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Efficacy of a polyherbal formula for controlling lice infestation in chickens and goats in backyard farming system in Romania

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Introduction: Lice infestation negatively impacts the welfare and productivity of domestic animals. This study aimed to evaluate the efficacy of a commercial polyherbal formula (PHF) containing *Asarum europaeum*, *Lavandula angustifolia*, *Artemisia absinthium*, and *Tanacetum vulgare* in managing lice infestations in chickens and goats within a backyard farming system in Romania.

Methods: The PHF was analyzed using high-performance liquid chromatography (HPLC) to determine its phytochemical composition. Chickens and goats with confirmed lice infestations were treated with the PHF by spraying it on the entire body. Treatments were administered three times at 14-day intervals for goats and at both 14- and 7-day intervals for chickens. Lice infestations were assessed in experimental groups before each treatment by counting the collected lice specimens.

Results: HPLC analysis identified key compounds in PHF: kaempferol (336.25 mg/ml), quercetin (88.38 mg/ml), epicatechin (43.98 mg/ml), and catechin (9.43 mg/ml). The PHF effectively controlled chewing lice in both chickens and goats, achieving a mortality rate of 76–98% in chickens and 96% in goats. However, the PHF was not effective against sucking lice in goats. A significant reduction in lice infestation (75% in chickens) was observed when the PHF was applied at 7-day intervals. No adverse reactions were noted in treated animals.

Discussion: The polyherbal formula demonstrated promising efficacy against chewing lice in chickens and goats, supporting its potential use as an alternative to chemical treatments, especially in organic farming settings. The findings suggest that this PHF could be a viable option for managing lice infestations in domestic animals, particularly where organic practices are prioritized.

KEYWORDS

lice, chicken, goat, plant extract, poultry, backyard

1 Introduction

Lice are wingless insects, typically ranging from 1 to 6 mm in length, that live as permanent parasites on the skin surface of mammals and birds (Benelli et al., 2018). They are classified into biting (chewing) lice (suborders Amblycera, Ischnocera, and Rhynchophthirina) and blood-sucking lice (suborder Anoplura), based on their feeding habits (Johnson and Clayton, 2003).

Chewing lice primarily feed on skin scales, feathers, lipids, and bacteria, while sucking lice feed on blood (Meguini et al., 2018). Both types can infest mammals, but birds typically host only chewing lice (Benelli et al., 2018; Meguini et al., 2018). Lice exhibit host specificity, and their life cycle follows incomplete metamorphosis. Females lay eggs, known as nits, which are attached to the hair and feathers of their host. Within 1–2 weeks, first-stage larvae hatch, followed by molting into second and third-stage larvae, before maturing into adult males and females. The entire life cycle lasts approximately 3–4 weeks. Infestations typically occur through direct contact between hosts, although indirect transmission is also possible, as lice can survive off the host for about 1–2 weeks (Benelli et al., 2018).

Lice infestations in animals can significantly impact productivity, causing skin irritations, itching, hair and feather loss, lichenification, scaling, and even anemia (Al-Badrani and Al-Muffti, 2021). Lice can also act as biological or mechanical vectors for other pathogens. For instance, they can transmit *Dipylidium caninum* in carnivores and the filarial heartworm *Sarconema eurycerca* in waterfowl (Seegar et al., 1976; Rousseau et al., 2022). Furthermore, *Pasteurella multocida* has been isolated from the gut of fowl lice (Derylo, 1970), and lice from cattle, goats, and pigs have been found to carry *Anaplasma* and *Rickettsia* DNA (Hornok et al., 2010).

Traditionally, lice infestations have been managed using synthetic insecticides such as pyrethroids, phoxim, and macrocyclic lactones. However, the emergence of drug-resistant ectoparasite populations has become a significant concern (McNair, 2015). Resistance has been observed across multiple drug classes, often through similar mechanisms within the same class (Houpt et al., 1988; Gao and Zhu, 2002; Gao et al., 2006). These resistant strains arise due to genetic mutations in parasite populations and given their short reproductive cycles and high reproductive rates, resistance can spread rapidly, especially in environments where antiparasitic treatments are frequently used (Wolstenholme et al., 2004; McNair, 2015).

Due to the increasing prevalence of antiparasitic drug resistance, particularly in livestock, researchers are actively exploring alternative strategies to control infestations. Future approaches may involve identifying new drug targets or adopting alternative control measures, such as investigating biological control methods like plant extracts and essential oils (McNair, 2015).

