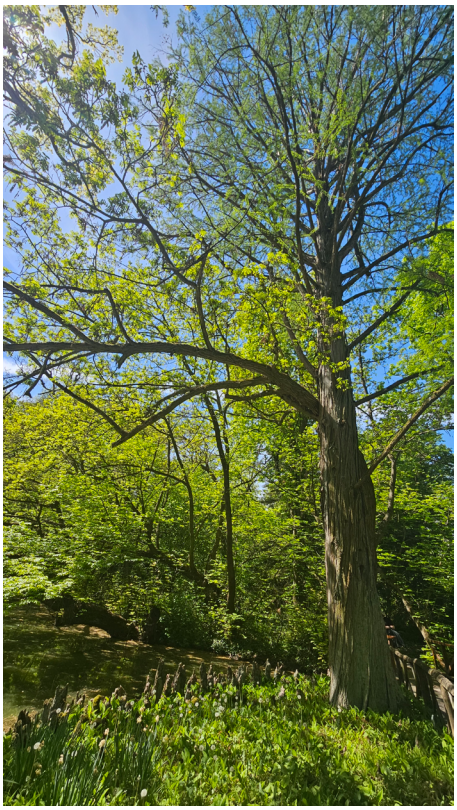

Cornus mas

Cornus florida
English name: Flowering dogwood
Estonian name: Õis-kontpuu
Family: Cornaceae
Natural distribution: Estonia – absent; Hungary – rare
Use: ornamental plant
Bee resource: pollen
Flowering (EE / HU): May / April

Telekia speciosum
English name: Yellow ox-eye
Estonian name: Kaunis teleekia
Family: Asteraceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): July–August / June–September

Epimedium sp.
English name: Barrenwort
Estonian name: Haldjatiib
Family: Berberidaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, small amount of pollen
Flowering (EE / HU): April–May / March–April

Taxodium distichum
English name: Bald cypress
Estonian name: Harilik sooküpress
Family: Cupressaceae
Natural distribution: Estonia – not present; Hungary – rare
Use: ornamental plant
Bee resource: insignificant for bees
Flowering (EE / HU): -


Taxodium distichum

Cercis griffithii
English name: Afghan redbud
Estonian name: Afgan juudapuu
Family: Fabaceae
Natural distribution: Estonia – absent; Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): April–May / March–April

Allium schoenoprasum
English name: Chives
Estonian name: Murulauk
Family: Liliaceae
Natural distribution: Estonia and Hungary – moderate
Use: herb
Bee resource: nectar
Flowering (EE / HU): June–July / May–June

Liriodendron tulipifera
English name: Tulip tree
Estonian name: Tulbipuu
Family: Magnoliaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): - / June

Salix babylonica
English name: Weeping willow
Estonian name: Babüloonia remmelgas
Family: Salicaceae
Natural distribution: Estonia – absent; Hungary – rare
Use: ornamental plant
Bee resource: pollen, small amount of nectar
Flowering (EE / HU): April–May / March–April

Euphorbia epithymoides
English name: Cushion spurge
Estonian name: Kuldne piimalill
Family: Euphorbiaceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: insignificant for bees
Flowering (EE / HU): April–May / March–April

Platanus × acerifolia
English name: London plane tree
Estonian name: Vahtralehine plaatan
Family: Platanaceae
Natural distribution: Estonia – moderate; Hungary – common
Use: ornamental plant
Bee resource: insignificant for bees
Flowering (EE / HU): May / March–April

Tilia cordata
English name: Small-leaved linden
Estonian name: Harilik pärn
Family: Tiliaceae
Natural distribution: Estonia and Hungary – moderate
Use: honey plant
Bee resource: nectar, pollen
Flowering (EE / HU): July / June–July

