



A Hybrid System for Medicinal Leech Cultivation: An Alternative Model to Enhance Reproductive Efficiency

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ABSTRACT

Background: The sustainable cultivation of medicinal leeches holds critical importance both for conserving natural populations and for ensuring a reliable and safe supply for medical applications. However, conventional rearing systems currently in use face several limitations, including high individual density, environmental stress, lack of traceability and low reproductive output. This study aims to evaluate the efficiency of a newly developed hybrid model designed to address the disadvantages associated with the traditional pond-based system.

Methods: The hybrid model provides a modular structure that enables individual monitoring while ensuring controlled temperature, humidity and substrate management. In this study, the effects of different protocols incorporating either fresh or reused peat substrates on cocoon number, offspring count and cocoon morphometry were compared. Intergroup differences were statistically analyzed, with a significance threshold set at $p < 0.05$.

Result: The results demonstrated that the hybrid model using fresh peat, particularly under Protocol A, yielded significantly higher cocoon and offspring production compared to all other groups ($p < 0.01$). In the hybrid model, the mean cocoon count per broodstock was 2.28 and the mean number of juveniles was 23.28; whereas in the traditional pond model, these values were 1.02 and 11.56, respectively. No significant differences in cocoon morphometry were observed between the groups ($p > 0.05$). Overall, the findings reveal that the hybrid model outperforms the conventional pond system in terms of both production efficiency and sustainability, emphasizing the critical role of substrate freshness and environmental stability in successful leech cultivation.

Key words: *Hirudo verbana*, Hybrid model, Reproductive efficiency, Substrate quality, Sustainable cultivation.

INTRODUCTION

Leeches are segmented worms belonging to the subclass Hirudinea within the phylum Annelida and exhibit considerable diversity in their ecological and biological characteristics. Although they are most commonly known as temporary blood-feeding ectoparasites of mammals, it has been reported that some species also parasitize amphibians, including frogs and tadpoles, as well as turtles and small fish (Moser *et al.*, 2009; Kriska, 2022). To date, more than 600 leech species have been described worldwide, inhabiting a wide range of ecosystems such as freshwater, marine, estuarine and moist terrestrial environments (Kaygorodova, 2024). In addition to their taxonomic diversity, leeches exhibit notable characteristics in terms of their reproductive biology. As hermaphroditic organisms, leeches possess both male and female reproductive systems within a single individual. Their typical life cycle consists of an egg stage developing within a cocoon, followed by a juvenile phase and a sexually mature adult stage (Copena and Gómez-Martín, 2024). This reproductive strategy is shaped by biologically sensitive processes that are highly responsive to environmental conditions, with substrate characteristics and microenvironmental stability recognized as key determinants of reproductive success. As integral components of ecological systems, leeches also occupy an important position in biomedical applications due to their distinctive biological properties. Some species that are obligate hematophagous organisms

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(particularly *Hirudo verbana* and *Hirudo medicinalis*) are capable of secreting a wide range of bioactive compounds during feeding on vertebrate blood (Karataş *et al.*, 2022). Owing to these properties, medicinal leeches have been widely used throughout history in the treatment of various clinical conditions, including circulatory disorders, hematoma, edema and wound healing (Montinari and Minelli, 2022; Zakian *et al.*, 2022).

In recent years, hirudotherapy practices have been addressed more systematically across medical, veterinary, cosmetic and pharmaceutical fields, leading to a steadily

increasing global demand for medicinal leeches (Lemke and Vilcinskas, 2020). This growing demand is largely met through the collection of leeches from natural habitats, exerting substantial pressure on wild populations. Factors such as overharvesting, environmental pollution and habitat loss have caused marked declines in leech populations, placing some species at risk of extinction (Firouzbakhsh, 2023). In this context, the inclusion of *Hirudo medicinalis* in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has subjected the international trade of medicinal leeches to regulatory control (CITES, 2017). Consequently, the sustainable production of medicinal leeches has become a strategic necessity, both for the conservation of biodiversity and for ensuring the continuity of legal and controlled trade (Kutschera and Elliott, 2014).

In response to this need, hirudiculture practices (medicinal leech farming) have been developed to enable the production of leech species independently of natural populations; however, the production protocols currently in use have not yet been fully standardized (Ceylan and Çetinkaya, 2021). The first systematic information on the maintenance and breeding of medicinal leeches under laboratory conditions was provided by Moore (1959). This classical approach was based on keeping leeches in glass containers under defined temperature and water parameters and feeding them periodically with blood. Nevertheless, this system entails significant limitations, including restricted production capacity, high maintenance requirements, prolonged fasting periods and yield losses associated with environmental stress (Moore, 1959).

Although recent years have witnessed an increasing number of attempts to develop improved production protocols for medicinal leeches, many technical and biological parameters (particularly those related to the incubation and breeding process) remain insufficiently clarified.

