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EDITOR-IN-CHIEF

EDITORIAL BOARD

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Editorial

Preface

Dear Reader,

From time to time, the life of a scientific journal presents moments that invite reflection on how knowledge is best communicated. During the preparation of the present issue, the Editorial Board identified several such questions, each related to the evolving practices of scholarly publishing in a digital environment.

The first issue concerns citation style and in-text referencing.

Different scientific disciplines and research traditions benefit from different citation formats. In some articles, author–year referencing supports readability by allowing readers to immediately recognize influential works based on the cited author and publication year. In other cases, particularly in technically dense or methodologically focused manuscripts, numbered reference systems enable a more fluent reading experience without interrupting the flow of the text. Recognizing these differences, the Editorial Board has decided to allow authors to choose the citation style that best serves the structure and purpose of their manuscript. Consequently, readers may encounter both Vancouver-style numbered references and author–year (e.g., Harvard-style) citations within this journal.

A second issue concerns the relationship between scientific conferences and journal publications.

The Editorial Board supports the integration of research originally presented at conferences into full-length journal articles, including the incorporation of visual materials such as figures and images derived from conference presentations. When used as explanatory elements rather than decorative additions, such visuals can significantly enhance clarity and transparency. While this approach may appear unconventional in comparison with traditional print-oriented formats, it enables a form of scientific communication with high informational value and facilitates the faithful transfer of research content from conference settings to archival journal publication.

A third consideration addresses the role of linked and supplementary data.

As a digital journal, we are not constrained by fixed page limits. Authors are therefore encouraged to include supplementary materials that are organically connected to the main article, such as extended datasets, methodological details, additional figures, or multimedia content. Readers who prefer a concise presentation may choose to focus exclusively on the main text, while those seeking deeper insight have immediate access to complementary materials. This structure supports both readability and scientific completeness without forcing a single mode of engagement.

Finally, editorial independence and transparency remain fundamental principles of this journal.

In cases where an author holds an editorial position within the journal, editorial handling and peer review are conducted independently and in accordance with standard journal procedures, ensuring the integrity and impartiality of the review process.

Through these editorial decisions, the journal aims to balance tradition with innovation, supporting diverse forms of scientific expression while maintaining rigorous standards of quality, transparency, and academic integrity.

So interesting articles! Thank you for submission.

Editor-in-Chief
Dr. János Körmendy-Rácz

APIS

Original Research

The Dr. Bódog Beck Félix Medal of Merit: Legacy, Recognition, and Contemporary Perspectives in International Apitherapy

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ABSTRACT

The Dr. Bódog Beck Félix Medal of Merit represents one of the highest distinctions in the field of international apitherapy. Established to honour the legacy of Dr Bódog Beck Félix (1868–1942), a pioneer of modern apitherapy, the award recognises individuals whose scientific and clinical contributions have significantly advanced the understanding and therapeutic application of bee-derived therapies. This article outlines the historical background and symbolic significance of the medal and documents the presentation of its third award at the Apimondia World Congress in Copenhagen. Particular attention is given to the professional achievements of the 2023 award recipient, Dr Dietrich Klinghardt, whose interdisciplinary work in biological medicine exemplifies the continuity between early apitherapy traditions and contemporary integrative medical approaches.

Keywords: apitherapy; Beck Bódog Félix; Apimondia; biological medicine; bee venom therapy

INTRODUCTION

Apitherapy, defined as the medical use of bee products for disease prevention and treatment, has progressed from empirically based practices to an increasingly structured and scientifically discussed therapeutic discipline. Among the early contributors to this field, Dr Bódog Beck Félix occupies a distinguished position as one of the founders of modern apitherapy. His clinical observations and therapeutic concepts laid important foundations for the systematic application of bee venom therapy and related apitherapeutic modalities [1].

Over recent decades, apitherapy has gained increasing scientific visibility through specialised journals and professional forums, reflecting its gradual integration into complementary and integrative medical discourse [2].

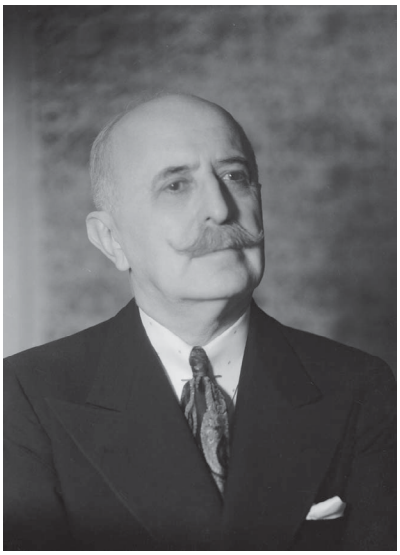

Figure 1: *Portrait of Dr Bódog Beck Félix (1868–1942).* Hungarian-born physician and one of the founders of modern apitherapy, whose clinical and scientific work laid the foundations of medicinal bee venom therapy.

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ESTABLISHMENT AND SIGNIFICANCE OF THE MEDAL

The Dr Bódog Beck Félix Medal of Merit was founded more than six years ago by Mihály Simics, a Canadian entrepreneur of Hungarian origin. The medal was designed by the Hungarian sculptor and medallist Mihály Fritz (Szeged, Hungary). Only ten medals were produced, underscoring the exclusivity and symbolic importance of the distinction.

The award is conferred upon individuals whose work demonstrates scientific credibility, clinical relevance, and sustained commitment to advancing apitherapy within an international and interdisciplinary context.

Figure 2: *The Dr Bódog Beck Félix Medal of Merit.*
Bronze medal designed by Hungarian sculptor and medallist Mihály Fritz. Only ten copies of the medal were produced, highlighting its exclusivity and symbolic importance within the international apitherapy community.

THE THIRD AWARD CEREMONY AT THE APIMONDIA WORLD CONGRESS

The third presentation of the Dr Bódog Beck Félix Medal of Merit took place during the ceremonial opening of the Apimondia World Congress in Copenhagen. Apimondia plays a central role in fostering international collaboration, scientific exchange, and professional integration within apitherapy and related disciplines [3].

The medal was presented by Dr János Körmendy-Rácz, Chair of the Apimondia Scientific Commission on Apitherapy and head of the Beck Bódog Award Committee.

Figure 3: *Presentation of the Dr Bódog Beck Félix Medal of Merit.*
Dr János Körmendy-Rácz (on the right), Chair of the Apimondia Scientific Commission on Apitherapy and head of the Beck Bódog Award Committee, presents the third medal to Dr Dietrich Klinghardt at the ceremonial opening of the Apimondia World Congress in Copenhagen.

The official certificate confirming the award was conferred by Dr Jeff Pettis, President of Apimondia. The ceremony was attended by several thousand participants from the international apicultural and medical communities.

Figure 4: *Presentation of the official certificate.*
Dr Jeff Pettis (on the left), President of Apimondia, presents the certificate confirming the award to Dr Dietrich Klinghardt during the formal ceremony.

ACCEPTANCE ADDRESS AND HISTORICAL CONTINUITY

In his acceptance address, Dr Dietrich Klinghardt emphasised the symbolic value of the medal as recognition of medical work that directly impacts human health. He paid tribute to Dr Bódog Beck Félix as a visionary who recognised the therapeutic potential of bees and their products at a time when such approaches were not widely accepted within mainstream medicine.

Dr Klinghardt also acknowledged the influence of Charles Mraz, a pioneer of bee venom therapy in the United States and the first recipient of the Beck Bódog Medal. This personal reference highlighted the continuity of apitherapeutic knowledge across generations and geographical regions.

Figure 5: *Acceptance address by Dr Dietrich Klinghardt.*
The award recipient delivers his ceremonial speech following the presentation of the Dr Bódog Beck Félix Medal of Merit at the Apimondia World Congress.

Note. Photographs reproduced with permission. Courtesy of the Apimondia Apitherapy Scientific Commission and private archival sources.

PROFILE OF THE AWARD RECIPIENT

Dr Dietrich Klinghardt is a Berlin-born physician who has spent most of his professional career in the United States. He is the founder and Medical Director of the Sophia Health Institute (Washington, USA) and the Klinghardt Neurobiology Institute (Germany). His integrative medical approach combines principles from apitherapy, immunology, endocrinology, toxicology, and neural therapy.

Dr Klinghardt's work focuses on complex chronic conditions, including neurodegenerative disorders, chronic pain syndromes, Lyme disease, and autism spectrum disorders, reflecting the systems-oriented philosophy that characterises contemporary biological medicine.

SCIENTIFIC CONTRIBUTION AT THE APIMONDIA CONGRESS

As an invited speaker at the Apimondia World Congress, Dr Klinghardt delivered a lecture entitled *"Treatment of Alzheimer's Disease and Lyme Disease with Apitherapy."* The presentation attracted an audience of approximately two thousand participants and generated considerable professional interest, emphasising the growing relevance of apitherapy within integrative approaches to chronic disease management.

CONCLUSION

The Dr Bódog Beck Félix Medal of Merit symbolises the continuity between the historical foundations of apitherapy and its contemporary scientific and clinical developments. The recognition of Dr Dietrich Klinghardt reflects the award's core values: respect for tradition, commitment to scientific inquiry, and dedication to improving human health through evidence-informed apitherapeutic practice.

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APIS

Original Research

Effects of various fall feeding sugar sources on survival, health, and productivity of honey bee colonies (Apis mellifera L.)

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Abstract: In Canada, beekeepers must supplement their colonies with sugar in the fall to ensure survival during winter, when floral resources are absent. Sucrose syrup is the predominant choice due to its high availability, chemical stability, and ease of use. However, upcoming revisions to Canada's organic standards will prohibit the use of conventional sugar syrup, necessitating a shift toward honey or organic sugar syrup as overwintering resources. The implications of this transition on colony survival, development, productivity, and pathogen prevalence remain insufficiently characterized.

This study evaluated honey bee (*Apis mellifera L.*) colonies fed with either conventional sugar syrup, organic sugar syrup, summer honey, or fall honey. Key parameters assessed included winter survival, colony development, honey production, and pathogen development (*Varroa destructor, Nosema spp.*, and six viruses).

Results indicate that organic sugar syrup, summer honey, and fall honey are viable alternatives to conventional sugar syrup for overwintering. However, precise colony weight management following fall feeding is critical to prevent starvation. In most cases, a minimum of nine honey frames per colony housed in a Langstroth single-brood chamber or four frames supplemented with 12L of organic sugar syrup supported successful overwintering, although adjustments may be required depending on colony size and winter severity.

No statistically significant differences were observed in brood and bee population development, honey production, or pathogen development across feeding treatments. These findings suggest that while organic beekeepers can effectively overwinter colonies using alternative carbohydrate sources honey-based feeding warrant careful consideration.

Keywords: honey, honey bee, organic, overwinter, sugar

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INTRODUCTION

Flower nectar is the main source of natural sugar harvested by foraging honey bees (*Apis mellifera L.*). The nectar is transported in the workers' crop to the colony, where it is processed by the bees. They reduce the water content to 16-20% humidity and add enzymes that break down complex sugars into simpler sugars, which are then stored as honey in the cells [7]. Sugar is used for energy production, which supports several metabolic functions, such as flight and thermoregulation, and can also be converted and stored as fat [34]. In temperate climates, like that of Canada, long winters, short blooming periods, and sometimes unfavorable weather conditions for bee activity, mean that colonies often require beekeepers' intervention with supplementary sugar or protein feedings [19]. The energy cost associated with wintering is very high, and the colony must have sufficient sugar reserves to survive until the return of food resources, which can be more than seven months in temperate climates. The thermoregulation cost during the winter months is 0.42 kg/week for colony survival in the absence of brood, and 0.84 kg/week once brood rearing begins in the colony [30]. In preparation for winter and spring, when nectar sources are scarce, beekeepers often supplement

their colonies with sugar to ensure their survival and development during the months without floral resources, thus preventing starvation. Beekeepers use various sugar sources to feed their colonies, including inverted syrup, fondant, and high-fructose corn syrup [2010]. The choice of sugar source depends on current practices in the country where beekeeping is practiced and the costs associated with different sugar sources. However, sugar syrup (from sugar cane or sugar beet) is the most used and recommended due to its abundance and simple, stable composition [31].

According to the Canadian Organic Standards of the Canadian Federation of Organic Agriculture (OFC), which apply to organic beekeepers, the primary food source for honeybees must be the nectar and pollen harvested by the colony. In cases of regional or seasonal food shortages, or for winter feeding of colonies, it is allowed to use, in order of preference: 1) organic honey from the operation; 2) organic sugar (e.g., inverted syrup, fondant); 3) non-organic honey from a conversion operation; or 4) non-organic sugar that is not genetically modified (COG, 2020). But in December 2019, the Interpretation Committee of the Standards (SIC) determined that the winter period alone cannot justify the annual recurrent feeding of bees with syrup. In 2025, article 7.1.11.1 regarding the feeding of honeybees in the Canadian Organic Standards will be revised. This revision is expected to eliminate non-organic sugar from the options available to organic beekeepers, unless data on the potential negative impacts of wintering bees with honey or organic sugar are provided.

Feeding colonies with honey during the winter, while practiced, is a less common technique in Canada. In fact, honey composition is dependent on floral sources, making it difficult to generalize the feeding method. Several risks associated with wintering bees on honey as the sole carbohydrate source, such as honey crystallization and dysentery in bees, have been identified in the past [2, 14, 17]. Honey crystallization is a natural process related to the glucose content that becomes supersaturated in certain types of honey (e.g., canola honey, *Brassica napus L.*). The winter mortality of colonies fed honey has been linked to insufficient reserves and honey crystallization [14], as crystallized honey in frames becomes unavailable to the bees. Among others, dandelion, maple, sweet clover, and alfalfa honeys have been associated with mortality due to crystallization [33, 16]. However, glucose solubility is influenced by other honey components, and current knowledge is insufficient to predict crystallization [5]. It seems that adding sucrose to honey reduces crystallization up to a total of 34% sucrose in the mixture [32]. Dysentery is also a problem associated with wintering bee colonies on certain honeys with excessively high moisture content [2]. Honeys produced late in the season, which the bees did not have time to properly process, such as aster or goldenrod honey, can also cause dysentery and mortality [33, 16, 24] . Some honeys, however, are beneficial for wintering bees, such as clover and buckwheat honey [33, 16]. It has also been demonstrated that a more complete nutrition of workers compared to a diet of sugar syrup can impact the quantity of *Nosema* spores per bee [4, 37] and may improve bee tolerance to pathogens [28]. Despite these observations, the overall understanding of honey as a winter feed remains incomplete. Few recent studies have investigated the potential of wintering bee colonies with honey, especially in climates similar to that of Canada. As a result, many questions remain unanswered regarding the feasibility and consequences of using honey instead of refined sugar, including its effects on colony survival, bee health, and post-winter productivity.

The main objective of this study is to assess the impact of the type of sugar used for feeding bee colonies on winter survival, bee health, and colony productivity. Different types of honey, organic sugar syrup, and conventional sugar syrup were characterized and administered to bee colonies in the fall to assess winter survival, winter sugar consumption, development of major bee pathogens (*Varroa destructor, Nosema spp.*, and six viruses), as well as colony development and honey production.

MATERIALS AND METHODS

To assess the impact of fall feeding types on the honeybee, the first experimental phase took place at the Centre de recherche en sciences animales de Deschambault (CRSAD). The objective of this phase was to compare traditional sugar syrup feeding methods (both conventional and organic) with honey feeding methods (summer honey and fall honey). This phase allowed for complete control of the experimental setup. The second experimental phase was conducted with two Quebec-based certified organic beekeepers. This phase aimed to evaluate the impact of feeding type in a commercial and organic context.

FIRST EXPERIMENTAL PHASE 2022-2023

Queen rearing and preparation of experimental colonies

The 2022-2023 experimental phase took place at CRSAD. In June 2022, 50 sister queens from the CRSAD-UL breeding program [22] were produced. These laying queens were introduced into 50 nuclei that had been pre-prepared with two frames of brood and one frame of honey/pollen. The nuclei were then randomly distributed across two apiaries located in the city of Pont-Rouge (Picard and 365 apiaries). On August 24, 2022, the colony strength was assessed to ensure the colonies were evenly distributed across the experimental groups. The number of developing worker bees (eggs, larvae, and pupae) was estimated by measuring the brood area (width x length) on both sides of the 10 frames in the brood chamber. The resulting rectangular area was multiplied by 0.8 to account for the elliptical shape of the brood pattern. A factor of 25 worker cells per 6.25 cm^2 was used to convert the area into the number of immature worker bees [12]. The adult bee population in each colony was also estimated visually [6] . On September

1, 2022, a sample of bees was collected for pathogen analysis (viruses and Nosema spp.), as well as an alcohol wash to assess the varroa infestation rate. The 50 colonies were then evenly distributed across the following five experimental groups.

Summer honey

In mid-July 2022, 80 frames of capped honey were harvested from colonies located at a single site in an agricultural area with meadows in the city of Pont-Rouge. These frames were stored in a cold room until feeding began. On September 15, for the colonies fed with summer honey, the lower brood chamber was rearranged by placing two frames of pollen at the edges and adding eight frames of capped honey in the center. The brood frames removed from the lower chamber were placed into a second super, which was installed above the first, separated by a queen excluder. The queen remained in the lower super. Three weeks later, after the brood had emerged, the second super was harvested to allow for wintering with a single super, as with the other experimental groups.

Fall honey

In early September, 80 frames of honey, partially capped, were harvested from hives located at a single site in an agricultural area with meadows in the city of Pont-Rouge. On September 15, colonies receiving fall honey underwent the same procedure as those receiving summer honey.

Organic syrup

The organic syrup was prepared using organic cane sugar from Brazil in bulk packages (Costco, Washington, USA). On September 15, 2022, the colonies receiving organic syrup were moved to a single super using a bee escape (Propolis etc., BE-1200), and each colony received 15 L of 2:1 sugar syrup in a Miller-type surface feeder. One week later, an additional 8 L of 2:1 sugar syrup were provided to complete the feeding.

Conventional syrup

The conventional sugar syrup was purchased in bulk as a liquid (Saint-Stanislas-de-Kostka, Quebec, Canada). On September 15, 2022, colonies receiving conventional syrup underwent the same procedure as those receiving organic syrup.

Mixture (50% fall honey and 50% organic syrup)

In early September, 40 frames of honey, partially capped, were harvested from hives located at a single site in an agricultural area with meadows in the city of Pont-Rouge. On September 15, for colonies receiving the mixture, four frames without brood or with brood that was about to emerge were removed from the brood chamber of each colony. These frames were replaced by four frames of fall honey. The removed frames were placed in a second super, which was positioned above the first and separated by a queen excluder. One week later (after the capped brood had emerged), the second super was harvested to allow for wintering with a single super, as with the other experimental groups. The colonies were then fed 12 L of organic sugar syrup (2:1 ratio) in a Miller-type surface feeder (Propolis etc., FE-1102).

Honey analysis

The melissopalynological analysis of the honey used for feeding was conducted by the company Bizzbilles (Baie-Saint-Paul, QC, Canada).

