



## Efficacy of plant-based remedies for *Varroa destructor* control in honey bee colonies: A preliminary study

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### ABSTRACT

The Western honey bee (*Apis mellifera* L.) plays a crucial role in global ecosystems and agriculture, yet the ectoparasitic mite *Varroa destructor* remains a primary cause of severe varroosis and significant colony morbidity and mortality. Current reliance on synthetic acaricides often leads to the development of mite resistance and raises concerns regarding chemical residues in hive products. This has created an urgent need for innovative and effective veterinary strategies for parasite control. This study investigated the efficacy of traditional plant-based remedies as an alternative treatment for *V. destructor* infestations in honey bee colonies. Herbal decoctions from medicinal plants (e.g., nettle, clove, linden, peppermint, lavender, sage, dandelion), known for their anti-parasitic properties, were applied to bee colonies via aerosol. *V. destructor* infestation levels were monitored monthly using a non-destructive CO<sub>2</sub>-induced narcosis method. Statistical analysis, specifically a Linear Mixed Model (LMM), revealed a significant decrease in mean *Varroa* infestation from 1.8% in 2021–1.1% in 2023 across the experimental colonies ( $p < 0.000001$  for year effect). Furthermore, the LMM confirmed significant differences in efficacy among the tested decoctions ( $p < 0.000001$  for Decoction label effect), with specific extracts (DIII and DV) demonstrating superior performance. These findings highlight a validated, ecologically derived phytotherapeutic approach for *Varroa* management, demonstrating its potential to significantly reduce mite populations over multiple years without adverse effects on honey bee health. This research provides valuable insights into natural alternatives for parasitic mite control in domesticated animals like honey bees.

### 1. Introduction

The Western honey bee (*Apis mellifera* L.) serves as a crucial managed animal species globally, vital for its pollination services and for beekeeping as a form of animal husbandry (Etkin, 2009; Boncristiani et al., 2020). However, honey bee populations worldwide face escalating threats from various factors, including pathogens, parasites, habitat degradation, and pesticide exposure, which contribute to significant colony morbidity and widespread losses across continents (Neumann and Carreck, 2010; Abrol and Abrol, 2012; Guzman-Novoa et al., 2016; Yang et al., 2023). These challenges directly impact animal welfare and the productivity of beekeeping operations, necessitating robust veterinary and management interventions.

Among these pervasive threats, the ectoparasitic mite *Varroa destructor* is recognized as the most significant biological factor driving honey bee colony collapse, often acting synergistically with other stressors to exacerbate decline (Guzman-Novoa et al., 2016; Hristov et al., 2020; Bruckner et al., 2021). Varroosis, the parasitic disease

caused by *Varroa* mites, represents a leading cause of morbidity and mortality in honey bee colonies globally, posing a critical challenge to apicultural health and productivity (Korená Hillařová et al., 2022, Terrestrial Animal Health Code, 2022). This obligatory external parasite infests both *Apis cerana* and *Apis mellifera* bees (Aldea and Bozinovic, 2020), completing its reproductive cycle exclusively within the honey bee brood. Mite infestation directly compromises bee health by consuming the fat body tissue, particularly in adult bees, while also feeding on hemolymph in pupae (Ramsey et al., 2019). This leads to physiological impairments such as weight loss, morphological deformities, reduced individual longevity, and significantly facilitating the transmission of viral pathogens (Sammataro et al., 2000; Chen and Siede, 2007). Without timely and effective therapeutic intervention, heavily infested colonies typically succumb to varroosis within a year, frequently exhibiting 100% mortality during autumn and winter periods (Martin, 2001; Wegener et al., 2016, Van Der Steen and Vejsnæs, 2021). The escalating pathological impact of varroosis underscores the urgent need for innovative and effective management strategies to

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safeguard honey bee health.

Effective parasite management in honey bee colonies is crucial, not only for apicultural productivity but also for broader ecological stability (Van der Sluijs and Vaage, 2016; Requier et al., 2023). While various acaricidal treatments for varroosis are available, conventional synthetic chemicals—such as amitraz, coumaphos, fluvalinate, and flumethrin—present significant challenges within veterinary practice. These agents can exert detrimental effects on bee colonies and frequently lead to undesirable residues in honey and other hive products, raising concerns for both food safety and animal welfare (Rosenkranz et al., 2010; Korená Hillayová et al., 2024). Such concerns are reflected in stringent regulations governing veterinary medicinal products, including those in Europe (e.g., EC, 2009a) and national monitoring by bodies like the State Veterinary and Food Administration in Central Europe. Crucially, the widespread and documented development of mite resistance to key synthetic acaricides (Korená Hillayová et al., 2022) highlights an urgent and growing need for novel, safer, and effective alternative veterinary treatments for varroosis control. This pressing need has stimulated global research efforts to reduce reliance on synthetic chemicals (Lorenz, 2016) and to explore integrated pest management (IPM) strategies.

In response to the critical demand for novel parasite control strategies, significant interest has emerged in re-examining and scientifically validating traditional and locally adapted approaches to animal health and parasite control (Cable et al., 2017). This interdisciplinary field, commonly termed ethnoveterinary medicine or applied ethnopharmacology, investigates the historical and contemporary use of natural resources, particularly plants, for their therapeutic and protective properties in animal contexts (Smith-Hall et al., 2012; Balick and Cox, 2020). This rich indigenous knowledge provides a valuable foundation for developing effective, environmentally sound, and economically viable veterinary treatments. The growing adoption of plant-derived compounds as acaricide alternatives in apiculture reflects their diverse biological activities, often associated with fewer side effects and lower environmental impact compared to synthetic options (Topal et al., 2020; Oliveira et al., 2025). Furthermore, traditional practices often highlight the holistic benefits of plants (e.g., peppermint, thymol, eucalyptus) for their general tonic, regenerative, and metabolism-enhancing properties, contributing to overall animal resilience—a crucial aspect of robust farm animal health (Rosado-Aguilar et al., 2017; Ahumada et al., 2022; Ozuicli et al., 2024; Salkova et al., 2024). While plants produce diverse primary and secondary metabolites (Evert, 2006; Anulika et al., 2016; Kandari, 2020; Bocso and Butnariu, 2022), the present study specifically focuses on the practical *in vivo* application and efficacy of these traditionally selected botanical compounds for *V. destructor* control within a working beekeeping system (Durazzo et al., 2021).

The primary objective of this research was to scientifically validate and evaluate the efficacy of traditional, plant-based ethnoveterinary remedies for *V. destructor* control in honey bee colonies. Specifically, our study focused on designing and testing novel, eco-friendly, and cost-effective acaricidal agents derived from diverse ethnoveterinary practices found across various regions. This research aimed to provide viable alternatives to conventional synthetic treatments for varroosis, thereby mitigating challenges associated with mite resistance and concerns regarding chemical residues. Our investigation capitalized on the rich phytopotential of regions abundant in plant species historically utilized in traditional medicine.