The antiparasitic effects of plant extracts have gained considerable attention in recent years. Numerous studies have shown promising results, demonstrating the potential efficacy of certain plant compounds in controlling parasitic infections (Al-Quraishy et al., 2012; Garcia-Bustos et al., 2019; Pop et al., 2019; Hartady et al., 2021; Coroian et al., 2022). Medicinal plants with a long history of traditional use for lice control, combined with their active ingredients, may offer a natural solution for external treatments (Al-Quraishy et al., 2012; Mund et al., 2017; Garcia-Bustos et al., 2019; Giannenas et al., 2020; Nobakht et al., 2022; Vakili et al., 2022; Challaton et al., 2023).

Therefore, this study aimed to assess the efficacy of a commercial mixed plant extract (polyherbal formula) in controlling natural lice infestations in chickens and goats raised under extensive conditions in Romania, given the limited number of studies available on this topic.

2 Materials and methods

2.1 Animals and experimental design

The insecticide efficacy of the polyherbal formula (PHF) was evaluated in natural infestations with lice in chickens (Experiments 1 and 2) and goats (Experiment 3). The experiments were conducted between June and July 2019. All animals were raised in backyard farm system. The chickens had access to outdoor areas with natural vegetation and were housed in simple coops that provided shelter, while the goats had basic shelter to protect them from rain, wind, and extreme temperatures. According to WorldClim (2024), the environmental temperature during the experimental period ranged from 16°C to 26°C, with moderate humidity levels typical for the summer months in this region (25). The animals were exposed to natural daylight cycles and their diet consisted of grazing on pasture, locally available feed (corn, wheat, barley and oats), and occasional supplementation (especially for the goats) with grains and hay. Clean water was provided *ad libitum*.

Experiment 1 included 15 chickens (14 hens and one rooster). The chickens were adult birds, aged between 1.5 to 2 years, with an average weight ranging from 1.8 to 2.5 kg. The PHF was applied three times at 14-day intervals. The chickens were examined for lice before each treatment, with lice counted in three body regions: dorsum, wings, and ventral body.

Experiment 2 involved 88 chickens (hens and roosters) divided in five households; the size of household ranged from 10 to 28 chickens. The chickens were 1 to 3 years old, with a body weight between 1.5 to 3 kg. Four households were allocated to treated group (n=4; 73 chickens) and one household to untreated group (n=1; 15 chickens). The PHF was applied three times at 7-day intervals. Five hens per household were examined for lice before each treatment, and lice were counted using the same method as in Experiment 1.

Experiment 3 included a small herd of 20 goats: 12 adult goats (2–5 years old, with an average bodyweight of 35 kg), and 8 kid goats (4–6 months old, with an average body weight of 15 kg). The PHF was applied three times at 14-day intervals. All goats were evaluated before each treatment and seven days after the final treatment. The evaluation was done by macroscopic examination of the skin followed by 5-minute coat brushing. The collected hairs and scales were put in a Petri dish and examined under a magnifier for lice. The lice were identified based on their morphology and counted (Wall and Shearer, 2001).

The prevalence of infested animals and the percentage of lice mortality was calculated for each experiment and time of the evaluation.

2.2 Polyherbal formula

The polyherbal formula (PHF) was a propylene glycol extract derived from *Asarum europaeum* (European wild ginger), *Lavandula angustifolia* (lavender), *Artemisia absinthium* (common wormwood), and *Tanacetum vulgare* (tansy). The extracts were obtained from ground dried plants through maceration in propylene glycol (16% dried plants in propylene glycol) for 14 days, followed by cold pressing. The PHF was analyzed using high-performance liquid chromatography.

The product was applied to chickens and goats by spraying the entire body. For chickens, approximately 5–6 pumps were applied from a distance of 10–15 cm. For goats, one pump was administered per 10 cm² from the same distance as in chickens.

2.3 Analytical characterization of the polyherbal formula

The polyherbal formula (PHF) was analyzed using high-performance liquid chromatography (HPLC) with a diode array detector (DAD). The system used was the Merck Hitachi L-7000 HPLC with the following components: interface D-7000, quaternary pump L-7100, solvent degasser L-7612, autosampler L-7200, and DAD detector L-7455. The analysis focused on identifying and measuring polyphenols and flavonoids, including chlorogenic acid, caffeic acid, quercetin, and kaempferol (purchased from Extrasynthese, Genay, France), as well as catechin, epicatechin, and isoquercetin (purchased from Rott GmbH, Karlsruhe, Germany).