Antiparasitic treatments and wintering

Along with feeding, a Hopguard® II treatment was applied to all colonies according to the manufacturer's recommendations. On October 27, 2022, the hives were treated with oxalic acid via dripping. The size of the cluster before winter was determined by estimating the total number of frames fully covered with bees when the temperature was below 10°C. The number of frames covered with bees on the top and bottom of the hive was noted, and the average was calculated for each colony (Büchler et al. 2013). The hives were weighed using a portable scale (capacity of 160 kg, minimum sensitivity of 0.1 kg). The hives were then moved to two wintering apiaries in the city of Pont-Rouge and wrapped with double-bubble reflective Thermofoil insulation, and a rigid R10 insulating panel made of extruded polystyrene was placed on the inner cover of each hive.

Evaluation of survival, colony development, and pathogens

In April 2023, the colony coverings were removed. Then, the cluster size was evaluated in the same way as in the fall, and the hives were weighed to estimate winter sugar consumption. Hives weighing less than 24 kg were fed with four litters of 2:1 syrup to prevent starvation, while all other colonies received 0.5 L of syrup to account for the stimulatory effect of spring feeding [1, 34, 28]. The strength of the colony was assessed by measuring the brood and estimating the population of worker bees (as described previously) in May and June 2023 to determine the spring development of the colonies. Bee samples were collected in May for pathogen analysis (viruses and Nosema spp.) as

well as alcohol washes to determine varroa infestation rates. The honey production of the colonies was estimated by subtracting the weight of the empty honey supers from the weight of the harvested honey supers at the end of August 2023.

Second experimental phase 2023-2024

Queen rearing and preparation of experimental colonies

The experimental phase of 2023-2024 took place at two certified organic beekeeping businesses in Quebec. During the first week of June, each beekeeper prepared 30 nuclei consisting of two frames of brood and one frame of honey/pollen, into which they introduced a young, mated queen of their operation. In mid-August, the CRSAD team visited both beekeepers to assess the colonies (brood and bee population) and collect samples for viral analyses, Nosema infection, varroa infestation rate, and syrup and honey analyses. The 60 colonies (30 hives per business) were then evenly distributed into the following three experimental groups.

Summer honey

In July, the beekeepers set aside partially capped honey frames for fall feeding. These frames were stored in a low relative humidity environment (40-45%) and at room temperature not exceeding 40°C. The beekeepers began feeding the colonies in mid-September 2023. In the summer honey feeding group, each colony's queen was located in the brood box. The brood box was then removed from the hive stand and replaced with a honey super containing nine frames of summer honey. One frame of brood, along with the queen, was then placed in the honey super. A queen excluder was positioned between the two supers. This way, the brood box was placed above the queen excluder and the honey super containing the queen (Figure 1). Three weeks later, when the brood had emerged, the brood box was removed.

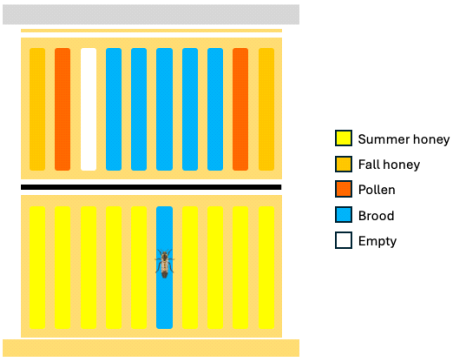

Figure 1. *Honey feeding technique for wintering using a single brood box, applied in the second experimental phase (© Laurence Plamondon).*

Organic syrup

Beekeeper #1's organic sugar syrup was prepared from organic cane sugar bags from Mexico (IAM, Saint-Hubert, QC, Canada). Beekeeper #2's organic sugar syrup was prepared from organic cane sugar bags from Colombia (Farinex, Boisbriand, QC, Canada). In early September, the colonies receiving syrup were moved to a brood box using a bee escape and were given an initial quantity of 2:1 sugar syrup in an individual feeder. One week later, the colonies received a second dose of 2:1 sugar syrup to complete the feeding. Each colony thus received a total of 24 L of syrup.

Conventional syrup

The conventional sugar syrup was purchased in bulk liquid form by Beekeeper #1 and prepared from sugar bags by Beekeeper #2. In early September, the colonies receiving syrup were moved to a brood box using a bee escape and were given an initial quantity of 2:1 sugar syrup in an individual feeder. One week later, the colonies received a second dose of 2:1 sugar syrup to complete the feeding. Each colony thus received a total of 24 L of syrup.

Syrup and honey analyses

The melissopalynological analysis of the honeys used for feeding during the second experimental phase was conducted by the company Bizzbilles (Baie-Saint-Paul, QC, Canada). The company Environex (Quebec City, QC, Canada) conducted the physicochemical analysis of the honeys and syrups.

Parasite control and wintering

Along with the feeding, the beekeepers applied a Thymovar® treatment. The CRSAD team completed the treatments with an oxalic acid drip treatment in early November 2023. At the same time, samples were collected for viral analysis, *Nosema* testing, and varroa infestation rates. The colonies were weighed, and the cluster size was

evaluated as previously detailed. The hives were then individually wrapped with double-bubble Thermofoil reflective insulation, and a rigid R10 insulation panel made of extruded polystyrene was placed on the inner cover of each hive.

Evaluation of colony survival, development, and pathogens

In May 2024, the CRSAD team returned to each beekeeper's site to assess the survival of the project colonies and evaluate the spring recovery. In mid-April, the colonies were weighed, and cluster size was assessed as previously described. Colonies weighing less than 17 kg received a frame of honey from the previous fall's brood box, as hive weights differed from the first year due to variations in materials and the absence of a cover during weighing. In mid-May, the colonies were evaluated for brood area as previously described. A sample of bees for pathogen analysis (viruses and Nosema spp.) and an alcohol wash for Varroa infestation rates were collected from each colony.

Virus analyses

Six common bee viruses were analyzed: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Deformed Wing Virus variants A and B (DWV-A, DWV-B), Israeli Acute Paralysis Virus (IAPV), and Kashmir Bee Virus (KBV). The bees were euthanized by placing them on dry ice. All samples were stored at -80°C until analysis. CRSAD carried out the viral analyses following the protocol described by Plamondon et al. (2024).

Nosema spp. counts

The intestines of 60 bees were collected and placed in a mortar for tissue grinding with a pestle. Then, 30 mL of distilled water was added and mixed until the solution became homogeneous. The macerate was transferred into a tube and vortexed. Nosema spores were quantified following the method by Fries et al. (2013). Two counts were performed for each sample, and the arithmetic mean of these counts was used to calculate the number of spores per bee: number of spores/bee = average number of spores for 5 squares / 5 squares / hemocytometer volume × dilution factor.

Statistical analyses

Statistical analyses were performed using R (v.4.2) (R Core Team, Vienna, Austria), with a significance level of 0.05. Variations in the ANOVA models, estimated with mixed linear models (nlme::lme [Pinheiro and Bates 2000]; lme4::lmer [Bates et al. 2015]) were conducted according to the experimental design of each variable. Fixed effects included the group and, when applicable, time and their interaction. Random effects included the apiary and the colony. Global tests for fixed effects were performed using the emmeans::joint_tests function (Lenth 2022). When a significant difference was found, pairwise comparisons were made using Tukey's adjusted tests (functions emmeans::emmeans and emmeans::pairs [Lenth 2022]). The normal distribution and homogeneity of variances were validated on the model residuals using histogram, and residual plots versus predicted values. In the presence of heteroscedasticity, heterogeneous variances were modeled based on the problematic factor. The spores and virus data were transformed using a log+1 transformation to meet the normality assumption. For spores data only, p-values come from the model with transformed data, while means and SE are derived from model using untransformed data. To test mortality differences, chi-square (χ^2) tests followed by Fisher's tests were used. The results are presented using ggplot2 (Wickham 2016).

Results

First experimental phase 2022-2023

Analyses of syrups and honeys

The analysis of the floral origin of the pollen grains from the honeys used for feeding the colonies in 2022 shows that they are monofloral honeys. The summer honey is a monofloral honey from the Brassicaceae family, as it exceeds the highest established threshold for this taxon, which is 80%. In this honey, the pollen from Brassicaceae plants were quite similar, which could be explained by a single floral source, likely of agricultural origin, such as canola or mustard (Supplementary file 1). The fall honey is a monofloral honey from the group of Eupatorium, asters, and goldenrods (Supplementary file 2). No crystallization of the honey was observed.

Winter mortality

In the fall of 2022, 46 colonies were prepared for outdoor wintering (Table 1). The following spring, 36 colonies had survived the winter period, resulting in a mortality rate of 21.7%. A significant difference in mortality is observed between the experimental groups ($\chi^2 = 19.877$, $p < 0.0001$), indicating that the type of feeding influences colony survival during winter. No dysentery was observed at the entrances of the hives.

Cluster size and colony weight before and after wintering

Cluster size is significantly affected by the group ($F_{4,32} = 3.704$, $p = 0.0138$), time ($F_{1,32} = 130.241$, $p < 0.0001$), and their interaction ($F_{4,32} = 5.926$, $p = 0.0011$). In the fall, the summer honey group and the fall honey group have a significantly smaller average cluster size compared to the organic syrup group (mean ± SE; 6.44 ± 0.581 frames,

Experimental groups	Number of wintered colonies	Number of surviving colonies in spring	Winter mortality (%)
Summer honey	9	5	44
Fall honey	9	3	67
Organic syrup	9	9	0
Conventional syrup	9	9	0
Mixture	10	10	0

Table 1. *Number of colonies wintered in the fall, number of colonies surviving in the spring, and winter mortality percentage for 2022-2023 in each experimental group.*

6.27 ± 0.583 frames, and 8.48 ± 0.581 frames, respectively). The average cluster size of the summer honey group is also significantly smaller than the conventional syrup group (8.24 ± 0.581 frames). In the spring, the summer honey group has a significantly smaller average cluster size compared to the organic syrup and fall honey groups (3.20 ± 0.647 frames, 5.62 ± 0.581 frames, and 6.02 ± 0.817 frames, respectively). Regarding the interaction, the fall honey group maintains a stable average cluster size, while the cluster size of the other groups decreases (Figure 2).

The weight of the hive is significantly affected by the group ($F_{4,32} = 17.194$, $p < 0.0001$) and time ($F_{1,32} = 297.364$, $p < 0.0001$), but is not affected by their interaction ($F_{4,32} = 0.669$, $p = 0.6184$). In the fall, the summer honey group and the fall honey group have a significantly lower average weight compared to the biological syrup and conventional syrup groups (mean ± SE; 32.5 ± 1.89 kg, 30.6 ± 1.90 kg, 42.3 ± 1.89 kg, and 40.6 ± 1.89 kg, respectively). The average weight of the mixed group is also significantly lower than the biological syrup group (36.2 ± 1.86 kg). In the spring,

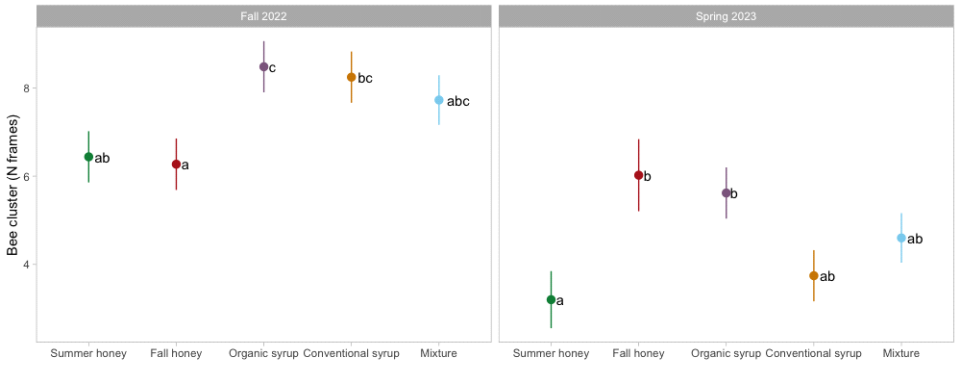

Figure 2. *Cluster size (number of frames covered by bees) in fall and spring, based on experimental groups (mean ± SE).* Different letters indicate statistically significant differences between groups. ($p < 0.05$).

the summer honey group and the fall honey group have a significantly lower average weight than the biological syrup and conventional syrup groups (22.9 ± 2.03 kg, 20.2 ± 2.39 kg, 30.1 ± 1.89 kg, and 29.8 ± 1.89 kg, respectively) (Figure 3).

Colony development

Bee population is significantly affected by the group ($F_{4,32} = 5.525$, $p = 0.0017$), time ($F_{1,32} = 114.867$, $p < 0.0001$), and their interaction ($F_{4,32} = 4.592$, $p = 0.0048$). In the spring, during June, the summer honey group and the fall honey group have a significantly lower average bee population than the other groups (mean ± SE; 7.145 ± 1.74 frames

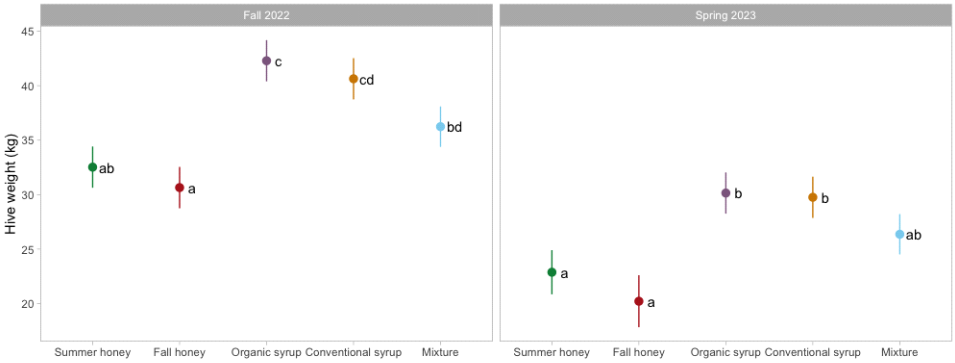

Figure 3. *Hive weight (kg) in fall and spring, based on experimental groups (mean ± SE).* Different letters indicate statistically significant differences between groups ($p < 0.05$).

and 5.120 ± 1.75 frames, respectively). Regarding the interaction, the summer honey group and the fall honey group show a lower average growth in bee population compared to the other groups (Figure 4).

Time significantly affects total brood ($F_{4,30} = 114.416$, $p < 0.0001$). The group ($F_{4,30} = 0.788$, $p = 0.5420$) and the interaction between group and time ($F_{4,30} = 1.869$, $p = 0.1419$) do not have a significant effect (Figure 5).

Honey production

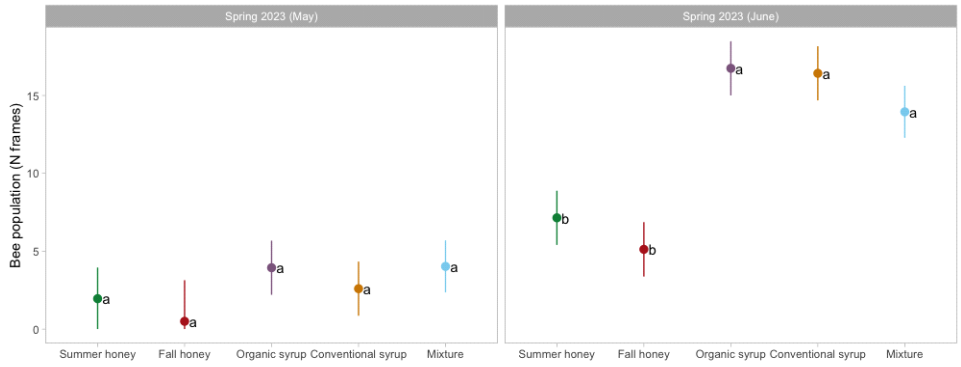

Figure 4. *Bee population (number of frames covered with bees) in May and June, based on experimental groups (mean ± SE).* Different letters indicate statistically significant differences between groups ($p < 0.05$).

Honey production from May to August 2023 is not significantly different depending on the type of feeding received in the previous fall ($F_{4,29} = 0.314$, $p = 0.8664$) (Figure 6).

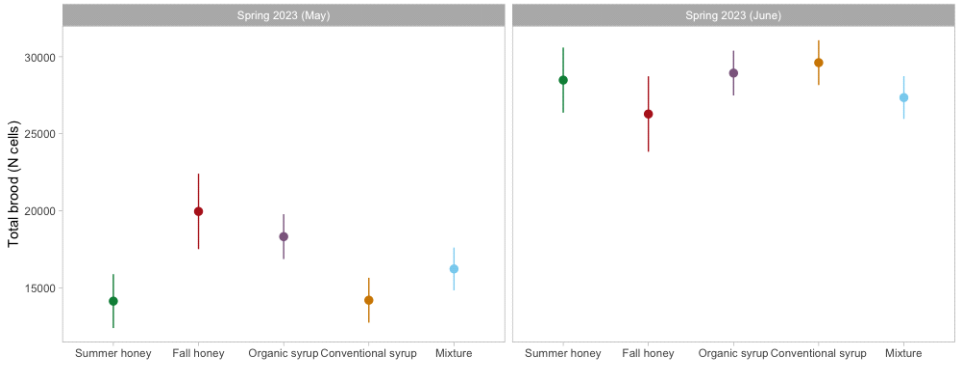

Figure 5. *Total brood population (number of cells of eggs, larvae, and pupae) in May and June, based on experimental groups (mean ± SE).*

Figure 6. *Honey production (kg) from May to August, based on experimental groups (mean ± SE).*

Pathogen development

Varroa destructor

Only five hives, spread across the different experimental groups, had a varroa infestation rate greater than 0% but less than 1% in the fall of 2022 and spring of 2023.

Second experimental phase 2023-2024

Analysis of syrups and honeys

The palynological analysis of the honey from beekeeper #1, used to feed the colonies in the experimental honey group, reveals that this honey contains several underrepresented species, including Epilobium and thistles. According to the Sawyer method, which accounts for the representativity of the pollen, no taxon exceeds the 45% threshold. However, the main nectar source is estimated to come from Epilobium at a concentration of 40.9%. This plant produces pollen that is very poorly represented in the honey compared to its nectar contribution. Thus, the honey would be classified as polyfloral since no single species stands out (Supplementary file 3). The sugar quantification shows that the honey from this beekeeper contains 40% fructose and 34% glucose, with sucrose, maltose, and lactose all present at less than 0.1%. No crystallization of the honey was observed (Supplementary file 5).

The analysis of honey from beekeeper #2 shows a significant proportion of Rubus species, but it is not sufficient to classify the honey as monofloral. However, it could be considered as a Rosaceae honey since the combined percentage of Rubus species and Geum species (which also belong to the same family) exceeds the general 45% threshold (Supplementary file 4). The sugar quantification for this honey shows 40% fructose and 32% glucose, with sucrose, maltose, and lactose present at less than 0.1% (Supplementary file 5). No crystallization of the honey was observed.

Winter mortality

Out of the sixty colonies prepared by the two organic beekeepers during the summer of 2023, two colonies became queenless before the fall feeding. In the fall of 2023, a total of 58 colonies were prepared for outdoor wintering. In the following spring, 55 colonies had survived the winter period, resulting in a mortality rate of 5.2%. The three colonies were from organic beekeeper #2 and were part of the honey-fed group. A significant difference in mortality is observed between the experimental groups ($\chi 2 = 7.0303$, $p = 0.02974$), indicating that the type of feeding influences colony survival during winter. Additionally, no dysentery at the colony entrances was identified.