The plants in our study are now scientifically recognized for containing compounds with documented acaricidal and insecticidal properties, such as *Lavandula angustifolia*, *Salvia officinalis* L., and *Taraxacum officinale* (Colin, 1990; Pohorecka, 2004; Nedec et al., 2015; Yaroshko et al., 2017; Ahumada et al., 2022). These botanical examples, along with many others, possess a demonstrated history of traditional use for controlling mites, ticks, and other arthropods. This work significantly contributes to the growing body of knowledge bridging traditional

ethnoveterinary wisdom with modern scientific validation, offering a transferable model for effective and sustainable parasitic mite management in apiculture globally.

## 2. Material and methods

### 2.1. Plant material: selection, collection and rationale

This study utilized specific aerial parts (leaves, stems, flowers, or a combination thereof) from eight plant species. These materials were sustainably collected from an experimental area in rural Slovakia, a region renowned for its rich and diverse flora with documented medicinal and pharmacological properties. Plant material collection was guided by the phenological cycle of Central Slovakian flora to ensure optimal yields of active compounds.

The selection of these plants was based on their historical use for parasitic control in traditional ethnoveterinary practices in Central Europe, as well as on scientific evidence of their acaricidal, insecticidal, or health-supportive effects on bees. The plant combinations were formulated to optimize synergistic effects and ensure the safety of the colonies by leveraging the complementary actions of key bioactive compounds, including flavonoids, phenolic acids, polysaccharides, and terpenoids. A detailed summary of the rationale for each plant's selection, including its known effects on bees and *Varroa* mites, and the key bioactive compounds responsible for these effects, is provided in Table 1.

### 2.2. Preparation, composition, and storage of decoctions








Plant decoctions were prepared by immersing collected plant parts in deionized water, maintained at a controlled temperature of 70 °C for 24 h. This temperature was chosen to facilitate the gentle extraction of a broad spectrum of polar compounds, including polysaccharides, glycosides, and phenolic compounds, while minimizing the degradation of other active constituents (see Table 1). Water was selected as the solvent because, in addition to its efficacy in extracting a broad spectrum of polar compounds, it facilitates a simple preparation process that can be easily replicated by beekeepers, unlike more complex methods involving oils or other solvents. To enhance extraction efficiency and ensure homogeneous solute release, the plant material was stirred during the 24-hour maceration period. Following maceration, the aqueous extracts were filtered through a fine mesh (cheesecloth) followed by a Whatman No. 1 filter paper to remove all solid plant residues. The filtrates were then transferred to sterile, dark glass bottles to prevent photodegradation and stored at 4 °C in a cool, dark environment until application to preserve the stability and efficacy of their active compounds.

### 2.3. Experimental setup and bee colonies


The research was conducted at the apiary near Horná Liešna, Slovakia (48°30'32.9"N 19°25'59.0"E, 535 m a.s.l.), a site with abundant natural floral resources. Ten experimental honey bee colonies (BH1–10) were used, all headed by "Singer" queens, which are recognized for their hygienic behavior and gentle temperament. While this hygienic trait provides some level of natural resistance, these lines are not fully resistant to *V. destructor* infestation and require regular treatment to ensure colony survival. Each colony was housed in an isolated B10 type hive (10 frames/hive). To ensure experimental consistency, colonies of approximately equivalent strength and initial total hive weight were selected. Initial hive weights were recorded on February 15, 2021; February 24, 2022; and February 17, 2023, with an average total hive weight of ~14 kg ± 500 g.

Due to the well-documented high mortality rates of honey bee colonies infested with *V. destructor* and left untreated, the study was conducted without a traditional placebo or negative control group. Omitting treatment for a control group would have resulted in an

**Table 1**  
Rationale for choosing plant materials and their active compounds for *V. destructor* suppression.

Plant species	Documented biological effects for <i>V. destructor</i>	Key bioactive compounds
	Traditionally used for immunomodulatory and anti-infective properties. Standardized extracts improve overall bee condition and support colony resilience ( Pohorecka, 2004).	Fatty acids, terpenoids (including eugenol), ethyl esters, terpenoids (apiol, eugenol), phenolic acids, amino acid, carotenoids, squalene, minerals, vitamins, flavonoids, acids are palmitic, linolenic, and nonadecanoic (see Lapinskaya and Kopyt'ko, 2008; Devkota et al., 2022; Bhusal et al., 2022).
	Extracts have demonstrated efficacy against <i>V. destructor</i> , suggesting a direct acaricidal or repellent action. Used as a traditional vermifuge (Yaroshko et al., 2017)	Flavonoids, phenolic acids (rosmarinic and caffeic acid), volatile compounds (menthol, menthone) (see Mahendran and Rahman, 2020)
	A significant herb in traditional medicine with documented toxicity against <i>V. destructor</i> . Its derivatives possess antibacterial, antifungal, and insecticidal properties (Maggi et al., 2010).	Tannins, saponins, flavonoids, high concentration of phenols (eugenol), alkaloids, glycosides, and flavonoids (see El-Maati et al. 2016; Anggrahini et al., 2021).
	Contains biologically active compounds known for disinfecting and antiparasitic therapeutic effects. Extract has shown to inhibit also <i>Nosema apis</i> development (Özüüçli et al., 2024).	Flavonoids, polyphenolic compounds, myrcene, bornyl acetate, cadinene camphene, <i>trans</i> -sabinyl acetate, guaiazulene, chamazulene, camphor, and linalool), essential oils, organic acids, resins, tannins, and phenols, bitter sesquiterpenoid lactones, flavonoids, other bitterness-imparting compounds, azulenes, phenolic acids, tannins and lignan (see Beigh and Ganai, 2017; Szopa et al., 2020; Batiha et al., 2020)
	Essential oils and extracts show strong antibacterial and antimicrobial activity, useful for overall hive hygiene (Ahumada et al., 2022).	Tannins, flavonoids, coumarins, anthocyanins, phytosterols, sugars, minerals, inalool, linalyl acetate, terpinen-4-ol, acetate lavandulol, ocimene, and cineole (see Mantovani et al., 2013; Prusinowska and Smigielski, 2014).
	Traditional use as a general tonic for bee vitality and immune support (Diaz et al., 2018).	Polysaccharides (inulin), phenolic acids (chlorogenic and chicoric acid), linalool, linalyl acetate and some other mono- and sesquiterpenes, sphingolipids, flavonoids like luteolin, triterpenoids like ursolic acid see ( Schütz et al., 2006; Fan et al., 2023; Singh et al., 2025)
	Studies show its essential oil is a potential and safe natural acaricide with no observed side effects on bees (Colin, 1990; Nedec et al., 2015).	Rosmarinic acid, luteolin 7-O-glukuronid, phenolic compounds, camphor, $\alpha$ -thujone, $\beta$ -thujone, borneol, and viridiflorol (see Miraj and Kiani, 2016; Ayoubi et al., 2024; Jakovljević et al., 2019).