Although it is well established that factors such as the depth of cocoon placement within moist peat substrate, orientation angle and surrounding environmental conditions directly influence reproductive success, the lack of standardized and scientifically grounded protocols for these processes complicates the resolution of practical problems encountered by producers and limits scientific productivity (Ceylan *et al.*, 2023). Therefore, experimental evaluation of production parameters and the development of applicable model-based approaches are of critical importance for achieving sustainable, controlled and high-yield medicinal leech production under closed-system conditions.

The aim of this study was to minimize the inherent limitations of closed-system pool-based leech cultivation and to enhance reproductive efficiency by describing a novel production system (referred to as a “hybrid model”) developed based on the classical laboratory-based hirudiculture approach and by comparatively evaluating its

effectiveness against the conventional closed-environment pool model. Within this framework, key production parameters (including the number of cocoons, cocoon weight, cocoon length, cocoon width and the number of hatchlings obtained from broodstock leeches reared in both systems) were analyzed and the effects of the two models on reproductive success and offspring yield were statistically compared. It is anticipated that the findings of this study will contribute to the development of more efficient, sustainable and standardizable production protocols for medicinal leech cultivation.

MATERIALS AND METHODS

This study was conducted at the Medicinal Leech Breeding and Research Laboratory, Department of Medical Parasitology, Faculty of Medicine, Kırşehir Ahi Evran University, Kırşehir, Türkiye, between March and July 2025.

Design and technical features of the hybrid model

The system defined as the “Hybrid Model” was designed to both enhance production efficiency in medicinal leech cultivation and to establish a biologically standardizable farming environment. Owing to its modular design, the system consists of detachable components. Its primary structural elements are illustrated in Fig 1 as follows: fully assembled configuration (A), disassembled components (B) and cross-sectional view (C). In 2024, a utility model application for this system was submitted for the first time by our group to the Turkish Patent and Trademark Office under application number 2024/021463. Within the scope of this application, the modular structure of the system, its traceability and its capacity to ensure controlled production conditions were evaluated and the model was deemed to possess significant potential for industrial applicability. The main components and functional features of the hybrid model are summarized below:

Lower body (1)

This is the primary load-bearing component of the system, manufactured from biocompatible polypropylene material. Structurally, its 30° inclined base design facilitates optimal collection of cocoons on the sieve (4). It is integrated with the upper body (2) *via* a threaded mechanism to ensure sealing and leak prevention.

Upper body (2)

A modular unit designed to facilitate access to the cocooning substrate and ease of cleaning. Integrated ventilation is provided through a perforated lid (8) on the top, allowing passive air circulation and reducing the risk of external contamination.

Support chassis (3)

A supporting frame made from biocompatible polypropylene. Positioned between the lower body (1) and the lower base frame (6), it enhances the mechanical

stability of the system. Additionally, it serves as a support platform for the sieve (4).

Sieve (4)

A component featuring 2 mm perforations that enables leeches to deposit their cocoons in a centralized manner. It is isolated using stoppers (7) to minimize contact with other system components and to reduce contamination risk.

Lower base frame (5)

A protective connector placed between the support chassis (3) and the upper base frame (6), designed to absorb shocks and vibrations.

Upper base frame (6)

Contains the locking mechanism that connects the upper and lower body components. Its threaded structure allows for easy assembly and disassembly, as well as modular cleaning.

Stoppers (7)

These isolate the sieve (4) from other system components, providing protection against microbial contamination. Their

polymer-coated surfaces offer support without restricting leech movement.

Lid (8)

An integrated ventilation cover mounted on the upper body. With a perforated structure providing 40% open surface area, it enables effective gas exchange while preventing the entry of foreign particles.

Since the design and component specifications of the model are protected under the utility model application, this section focuses solely on the system's functional principles.

Experimental procedure and production protocols

Experimental design and groups: Two different leech cultivation models were compared in this study:

Pool Model (Control Group) (Fig 2A):

- Comprised 123 broodstock leeches (2-7 g) (n = 123).
- Both new and used peat were tested.
- Protocol A was implemented.

Hybrid Model (Experimental Groups) (Fig 2B):

- Comprised four separate groups, each containing 7 broodstock leeches (2-7 g) (n = 7/group).