Cluster size and colony weight before and after wintering

Cluster size is significantly affected by time ($F_{1,52} = 12.037$, $p = 0.0011$). The group ($F_{2,52} = 1.205$, $p = 0.3078$) and the interaction between group and time ($F_{2,52} = 0.924$, $p = 0.4034$) have no significant effect (Figure 7).

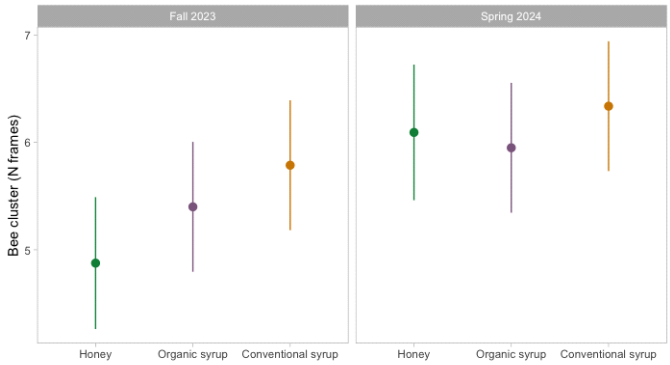

Figure 7. *Cluster size (number of frames covered by bees) in fall and spring, based on experimental groups (mean ± SE).*

Hive weight is significantly affected by the group ($F_{2,54} = 34.347$, $p < 0.0001$) and time ($F_{1,54} = 765.134$, $p < 0.0001$), but their interaction is not significant ($F_{2,54} = 0.339$, $p = 0.7139$). The honey group is significantly lighter than the organic syrup group and the conventional syrup group in both fall (mean ± SE; 26.2 ± 1.49 kg, 30.6 ± 1.47 kg, and 31.1 ± 1.47 kg, respectively) and spring (16.3 ± 1.48 kg, 20.3 ± 1.47 kg, and 20.4 ± 1.47 kg, respectively) (Figure 8).

Colony development

The bee population and brood size are not significantly different based on the type of feeding received the previous fall ($F_{2,50} = 1.872$, $p = 0.1644$; $F_{2,50} = 0.712$, $p = 0.4955$; respectively) (Figure 9).

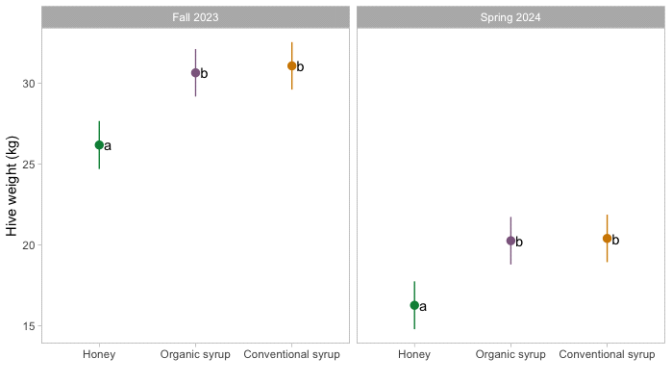

Figure 8. *Hive weight (kg) in fall and spring, based on experimental groups (mean ± SE).* Different letters indicate statistically significant differences between groups ($p < 0.05$).

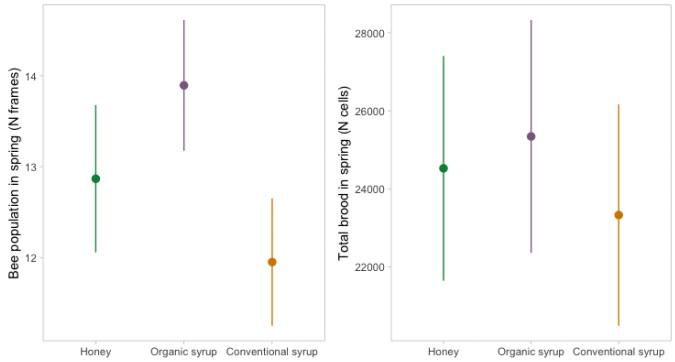

Figure 9. *Bee population (number of frames covered with bees) on the left graph and total brood (number of cells with eggs, larvae, and pupae) on the right graph in spring, based on experimental groups (mean ± SE).*

Pathogen development

Varroa destructor

All colonies had a varroa infestation rate of 0% in summer 2023, and only 2 colonies had an infestation rate greater than 0%, but less than 1%, in spring 2024.

Viruses

No colonies tested positive for IAPV and KBV. All tested viruses were unaffected by the group (ABPV : $F_{2,54.06} = 0.534$, $p = 0.5894$; BQCV : $F_{2,54} = 1.271$, $p = 0.2888$; DWV-A : $F_{2,54.21} = 0.950$, $p = 0.3930$; DWV-B : $F_{2,54.01} = 0.971$, $p = 0.3852$) nor by the interaction between group and time (ABPV : $F_{2,55} = 0.574$, $p = 0.5665$; BQCV : $F_{2,55} = 0.997$, $p = 0.3755$; DWV-A : $F_{2,55} = 1.181$, $p = 0.3145$; DWV-B : $F_{2,55} = 0.965$, $p = 0.3874$). However, all tested viruses were affected by the sampling time (ABPV : $F_{1,55} = 52.311$, $p < 0.0001$; BQCV : $F_{1,55} = 11.749$, $p = 0.0012$; DWV-A : $F_{1,55} = 58.309$, $p < 0.0001$; DWV-B : $F_{1,55} = 116.542$, $p < 0.0001$) (Figure 10).

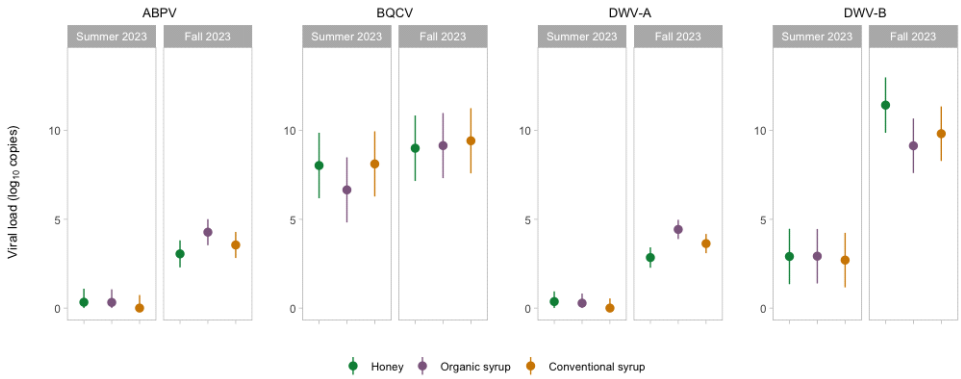

Figure 10. *Viral load (log10 copies) for different viruses in summer and fall, based on experimental groups (mean ± SE).*

Nosema spp.

The presence of *Nosema* spores is unaffected by the group ($F_{2,54.59} = 0.019$, $p = 0.9814$) nor by the interaction between group and time ($F_{4,106.42} = 0.895$, $p = 0.4696$). However, the effect of time is significant ($F_{2,107.02} = 16.170$, $p < 0.0001$) (Figure 11).

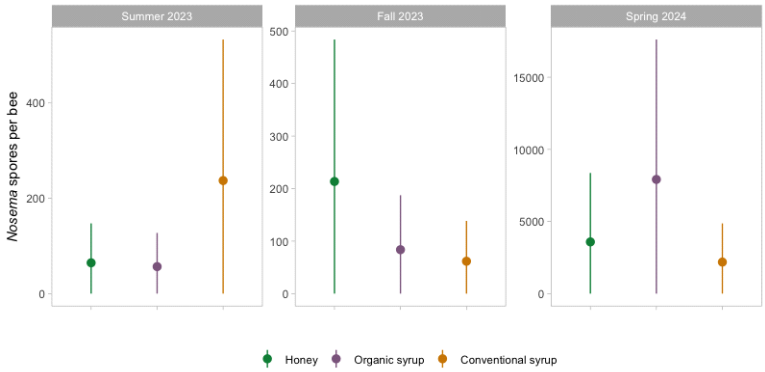

Figure 11. *Number of Nosema spp. spores per bee in summer, fall and spring, based on experimental groups (mean ± SE from model using untransformed data).*

DISCUSSION

In this project, we tested the impact of the type of feeding sugar provided to bee colonies in the fall on colony survival, health, and productivity. The first phase aimed to develop feeding techniques using honey and syrup, while the second phase focused on implementing and assessing the feasibility of different feeding techniques with two organic beekeepers. Our results show that it is possible to overwinter colonies with honey, but special attention must be given to ensure that colonies have enough honey, which should be at least nine frames of honey. Furthermore, no differences were observed between organic and conventional sugar in terms of survival, colony development, and pathogens.

Colony size, hive weight, and winter mortality

The high winter mortality observed in the groups fed summer honey (44%) and fall honey (67%) during the first phase of the project can be explained by the fact that eight frames of honey are insufficient to sustain the colonies until spring when resources return. Indeed, the weight of colonies fed summer or fall honey was 4.5 kg lower compared to colonies fed conventional or organic syrup immediately after the fall feeding. This weight difference also persisted in the spring, with colonies fed honey weighing on average 3.8 kg less than those fed conventional or organic syrup. It appears that the addition of organic syrup in the mixed group helped achieve a colony weight and size that allowed better survival over the winter compared to the group fed only fall honey.

Winter weight loss is similar across all experimental groups. On average, colonies lost 10.6 kg. This weight loss is comparable to the average weight loss of the last five years for CRSAD colonies overwintered outside, which was 9.3 ± 1.4 kg. The similar weight loss across all groups suggests that the mortality of colonies in the honey-fed groups could potentially have been avoided if the colonies had received more honey in the fall. The second phase of the project suggests that adding a ninth frame of honey improved the winter survival of honey-fed colonies, with 100% survival in the first beekeeper's colonies and 89% survival in the second beekeeper's colonies. The weight of two of the three colonies that died over the winter with the second beekeeper (12.2 and 11.3 kg) suggests that these colonies died from starvation. The weight of the third colony (21.6 kg) indicates that this colony died before winter, likely from an unknown cause, having consumed little to none of its stores. It is possible that overwintering the colonies with two supers, rather than one, could have reduced the risk of food shortages for the colonies during the winter in the honey-fed groups.

In this project, we chose to test overwintering colonies with a single brood box to verify its feasibility, knowing that this technique is recommended in Quebec, while also reducing economic implications for beekeepers. The results show that it is possible to overwinter colonies fed with conventional syrup, organic syrup, summer and fall honey, and a mixture of fall honey and syrup in Quebec. However, special attention should be paid to the weight of the colonies after feeding to ensure that sugar stores are sufficient. It is important to note that in the winter of 2023-2024, the average temperature across the province was 5.2°C higher than the reference average (ECCC 2024). However, winter temperatures vary from year to year, which affects the consumption rates of bees overwintered outside. Further research in different regions of Quebec will need to be conducted to determine the safe target weight for colonies in the fall to ensure winter survival, regardless of weather conditions.

Colony development and honey production

In the first phase of the project, the colonies were evaluated for bee population and brood development in May and then again in June. The average amount of brood was the same across all groups and increased between the two evaluations, regardless of the type of feeding received in the fall. The number of bees also increased for all groups between May and June. These results are typical of the development of a bee colony during the beekeeping season in Quebec, where the bee population doubles between May and June (CRAAQ 2020). The bee population was similar across all groups in May. However, in June, the honey-fed groups had a significantly lower adult bee population compared to the other groups. It would have been interesting to continue evaluations beyond June to determine if this difference persisted over time. It is important to note that the evaluation of the adult bee population in a colony is a visual estimate of the frame coverage by the bees. Additionally, the estimation of bee population can vary with weather conditions, as well as the time of day when evaluations are done, and results may vary considerably (Chabert et al. 2021, Dainat et al. 2020, Hernandez et al. 2020). The amount of brood is a variable that is not affected by weather conditions or the timing of the evaluation. Thus, the similar amount of brood between the groups in June suggests that an evaluation of the adult bee population in July might yield similar results across the groups.

Honey production during the 2022 beekeeping season, with an average of 38 kg per colony, indicates that foraging effort was the same across all colonies throughout the beekeeping season and that the average honey harvest per colony is higher than the Quebec average for 2022, which was 29.8 kg per colony (ISQ 2022). The results from the second phase, with the organic beekeepers, also show no impact of the type of feeding received in the fall on brood and adult bee populations in the spring. The absence of differences between organic and conventional sugar can be explained by the fact that both organic and conventional syrups contain the same amount of sucrose. Indeed, syrup consumption rates are similar when sugar concentrations are the same (Pridal et al. 2023). No difference was observed between summer and fall honey regarding the evaluated colony parameters. We can conclude that the type of feeding in the fall had no impact on colony development and production in the following season.

Pathogen development

Varroa infestation was not influenced by the type of feeding given in the fall and remained below the treatment threshold (MAPAQ 2024) in all groups, for both phases of the project. It was in the second phase of the project that the development of Nosema disease and the presence of viruses were evaluated. The average number of spores per bee remained below the economic damage threshold of one million (Bailey and Ball 1991), and no impact of the type of feeding was detected. Previous studies conducted in cages have demonstrated the impact of more complete nutrition of workers compared to a diet of sugar syrup on the quantity of Nosema spores per bee (Basualdo et al. 2014, Zheng et al. 2014). It is primarily the abundance and diversity of pollen, rather than the sugar source, that may improve bee tolerance to pathogens (Holt and Grozinger 2016). Since the colonies in this project had access to the same environmental pollen sources, this may explain why they did not show differences in infestation levels for varroa, Nosema spores, or viral load.

CONCLUSION AND LIMITATIONS OF THE STUDY

This project aimed to compare the survival, development, productivity, and major pathogens of bee colonies that received different sugar sources in the fall. Few differences were noted between the experimental groups for the evaluated variables. However, it is already known that, compared to feeding with honey, feeding bee colonies with sucrose syrup results in differences in fat content and the expression of over a hundred genes in the fat body of winter bees (Quilan et al. 2023, Wheeler and Robinson 2014). Thus, it appears that at the physiological level, the nutritional status differs between bees fed with syrup and those fed with honey. Our results show that at the colony level, there is little impact of the type of sugar given to bees in the fall on performance and disease resistance in the following season. However, the implications of the physiological differences highlighted in previous studies could be further investigated to clearly establish the ideal nutritional status for the honeybee before winter.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APIS

Original Research

In vitro assessment of Tropilaelaps mercedesae survival across different substrates

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ABSTRACT

Tropilaelaps spp. are parasitic mites that feed and reproduce within honey bee brood (*Apis spp.*), causing significant damage to *Apis mellifera* colonies. While traditionally believed to be incapable of surviving without brood, recent findings suggest *T. mercedesae* may persist during broodless periods. This study aimed to investigate the survival potential of T. mercedesae on various matrices in the absence of brood, with a focus on understanding possible mechanisms supporting its persistence and spread.

An in vitro survival experiment was conducted using *T. mercedesae* mites placed on three matrix types: live adult bees (*A. dorsata / A. mellifera*), decomposing pupae, and decomposing adults. Mite survival was monitored over time under controlled conditions. Survival duration was recorded and analysed using Log Rank tests and visualized with Kaplan Meier survival curves to identify differences in survival across matrices.

Mites survived for over 96 hours on live adult *A. mellifera*, over 144 hours on decomposing pupae, and up to 168 hours on decomposing adults. These findings demonstrate the mite's ability to survive for extended periods without access to live brood, challenging existing assumptions about its biology and survival limits.

This study highlights a potential survival mechanism of *T. mercedesae* outside brood environments, which may contribute to its spread through previously considered low-risk pathways, such as queen/package bee trade and used beekeeping equipment. These findings underscore the need for updated biosecurity protocols and further research into transmission dynamics and control strategies.

Keywords: *Apis mellifera, Tropilaelaps,* survival transmission, honey bee mites, broodless survival, parasitic mites, matrix-dependent viability.

INTRODUCTION

Western honey bee (*Apis mellifera*) queens, packages and colonies are frequently transported on a local, national, and international level as an agricultural pollinator and for the production of bee hive products such as honey, wax and propolis. This movement of honey bees has allowed for the global transmission and spread of multiple honey bee pests and pathogens such as *Varroa destructor*, small hive beetle (*Aethina tumida*) and American foulbrood (*Paenibacillus larvae*) (Neumann & Ellis, 2008; Roberts & Anderson, 2015; Papić et al. 2021). The ectoparasites *V. destructor* and *Tropilaelaps spp.* have jumped species from their natural honey bee hosts Apis cerana and Apis dorsata respectively to parasitise all Apis species of honey bee (Oldroyd, 1999; de Guzman et al. 2017). Both mites have spread outside of the ranges of their natural hosts, with *V. destructor* now being found on every continent and in almost every country where *A. mellifera* is present (Traynor et al. 2020; Owen et al. 2021). Currently the spread of *Tropilaelaps spp.* is not fully verified and until recently it was believed to be restricted to Asia (Chantawannakul et al. 2015). However, the presence of *T. mercedesae* has recently been confirmed in colonies of A. mellifera in the Krasnador and Rostov-on-don regions of Russia and the Samegrelo-Zemo Svaneti region of Georgia, thus confirming its presence in Europe for the first time (Brandorf et al. 2024; Janashia et al. 2024; Namin et al. 2024).

V. destructor causes physical damage to honey bee brood and vectors viruses during feeding, causing significant colony losses and is considered a major threat to global beekeeping (Rosenkranz et al. 2010). *Tropilaelaps spp.* are similar to *V. destructor* in that they primarily reproduce within sealed brood cells. Of the

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four know species of *Tropilaelaps* (*T. mercedesae, T. clareae, T. koenigerum and T. thaii*) *T. mercedesae* is the most widespread and is regarded as a more damaging parasite of *A. mellifera* than *V. destructor* (Anderson & Morgan, 2007). During feeding *Varroa* mites will open one or two large wounds on the brood to facilitate communal feeding of mites present within the sealed cell (Kanbar & Engels, 2005). In contrast *T. mercedesae* feed on both pre and post capped stages of brood, utilising multiple feeding sites, which can then go on to form wounds and deformities in the adult bee causing higher brood mortality than *V. destructor* infestations (Phokasem et al. 2019). Both *V. destructor* and *T. mercedesae* have been shown to vector viruses when they feed (Dainat et al. 2008; MinOo et al. 2018). When *T. mercedesae* vector deformed wing virus (DWV) there is a significant reduction in the longevity and emergence weight of parasitised hosts, plus an increase to the level of DWV load and associated clinical symptoms (Khongphinitbunjong et al. 2016). *T. mercedesae* are a major vector of honey bee pathogens and Turong et al. (2021) demonstrated that 100% of *T. mercedesae* mites they examined harboured DWV compared to only 81.8% of *V. destructor* mites examined in the same study. Israeli acute paralysis virus (IAPV) (47.4%), sac brood virus (SBV) and *Ascosphaera apis* (31.6%) were also prevalent honey bee pathogens detected in *T. mercedesae* (Turong et al. (2021). *T. mercedesae* are known to reproduce more quickly than *V. destructor* (Buawangpong et al. 2015) and unmated females have the ability to reproduce via deuterotokous parthenogenesis, producing both male and female offspring (de Guzman et al. 2018). The smaller size, increased mobility and shorter phoretic phase of *Tropilaelaps spp.*, coupled with the similarities between the visual symptoms of infestation, makes their detection and management more difficult than that of *V. destructor* (Pettis et al. 2013; Gill et al. 2024). To combat the high level of colony mortality caused by *Tropilaelaps spp. A. mellifera* colonies kept in infested areas require continual prophylactic treatment with miticides (Rinderer et al. 1994).