**Table 1 (continued)**

Plant species	Documented biological effects for <i>V. destructor</i>	Key bioactive compounds
	Traditionally recognized for supporting bee health and parasite control. Contains thymol, a compound used in natural remedies against varroosis (Colin, 1990).	Mucilaginous polysaccharides, flavonoid glycosides (tiliroside), tricosane, nonanal, octadeca-9,12-dienoic acid, among terpenoids, linalool, menthone, borneol, menthol, terpinen-4-ol, p-cymene-8-ol, carvone, E-anethole, thymol of E- $\beta$ -damascenone, methyl eugenol, neryl acetone, $\delta$ -amorphene, linal, caryophyllene oxide, humulene epoxide II, epi- $\alpha$ -muurolol, kaurene (see ( European Medicines Agency [homepage on the Internet], 2012).

unacceptable level of colony loss, directly conflicting with the ethical principles of minimizing harm to research subjects (Spivak and Reuter, 2001). Therefore, the experimental design was focused on a comparative evaluation of different herbal treatments rather than a placebo-controlled trial. This approach aligns with ethical research practices in apiculture, where the welfare of the bee colonies is prioritized.

#### 2.4. Composition and application of decoctions

The plant-based solutions were administered as an aerosol using a manual Festa pressure sprayer (with a 5-liter capacity) to ensure uniform distribution within the bee colonies. The sprayer was manually pressurized to a constant pressure of 3 bars, and the adjustable nozzle was calibrated to produce a droplet size in the range of 100–150  $\mu$ m. Details of the specific plant materials and their combinations are provided in Table 2.

The herbal decoctions were sprayed directly onto bees within hives during routine monthly inspections from March to November. The decoction was applied by carefully removing each frame one by one and lightly spraying the bees on both sides of the frame. This ensured thorough coverage of the entire bee population within the colony. A single solution was applied per hive during each monthly inspection. The six distinct herbal extract combinations (DI-DVI) were systematically rotated on a monthly basis to optimize therapeutic efficacy and mitigate the potential for *V. destructor* to develop resistance. The specific composition and application schedule for each decoction is detailed in Table 3. This approach, with its practical beekeeping context, was designed to evaluate the long-term cumulative effects of the treatment.

#### 2.5. Assessment and quantification of Varroa infestation levels

*V. destructor* infestation levels were quantified using a non-destructive adult bee infestation estimation method, which was

**Table 2**  
Plant species, parts used, and their quantities per 5 L decoction.

Plant	The plant part used	Weight	Herb designation
Nettle	The whole plant	200 g	A
Peppermint	Leaf and stem	200 g	B
Clove	Flowers	20 g	C
Wormwood	Leaves	20 g	D
Lavender	Flowers	5 g	E
Dandelion	Flowers and leaves	100 g	F
Sage	Leaves	5 g	G
Linden	Flowers	100 g	H

**Table 3**  
Decoction formulations, application schedule, and composition.

Decoction formula	Application period	Number of applications	Composition (by herb designation)
DI	March, October, November	3x, 3x, 2x	A, B, C
DII	April, May	4x, 6x	A, B, C, F,
DIII	June	6x	A, B, C, D, E, F, G
DIV	July	4x	A, B, C, D, E, G,H
DV	August	4x	A, B, C, D, E, H
DVI	September	5x	A, B, C, D, E

selected for its suitability for repeated field sampling and its alignment with ethical animal welfare principles. Infestation rates were determined at the beginning (pre-treatment) and end of each monthly application cycle.

The non-destructive sampling technique involved CO<sub>2</sub>-induced narcosis to temporarily sedate bees and induce mite detachment (Aliano and Ellis, 2005). For each sample, approximately 100 adult bees were collected from a single brood frame (Lee et al., 2010) and placed into a 900 ml glass jar equipped with a sieve at the bottom. Food-grade CO<sub>2</sub> was introduced into the jar via the lid for 15–20 s. Once sedated, the bees were gently agitated for 30 s to facilitate mite detachment. Mites fell through the sieve to the bottom of the jar.

Bees were then carefully counted and visually inspected for any remaining mites before being gently returned to their colonies. The dislodged mites were emptied onto a white counting surface and enumerated using a magnifying glass and a Levenhuk D85L LCD digital microscope. The *Varroa* infestation rate (mites per 100 bees) was calculated using the following formula (De Jong et al., 1982):

$$\text{Infestation rate (\%)} = \frac{\text{number of bees sampled}}{\text{number of mites}} \times 100 \quad (1)$$

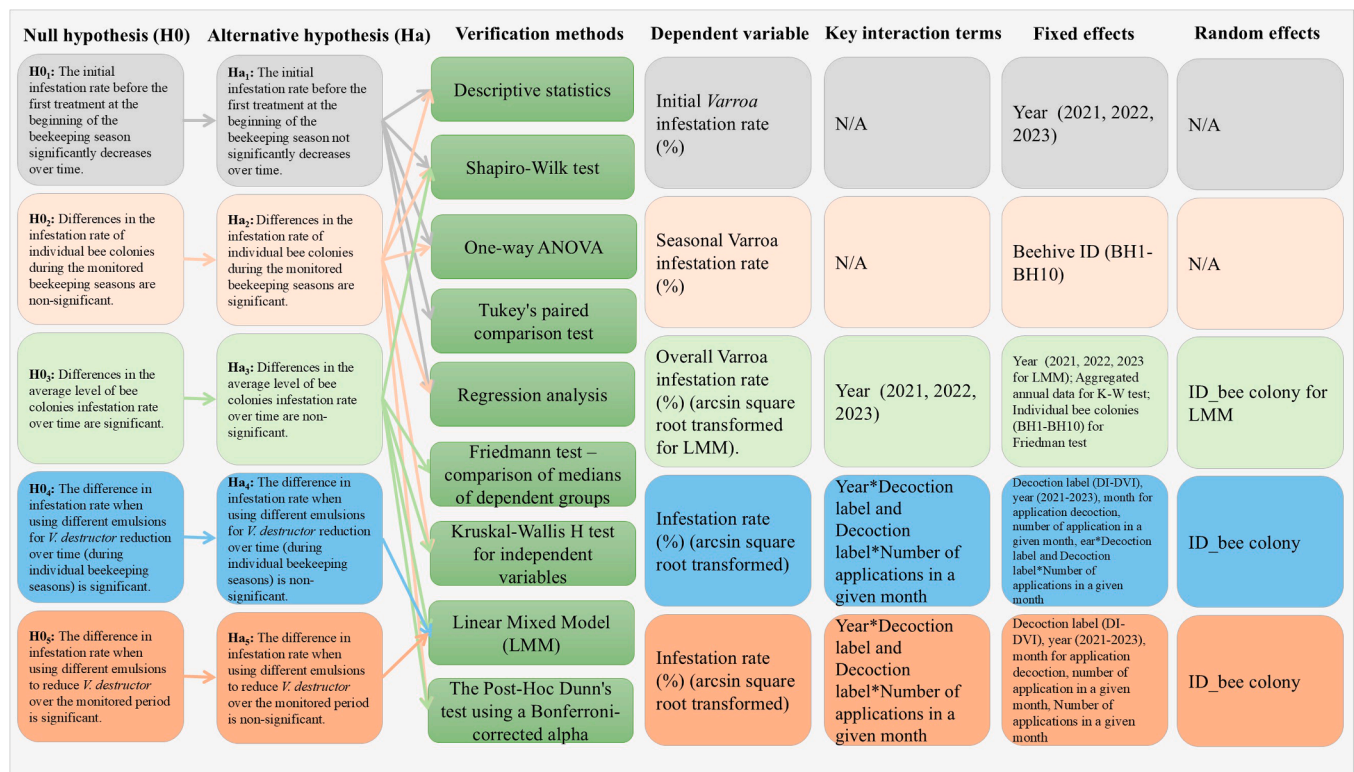
This study focused on evaluating the efficacy of the experimental