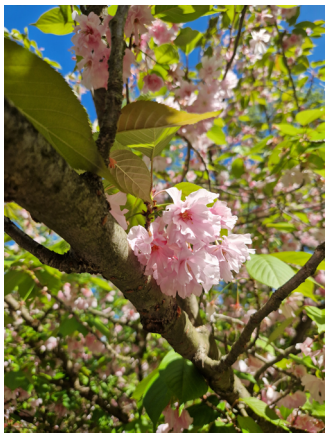
Prunus padus
English name: Bird cherry
Estonian name: Harilik toomingas
Family: Rosaceae
Natural distribution: Estonia and Hungary – moderate
Use: ornamental plant
Bee resource: insignificant for bees
Flowering (EE / HU): May–June / April

Prunus padus

Rosa foetida
English name: Austrian briar
Estonian name: Kollane kibuvits
Family: Rosaceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: pollen
Flowering (EE / HU): June / April–May

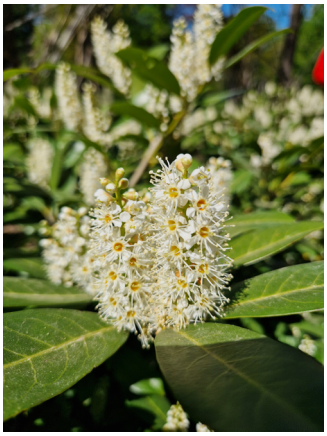
Rosa foetida

Prunus serrulata
English name: Japanese cherry
Estonian name: Peensaagjas kirsipuu
Family: Rosaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: small amount of nectar
Flowering (EE / HU): May / April



Prunus serrulata

Prunus laurocerasus
English name: Cherry laurel
Estonian name: Loorberkirsipuu
Family: Rosaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): May–June / April–May



Prunus maackii
English name: Manchurian cherry
Estonian name: Amuuri toomingas
Family: Rosaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): May–June / April–May

Malus sp.
English name: European apple tree
Estonian name: Õunapuu
Family: Rosaceae
Natural distribution: Estonia and Hungary – moderate
Use: fruit tree
Bee resource: nectar, pollen
Flowering (EE / HU): May–June / April–May

Prunus laurocerasus

Foeniculum vulgare
English name: Fennel
Estonian name: Harilik apteegitill
Family: Apiaceae
Natural distribution: Estonia and Hungary – rare
Use: medicinal plant
Bee resource: nectar
Flowering (EE / HU): August–September / July–August

Fallopia sachalinensis
English name: Giant knotweed
Estonian name: Sahhalini pargitatar
Family: Polygonaceae
Natural distribution: Estonia and Hungary – invasive species
Use: biogas production
Bee resource: nectar
Flowering (EE / HU): August–September / July–October

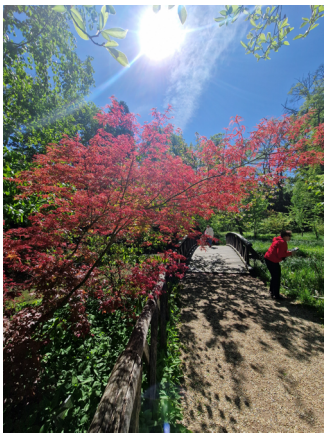

Fallopia sachalinensis

Eranthis hyemalis
English name: Winter aconite
Estonian name: Talvine lumekupp
Family: Ranunculaceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar
Flowering (EE / HU): March–April / February–March

Acer pseudoplatanus
English name: Sycamore maple
Estonian name: Mägivaher
Family: Aceraceae
Natural distribution: Estonia – rare; Hungary – moderate
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): May / April–May


Acer pseudoplatanus

Acer palmatum
English name: Japanese maple
Estonian name: Kämmalvaher
Family: Aceraceae
Natural distribution: Estonia and Hungary – rare
Use: ornamental plant
Bee resource: nectar, pollen
Flowering (EE / HU): May–June / April–May


Acer palmatum

LIMITATIONS

This study has several limitations that should be considered when interpreting the results. First, data collection was restricted to a single field visit, which limited both temporal representativeness and the ability to capture variability in plant phenology. Second, due to time constraints, it was not possible to survey the entire area of the National Botanical Garden and Institute of Ecology and Botany; observations were therefore confined to selected sections of the garden.