The stationary phase used was a NUCLEODUR C18 Gravity column (3 µm, 150 x 3 mm, Macherey-Nagel) with a flow rate of 0.750 ml/min. Gradient elution was performed with acetonitrile-phosphate buffer (10 mM, pH 2.5), increasing the organic modifier concentration from 8% to 35% over 25 minutes. A sample volume of 50 µl was injected using loop injection mode after a ten-fold dilution. The best chromatogram extraction was observed at 205 nm. The method was validated for precision, linearity, and accuracy, and the limits of detection (LOD) and quantification (LOQ) were determined for each standard used (Varga et al., 2020).

2.4 Statistical analysis

The data were statistically analyzed using Epitools and MedCalc softwares. The prevalence of positive animals was calculated as the percentage of positive animals from the total evaluated animals, along with its 95% confidence interval. Differences in prevalence among time points of evaluation and between treated and untreated groups were determined by the chi-square test, corrected by Yates. The average and standard error of the mean were calculated for the number of lice per animal (chicken or goat). A one-way ANOVA was used to assess statistical differences among the evaluation time points and between treated and untreated groups. A P value of 0.05 or lower was considered statistically significant.

3 Results

3.1 HPLC analysis

The results of the analytical characterization of the PHF by high-performance liquid chromatography (HPLC) are presented in Table 1, with the typical chromatogram of the tested product shown in Figure 1. Four polyphenols were identified in the PHF: catechin (9.43 µg/ml), epicatechin (43.98 µg/ml), kaempferol (336.25 µg/ml), and quercetin (88.38 µg/ml) (Table 1).

TABLE 1 Summarized results of the validation parameter and characterization of the polyherbal formula by high-performance liquid chromatography.

	Linearity range (µg/ml)	Correlation coefficient	Accuracy (%)	LOD (µg/ml)	LOQ (µg/ml)	HF (µg/ml)
Chlorogenic acid	2.35–23.30	0.998	101.21	0.78	2.33	BLQ
Caffeic acid	4.02–20.1	0.998	102.84	0.80	2.45	BLQ
Quercetin	6.15–30.76	0.997	101.95	1.88	5.71	88.38
Kaempferol	12.32–30.78	0.995	103.15	3.4	10.38	336.25
Catechin	5.56–27.60	0.996	102.86	1.85	5.52	9.43
Epicatechin	6.01–30.08	0.988	103.24	1.72	5.65	43.98
Isoquercetin	3.76–30.92	0.997	101.44	1.07	3.26	BLQ

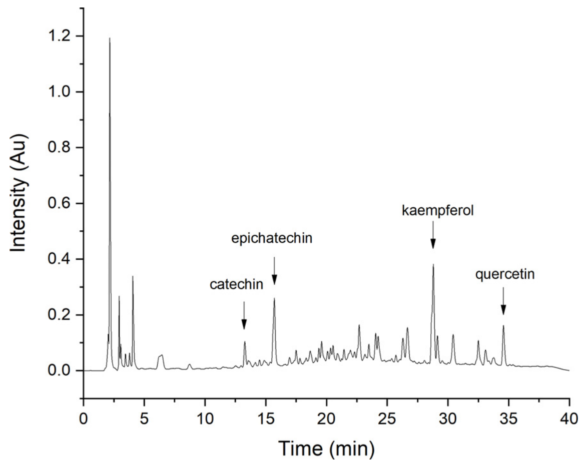

FIGURE 1
The chromatogram of the PHF.

3.2 Efficacy in chickens

The following lice species were identified in chickens: *Menopon gallinae*, *Goniodes dissimilis*, and *Menachantus stramineus* (Figure 2). In Table 2 (Experiment 1) and Table 3 (Experiment 2), the number of positive chickens and the number of lice per chicken at each evaluation time point after PHF application are presented. No adverse reactions were observed after any of the treatments with PHF in either experiment.

In Experiment 1, where PHF was applied at 14-day intervals, half of the evaluated chickens tested negative for lice after the third treatment. The number of lice per positive chicken decreased significantly ($p = 0.012$) from 7 lice on day 0 to 1.7 lice on day 28 (Table 2). In Experiment 2, where PHF was applied at 7-day intervals, better results were achieved based on lice population mortality. The number of positive chickens decreased significantly ($p < 0.00001$) from 100% on day 0 to 25% on day 14. Additionally, the number of lice per chicken decreased notably ($p < 0.001$) from 18.1 lice on day 0 to 0.25 lice on day 14 (Table 3). In the untreated group, both the number of positive chickens and the number of lice/chicken remained constant throughout the experimental period. Significant differences ($p \leq 0.05$) between the treated and untreated groups were observed after the first treatment (Table 3).