treatments against the observed infestation dynamics, rather than relying on a strict adherence to pre-defined intervention thresholds.

2.6. Statistical analysis

All statistical analyses were conducted using StatSoft Statistica, version 14.0.0, with a significance level of  $\alpha = 0.05$ . A comprehensive database was compiled, including *V. destructor* infestation levels, mite fall, treatment dates, number of treatments, and decoction types. The evaluation of differences in the effectiveness of ecological emulsions for treating bee colonies, as well as seasonal and interannual infestation dynamics, was performed by formulating and testing specific statistical hypotheses (details on hypothesis testing are provided in Fig. 1, summarizing the methods used). Specifically, for the analysis of initial *Varroa* infestation (Hypothesis H0<sub>1</sub>/Ha<sub>1</sub>), data normality was assessed via the Shapiro-Wilk test; for normally distributed data, a one-way ANOVA was performed, followed by Tukey HSD post-hoc tests for pairwise comparisons across years (2021, 2022, 2023), with regression analysis additionally performed to identify infestation rate trends over time. For the analysis of Seasonal *Varroa* Infestation Dynamics within Individual Bee Colonies (Hypothesis H0<sub>2</sub>/Ha<sub>2</sub>), data normality for each hive and year was again assessed using the Shapiro-Wilk test; for normally distributed data, one-way ANOVA was performed to compare mean infestation among colonies within a given year, while in cases of non-normal distribution, non-parametric Kruskal-Wallis H tests were used with Post-Hoc Dunn’s test (with Bonferroni correction) for pairwise comparisons, and regression analysis was also applied to quantify seasonal trends for individual beebees.

For the analysis of interannual trends and treatment effectiveness (Hypotheses H0<sub>3</sub>/Ha<sub>3</sub>, H0<sub>4</sub>/Ha<sub>4</sub>, H0<sub>5</sub>/Ha<sub>5</sub>), given the longitudinal nature of the data with repeated measurements on individual bee colonies over multiple years and months, a Linear Mixed Model (LMM) was employed. The dependent variable, infestation rate (%), was transformed using the arcsin square root transformation (arcsin



**Fig. 1.** Research hypotheses and verification methods. The diagram provides a structured overview of the study’s hypotheses, and the methodology used to test them. Each hypothesis is detailed regarding its statistical approach, dependent variables, fixed effects, key interaction terms, and random effects.

$\sqrt{\text{percentage}/100}$ ) to meet the assumptions of normality and homoscedasticity. Fixed effects in the model included year, decoction label, month, number of applications in a given month, and their relevant two-way interactions (year *decoction label* and *decoction label* number of applications in a given month), with the ID\_bee colony included as a random effect to account for the dependency of observations within the same bee colony over time. In addition to the LMM, for the assessment of aggregated interannual trends (as part of Hypothesis H0<sub>3</sub>/Ha<sub>3</sub>), Shapiro-Wilk tests were performed on the pooled infestation data for each colony across all three years; if non-normal, non-parametric Kruskal-Wallis H tests were used to compare overall infestation rates between years, followed by Post-Hoc Dunn's test (with Bonferroni correction), and the non-parametric Friedman test was also applied to evaluate changes over time in individual colonies for non-parametric data.

### 3. Results

#### 3.1. Initial Varroa infestation: baseline and significant decline (2021–2023)

Initial *V. destructor* infestation levels were monitored in all experimental bee colonies (BH1–BH10) at the commencement of the 2021, 2022, and 2023 beekeeping seasons, prior to any treatment application. Baseline infestation rates across all colonies were consistently low, remaining below 1.2 %. In 2021, the highest initial infestation recorded was 1.2 % (BH3 and BH8), with the lowest at 0.6 % (BH6); other colonies ranged from 0.7 % to 1.1 %. Initial infestations generally decreased in subsequent years, ranging from 0.1 % to 1.0 % in 2022, and from 0.0 % to 0.8 % in 2023 (Fig. 2a). An overall consistent downward trend in initial infestation was observed across colonies BH1–10 throughout the monitored seasons (Fig. 2b).

Statistical analysis corroborated this observed decline. The Shapiro-Wilk test confirmed normal distribution for initial infestation data in each respective year. A subsequent one-way ANOVA revealed a statistically significant difference in mean initial infestation rates across the beekeeping seasons ( $p = 0.000282931$ ), with a large effect size ( $f = 0.91$ ). Tukey's HSD post-hoc test further identified significant decreases in initial infestation between the 2021–2022 and 2021–2023 periods. These findings were additionally supported by regression analysis (Fig. 2c). Based on these results, hypothesis H0<sub>1</sub>, positing a significant decrease in the initial infestation rate of bee colonies since the study's inception, was accepted.