Understanding the mechanisms by which *Tropilaelaps spp.* may be transmitted is crucial to controlling their global spread. There is conflicting evidence as to whether *Tropilaelaps spp.* can survive and feed on adult bees. Rinderer et al. (1994) reported that *Tropilaelaps spp.* did not feed on adult *A. mellifera* and were therefore unable to survive for longer than 3 days without the presence of brood. However, possible feeding of *Tropilaelaps* mites at the soft membranes of the wing axillaries was reported by de Guzman et al. (2017). Equally under laboratory conditions Koeniger & Muzaffar (1988) observed *Tropilaelaps* survive for more than 5 days on *A. mellifera* pupae. Pettis & Chaimanee (2019) also observed *T. mercedesae* survive on *A. mellifera* and *A. cerana* larvae for 9-10 days and 5 days respectively under laboratory conditions. Less is known about the survival of *Tropilaelaps spp.* on their natural hosts *A. dorsata*. However, during migration *A. dorsata* colonies will 'bivouac' and not produce comb or brood for several weeks at a time (Robinson, 2012) with *Tropilaelaps* mites surviving by some unknown mechanism.

Beekeeping equipment and hive products pose an additional transmission route, and the World Organisation for Animal Health (OIE) recommends restricting the trade of honey bee products which are produced in colonies infested by *Tropilaelaps* mites (OIE, 2024). However, only limited research has been undertaken to assess the survival of *Tropilaelaps* mites on beekeeping equipment and hive products. Khongphinitbunjong et al. (2019) found that *T. mercedesae* were able to survive for up to three days in dry pollen and up to six days in honeycomb and Pettis & Chaimanee (2019) found that *Tropilaelaps* mites did not survive for longer than 3 days on honey/pollen, sugar candy and royal jelly.

This study set out to examine the survival of *T. mercedesae* on a range of matrices that represent transmission scenarios that might occur during the movement of honey bees (*A. mellifera* and *A. dorsata*), beekeeping equipment and hive products.

MATERIAL AND METHODS.

Study area and duration: The study was undertaken at Chaing Mai University, Thailand in July 2024.

Mite collection.

Adult mites were collected from colonies located at Chiang Mai University. Brood frames from colonies where phoretic mites were observed were selected from 10 colonies. Sealed brood was uncapped using forceps and mites were collected either with an entomological aspirator (pooter) or a slightly moist fine tipped paintbrush. Mites could be encouraged to leave the brood cells by blowing over the uncapped cells or by tapping the side of the brood frame on a solid surface. Mites were visually examined and confirmed as *T. mercedesae* and pooled in a sealable plastic container (350 ml) with freshly obtained 5th instar *A. mellifera* larvae prior to being individually transferred to treatment containers.

Survival study.

Sealable plastic containers (350 ml) were prepared with matrices and three replicates were used in each treatment group. The treatment groups comprised of

- An empty container (control),
- Five *A. dorsata* adults collected from a wild colony at Chiang Mai University,
- Five *A. mellifera* pupae at the pink-eyed pupal stage,
- Five newly emerged *A. mellifera* adults,

- Five newly emerged freshly euthanised *A. mellifera* adults.

A sugar cube was glued to the side of containers containing live bees to act as a feeder and a ventilation hole covered with 0.025 mm nylon mesh was also added. *A. mellifera* pupae at the pink eyed development stage and adults were collected from frames and colonies that were randomly selected from ten colonies at Chiang Mai University. Pupae and adult bees were pooled in sealed containers before being assigned to replicate containers. Thirty *T. mercedesae* mites (n = 90 mites per treatment) were carefully individually introduced to treatment containers with a fine-tipped paintbrush. Containers were then sealed with parafilm and randomly placed in an incubator at 34 ^{O}C and 60% R.H. to limit possible spatial effects. *T. mercedesae* mortality was assessed every 24 hours until all the mites were dead. Mites were deemed dead if they were immobile, could not be encouraged to move when gently stimulated with a fine tipped paintbrush and were observed to have their legs curled up beneath them. It should be noted that the pink-eyed pupae and dead A. mellifera adults were not replaced and decomposed throughout the trials.

STATISTICAL ANALYSIS.

All statistical analysis was performed using R Studio (Antoch, 2008). Since the data were not normally distributed non-parametric tests were used to assess *T. mercedesae* survival between different treatments. Treatments were compared using Log Rank tests and visualized with Kaplan Meier survival curves (Fig 1.). The level of significance

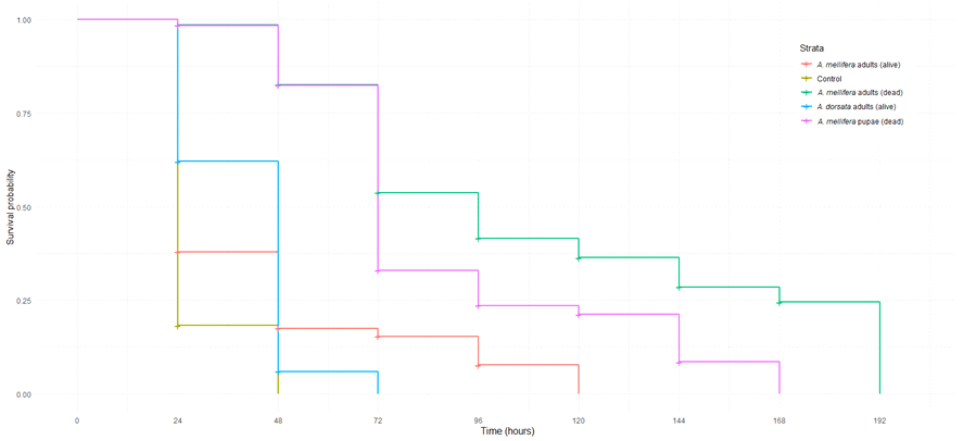

Figure 1. Kaplan-Meier survival curve of *T. mercedesae* mites across five treatments: live *A. mellifera* adults, dead *A. mellifera* adults, dead *A. mellifera* pupae, live *A. dorsata* adults, and empty control ($p < 0.0001$; n = 30 mites per treatment).

was $p < 0.0001$.

RESULTS.

The mean survival time of *T. mercedesae* mites was 13.3 hours in the control treatment, 15 hours in the treatment group containing live *A. mellifera* adults, 21.8 hours in the treatment group containing live *A. dorsata* adults, 54.6 hours in the treatment group containing dead *A. mellifera* pupae and 55.6 hours in the treatment group containing dead *A. mellifera* adults. The final *T. mercedesae* died after 168 hours in the treatment group containing dead *A.*

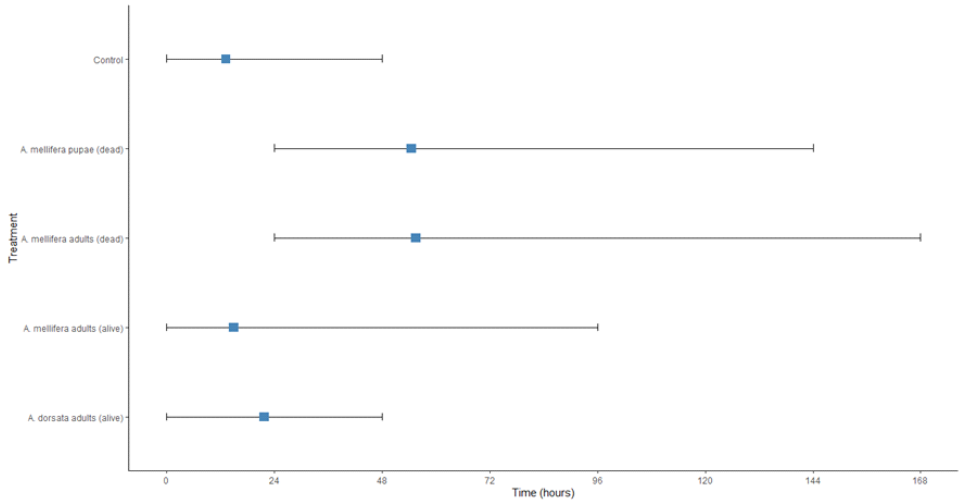

Figure 2. Diagram showing the survival times of *T. mercedesae* with blue squares showing mean survival time.

mellifera adults and survived for significantly longer than mites in the other treatment groups (Fig 2.).

A log rank test (Wilcoxon - Breslow) determined that the survival distributions between the differing treatments were statistically significantly ($\chi2(4) = 478.71$, $p < 0.0001$). Pairwise comparison using the Log Rank test showed that there was a statistically significant difference in survival distributions between all treatment groups apart from live *A.*

	Live *A. mellifera* adults	Control	Dead *A. mellifera* adults	Live *A. dorsata* adults
Control	$p<0.001$	-	-	-
Dead *A. mellifera* adults	$p<0.0001$	$p<0.0001$	-	-
Live *A. dorsata* adults	$p = 0.0776$	$p<0.0001$	$p<0.0001$	-
Dead *A. mellifera* pupae	$p<0.0001$	$p<0.0001$	$p<0.01$	$p<0.0001$

Table 1. *p-values* from pairwise comparison using the Log Rank test.

dorsata adults vs live *A. mellifera* adults and p values are shown in Table 1.

DISCUSSION.

T. mercedesae mites experienced high mortality in the control group, with 90% of mites not surviving for more than 24 hours in the treatment which consisted of an empty container with no food source, and these results corroborate those of Koeniger & Muzaffar (1988) and Pettis & Chaimanee (2019). However, *T. mercedesae* survived for longer than previously observed in other studies in the treatment groups containing dead *A. mellifera* adults, dead *A. mellifera* pupae and live *A. mellifera* adults (Koeniger & Muzaffar, 1988; Pettis & Chaimanee, 2019). De Guzman et al. (2017) observed the apparent feeding of *T. mercedesae* on adult *A. mellifera* where the mites mouthparts appeared to pierce the soft membrane of the wing axillaries and this was accompanied by a pumping or pulsating motion of the opisthosoma. Feeding on live adult bees was not observed during this study, however a small proportion of mites (~10%) were able to survive for significantly longer on live adult *A. mellifera* than mites in the control group, which could suggest that these mites were able to feed on the adult bees. Another possibility is that *Tropilaelaps*, like Braula coeca, may receive food during trophallaxis between bees. Koeniger & Muzaffar (1988) observed the "conspicuous reactions of mites" during trophallaxis between *A. dorsata* and suggested that mite feeding might be taking place. This theory might also explain why the survival distribution between the live *A. mellifera* and live *A. dorsata* treatments was not statistically significantly different, although both groups did survive for significantly longer than the control group. Mites did not survive for longer than 48 hours in the treatment group containing live *A. dorsata* adults, which is again in line with the findings of Koeniger & Muzaffar (1988). Koeniger & Muzaffar (1988) observed injuries and lost appendages on *T. mercedesae* in their A. dorsata treatment groups, whereas the mites in other treatment groups were uninjured. The dead mites in this study were not examined for injuries, however *T. mercedesae* survived for up to 48 hours longer on live *A. mellifera* when compared to live *A. dorsata* and this may be due in part to the parasite host relationship and grooming behaviours of *A. dorsata*.

T. mercedesae mites survived for up to 168 hours in the treatment group containing dead *A. mellifera* pupae and up to 192 hours in the treatment group containing dead *A. mellifera* adults and it should be noted that dead pupae and adults were not replaced throughout the trial but instead decomposed. It is unclear if Pettis & Chaimanee (2019) replenished the *A. mellifera* larvae used in their study to achieve mite survival of 9-10 days (216 – 240 hours) or allowed them to decompose, however Koeniger & Muzaffar (1988) replaced *A. mellifera* pupae every 3 days and observed mite survival of up to 120 hours. Mites were observed spending the majority of their time on the dead pupae and dead bees and appeared to feed on the exudate created during decomposition. This finding suggests that *T. mercedesae* could be transported in scenarios where live *A. mellifera* brood and adults are not present, such as in used beekeeping equipment containing decaying brood and bees or in queen shipments and packages where bees have died during the caging / packaging and transportation process. Feral colonies have been transported to countries outside of the current distribution of *T. mercedesae* on shipping containers, boats, and airplanes (Heersink et al. 2016) and it has been assumed that broodless colonies and colonies that have died at sea do not pose a transmission risk for *Tropilaelaps spp.* (EFSA, 2013). Our findings suggest that aside from the obvious transmission root of brood within feral *Apis spp.* colonies *T. mercedesae* could survive in a broodless scenario on live adult bees or on decaying brood or adult bees that have died during transportation.

T. mercedesae can survive during broodless periods in *A. mellifera* colonies (Brandorf et al. 2024) and during broodless periods on their natural hosts *A. dorsata* during bivouacking (Robinson, 2012), but the mechanisms by which they are able to do so are little researched and not understood. This study has demonstrated *T. mercedesae* ability to survive on decaying *A. mellifera* brood and bees and live newly emerged *A. mellifera* bees, and these findings coupled with that of Robinson (2012) and Brandorf et al. (2024) demonstrate that the transmissibility of *T. mercedesae* is higher than previously evaluated. Given the increasing global spread of *T. mercedesae* and its recent incursion into regions bordering areas which produce significant amounts of honey each year such as Ukraine and Türkiye (Popescu et al. 2024) and given that political and socioeconomic factors in these regions could impact

successful monitoring for *Tropilaelaps spp*. it is important that beekeepers and authorities globally are vigilant to the threat that the transmission of *Tropilaelaps* poses.

CONCLUSION.

In conclusion, this study demonstrates that *T. mercedesae* can survive significantly longer on decaying *A. mellifera* brood and adults than previously documented, with survival of up to 192 hours. A small proportion of mites also persisted on live adult bees, suggesting possible feeding or trophallactic interactions. These findings highlight that *T. mercedesae* may be transmitted not only through brood but also via dead bees or brood in hive products and equipment. Such survival potential in broodless or decomposing environments broadens known transmission pathways and underscores the need for heightened biosecurity measures. This expanded understanding of *T. mercedesae* survivability should inform global management and trade policies to curb its spread.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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APIS

Conference Abstract/Poster/Slides

When the Patient Is the Control: A Pragmatic Framework for Early-Phase Evaluation of Complex, Low-Risk Clinical Interventions

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ABSTRACT

Background

Parallel control groups, particularly within randomized controlled trials, are widely regarded as the gold standard of clinical evidence. While indispensable for confirmatory and high-risk investigations, this paradigm may be ill-suited for early-phase evaluation of complex, multi-component, and low-risk interventions operating within real-world clinical systems.

Objective

This paper proposes a pragmatic methodological framework for evaluating such interventions without reliance on parallel control groups, while maintaining scientific rigor and ethical proportionality.

Methods

We synthesize methodological principles from longitudinal within-subject designs, complex systems theory, and risk-based research ethics. The framework rests on three core pillars: (1) the use of patients as their own controls through stable baseline and pre–post comparisons, (2) black-box, output-oriented validation prioritizing reproducible clinical outcomes over early mechanistic isolation, and (3) safety-first justification grounded in the absence of known adverse effects and low iatrogenic risk.

Results

We demonstrate that, under clearly defined conditions, control-free and within-subject designs can provide valid exploratory evidence, address common methodological criticisms—including placebo effects, natural disease course, and regression to the mean—and serve as a coherent first step in a phased research trajectory.

Conclusion

The absence of a parallel control group does not imply the absence of methodological control. When applied proportionately and transparently, pragmatic, control-free frameworks can generate meaningful, reproducible clinical insights while guiding subsequent mechanistic and controlled investigations. This approach supports methodological pluralism and aligns evidentiary standards with intervention complexity, risk profile, and research objectives.

Keywords: evidence-based medicine; naturopathy; placebo effect; self-controlled study design; randomized controlled trial; pre–post analysis

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1. INTRODUCTION

Randomized controlled trials (RCTs) are widely regarded as the gold standard of clinical evidence. Their strength lies in isolating causal effects by minimizing bias through randomization and parallel control groups. [1,2] However, this methodological framework was developed primarily for single-component, pharmacological, and higher-risk interventions. When applied uncritically to complex, multi-component, and low-risk interventions, the requirement for a classical control group may become not only impractical but methodologically misleading. [6,7]

In real-world clinical practice, many interventions operate as integrated systems rather than as isolated variables. Behavioral, rehabilitative, educational,

and certain non-invasive therapeutic approaches exert their effects through interacting biological, psychological, and contextual mechanisms. [6,7] In such settings, strict separation of components or the construction of an inert placebo condition may be artificial and may fail to reflect the actual conditions under which the intervention is intended to function. [6]

The aim of this paper is not to argue against controlled research, but to propose a pragmatic methodological framework for early-phase and exploratory evaluation of complex, low-risk clinical interventions. Under well-defined conditions, this framework offers a coherent and scientifically defensible alternative to parallel control group designs, without precluding subsequent confirmatory research. [8,9]

2. The Proposed Pragmatic Control-Free Evaluation Framework (Overview)

The framework proposed in this paper is designed for the early-phase evaluation of complex, low-risk clinical interventions operating within real-world clinical systems. Rather than relying on parallel control groups, methodological control is achieved through the structured integration of longitudinal observation, system-level outcome validation, and explicit risk-based justification. [6,8]

The framework consists of three interdependent pillars:

(1) Within-subject control through stable baselines and longitudinal comparison, whereby each patient serves as their own reference point over time. Control is embedded within the temporal structure of observation rather than between-group allocation.

(2) Black-box, output-oriented validation, in which interventions are evaluated as functional systems based on reproducible clinical outcomes, without requiring early mechanistic isolation of individual components.

(3) Safety-first proportionality, whereby the absence of known serious adverse effects and low iatrogenic risk justifies greater methodological flexibility in exploratory research phases. [6,12,13]

These elements function not as independent alternatives but as a coherent evaluative system. Baseline stability constrains natural fluctuation, reproducible outputs strengthen inferential confidence, and safety considerations define the appropriate evidentiary threshold. [10,12] This perspective is consistent with broader methodological guidance emphasizing the need to adapt evaluative strategies to the complexity and contextual embeddedness of clinical interventions.