3.3 Efficacy in goats

The following lice species were identified in goats: *Linognatus stenopsis* (sucking louse) and *Bovicola caprae* (chewing louse) (Figure 3).There were no adverse clinical reactions recorded after PHF application in goats. The number of positive goats did not decrease significantly ($p = 0.49–0.88$) after treatment with PHF, regardless of the louse species (Table 4). However, a significant reduction ($p < 0.001$) in the number of lice per goat was observed for *B. caprae* after the first treatment, with lice counts dropping from 31.5 to 5.43 and remaining relatively stable thereafter (5.43 on day 14, 3.63 on day 28, and 1.25 on day 35) (Table 4).

The number of sucking lice per infested goat remained constant throughout the experimental period (Table 4).

4 Discussions

Lice infestation in animals is often neglected by farm owners due to the low frequency of severe symptoms. However, lice directly impact the overall condition and feed intake of animals, leading to reduced productivity (Al-Quraishy et al., 2012).

Medicinal plants have been widely utilized since ancient times, and their use is experiencing a resurgence in modern livestock management. This is partly due to the decreasing effectiveness of synthetic compounds and the growing awareness of their adverse effects (Giannenas et al., 2020). As a result, herbal extracts are increasingly seen as promising alternatives for prophylactic or therapeutic interventions (Hartady et al., 2021; Challaton et al., 2023).

The widespread use of chemical compounds in animal products can lead to chemical residues, which contribute to the rise of drug-resistant microorganisms in humans (Mund et al., 2017). Recently, consumer preference for organic animal products has significantly increased, driven by greater awareness of dietary choices (Napolitano et al., 2009). Regulations on organic certification limit the use of synthetic compounds in poultry farming (Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on Organic Production and Labelling of Organic Products and Repealing Council Regulation (EC) No 834/

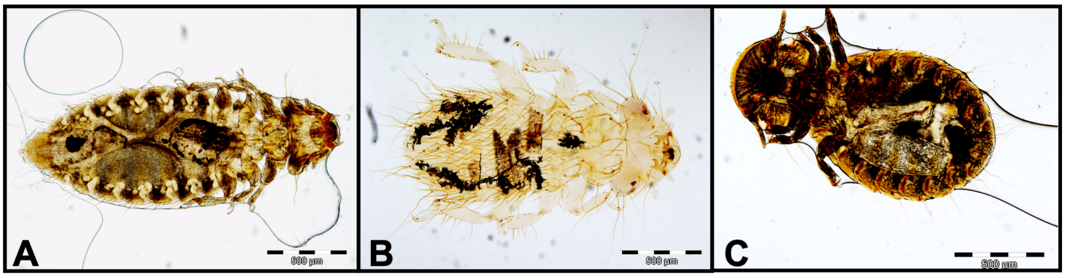

FIGURE 2
Lice species identified in chickens: *Menopon gallinae* **(A)**, *Goniodes dissimilis* **(B)**, *Menachantus stramineus* **(C)**.

TABLE 2 Prevalence of lice infestation and the number of lice per chickens after treatment with the polyherbal formula applied at 14 days interval (Experiment 1).

	Day 0	Day 14	Day 28	χ_{df}/F_{df}	p
Positive chickens/total (prevalence; 95% CI)	6/6 (100%; 56.6–100.0)	5/6 (83.3%; 43.7–97.0)	3/6 (50.0%; 18.8–81.2)	$2.2154_{(2,18)}$	0.33
Number of lice/chickens (95% CI)	7 ± 1.52 (2.8–11.2)	2.8 ± 0.95 (0.2–5.8)	1.7 ± 0.84 (−0.5–3.8)	$6.22_{(2,14)}$	0.012

Day 0 – 1st treatment; Day 14 – 2nd treatment; Day 28 – 3rd treatment. 95% CI: 95% Confidence interval 0.05.

2007, 2018), which has led to growing interest in natural herbal products as alternatives for disease prevention and management.

Based on these considerations, our study aimed to evaluate the effectiveness of a commercially available plant extract (a mixture of European wild ginger, lavender, common wormwood, and tansy) in controlling natural lice infestations in chickens and goats raised in extensive farming systems in Romania.