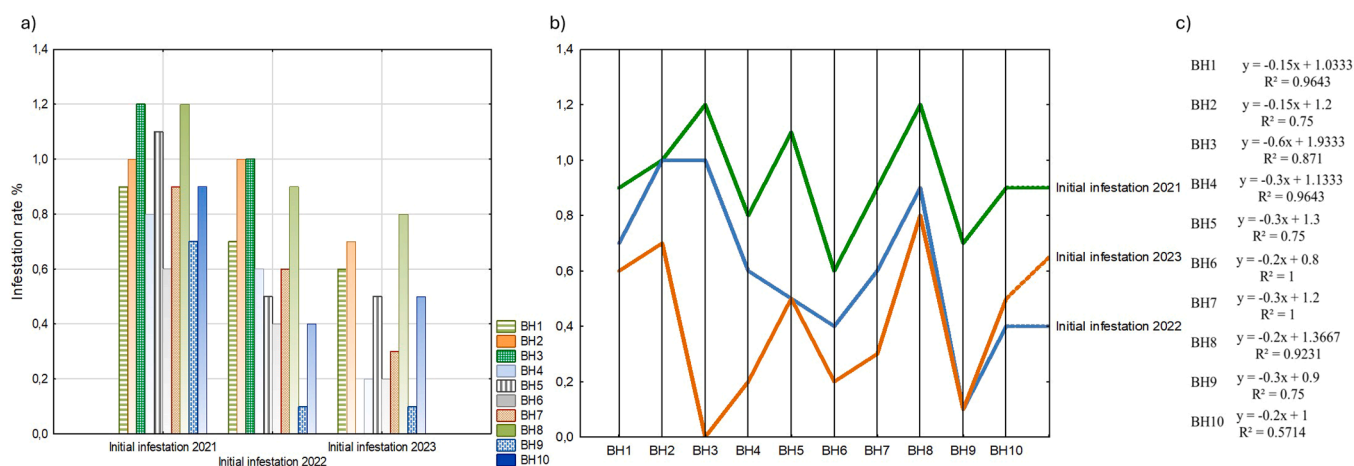
#### 3.2. Seasonal Varroa infestation dynamics in individual bee colonies (2021–2023)

This section elucidates the seasonal progression of *V. destructor* infestation rates within individual bee colonies (BH1–BH10) across the 2021, 2022, and 2023 beekeeping seasons. Distinct patterns in infestation peaks and subsequent declines were observed each year, alongside variations among individual hives (Fig. 3a–i).

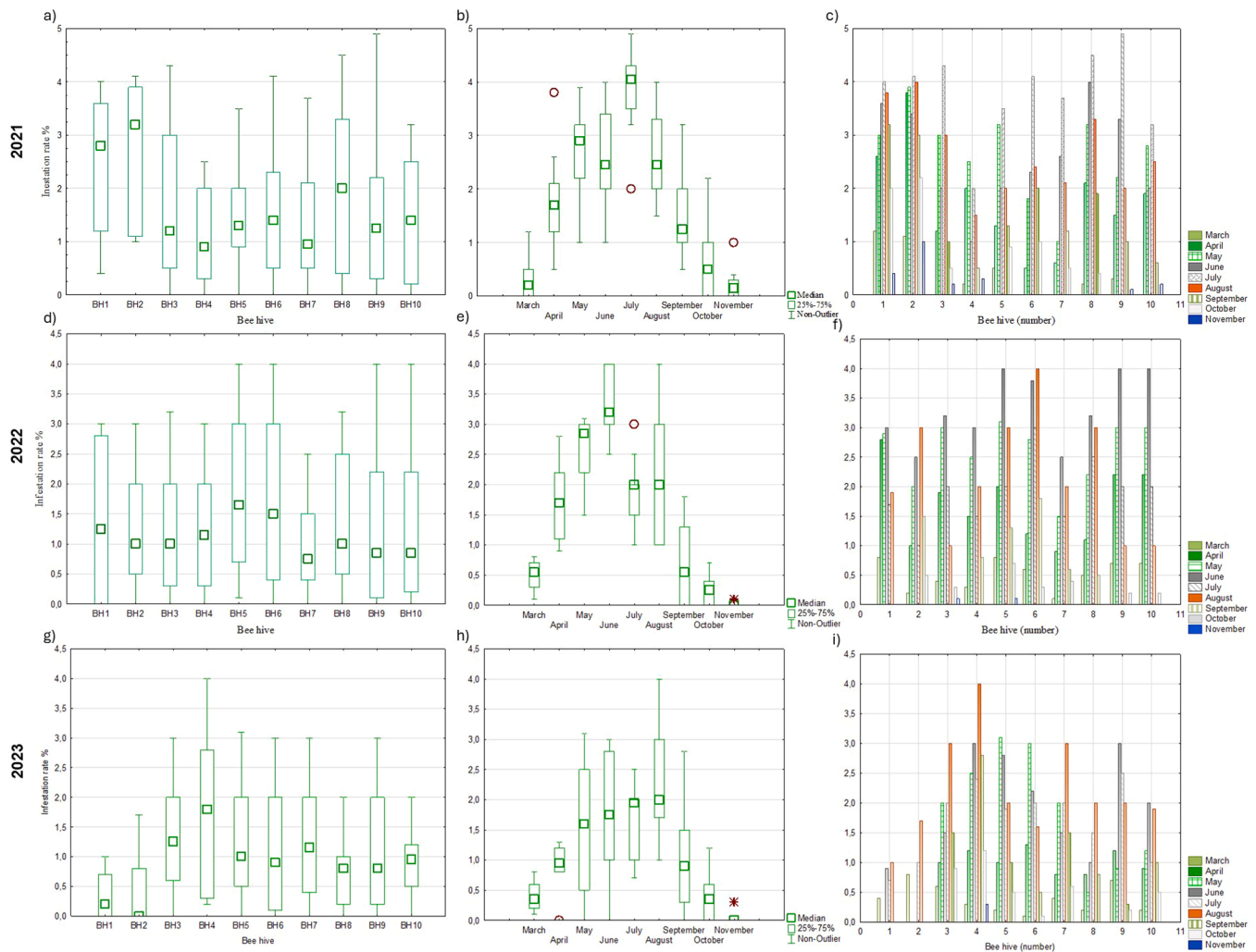
In the 2021 season, the highest infestation rates (3.2 %–4.3 %) primarily occurred in July for colonies BH1–3 and BH5–10, coinciding with peak bee emergence (Fig. 3a–c). Colony BH4, exceptionally, peaked earlier in May at 2.5 %. Infestation generally declined to minimal levels (0.0 %–1.0 %) by November for most hives, although some (BH3, BH6, BH7, BH10) reached their minimums in March. The overall mean infestation for all hives in 2021 was 1.8 %. Descriptive statistics for individual beehives (BH1–BH10) are provided in Table S1. Regression analysis (Table S2) indicated a significant seasonal decrease only in BH4, with other colonies showing non-significant reductions. Statistical analysis, primarily using the Shapiro-Wilk test, confirmed normal data distribution for most individual hives (BH1, BH3–BH10) in 2021 (Table S3). A one-way ANOVA revealed no statistically significant difference in mean infestation among these normally distributed colonies ( $F = 1.051612$ ,  $p = 0.406$ ). For BH2, where data were not normally distributed, the Kruskal-Wallis H test similarly showed no significant difference in median infestation. Consequently, hypothesis H0<sub>2</sub>, which posited no significant difference in infestation rates among individual colonies, was accepted for 2021.