The framework is explicitly intended for exploratory, pragmatic, and hypothesis-generating research. It is positioned as an initial phase within a broader research trajectory that may subsequently incorporate mechanistic studies and parallel-group controlled trials where appropriate. [8,9]

2.1 Framework-at-a-Glance: Structural Summary

For clarity, the proposed framework can be summarized as a sequence of evaluative steps that together approximate key functions of a parallel control group through structural and temporal control mechanisms:

Step 1. Risk Profiling of the Intervention

The intervention is characterized with respect to invasiveness, reversibility, and known adverse effects. Only low-risk, non-invasive interventions qualify for control-free exploratory evaluation. [12,13]

Step 2. Baseline Stability Assessment

A sufficiently long and well-documented baseline period is established to confirm temporal stability of the target condition prior to intervention. [10]

Step 3. Within-Subject Longitudinal Observation

Each participant serves as their own control through repeated measurements over time, embedding control within the temporal structure rather than between-group comparison. [3-5]

Step 4. Output-Oriented (Black-Box) Evaluation

The intervention is assessed as a functional system based on reproducible, clinically meaningful outcomes, without requiring early isolation of individual causal components. [6,7]

Step 5. Reproducibility Across Individuals or Contexts

Consistent patterns of change across multiple participants, settings, or implementations are used to strengthen inferential confidence. [14]

Step 6. Safety Monitoring and Escalation Criteria

Continuous monitoring for adverse effects is maintained, and predefined criteria guide progression toward mechanistic studies or controlled trials where warranted. [12,13]

Together, these steps constitute a coherent, proportionate evaluative pathway for early-phase investigation. Methodological control is achieved through temporal structure, reproducibility, and risk-based justification rather than through parallel group allocation.

2.2 Positioning the Framework Among Existing Clinical Research Designs

The methodological framework proposed here does not seek to replace established experimental designs but to occupy a specific and clearly delimited position within the broader landscape of clinical research methodologies. Its primary function is exploratory rather than confirmatory, and its evidentiary claims are intentionally constrained by scope, risk profile, and research objective.

Randomized controlled trials (RCTs) remain the preferred design for confirmatory efficacy testing, particularly when interventions carry significant risk, produce irreversible effects, or require precise causal attribution for regulatory or guideline purposes. [1,2] The present framework does not challenge this role and should not be interpreted as an alternative pathway to definitive efficacy claims.

N-of-1 trials similarly rely on within-subject comparison but typically involve randomized or counterbalanced alternation between intervention and control conditions, often with the goal of individual-level treatment optimization. [3,4] In contrast, the proposed framework does not require treatment withdrawal, crossover, or blinding, and is oriented toward identifying reproducible system-level effects across individuals rather than optimizing decisions for a single patient.

Single-case experimental designs (SCEDs) and other time-series methodologies emphasize controlled phase manipulation and formal interruption of exposure. [5] While sharing an emphasis on longitudinal structure, the present framework adopts a more pragmatic stance, prioritizing feasibility and ecological validity over strict phase control when interventions are embedded in real-world clinical systems.

Observational cohort studies typically aim to describe associations or natural histories at the population level. By contrast, the framework proposed here embeds intentional intervention and prospective outcome tracking, while achieving methodological control through temporal structure and baseline stability rather than through group-level comparison.

In summary, this framework occupies an intermediate methodological space: more structured and inferentially constrained than uncontrolled observation, yet more flexible and proportionate than parallel-group experimental designs. It is explicitly designed for early-phase evaluation of complex, low-risk interventions where mechanistic isolation or inert placebo conditions are impractical, premature, or ethically unnecessary. [6,8]

By clearly delineating its intended scope and limitations, the framework supports methodological pluralism while preserving the distinction between exploratory evidence generation and confirmatory causal inference.

2.3 Minimal Analytical Considerations in Control-Free, Within-Subject Designs

The analytical approach appropriate for control-free, within-subject evaluations differs fundamentally from that used in parallel-group confirmatory trials. The primary objective is not precise estimation of population-level effect sizes but the identification of stable, reproducible patterns of change temporally associated with intervention exposure. [15]

Accordingly, emphasis is placed on longitudinal structure rather than cross-sectional comparison. Repeated measurements over time allow assessment of baseline stability, trajectory change, and durability of observed effects. [10] Analyses that model within-subject trends—such as repeated-measures approaches, mixed-effects models, or simple slope comparisons—are generally more informative than single pre–post contrasts.

Visual inspection of individual trajectories, combined with quantitative summaries, plays an important role in distinguishing systematic change from random fluctuation. Consistency of direction, timing, and persistence of change across participants is prioritized over statistical significance testing based on group-level null hypotheses. [15]

The framework explicitly de-emphasizes reliance on isolated p-values derived from single time-point comparisons. Instead, inferential confidence is strengthened through convergence of multiple indicators, including baseline stability, temporal alignment with intervention onset, reproducibility across individuals or settings, and maintenance of effects over time. [14]

Where appropriate, sensitivity analyses may be used to examine the robustness of observed patterns to alternative baseline definitions or analytic assumptions. However, analytical complexity should remain proportionate to study aims and data structure, avoiding overfitting or false precision in exploratory contexts. [15]

These analytical principles are intended to support transparent, proportionate interpretation of exploratory findings rather than to substitute for the rigorous statistical requirements of confirmatory trials.

2.4 Illustrative Application of the Framework (Hypothetical Example)

The following example is intended solely to illustrate the logical application of the proposed framework and does not constitute empirical evidence or a clinical recommendation.

As an illustrative case, consider an intervention situated within an apitherapeutic context, in which the core component is the regular oral consumption of royal jelly. According to publicly available regulatory classifications, royal jelly is generally categorized in most jurisdictions as a food product or dietary supplement rather than as a medicinal product. Its consumption is not associated with known serious adverse effects, and the available evidence suggests a low iatrogenic risk profile.

On this basis, a non-invasive intervention involving royal jelly consumption is consistent with the eligibility criteria articulated in the present framework for control-free, exploratory evaluation. Provided that all additional methodological conditions are met—including the establishment of a sufficiently long and well-documented baseline period, longitudinal within-subject observation, and the predefinition and reproducible measurement of clinically relevant outcomes—a royal jelly–based intervention may, under the present terminology, be examined using a control-free, within-subject evaluative design.

In this context, the primary objective of evaluation is not the early isolation of specific biochemical or pharmacological mechanisms, but rather the assessment of whether the intervention, considered as a functional system within a defined clinical context, produces stable and reproducible changes in relevant outcomes. The favorable safety profile, reversibility, and low risk associated with the intervention justify the application of a pragmatic, output-oriented approach in the initial evaluative phase, while allowing for subsequent progression to more mechanistic or controlled study designs should the findings warrant further investigation.

3. THE PATIENT AS THEIR OWN CONTROL: WITHIN-SUBJECT DESIGNS

In longitudinal clinical research, participants may serve as their own controls through within-subject (pre–post) comparisons. This approach is well established in multiple research domains, including psychology, rehabilitation medicine, and physiology, yet it is often undervalued in debates dominated by parallel-group RCT paradigms. [3,5]

The methodological validity of within-subject control relies on several key conditions. First, the baseline state must demonstrate temporal stability prior to intervention. Second, the observed change must occur in temporal association with the intervention. Third, similar patterns of change must be observed across multiple participants. When these conditions are met, the likelihood that the observed effect is attributable to random fluctuation alone is substantially reduced. [5,10]

Within-subject designs offer several methodological advantages. By controlling for inter-individual variability, they increase sensitivity to change, particularly in heterogeneous populations. They are also ethically advantageous when withholding an intervention is undesirable or impractical. Importantly, the absence of a parallel control group does not imply the absence of control; rather, control is embedded within the temporal structure of the observation. [3]

This approach does not claim to eliminate all sources of bias, nor does it replace randomized designs in confirmatory research. Instead, it provides a legitimate evidentiary framework for early-stage investigation, hypothesis generation, and real-world validation of complex interventions. [6]

4. BLACK-BOX VALIDATION IN COMPLEX SYSTEMS

Complex biological and psychosocial systems are characterized by nonlinearity, feedback loops, and emergent behavior. In such systems, the relationship between input and output cannot always be decomposed into a single dominant causal pathway. [6,7] Attempting full mechanistic isolation at an early investigative stage may therefore obscure, rather than clarify, functional effectiveness. [6]

A black-box approach evaluates an intervention as a functional system, focusing on reproducible outputs rather than complete mechanistic decomposition. This methodology is widely accepted in engineering, systems science, and applied research, where functionality and reliability are often established prior to full explanatory modeling. [7]

In clinical research, black-box validation does not imply disregard for mechanisms. Rather, it reflects a phase-sensitive research strategy in which consistent, clinically meaningful outcomes provide the empirical foundation upon which mechanistic hypotheses can later be built. [6,8] Reproducible effects across settings, populations, or implementations strengthen the inference that the intervention operates as a coherent system, even if the relative contribution of individual components remains uncertain. [6,7]

By prioritizing output-first validation, researchers can identify interventions worthy of further mechanistic and controlled investigation, thereby allocating resources more efficiently and aligning methodology with the nature of the system under study without presupposing which components or pathways are responsible for observed effects. [8]

5. SAFETY AND DEFENSIBILITY AS METHODOLOGICAL FACTORS

Risk is a central determinant of appropriate research methodology. Invasive or high-risk interventions justifiably demand stringent evidentiary thresholds before implementation. [12,13] Conversely, non-invasive interventions with no known serious adverse effects occupy a fundamentally different ethical and methodological space. In such contexts, the primary objectives of early-phase research are often to establish feasibility, observe potential benefit, and confirm safety rather than to deliver definitive causal proof. [12]

When an intervention is characterized by the absence of known side effects, low iatrogenic risk, and reversibility, exploratory study designs without parallel control groups may be methodologically justified, particularly in early-phase research. [13] In such contexts, the primary objectives are to establish feasibility, observe potential benefit, and confirm safety rather than to deliver definitive causal proof.

Safety considerations therefore function not only as ethical constraints but also as methodological enablers. Lower-risk profiles permit greater flexibility in study design, including the use of observational, within-subject, and pragmatic approaches, provided that transparency and proportional interpretation are maintained. [12,13]

This principle of proportionality between risk and evidentiary burden is already embedded in phase-based clinical research frameworks. Early-phase studies are not intended to provide definitive efficacy claims but to generate signals that justify or refute further investigation under more controlled conditions. [12]

Importantly, acknowledging safety as a methodological factor does not imply reduced rigor. Rather, it reflects alignment between the level of methodological control required and the potential consequences of error. In low-risk settings, insisting on maximal methodological constraint at the earliest stages may impede learning without providing commensurate gains in patient protection or scientific validity. [13]

Within the proposed framework, safety monitoring remains mandatory regardless of perceived risk. The absence of adverse effects constitutes a relevant empirical finding and contributes to the overall evidentiary assessment, informing decisions about escalation to mechanistic studies or parallel-group trials where appropriate. [12,13]

6. COMMON CRITICISMS OF CONTROL-FREE DESIGNS AND METHODOLOGICAL RESPONSES

The absence of a parallel control group in exploratory clinical studies frequently invites a set of recurring methodological criticisms. These concerns are legitimate and must be addressed explicitly. However, their presence does not automatically invalidate within-subject or pragmatic designs when such approaches are applied within clearly defined boundaries. [1,2].

6.1 Placebo Effects

One of the most common objections is that observed improvements may be attributable to placebo effects rather than to the intervention itself. Placebo responses are well documented and can influence outcomes across a wide range of clinical contexts, including randomized controlled trials. [11]

Importantly, the presence of placebo effects does not uniquely undermine within-subject designs. Expectancy and contextual influences are not eliminated by randomization alone and may contribute meaningfully to observed outcomes in both controlled and uncontrolled settings. [11]

In complex, multi-component interventions, the distinction between “specific” and “non-specific” effects may be conceptually artificial. Engagement, expectation, and therapeutic context are often integral components of how such interventions function in real-world clinical systems. [6,7]

From a pragmatic perspective, the relevant question is therefore not whether placebo mechanisms contribute, but whether the intervention reliably produces clinically meaningful outcomes without causing harm. Stable baselines and repeated observations further reduce the likelihood that short-lived expectancy effects alone account for sustained or reproducible changes across individuals. [10]

6.2 Natural Course of the Condition

Another common concern is that observed improvements may reflect the natural progression or spontaneous remission of the condition rather than an intervention-associated effect. This possibility must be considered carefully, particularly in conditions characterized by fluctuating or self-limiting trajectories. [10]

Within-subject designs address this concern by emphasizing temporal structure. When a condition demonstrates stability or chronicity prior to intervention, and improvement consistently coincides with intervention onset across multiple cases, the plausibility of spontaneous change as the sole explanation diminishes. [10]

While such temporal association does not establish definitive causality, it supports the presence of a meaningful intervention-related signal that warrants further investigation under more controlled conditions. [8]

6.3 Regression to the Mean

Regression to the mean is a statistical phenomenon that can create the appearance of improvement when participants are selected based on extreme values, a limitation that is especially relevant in studies lacking randomization. [10]

As noted above, regression effects are most pronounced when measurements are taken at a single extreme time point. Longitudinal observation with repeated measurements substantially mitigates this risk by allowing confirmation of baseline stability prior to intervention exposure. [10]

When improvements persist beyond initial post-intervention assessments and are observed consistently across multiple individuals, regression to the mean alone becomes an insufficient explanation for the observed patterns of change. [14]

6.4 Selection Bias and Generalizability

Self-selection and non-random sampling can limit generalizability, a limitation that is openly acknowledged in exploratory and pragmatic study designs. [14]

The primary aim of such studies is not population-level inference but the identification of reproducible patterns,

feasibility, and safety under real-world conditions. In this context, heterogeneous, non-idealized populations may enhance rather than undermine external validity. [8]

Repeated implementation across different settings or populations can function as a form of pragmatic replication, complementing—rather than replacing—later controlled studies designed for definitive causal inference. [6,9]

7. TRANSPARENCY, REPORTING, AND METHODOLOGICAL RIGOR

Exploratory and control-free study designs place particular responsibility on transparent reporting and explicit delineation of methodological boundaries. The flexibility afforded by pragmatic, within-subject approaches does not imply reduced rigor but instead shifts the emphasis toward clarity, reproducibility, and proportional interpretation of findings. [1,2]

Studies conducted within the proposed framework should clearly document the duration and stability of baseline observation, including justification for baseline length and evidence supporting temporal stability prior to intervention exposure. Transparent reporting of baseline characteristics is essential for assessing susceptibility to alternative explanations such as natural disease course or regression to the mean. [10]

Outcome measures, assessment frequency, and criteria for meaningful change should be specified a priori where feasible, even in exploratory contexts. Clear definition of outcomes supports interpretability and reduces the risk of selective reporting or post hoc inference. [1,9]

Longitudinal data presentation is strongly encouraged. Display of individual trajectories alongside summary trends allows independent assessment of temporal patterns, variability, and durability of observed effects and is particularly informative in within-subject designs. [15]

Selective reporting of isolated pre–post contrasts should be avoided. Instead, convergence across multiple indicators—such as baseline stability, temporal alignment with intervention onset, reproducibility across individuals or settings, and maintenance of effects over time—should guide interpretation. [14,15]

Safety monitoring and adverse event reporting are required regardless of perceived intervention risk. The absence of observed adverse effects constitutes a relevant empirical finding and should be explicitly reported rather than assumed. [12,13]

Authors should clearly acknowledge the exploratory nature of findings derived from control-free designs and refrain from definitive causal or efficacy claims. Limitations related to selection bias, generalizability, placebo effects, and alternative explanations should be explicitly addressed. [14]

Where exploratory findings suggest consistent and clinically meaningful benefit, the framework encourages predefinition of escalation criteria guiding progression toward mechanistic studies or parallel-group controlled trials. In this way, control-free evaluation functions not as an endpoint but as a structured entry point into a phased research trajectory. [6,8]

Through explicit reporting standards and proportional interpretation, methodological rigor can be preserved while allowing flexible, real-world evaluation of complex, low-risk clinical interventions. [1,2]

8. SCOPE, LIMITS, AND APPROPRIATE USE OF CONTROL-FREE FRAMEWORKS

Having outlined principles for application, transparency, and rigor, it is essential to define the boundaries within which the proposed framework remains appropriate. Clear articulation of scope and limits is a defining feature of defensible methodology and is particularly important for exploratory and control-free research approaches. [1,2]

The framework proposed here is not intended to replace randomized controlled trials, nor to serve as a universal evaluative strategy. Instead, it occupies a delimited position within a phased research trajectory, aligned with intervention complexity, risk profile, and research objectives. [6,8]

8.1 When Control-Free or Within-Subject Designs Are Appropriate

Control-free or within-subject designs may be methodologically justified when interventions are non-invasive, reversible, and associated with low iatrogenic risk. In such contexts, early-phase research aims are typically exploratory, pragmatic, or hypothesis-generating rather than confirmatory. [12,13]

Additional conditions supporting appropriateness include demonstrable baseline stability of the target condition prior to intervention, intervention complexity that renders component isolation impractical or artificial, and the absence of ethically acceptable inert placebo conditions. [6,7]

When these conditions are met, within-subject control, output-oriented evaluation, and safety-first proportionality together provide a coherent and defensible evidentiary framework for early-stage investigation. [8,12]

8.2 When Parallel Control Groups Are Necessary

Parallel control groups remain essential when interventions carry significant biological or psychological risk, when effects are irreversible, or when small effect sizes require precise causal attribution. In such cases, the potential consequences of error justify higher evidentiary thresholds and stricter methodological constraints. [12,13]

Similarly, regulatory approval, guideline development, or definitive efficacy claims require controlled designs capable of isolating causal effects with high internal validity. Under these circumstances, control-free approaches are insufficient and should be regarded solely as preliminary or hypothesis-generating steps. [1,2]

8.3 From Exploratory Evidence to Controlled Research

Exploratory studies conducted within the proposed framework should be understood as components of a broader research trajectory rather than as endpoints. Their primary functions are to establish safety, feasibility, and reproducible signals of potential benefit under real-world conditions. [8]

Findings generated through control-free evaluation can inform the design of subsequent mechanistic studies or controlled trials, including selection of outcome measures, identification of responsive populations, and determination of appropriate control conditions. [6,9]

By explicitly defining escalation criteria and maintaining proportional interpretation of early findings, the framework supports methodological continuity while preserving the distinction between exploratory evidence generation and confirmatory causal inference. [14]

CONCLUSION

The requirement for a parallel control group has become deeply ingrained in clinical research practice, often functioning as a methodological default rather than a context-sensitive choice. While randomized controlled trials remain indispensable for confirmatory research and for interventions associated with substantial risk, their uncritical application to all forms of clinical inquiry risks conflating rigor with rigidity.

This paper proposes a pragmatic methodological framework for the early-phase evaluation of complex, low-risk clinical interventions in which patients serve as their own controls. By integrating within-subject longitudinal observation, output-oriented black-box validation, and safety-first proportionality, the framework demonstrates that the absence of a parallel control group does not imply the absence of methodological control. Instead, control is achieved through temporal structure, reproducibility of outcomes, and explicit alignment between evidentiary demands and intervention risk.

Crucially, the framework does not reject mechanistic investigation or controlled research. Rather, it articulates a phased research logic in which reproducible, real-world clinical outcomes establish the empirical foundation upon which mechanistic understanding and confirmatory trials can later be built. In this way, exploratory evidence generation is positioned not as an endpoint, but as a structured and ethically proportionate entry point into a broader research trajectory.

By reframing control as a structural and contextual concept rather than a binary requirement, this approach supports methodological pluralism while preserving scientific accountability. It offers a defensible pathway for evaluating interventions that operate within complex human systems, where functionality, safety, and reproducibility may justifiably precede full mechanistic explanation.