The efficacy of natural alternatives for treating lice infestations in animals is supported by a limited number of studies, which have investigated various herbal remedies such as neem, lavender, clove, cinnamon, and star anise (Al-Quraishy et al., 2012; Pumnuan et al., 2020). Lavender's insecticidal properties have been studied in donkeys and humans (Canyon and Speare, 2007; Talbert and Wall, 2012; Sands et al., 2016). For example, a study by Ellse et al. (2013) using lavender oil in donkeys reported a significant reduction in chewing louse populations. Similarly, Cotticelli et al. (2023) demonstrated that neem seed extract reduced *Linognathus stenopsis* infestations in goats by 69.1% to 100%. The treatment involved eight administrations at 7-day intervals, and the authors suggested that efficacy may be dose-dependent.

In our study, the tested PHF was not effective against sucking lice but showed promising results in controlling chewing lice in both chickens and goats. Previous studies have hypothesized and demonstrated that essential oils can inactivate lice by affecting their nervous system (Veal, 1996; Mills et al., 2004). For instance, Terpen-4-ol and 1,8-cineole, found in tea tree oil, inhibit acetylcholinesterase (Veal, 1996). Essential oils may also block spiracles, mechanically suffocating the lice (Ellse et al., 2013). Based on these mechanisms, both types of lice should theoretically be affected. Additionally, phenolic acids from alcoholic plant extractions penetrate and accumulate in the skin (Nowak et al., 2021). These plant extracts, when accumulated in the skin, can be ingested by chewing lice that feed on scales, hair, and feathers of the host.

In the present study, a statistically significant mortality rate of 76-98% was observed in chewing lice on chickens and 96% in goats. The number of infested animals also decreased significantly in chickens when the PHF was applied at 7-day intervals, with a 75% reduction in positive animals. When the PHF was applied at 14-day intervals, the reduction in positive animals ranged from 25% (goats) to 50% (chickens).

Experiments 1 (chickens) and 3 (goats) were conducted concurrently, following the manufacturer's recommended treatment protocol of 14-day intervals. After analyzing the data, it was decided to repeat the experiment with chickens, applying the treatment every 7 days. This resulted in superior therapeutic efficacy, with a 75% reduction in lice prevalence compared to 50%

TABLE 3 Prevalence of lice infestation and the number of lice per chickens after treatment with the polyherbal formula applied at 7 days' interval (Experiment 2).

	Day 0	Day7	Day 14	χ_{df}/F_{df}	p
Positive chickens/total (prevalence; 95% CI)					
Treated group	20/20 (100%; 83.9–00.0)	6/20 (30.0%; 14.6–51.9)	5/20 (25.0% 11.2–46.9)	$25.38_{(2,61)}$	< 0.00001
Untreated group	5/5 (100%; 56.6–100.0)	5/5 (100%; 56.6–100.0)	5/5 (100%; 56.6–100.0)	$0.0_{(2,15)}$	1.0
t_{df}	$0.01_{(2,1)}$	3.75	4.40		
P	0.92	0.05	0.04		
Number of lice/chicken ± SEM (95% CI)					
Treated group	18.1 ± 1.77^b (11.3–22.7)	0.6 ± 0.25^a (0.04–1.1)	0.25 ± 0.1^a (0.04–0.5)	$97.72_{(2,57)}$	< 0.001
Untreated group	14.2 ± 2.63^a (6.9–21.5)	14.6 ± 2.56^a (7.5–21.7)	16.4 ± 1.94^a (11.0–21.8)	$0.24_{(2,12)}$	0.79
t_{df}	$−1.214_{8,1}$	$−5.461_{4,1}$	$8.32_{4,0}$		
P	0.26	0.006	0.001		

Day 0 – 1st treatment; Day 7/14 – 2nd treatment; Day 14/28 – 3rd treatment; 95% CI: 95% Confidence interval.
Lower case letters indicate statistical values.

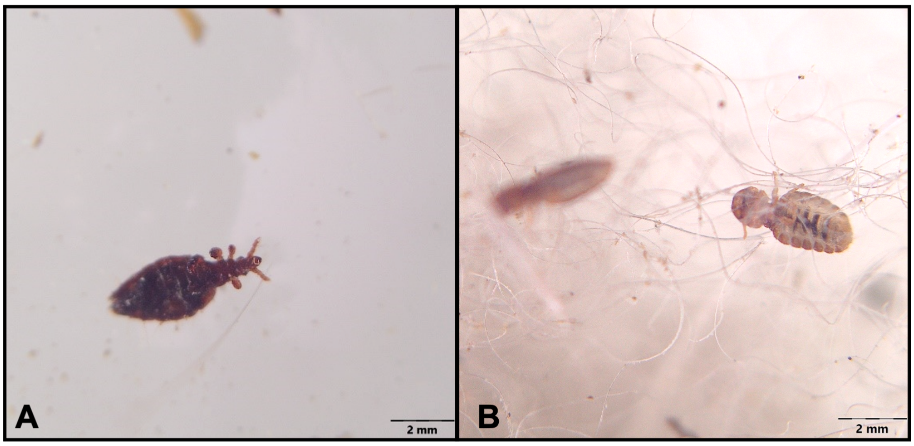

FIGURE 3
Lice species identified in goats: *Linognatus stenopsis* **(A)** and *Bovicola caprae* **(B)**.