During the 2022 season, colonies BH1 and BH3–BH10 reached their highest infestation rates (2.5 %–4.0 %) in June (Fig. 3d–f), while BH2 peaked later in August at 3.0 %. By November, infestation dropped to minimal levels (0.0 %–0.1 %) across all hives. The average infestation for all hives in 2022 was 1.46 %, representing a 0.34 % decrease from 2021 (Table S1). Regression analysis (Table S2) indicated significant seasonal decreases in infestation for BH1, BH3–BH5, BH9, and BH10, with non-significant decreases in the remaining colonies (BH2, BH6–BH8). Similar to 2021, Shapiro-Wilk tests confirmed normal data distribution for most hives (BH2–BH10) in 2022 (Table S3). A one-way ANOVA showed no statistically significant difference in mean infestation among these colonies ( $F = 0.466$ ,  $p = 0.876$ ). For BH1 (non-normally distributed), the Kruskal-Wallis H test again showed no significant difference in median infestation relative to other hives. Thus, hypothesis H0<sub>2</sub> was also accepted for 2022.

In 2023, the highest infestation rates (1.0 %–4.0 %) were primarily



**Fig. 2.** Assessment of initial infestation rate dynamics. a) Bar chart shows a general decreasing trend in initial infestation rates for most beehives from 2021 to 2023, with many falling below 0.4 % by 2023. b) Line graph depicts a significant overall decline in initial infestation rates from 2021 to 2023 across the beehive population, with 2021 showing the highest rates and 2023 the lowest. c) Regression equations quantify linear relationships within each beehive's data, revealing generally negative slopes indicating decreasing trends.



**Fig. 3.** Seasonal and hive-specific infestation rates in bee colonies (2021–2023). Box plots (a, d, g) illustrate infestation rate distributions across 10 bee hives (BH1–BH10), showing most medians between 1.0 % and 2.5 % and BH1 consistently lower. Box plots (b, e, h) display monthly infestation trends (March–November), with higher median rates from May to August. Bar charts (c, f, i) compare infestation rates per hive by month, consistently showing higher levels in July and August. Red symbols indicate outliers or notable points.

observed in August for colonies BH1–BH4 and BH7–BH8 (Fig. 3g–i). BH5 (3.1 %) and BH6 (3.0 %) peaked in May, while BH9 (3.0 %) and BH10 (2.0 %) peaked in June. All hives showed a reduction to minimum infestation levels (0.0 %–0.3 %) by November. The mean infestation for all hives in 2023 was 1.1 %, indicating a 0.7 % decrease from the 2021 season (Table S1). Regression analysis (Table S2) revealed significant seasonal decreases in infestation only for BH6 and BH9, with other colonies experiencing non-significant declines. For 2023, Shapiro-Wilk tests confirmed normal distribution for data from most hives (BH3–BH10) (Table S3). A one-way ANOVA showed no statistically significant difference in mean infestation among these colonies ( $F = 1.15958$ ,  $p = 0.338$ ). However, for BH1 and BH2 (non-normally distributed), the Kruskal-Wallis H test followed by Dunn’s post-hoc test identified significant differences in mean ranks between BH1 and BH4, and BH2 and BH4. Despite these specific differences, the overall acceptance of hypothesis  $H_{02}$  for the 2023 season suggests that individual hive performance within the season was generally not significantly different.

### 3.3. Interannual trends in *Varroa* infestation (2021–2023)

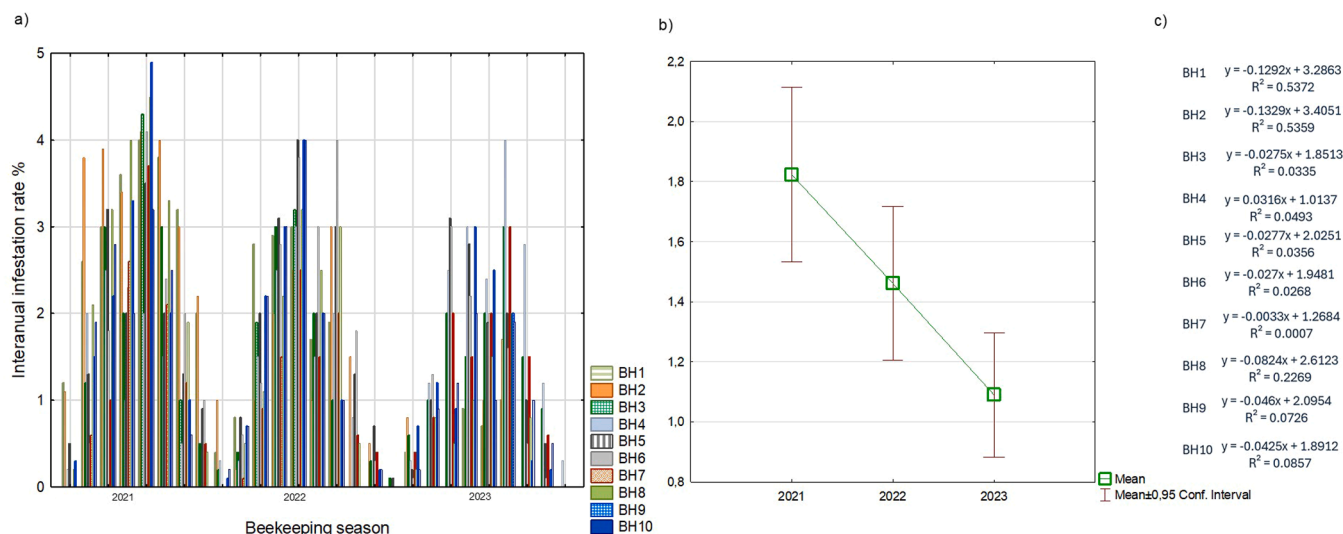
Building upon the seasonal analyses, the overall interannual *V. destructor* infestation rate across all monitored bee colonies (BH1–BH10) was further evaluated from 2021 to 2023. This overarching

analysis considered the collective data from individual colonies, acknowledging the generally non-significant differences in seasonal infestation observed among them.

Over the entire study period, colonies BH7 (1.1 %) and BH10 (1.2 %) exhibited the lowest average infestation rates, while BH2, BH5, and BH6 recorded the highest average rates at 1.5 %. The maximum observed infestation across all years was 4.9 % in BH9, with BH7 showing the lowest maximum at 3.7 % (Fig. 4a). Detailed descriptive statistics for this aggregated period are presented in Table S4.

To assess the overall interannual trend, Shapiro-Wilk tests (Table S5) were first performed on the pooled infestation data for each colony across all three years. As the data generally indicated non-normal distribution (e.g., BH1:  $W = 0.87$ ,  $p = 0.003$ ; BH2:  $W = 0.87$ ,  $p = 0.004$ ), non-parametric tests were utilized. A Kruskal-Wallis H test (Table S5) on the interannual infestation rates across individual colonies revealed a non-significant difference ( $\chi^2(9) = 1.19$ ,  $p = 0.999$ ), indicating that no single colony consistently performed drastically differently from others over the entire three-year period.