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APIS

Conference Abstract/Poster/Slides

From the Hive to the Clinic: Clinical Case Studies Illustrating the Use of Propolis in Dentistry

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ABSTRACT

Background

Propolis has been increasingly investigated in dentistry due to its antimicrobial, anti-inflammatory, analgesic, and regenerative properties. Several clinical applications have been reported; however, detailed case-based documentation remains limited.

Objective

This article presents a series of clinical case studies originally introduced at an international conference and subsequently expanded to provide a comprehensive description suitable for a scientific readership. The aim is to illustrate the clinical use of propolis-based treatments across different dental disease groups.

Materials and Methods

Clinical cases from daily dental practice were retrospectively analyzed. The cases included patients aged between 14 and 75 years and covered five main clinical categories: endodontic infections, periodontal disease, deep carious lesions, prosthetic-related fungal infections, and post-extraction wound management. Propolis was applied mainly as a 5% solution, either alone or in combination with other biomaterials such as calcium hydroxide, hydroxyapatite, or calcium alginate. Clinical and radiographic follow-up ranged from one week to one month, depending on the indication.

Results

In endodontic cases, propolis demonstrated effective antibacterial and anti-inflammatory activity, contributing to pain reduction and periapical healing. Periodontal applications showed visible improvement in gingival inflammation within one week. In deep carious lesions, propolis-supported treatments promoted pulp vitality and dentin bridge formation after one month. Prosthetic cases involving Candida albicans infection exhibited marked mucosal healing within 7–15 days. Post-extraction use of propolis combined with calcium alginate supported normal wound healing and helped prevent alveolar osteitis**.**

Conclusions

The presented case studies suggest that propolis is a safe and versatile adjunctive agent in various dental treatments. When standardized and properly applied, propolis may support infection control, inflammation reduction, and tissue regeneration. Further controlled clinical studies are warranted to confirm these observations and to establish standardized treatment protocols.

Keywords: Propolis; apitherapy; dentistry; endodontic infections; periodontal disease; deep carious lesions; oral wound healing; clinical case studies

INTRODUCTION

Propolis has a long history of medicinal use and has been extensively described in the apitherapy literature for its immunomodulatory, antimicrobial, anti-inflammatory, and regenerative properties. Foundational works have documented its biological activity and clinical relevance across a wide range of medical applications (Harnaj, 1982; Chung, 2015).

In the field of dentistry, propolis has been specifically investigated for oral and periodontal applications, including caries management, endodontic disinfection,

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periodontal therapy, and mucosal healing. These applications have been systematically reviewed and addressed in dedicated monographs and review articles focusing on oral healthcare (Fearnley & Wander, 2010; Zulhendri et al., 2021). Within this historical and scientific context, the present article presents a series of clinically documented cases that visually and clinically illustrate therapeutic effects of propolis that have been repeatedly described in the scientific and apitherapy literature over several decades.

Propolis exerts a broad spectrum of biological activities that are particularly relevant in dentistry, where microbial infection, inflammation, and tissue regeneration are closely interconnected. Its antimicrobial properties target oral biofilms and pathogenic microorganisms, while its anti-inflammatory and immune-modulatory effects support tissue healing and regeneration (Zulhendri et al., 2021). These combined mechanisms make propolis applicable across a wide range of dental conditions, including caries, endodontic infections, periodontal diseases, pulp-related pathologies, and post-surgical wound healing. A conceptual overview of these mechanisms and their clinical relevance is presented in Figure 1.

From a biological perspective, propolis is a complex resinous substance collected by honeybees from plant exudates and mixed with wax and salivary enzymes. It is primarily used by bees to seal cracks and gaps within the hive and to protect the colony against microbial threats. Its chemical composition and physical properties vary considerably depending on the botanical origin, which also influences its colour, ranging from yellow and brown to red or black. Owing to its antimicrobial, antioxidant, and anti-inflammatory properties, propolis has attracted increasing interest for biomedical and dental applications. In endodontic research, natural medicaments such as propolis have demonstrated significant anti-inflammatory effects compared with conventional materials (Sabir & Tabbu, 2004), as well as favourable biocompatibility, with lower cytotoxicity and reduced oxidative DNA damage in fibroblast cells relative to commonly used chemical disinfectants (Zulhendri et al., 2018).

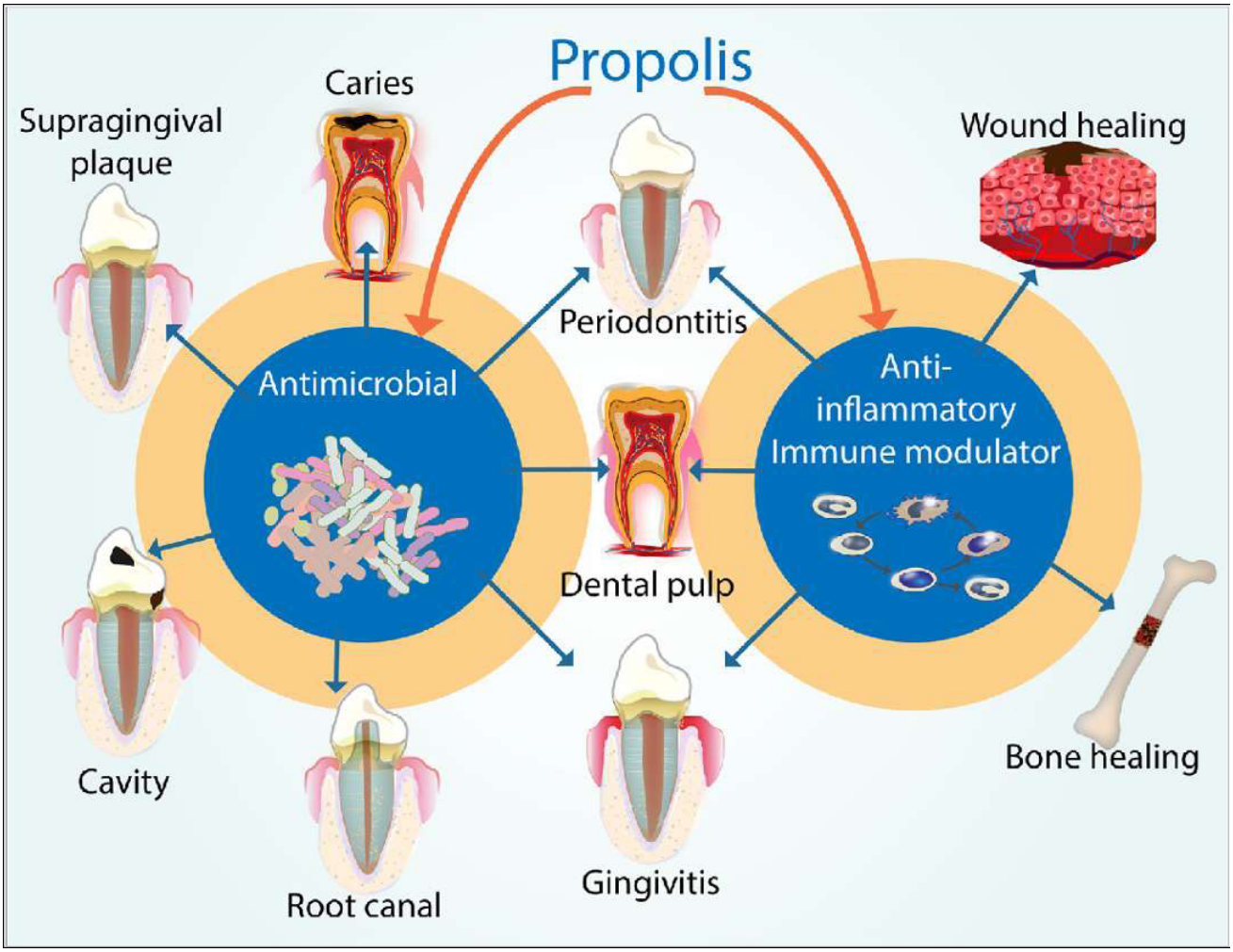


Figure 1. *Conceptual overview of the main biological actions of propolis and their relevance in dental diseases*. The diagram illustrates the dual antimicrobial and anti-inflammatory/immune-modulatory effects of propolis and their clinical relevance across major dental and oral conditions, including supragingival plaque, caries, root canal infections, gingivitis, periodontitis, dental pulp involvement, wound healing, and bone healing. This conceptual framework provides the biological rationale for the clinical case studies presented in the article.

Beyond its traditional use, propolis has increasingly been investigated within evidence-based biological and clinical frameworks in dentistry. In endodontics, experimental and in vitro studies have shown that propolis exhibits significant antimicrobial and anti-inflammatory effects while maintaining favourable biocompatibility. These properties support its potential use as an adjunctive intracanal medicament, particularly in situations where tissue preservation is critical. In this context, Jahromi et al. demonstrated that propolis exhibits significant antimicrobial activity against common endodontic pathogens and may serve as an effective alternative or adjunctive agent for root canal disinfection, with particular efficacy against *Enterococcus faecalis* (Jahromi et al., 2012).

In the management of deep carious lesions, where complete caries removal may jeopardize pulp vitality, both clinical and biological evidence support the use of natural materials. A randomized controlled clinical trial by Anani et al. (2023) demonstrated that natural materials applied to deep carious dentin showed significant antibacterial activity and promoted remineralization, with outcomes comparable or superior to synthetic alternatives. Importantly, preservation of pulp vitality was achieved under clinical conditions, providing high-level evidence for biologically oriented and minimally invasive caries management strategies.

At the cellular level, propolis-derived compounds have also been shown to actively support regenerative processes within the dentin–pulp complex. Liu et al. (2019) reported that caffeic acid phenethyl ester (CAPE), a major bioactive component of propolis, significantly upregulated vascular endothelial growth factor (VEGF) expression and production in odontoblastic cells without inducing cytotoxic effects. As VEGF plays a central role in angiogenesis and pulp tissue repair, these findings provide a mechanistic basis for the regenerative potential of propolis in pulp preservation and healing.

Taken together, these experimental, clinical, and mechanistic data provide a solid scientific foundation for the clinical case series presented in this article. The following cases should therefore be interpreted as practical clinical illustrations of well-documented biological effects of propolis when applied under routine dental practice conditions, rather than as isolated or exploratory findings.

PROPOLIS

Figure 2A. *Bees fill the gaps of the hive with a mixture of wax and propolis*. The colour of propolis depends on the dominant plant source and may range from brown and yellow to red or even black. In Uruguay, bees predominantly collect poplar-type propolis, which is characterized by a brownish colour. One of the most effective methods for harvesting propolis intended for food-grade and biomedical applications is the use of a food-grade propolis collection grid.

Figure 2B. *Some companies produce easy to handle products*

ROOT CANAL TREATMENT 1

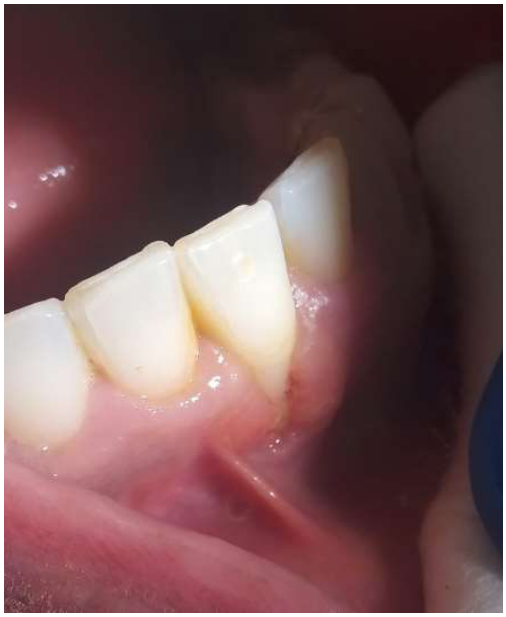


Figure 3. *Preoperative clinical image of a 25-year-old female patient presenting with pain in tooth 31.*
The patient attended the dental clinic complaining of localized pain. Clinical examination suggested endodontic involvement.

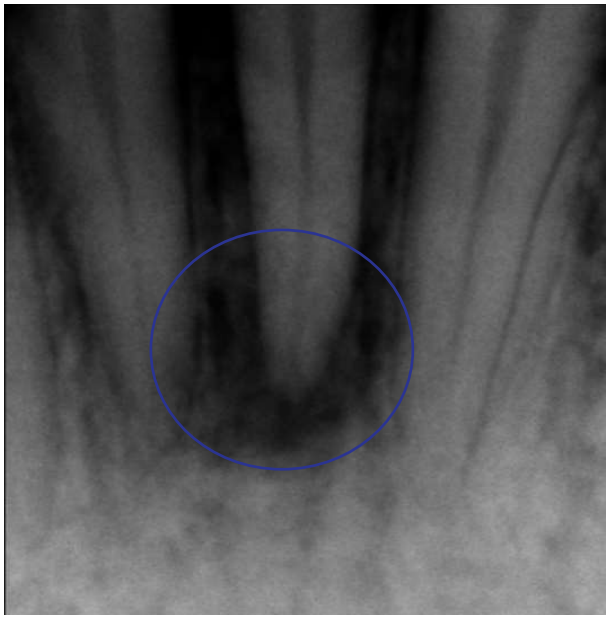


Figure 4. *Initial radiographic image showing a periapical area of infection associated with tooth 31.*
The radiograph demonstrates a radiolucent area consistent with periapical inflammation and infection.

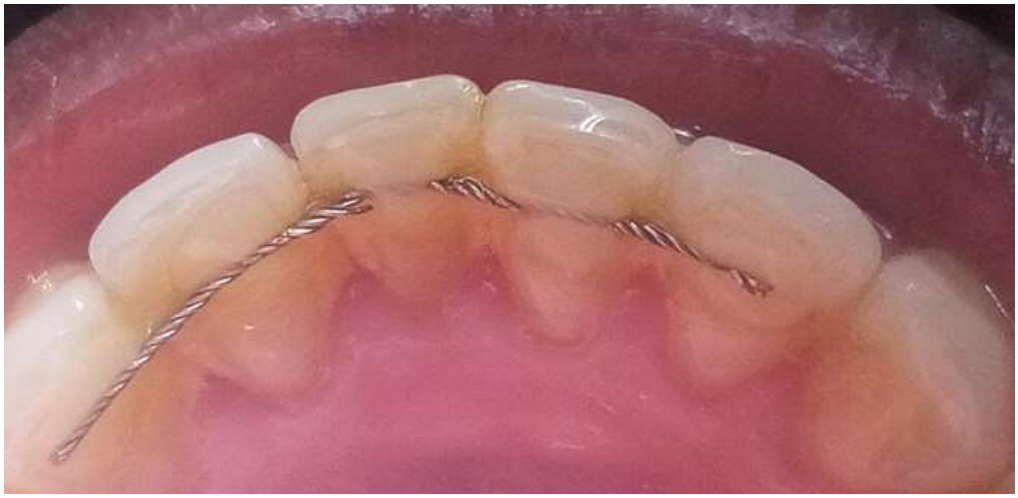


Figure 5. *Buccal view of the affected tooth and surrounding soft tissues.*
The image illustrates the clinical condition of the area prior to treatment.

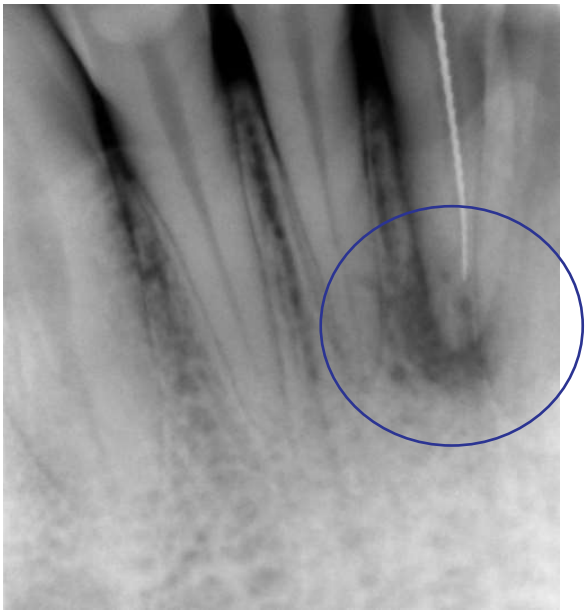


Figure 6. *Radiographic image obtained during working length determination.*
The image shows the root canal during conductometry, with an initial reduction of the infected periapical area.

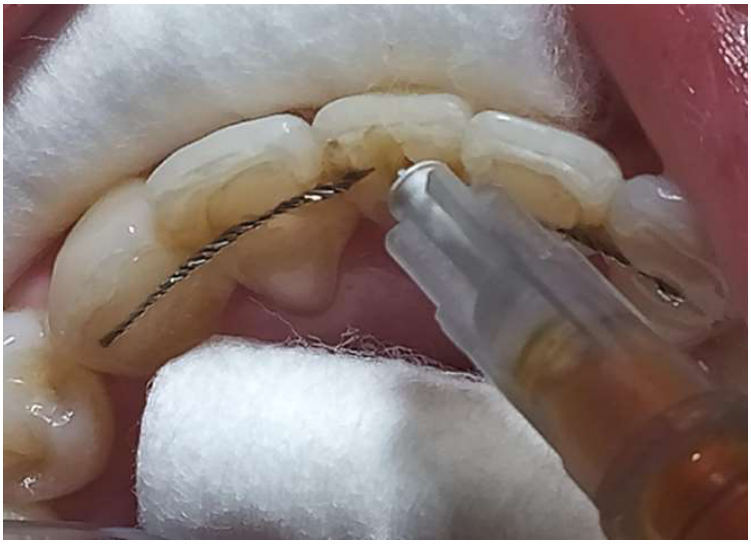


Figure 7. *Placement of a 5% propolis solution into the root canal.*
The propolis solution was used as an intracanal medicament to provide antibacterial, analgesic, and anti-inflammatory effects.