TABLE 4 Prevalence of lice infestation and the number of lice per goat after treatment with the polyherbal formula applied at 14 days interval (Experiment 3).

	Day 0	Day 14	Day 28	Day 35	χ_{df}/F_{df}	*p*
Chewing lice						
Positive goats/total (prevalence; 95% CI)	6/8 (75.0%; 40.9–92.9)	6/8 (75.0%; 40.9–92.9)	6/8 (75.0%; 40.9–92.9)	6/8 (75.0%; 40.9–92.9)	$0.68_{(3,29)}$	0.88
Number of lice/goat ± SEM (95% CI)	31.5 ± 8.61[b] (9.36–53.64)	5.43 ± 2.11[a] (0.26–10.60)	3.63 ± 1.25[a] (0.66–6.59)	1.25 ± 0.41[a] (0.28–2.22)	$12.74_{(3,25)}$	< 0.001
Suckling lice						
Positive goats/total (prevalence; 95% CI)	4/8 (50.0%; 21.5–78.5)	2/8 (25.0%; 7.2–59.1)	4/8 (50.0%; 21.5–78.5)	5/8 (62.5%; 30.6–86.3)	$2.42_{(3,29)}$	0.49

Day 0 – 1st treatment; Day 14 – 2nd treatment; Day 28 – 3rd treatment; Day 35 – 7 days after the 3rd treatment.

with the 14-day interval. Additionally, applying the treatment every 7 days led to a 98.6% reduction in the number of lice per chicken, compared to a 75% reduction with the 14-day interval. Based on these findings, we recommend administering at least five treatments at 7-day intervals to enhance the therapeutic outcomes of the polyherbal formula.

5 Conclusions

The polyherbal formula shows potential as a viable alternative to chemical treatments for managing chewing lice infestations in chickens and goats, especially when applied at 7-day intervals. Its plant-based composition makes it particularly suitable for organic farming, aligning with European regulations that restrict the use of synthetic chemicals in animal therapies. Although the study was narrowly focused on a specific formula and specific animal populations, this was intentional to explore the product's practical effectiveness in real-world, small-scale farming conditions. By conducting the research in these conditions, we aimed to mimic the scenarios in which smallholders and organic farmers operate, ensuring the findings are directly applicable to such contexts. The expected efficacy of the polyherbal formula was a hypothesis based on existing evidence of the benefits of plant-based treatments; however, this study provides valuable confirmation of its effectiveness under real-world conditions. The findings suggest that, with minor adjustments to enhance efficacy, the polyherbal formula could play an important role in reducing reliance on synthetic insecticides. This is especially relevant in preventing chemoresistance in lice populations – a growing concern in both organic and conventional farming. By focusing on the narrow scope of backyard farms and organic methods, the study underscores the importance of developing sustainable, low-impact solutions for pest management, contributing to long-term agricultural resilience.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The collection of ectoparasites was conducted in the shortest possible time, without causing pain or distress to the animals, and their daily activities were not affected. No experimental infection was performed, as the animals were naturally infested. In accordance with Directive 2010/62/EU and Romanian Law 43/2014 on the protection of animals used for scientific purposes, the procedures were non-invasive, in-volving macroscopic examination and brief brushing of the animals. Informed consent was obtained verbally from all animal owners for sample collection, product use, and publication of results. As the study was conducted on backyard farms and complied with national and EU regulations, formal ethical approval from an institutional committee was not required. Nevertheless, all efforts were made to ensure the welfare of the animals throughout the study. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MC: Data curation, Writing – original draft, Writing – review & editing. C-CH: Data curation, Investigation, Writing – review & editing. EV: Data curation, Investigation, Methodology, Validation, Writing – review & editing. IF: Data curation, Investigation, Methodology, Validation, Writing – review & editing. MF: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing – review & editing. AG: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

Author MF was employed by SC Promedivet SRL.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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