An important limitation of this study arises from the nature of the botanical garden setting. The plant taxa presented do not necessarily represent the most typical or widely occurring species in Hungary, as botanical gardens often include rare, ornamental, or non-native taxa. Consequently, the observed species may not fully reflect the composition of natural or agricultural bee forage resources.

This may also influence the reported flowering periods and apicultural relevance, which can differ from those of the same or related species under natural conditions. The relatively frequent occurrence of taxa categorized as “rare” in this study is therefore partly a result of the curated selection within the garden.

For this reason, the findings should be interpreted with caution, and further citizen science–based studies conducted in more representative, natural habitats would be necessary to validate and extend the results. Additionally, only plant species that had reached a suitable phenological stage for presentation at the time of the visit (late April) were included, potentially excluding other bee-relevant taxa that emerge earlier or later in the season. The photographic

documentation was carried out using mobile phones rather than professional imaging equipment, which may have affected image quality and consistency.

Finally, the data integration and synthesis process was conducted within a limited timeframe, with approximately two evening sessions available for collaborative analysis. This constrained period may have reduced the depth of cross-validation and harmonization among the datasets produced by the different working groups.

DISCUSSION

The results demonstrate that even a short-term, structured field survey can provide valuable insights into bee-relevant plant diversity and flowering phenology. The observed differences in flowering periods between Hungary and Estonia reflect regional climatic conditions and highlight the importance of temporal planning in beekeeping practices. In several cases, earlier flowering in Hungary compared to Estonia was evident, particularly among early-season species.

The relatively high proportion of rare or ornamental species in the dataset is consistent with the botanical garden setting and should be interpreted accordingly. Nevertheless, the findings illustrate the diversity of potential nectar and pollen sources and underline the importance of species selection in managed and semi-natural environments. Despite this, the dataset provides a useful reference framework for comparative observations and educational applications.

Importantly, the study confirms the effectiveness of this collaborative approach in applied ecological data collection. The integration of multilingual documentation enhances both the educational value and the practical applicability of the results, while data reliability was supported by cross-group validation and expert guidance during the field observations.

An additional important outcome of the study is the practical application of a structured data collection methodology by the participating educators. Through coordinated group work within a limited timeframe, participants experienced a complete workflow of field data collection, processing, and synthesis. This hands-on approach may support the independent application of similar methods in their future teaching and professional practice.

From a broader perspective, the comparative observations may also be relevant in the context of potential future environmental changes. If conditions in Estonia were to shift towards those currently observed in more southern regions, plant species typical of Central European bee forage systems could become increasingly relevant. In this context, the present observations may serve as a preliminary reference for understanding how such species might contribute to future forage availability.

Strengths

This study demonstrates several strengths. The collaborative, practice-based approach, involving beekeeping educators as active data collectors, enabled efficient and multidisciplinary documentation of bee-relevant plant species. The division of tasks among working groups (taxonomy, language mapping, photographic documentation, and ecological interpretation) increased both the depth and reliability of the collected data, while also providing participants with a transferable methodological framework for future use.

Furthermore, the multilingual data integration (Estonian, English, and Latin nomenclature) enhances the usability of the dataset across different professional and educational contexts. The study also benefits from its international and applied perspective, as the field observations conducted in Hungary directly contribute to the knowledge base supporting Estonian beekeeping education.

Finally, the framework of the Erasmus+ programme provided a structured environment for knowledge exchange, strengthening the link between field-based ecological observations and practical apicultural training.

CONCLUSION

This study demonstrates that structured field observations can effectively document bee-relevant plant species and support knowledge transfer in beekeeping education. The integration of multilingual plant data and phenological information provides a practical basis for understanding regional differences in forage availability.

In addition, the study highlights the value of structured group-based learning, where participants not only contribute to data collection but also gain practical experience in applying a reproducible methodology.

Despite its limitations, the study highlights the value of international collaboration within the Erasmus+ framework in supporting applied ecological learning and pollinator-friendly practices.

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