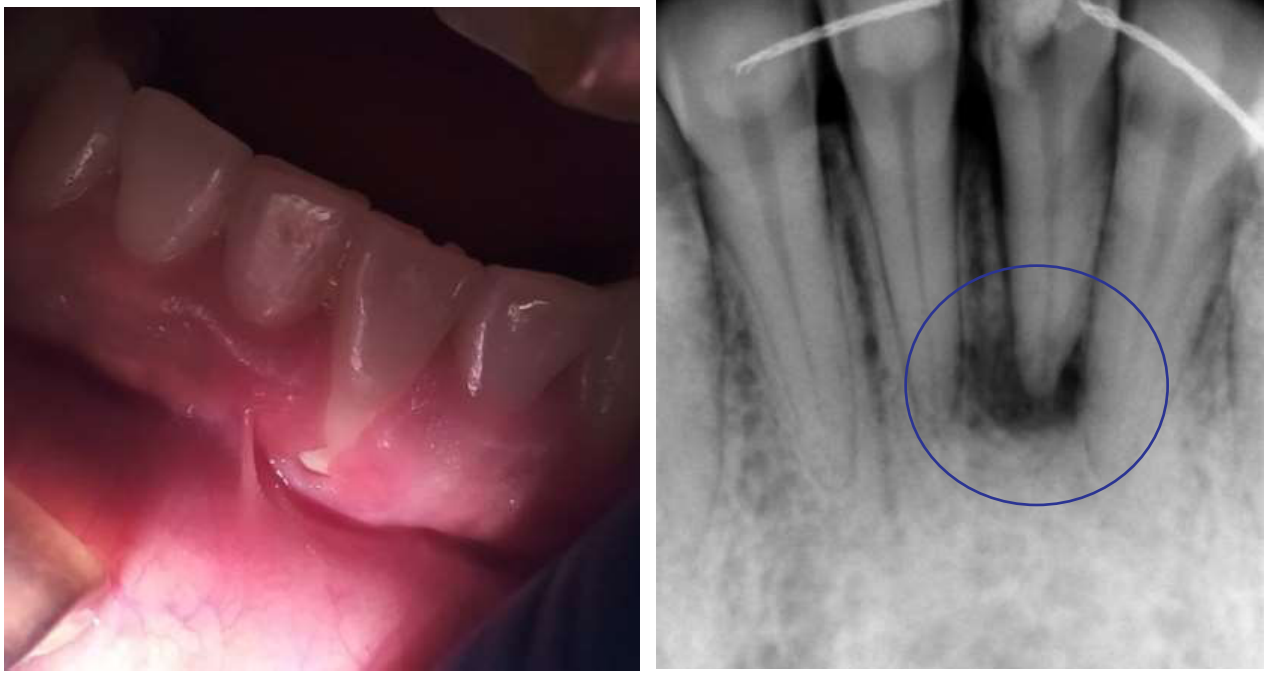


Figure 8. *Clinical and radiographic images one week after the initiation of endodontic treatment.*
Early signs of healing can be observed, including a reduction in inflammatory changes.

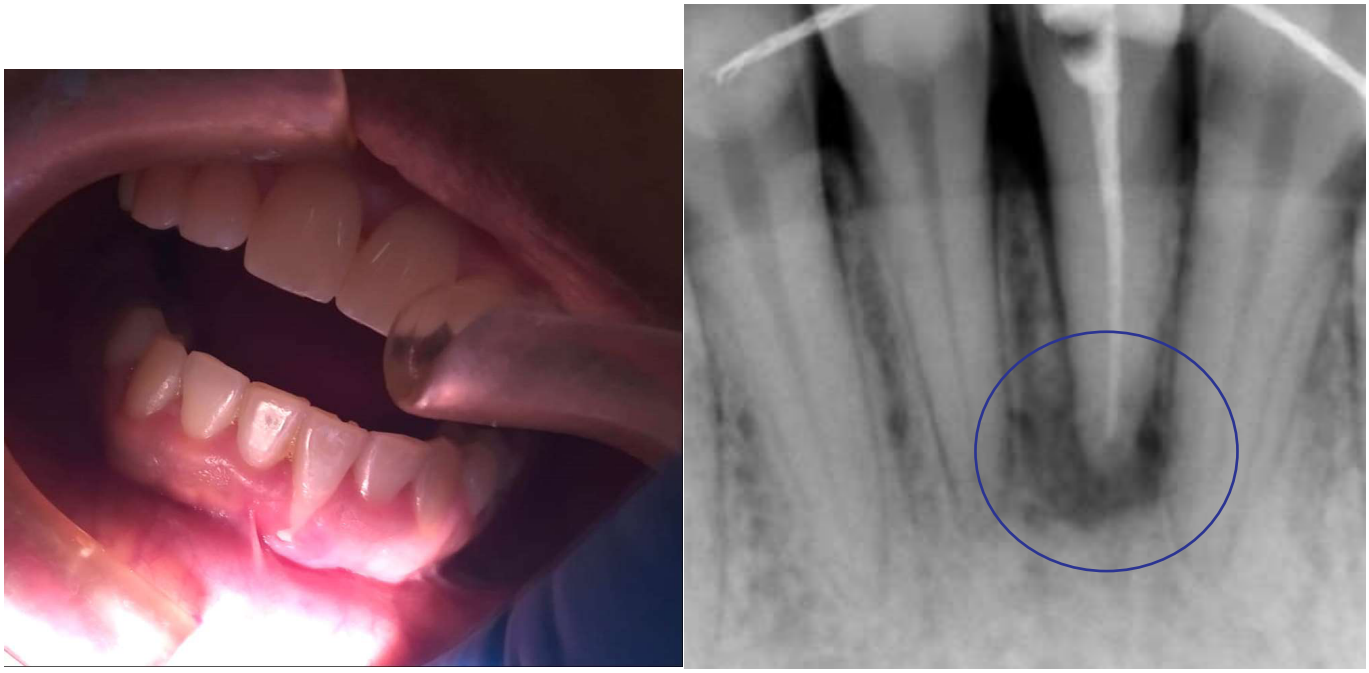


FIGURE 9. *Final clinical and radiographic images showing completed root canal obturation and bone regeneration.*

The images demonstrate successful endodontic treatment.

IMAGE-BASED SUMMARY OF ROOT CANAL TREATMENT IN THE PRESENT CASE

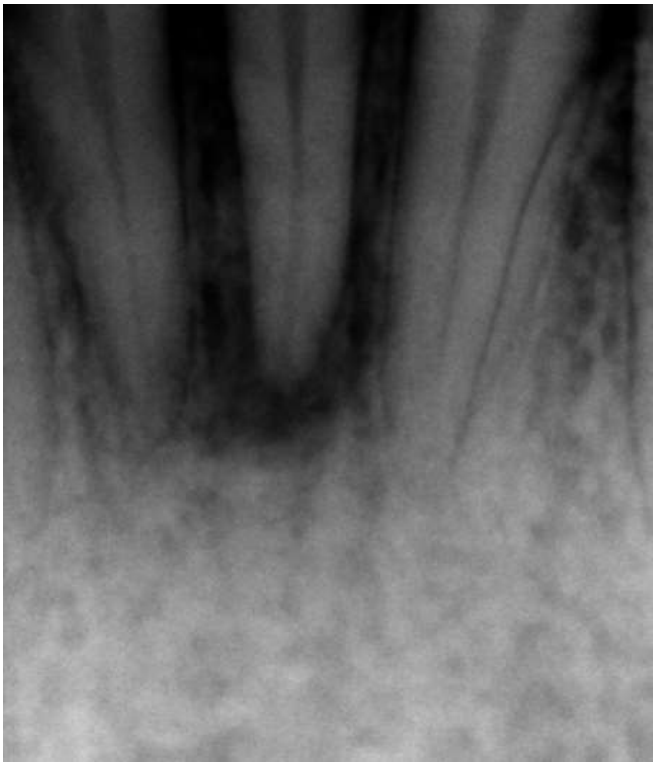


FIGURE 10. Before treatment

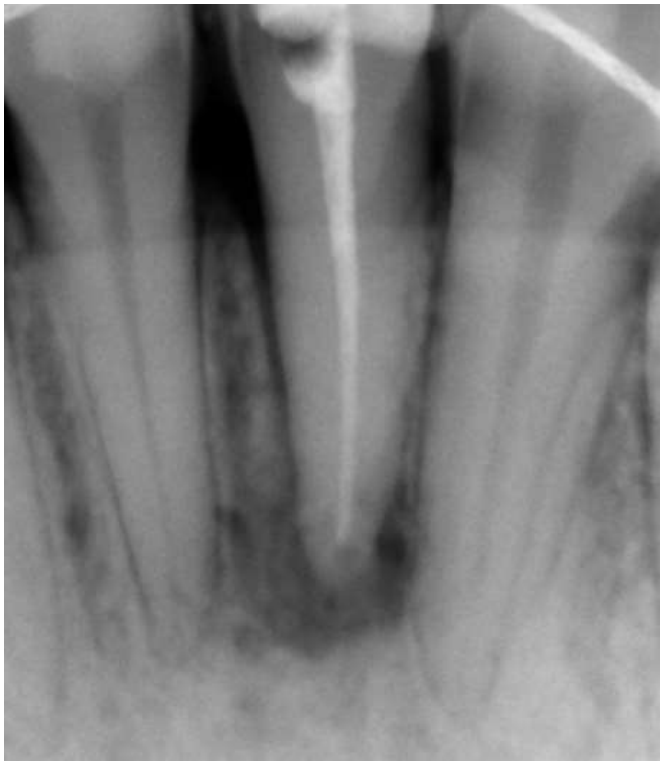


FIGURE 11. After 2 weeks treatment

ROOT CANAL TREATMENT 2

Male, 13 years old. Treatments on day 1, 7, 14. Check follow up after one year.

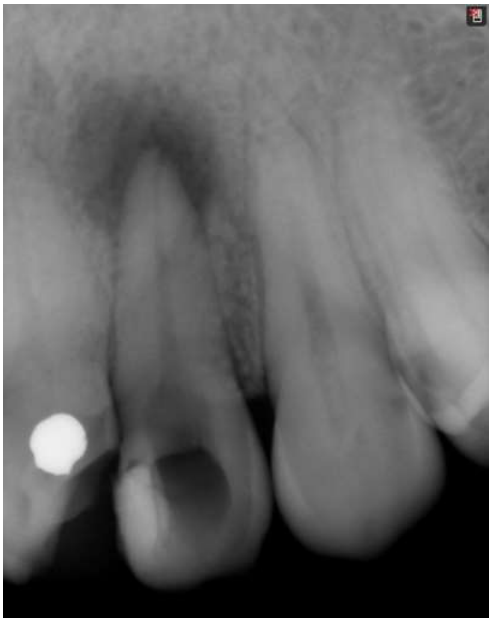


Figure 12. *Before treatment (day 1)*

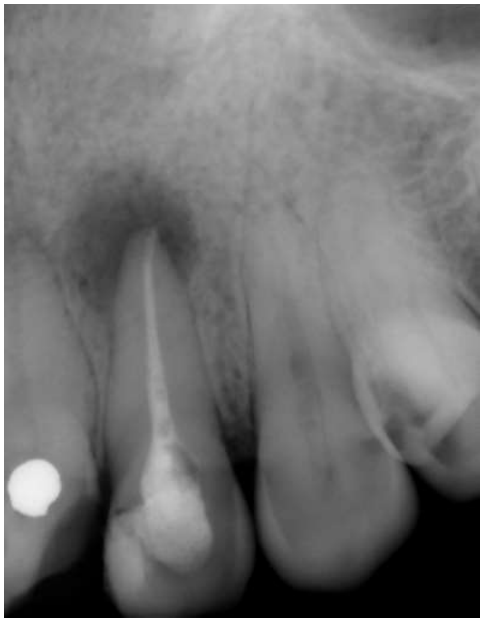


Figure 13. *After treatment (day 14)*

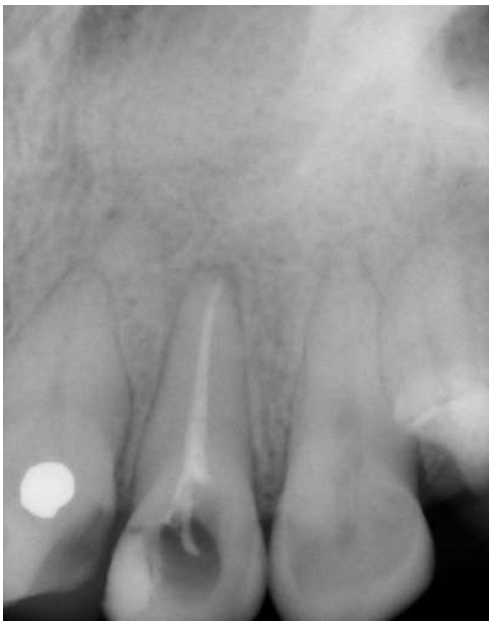


Figure 14. *Follow up: One year after treatment*

PERIODONTAL TREATMENT 1

Male, 40 years old

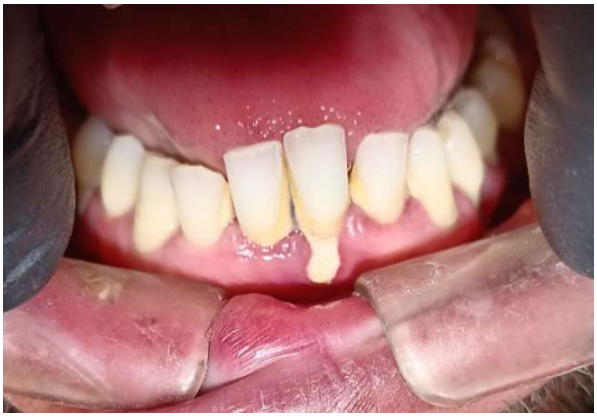
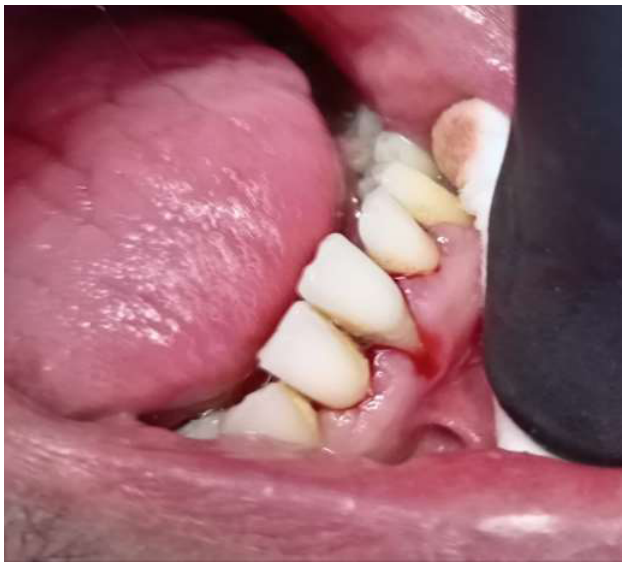


Figure 15. *Preoperative clinical image obtained at the patient's initial presentation.*

Figure 16. *Ultrasonic activation during root canal treatment followed by the placement of a 5% propolis solution.*



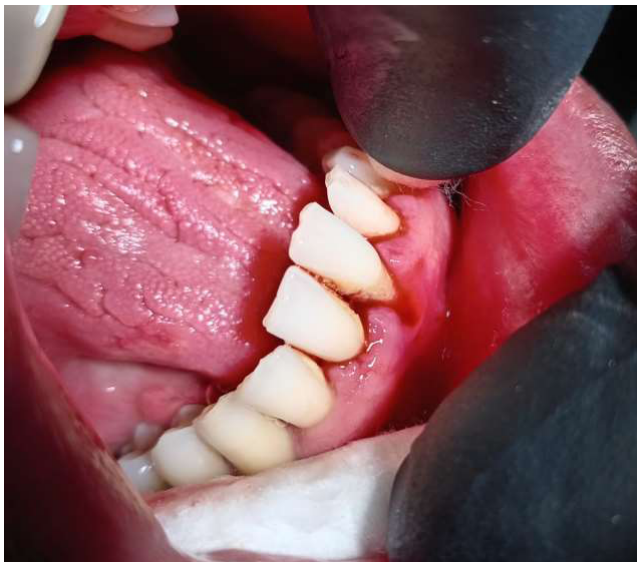


Figure 17. *Preoperative clinical imagea obtained during the treatmernt*

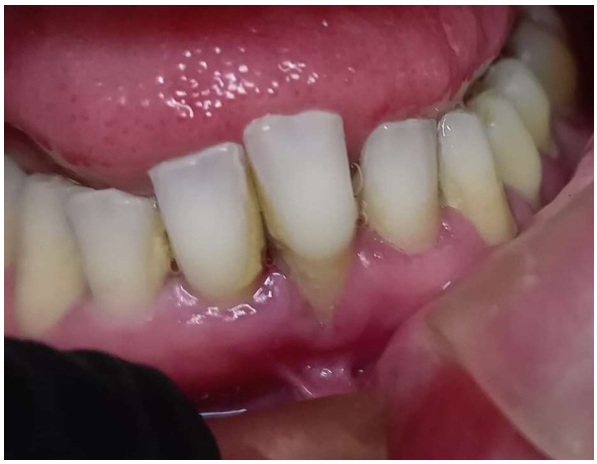


Figure 18. *Final clinical image obtained one week after completion of the treatment.*

PERIODONTAL TREATMENT 2

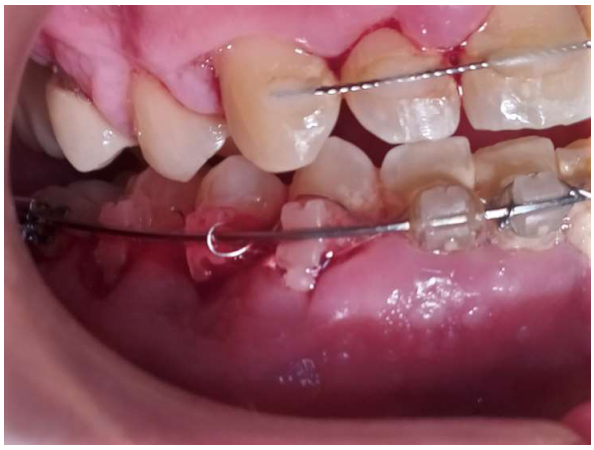


Figure 19. *Intraoral clinical photograph showing the dentition with fixed orthodontic appliances in place at the patient's initial presentation.*

Figure 20. *Intraoral clinical photograph demonstrating localized gingival inflammation in the anterior region during orthodontic treatment, followed by topical application of a 5% propolis solution.*

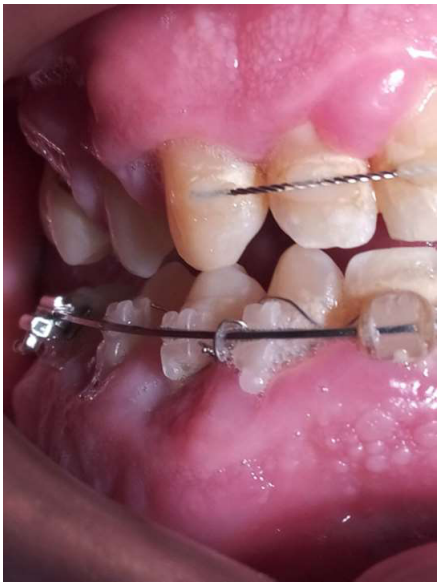


Figure 21. Intraoral clinical photograph obtained at follow-up, showing improvement of the gingival condition.