Subsequently, the non-parametric Friedman test (Table S5) was employed for individual colonies to assess the significance of infestation changes between 2021 and 2023. Most colonies showed a decrease in infestation, with statistically significant decreases observed in BH1 ( $\chi^2 = 11.7$ ,  $p = 0.00$ ) and BH2 ( $\chi^2 = 13.38$ ,  $p = 0.00$ ). In contrast, BH4



**Fig. 4.** Interannual infestation rates and trends. a) Bar chart displays the "Interannual infestation rate%" for ten beehives (BH1-BH10) from 2021 to 2023. It shows significant year-to-year and hive-to-hive variability in infestation rates, with some reaching nearly 5% and fluctuating across the seasons. b) Line plot with error bars, presents the "Mean" infestation rate (average across all beehives) from 2021 to 2023. It reveals a clear downward trend in the mean infestation: approximately 1.8% in 2021, decreasing to 1.45% in 2022, and about 1.1% in 2023, indicating an overall improvement. c) Linear regression equations ( $y = mx + b$ ) and  $R^2$  values for each beehive (BH1-BH10). These statistics quantify the specific trend of infestation over time for each hive, with varying slopes (indicating increase or decrease) and  $R^2$  values (indicating the strength of the linear fit).

exhibited a non-significant increase ( $\chi^2 = 2.47$ ,  $p = 0.29$ ), and other colonies (BH3, BH5-BH10) recorded only non-significant decreases in infestation over the three seasons. These trends are quantitatively supported by the regression equations presented in Fig. 4c.

Furthermore, a Linear Mixed Model (LMM), which accounted for repeated measurements within bee colonies, confirmed a highly significant overall effect of year on the infestation rate ( $F = 18.53794$ ,  $p < 0.000001$ ). This robust finding underscores a pervasive and statistically strong reduction in *Varroa* infestation across the entire study period, independent of individual hive variability.

Finally, aggregated data for all bee colonies were statistically tested for each beekeeping season (2021, 2022, 2023) (Fig. 4b). Shapiro-Wilk tests confirmed that the overall infestation rate data for each year were not normally distributed (e.g., 2021:  $W = 0.936$ ,  $p = 0.0002$ ). A Kruskal-Wallis H test on these aggregated yearly data demonstrated a significant difference ( $\chi^2(2) = 7.52$ ,  $p = 0.023$ ) in the overall infestation rates between the beekeeping seasons. Subsequent Post-Hoc Dunn's test (with Bonferroni correction,  $\alpha = 0.017$ ) specifically identified a significant decrease in infestation between the 2021 and 2023 beekeeping seasons. This crucial finding led to the acceptance of hypothesis H0<sub>3</sub>, confirming an overall significant reduction in *Varroa* infestation across the bee colonies during the study period.

### 3.4. Effectiveness of herbal decoctions on *Varroa* infestation (2021–2023)

Throughout the 2021, 2022, and 2023 beekeeping seasons, all experimental bee colonies received treatment with six distinct plant-based decoctions (DI–DVI), prepared from freshly collected plant materials and applied monthly from March to November. The effectiveness of these individual decoctions in reducing *V. destructor* infestation rates, and how this effectiveness varied over time and with application frequency, was rigorously evaluated using a Linear Mixed Model (LMM). The dependent variable, infestation rate (%), was arcsin square root transformed for analysis. The LMM included Decoction label, year, Month, Number of applications in a given month, and their relevant two-way interactions (year\*Decoction label and Decoction label\*Number of applications in a given month) as fixed effects, and ID\_bee colony as a random effect to account for repeated measurements within bee

colonies.

#### 3.4.1. Overall efficacy of decoctions and interannual variability

The Linear Mixed Model revealed a highly statistically significant main effect of Decoction label ( $F = 34.21101$ ,  $p < 0.000001$ ). This indicates that, on average across all years and application frequencies, different herbal decoctions had a significantly different impact on *Varroa* infestation. This robust finding supports the acceptance of hypothesis H0<sub>4</sub>, confirming that the effectiveness of various emulsions in reducing *V. destructor* is significant.

Furthermore, a highly significant interaction between year and Decoction label was observed ( $F = 4.10520$ ,  $p = 0.000031$ ). This crucial finding demonstrates that the effectiveness of the various decoctions in reducing infestation varied significantly across the study years (2021, 2022, 2023). For instance, while some decoctions (e.g., DI) maintained relatively stable (albeit higher) infestation rates across years, others (e.g., DIII, DV) showed more pronounced improvements in reducing infestation in later years. Specifically, in 2021, Decoctions DIV and DV appeared most effective, but their relative performance shifted in 2022 and 2023, where DIII and DV generally exhibited lower infestation rates.

#### 3.4.2. Impact of application frequency and cumulative efficacy

The LMM also identified a highly significant interaction between Decoction label and Number of applications in a given month ( $F = 17.76602$ ,  $p < 0.000001$ ). This suggests that the optimal number of applications for a given month varied depending on the specific decoction used. For example, some decoctions may have achieved their best efficacy with fewer applications, while others required more frequent application to reduce infestation.

The cumulative impact of the combined application of these diverse decoctions on the overall year-on-year decrease in the infestation rate was statistically significant, as evidenced by the significant main effects and interactions within the LMM, particularly when considered alongside the significant year effect discussed in 3.3. This comprehensive LMM analysis thus supports the acceptance of hypothesis H0<sub>5</sub>, confirming a significant overall effect of the emulsions on *V. destructor* reduction over the monitored period and highlighting the positive, cumulative impact of the phytotherapeutic approach over time.

## 4. Discussion

### 4.1. Summary of key findings and study context

The escalating impact of *V. destructor* as a primary ectoparasite of *A. mellifera* underscores a critical challenge in veterinary parasitology, demanding ecologically sound prophylactic and therapeutic strategies. Current reliance on synthetic acaricides often leads to mite resistance (Van Der Steen and Vejsnæs, 2021) and the potential for undesirable chemical residues in hive products. Our study addresses these issues by demonstrating the substantial potential of plant extracts, or "green pesticides," as a promising, cost-effective, and environmentally compatible alternative (Koul et al., 2008; Bava et al., 2023). This methodology, centered on naturally derived compounds, aims not only to reduce mite populations but also to enhance overall bee colony health, aligning with a holistic, safe, and environmentally compatible approach to apiary management.