Figure 22. *Intraoral clinical photograph providing an overall view of the dentition at follow-up during orthodontic treatment, demonstrating stable soft tissue conditions.*

OPERATIVE DENTISTRY

Deep carious treatment

Male, 14 Years old

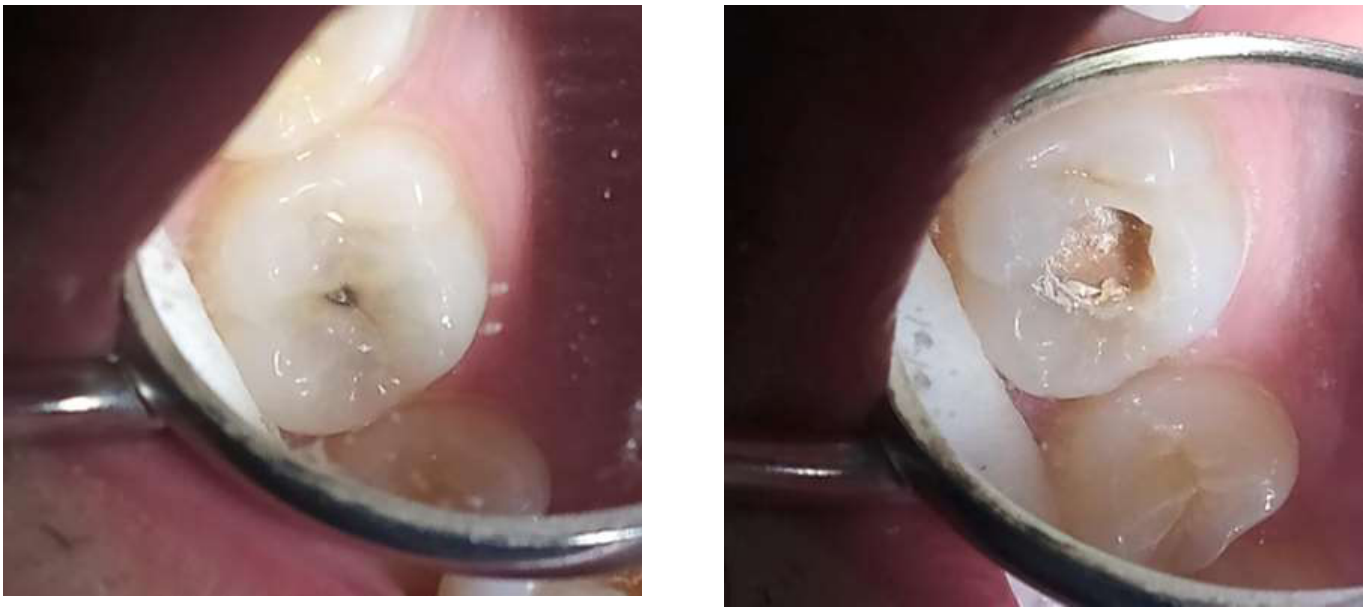


Figure 23. *Clinical image of a deep carious lesion prior to treatment and after caries removal, illustrating the extent of the lesion and exposure of the underlying dentin.*

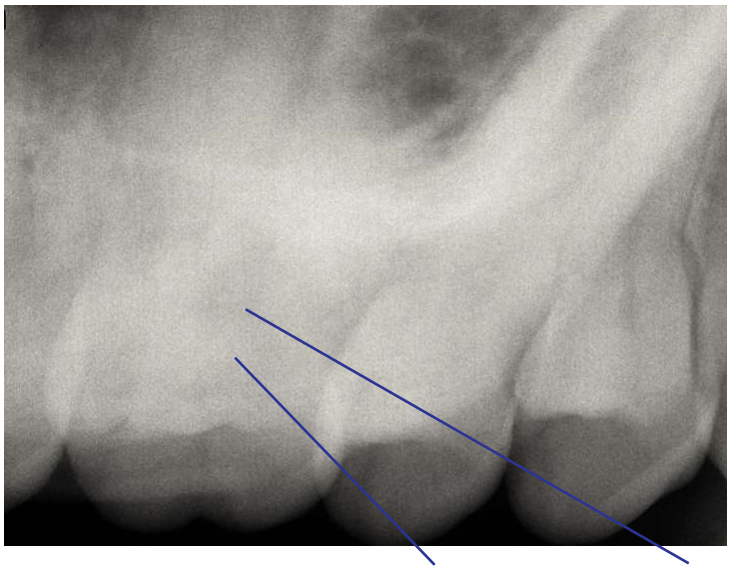


Figure 24. *Rx initial.* Distance between carious lesion and dental pulp

Figure 25. *Place propolis solution with hidroxiapatite*

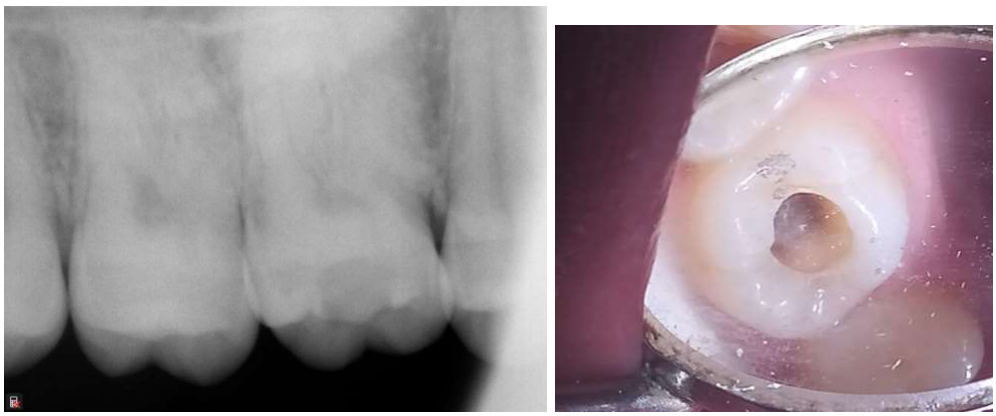


Figure 26. *One month later*. Radiographic appearance suggestive of dentin bridge formation.

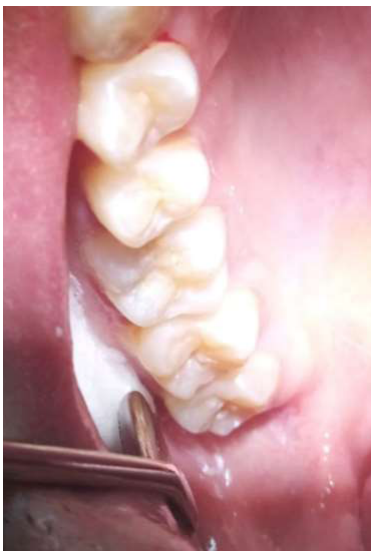


Figure 27 . *End of the treatment*

PROSTHETIC DENTISTRY

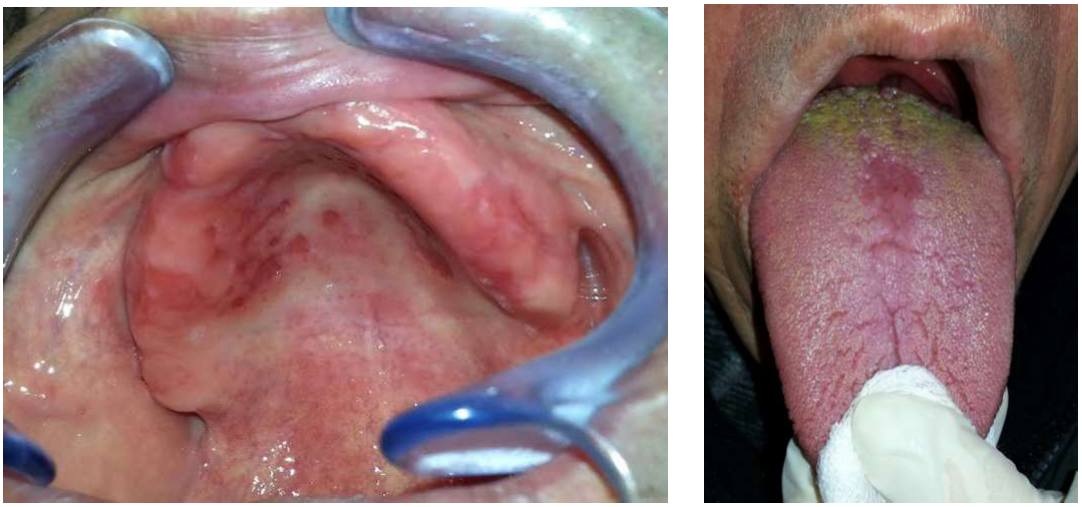


Figure 28 . *Clinical images of a 75-year-old male patient presenting with sub-prosthetic Candida albicans infection.*
Inflammation and fungal colonization were observed beneath the dental prosthesis

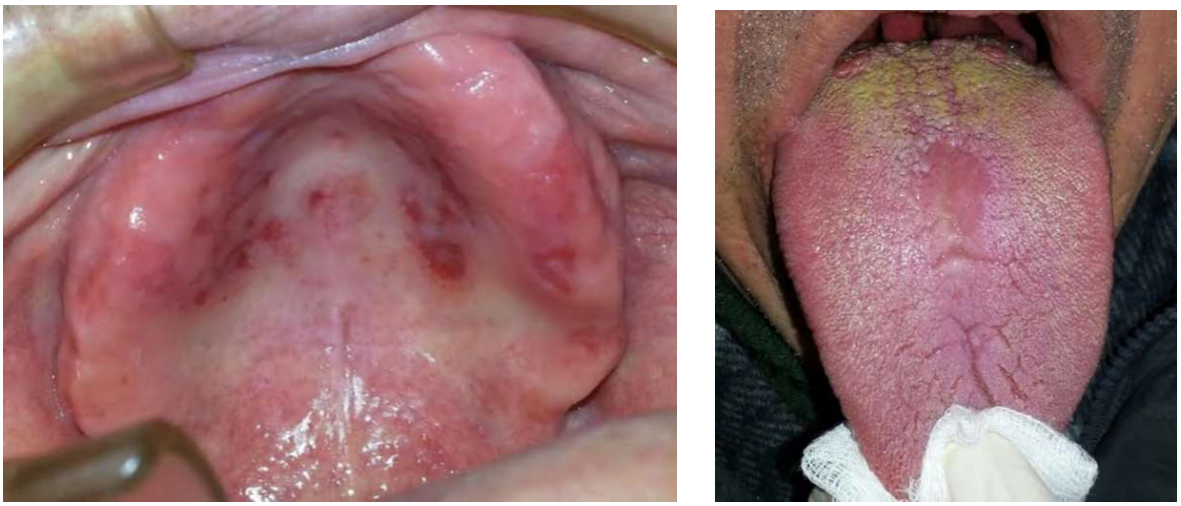


Figure 29. *Clinical image one week after treatment with a 5% propolis solution.*
A marked reduction in inflammation and fungal signs can be observed, in agreement with the documented antifungal properties of propolis (Zulhendri et al., 2021).

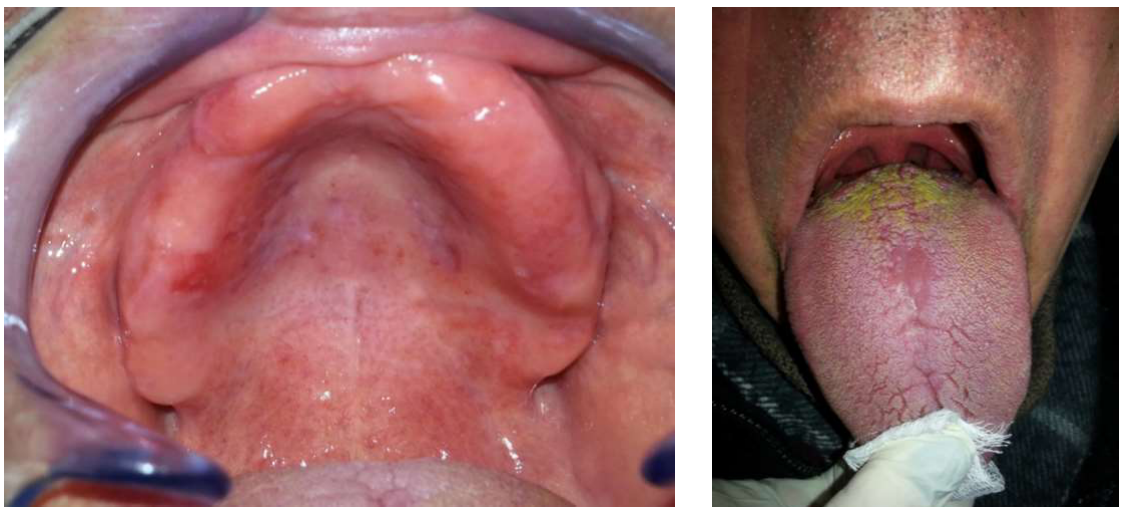


Figure 30. *Clinical image fifteen days after treatment.*
Further healing and restoration of healthy mucosal tissue are evident.

ALGINATE POST EXTRACTION

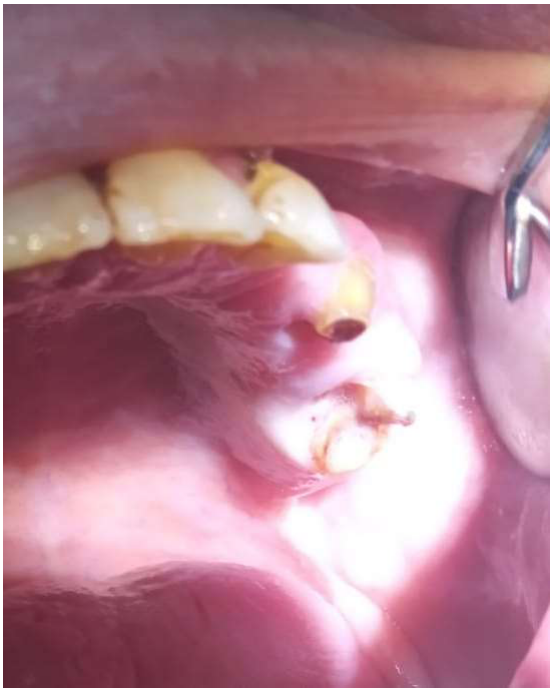


Figure 31. *Clinical image of a tooth indicated for extraction due to extensive caries.* The tooth exhibited severe structural damage requiring removal.

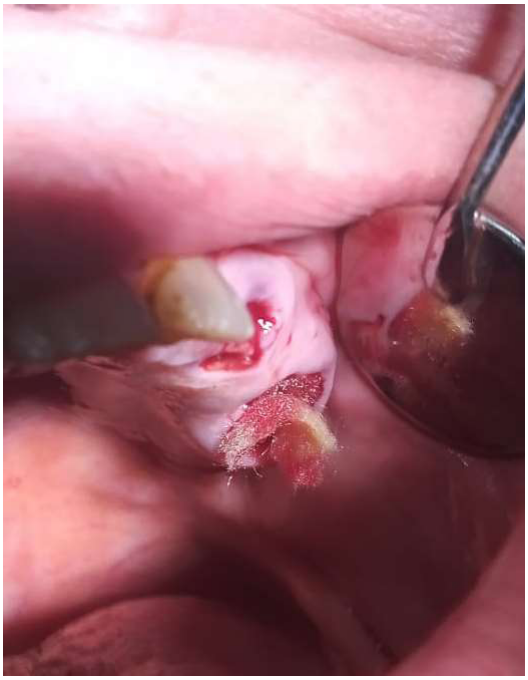


Figure 32. *Placement of calcium alginate combined with propolis into the extraction socket. The material w*as used to prevent dry socket and to support wound healing, consistent with previous reports on the biocompatibility of propolis.

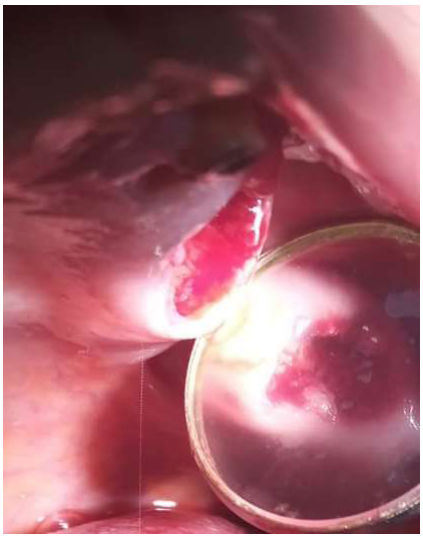


Figure 33. *Clinical image of the extraction site with calcium alginate and propolis in place.* The image demonstrates correct placement of the material within the alveolus.

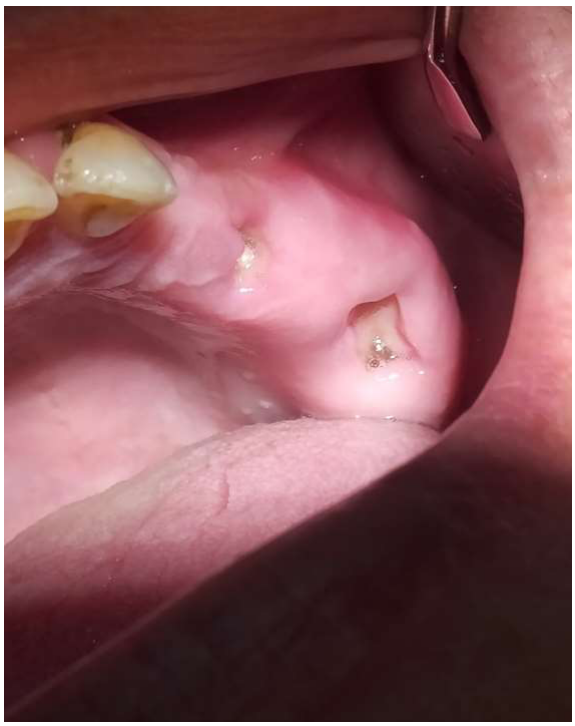


Figure 34. *Postoperative clinical image one week after tooth extraction.* Normal healing of the extraction site can be observed.

CONCLUSIONS

The present article expands previously reported conference cases into a structured clinical case series, providing a clearer and more detailed understanding of the practical application of propolis in dentistry. Across multiple dental disease groups—including endodontic infections, periodontal disease, deep carious lesions, prosthetic-related fungal infections, and post-extraction wound management—propolis demonstrated consistent antimicrobial, anti-inflammatory, and tissue-supportive effects when applied under routine clinical conditions.

The clinical observations suggest that propolis can serve as a valuable adjunctive agent in dental therapy, particularly in situations where infection control, inflammation reduction, and tissue regeneration are closely interconnected. The use of a standardized 5% propolis solution, either alone or in combination with established biomaterials, was associated with favorable clinical and radiographic outcomes over short-term follow-up periods.

Importantly, the cases presented should be interpreted as clinical illustrations of biological effects that have been extensively described in the scientific and apitherapy literature rather than as isolated or novel findings. Their value lies in demonstrating how these documented properties can be translated into everyday dental practice.

Despite the positive outcomes observed, the present work is limited by its descriptive, case-based design and the absence of control groups or long-term follow-up. Therefore, the findings should be considered exploratory. Future prospective, controlled clinical studies are required to further evaluate efficacy, define optimal indications, and establish standardized treatment protocols.

In conclusion, when quality, standardization, and appropriate clinical indication are ensured, propolis represents a safe and versatile complementary option in contemporary dental care. Its integration into evidence-based treatment strategies may contribute to improved clinical outcomes and patient-centered approaches in dentistry.

ETHICS STATEMENT

All clinical procedures were conducted in accordance with the ethical standards of routine dental practice. Written informed consent was obtained from all patients for the use of anonymized clinical and radiographic images for scientific publication.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST STATEMENT

The author is a practicing dentist and reports that the clinical cases presented in this article originate from his own private dental practice. The author declares that he has no financial, commercial, or personal relationships with any company or organization that could be perceived as a potential conflict of interest in relation to this work.

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