The experiment, conducted over three years, demonstrated a crucial and statistically significant year-on-year decrease in overall *Varroa* infestation, notably without the use of conventional synthetic acaricides. This robust long-term decline was powerfully evidenced by the highly significant main effect of "Year" in our Linear Mixed Model (LMM) analysis. The LMM also confirmed that our different herbal decoctions had a variable yet significant impact on infestation. Crucially, the analysis revealed significant interactions between the "Year" and "Decoction label," as well as between the "Decoction label" and the "Number of applications" in a given month. These time-dependent variations underscore the dynamic nature of field conditions and highlight that optimal application frequency is not universal, but specific to each decoction's unique composition, volatility, and mode of action.

### 4.2. Phytotherapeutic mechanisms and plant-specific rationale

Our plant selection was guided by an ethnopharmacological approach, leveraging traditional knowledge of plants for their medicinal and pesticidal properties. Plant species contain diverse phytochemical constituents—such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes—which offer various pharmacological properties. These compounds, identified through centuries of traditional knowledge, are compelling candidates for novel anti-varroosis treatments. Our study validates traditional remedies within a modern pharmacological framework by detailing the specific roles of each plant component.

The choice to use plant decoctions over more concentrated forms, such as essential oils, was deliberate for both ethnopharmacological and practical reasons. The preparation of decoctions is a traditional ethnoveterinary practice that is widely accessible and easily replicated by beekeepers, even without specialized equipment. The hot water extraction method we employed is particularly effective at releasing a broad spectrum of heat-soluble compounds, including a wide array of minerals, vitamins, and flavonoids that offer antioxidant and anti-inflammatory benefits. This comprehensive approach ensures that the decoctions provide holistic support for the overall health and vitality of the bee colony, thereby indirectly strengthening their resilience against *Varroa* infestation (Bhusal et al., 2022; Lapinskaya and Kopyt'ko, 2008). This approach not only addresses the ecological and residue-related issues of synthetic treatments but also offers a realistic, cost-effective tool for apiary health management.

The inclusion of peppermint (*Mentha × piperita*), clove (*Syzygium aromaticum*), wormwood (*Artemisia absinthium*), and sage (*Salvia officinalis*) was based on their documented anti-parasitic properties. Peppermint's principal volatile compounds, menthol and menthone, possess potent antimicrobial and biopesticidal properties. Studies have directly evaluated its antiparasitic effects in decoction form and demonstrated its ability to reduce parasite burden (Dejani et al., 2014). Similarly, clove was selected for its anthelmintic and antimicrobial properties, with its

primary active component, eugenol, documented to be toxic to mites (Maggi et al., 2010). The mechanism involves tannins, saponins, and eugenol disrupting the outer layers of parasites (Anggrahini et al., 2021). Extracts from wormwood and sage are also known for their anti-parasitic and disinfecting properties (Özüüçli et al., 2024; Ayoubi et al., 2024).

Beyond direct parasiticidal action, our approach also aimed to bolster overall bee health and resilience. The decoction of stinging nettle (*Urtica dioica*), known in traditional medicine for enhancing immune function, provides a rich profile of minerals, vitamins, and terpenoids (Pohorecka, 2004). The decoction of common dandelion (*Taraxacum officinale*) efficiently extracts polysaccharides like inulin and phenolic acids such as chlorogenic acid, which contribute to its antioxidant, antiviral, and immunostimulatory effects (Diaz et al., 2018). Finally, linden flowers (*Tilia platyphyllos* and *Tilia cordata*) yield mucilaginous polysaccharides and flavonoid glycosides like tiliroside when decocted, supporting bee health and parasite control (European Medicines Agency homepage on the Internet, 2012; Colin, 1990).

Our plant selection was guided by an ethnopharmacological approach, leveraging traditional knowledge of plants for their medicinal and pesticidal properties. Plant species contain diverse phytochemical constituents—such as glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes—which offer various pharmacological properties. These compounds, identified through centuries of traditional knowledge, are compelling candidates for novel anti-varroosis treatments. Our study validates traditional remedies within a modern pharmacological framework by detailing the specific roles of each plant component.

The choice to use plant decoctions over more concentrated forms, such as essential oils, was deliberate for both ethnopharmacological and practical reasons, as hot-water extraction is a widely accessible traditional practice that is easily replicated by beekeepers.

### 4.3. Strengths, limitations, and future directions

Our study's unique focus on in-situ treatment and infestation assessment directly within the apiary is a key strength. This approach effectively bridges the translational gap between fundamental scientific research and practical, field-based veterinary application, using methods that are simple and accessible to beekeepers.

However, a primary limitation of our study is the absence of an untreated control group, as detailed in the methodology. This design decision was based on ethical considerations and the established high mortality rates of untreated colonies under local *Varroa* pressure (Spivak and Reuter, 2001). Additionally, we recognize that our CO<sub>2</sub> application method for monitoring mite populations has lower diagnostic accuracy compared to a sugar roll test (Bava et al., 2022). This method, however, was a deliberate choice for our long-term field study due to its non-destructive nature and practicality, which were essential for minimizing stress and mortality to the colonies.

For future research, we recommend focusing on the underlying mechanisms of action of these decoctions on mite physiology and bee immunity. It would also be beneficial to explore the synergistic potential of these plant-based decoctions when combined with other ecological *Varroa* control products. Continued interdisciplinary research and knowledge dissemination are paramount to realizing the full potential of these natural solutions in modern beekeeping.

## 5. Conclusions

Our study underscores the critical role of ethnopharmacological approaches in addressing the global challenge posed by *V. destructor*. Our findings provide evidence for a sustainable, natural alternative to synthetic acaricides, which are increasingly ineffective due to mite resistance and leave undesirable chemical residues in hive products.

We successfully validated the potential of using eco-friendly

phytotherapeutic decoctions derived from locally sourced medicinal plants to effectively control *V. destructor*. This approach is a significant step forward as it's simple, cost-effective, and environmentally benign. The use of a water-based extraction method, rather than concentrated essential oils or synthetic chemicals, is a key factor that significantly reduces the risk of chemical residues in honey and beeswax. The active compounds, being water-soluble and often volatile, are less likely to accumulate in lipid-rich hive products. This characteristic enables safe application, even during the honey flow, thereby protecting both the quality of bee products and consumer health. By successfully bridging traditional wisdom with modern scientific inquiry, our research paves the way for improved apiculture health, enhanced bee product quality, and more environmentally responsible apiculture.

#### CRediT authorship contribution statement

**Lubomír Korený:** data curation, formal analysis, investigation, writing – original draft, methodology, writing – review & editing; **Michaela Korená Hillayová:** conceptualization, data curation, formal analysis, investigation, visualization, writing – original draft, methodology, funding acquisition, writing – review & editing; **Jaroslav Škvarenina:** conceptualization, writing – original draft, writing – review & editing, funding acquisition, supervision.

#### Clinical trial number

not applicable.

#### Ethics approval

Not applicable.

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#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.vetpar.2025.110629](https://doi.org/10.1016/j.vetpar.2025.110629).

#### Data availability

The data presented in this study are available on request from the corresponding author.

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