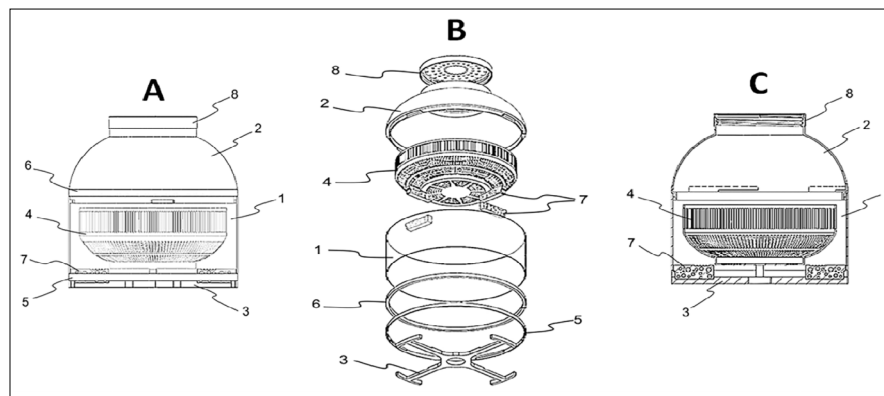
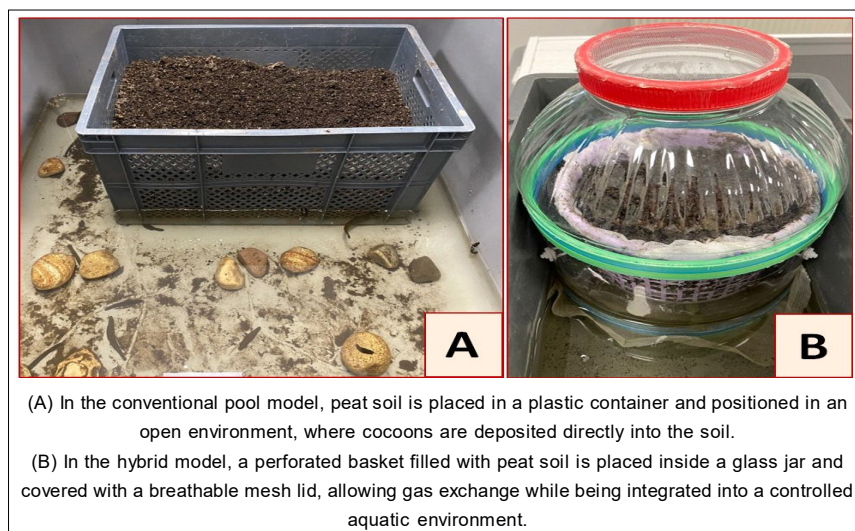


Fig 1: Assembled view (A), Disassembled components (B) and Cross-Sectional diagram (C) of the Hybrid model.



(A) In the conventional pool model, peat soil is placed in a plastic container and positioned in an open environment, where cocoons are deposited directly into the soil.
(B) In the hybrid model, a perforated basket filled with peat soil is placed inside a glass jar and covered with a breathable mesh lid, allowing gas exchange while being integrated into a controlled aquatic environment.

Fig 2: Two distinct systems used for medicinal leech cultivation.

- Both new and used peat were tested.
- Four different protocols were implemented (A, B, C, D).

Production protocols and environmental conditions

In this study, four different production protocols were applied, differing in terms of water exchange frequency, cocoon collection interval and the type of peat substrate used (Table 1). All experimental groups were monitored under controlled laboratory conditions for a total of 20 weeks. Throughout the experimental period, ambient temperature was maintained between 24-26°C and relative humidity was kept constant at 70-80%.

Culture units were maintained under natural photoperiod conditions and positioned in a low-light environment to prevent direct light exposure. The water used in both production systems was dechlorinated and equilibrated to room temperature prior to use.

The rearing substrate consisted of a peat mixture containing cocopeat and perlite, with a reported pH range of 5.5-6.5 according to the manufacturer's technical specifications. This peat type was selected as the substrate material due to its high water-holding capacity, porous structure and favorable drainage and aeration properties. In this context, new peat refers to peat obtained directly from the manufacturer and used for the first time in leech cultivation, whereas used peat denotes peat that had previously been employed in leech rearing and reused without any disinfection treatment.

Measured variables

During the study period, the following parameters were recorded

- **Total number of cocoons:** The total number of cocoons produced per group during the observation period.
- **Total number of hatchlings:** The total number of hatchlings obtained per group.
- **Number of cocoons per broodstock:** The mean number of cocoons produced per broodstock leech within each group.
- **Number of hatchlings per broodstock:** The mean number of hatchlings obtained per broodstock leech within each group.
- **Cocoon morphometrics:** Cocoon weight (g), length (mm) and width (mm), measured using calibrated instruments under standard laboratory conditions.

All numerical variables were evaluated both on a group basis and as values normalized per broodstock individual.

Statistical analysis

All statistical analyses of the data obtained in this study were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Prior to analysis, the distribution characteristics of the data related to cocoon number, hatchling number, number of cocoons and hatchlings per broodstock, as well as cocoon morphometric measurements (weight, length and width), were assessed. Given the total sample size of 130 individuals (control group: $n = 123$; experimental groups: $n = 7$ per group), the assumption of normality was tested using the Kolmogorov-Smirnov test.

As the normality assumption was not met ($p < 0.05$), non-parametric statistical methods were employed for between-group comparisons. Accordingly, the Kruskal-Wallis H test was used to evaluate whether statistically significant differences existed among production models, protocols and peat types with respect to cocoon number, hatchling number and morphometric parameters.

In all statistical analyses, the level of significance was set at $p < 0.05$. Results were expressed as mean $\pm$ standard deviation (SD) where appropriate.

RESULTS AND DISCUSSION

The effects of different rearing protocols and peat substrate types on cocoon and hatchling production parameters in medicinal leech cultivation are presented in Table 2. In this study, four different protocols were evaluated within the hybrid model, while the classical pool model was used as the control group.

In the hybrid model groups using new peat, Protocol A yielded a mean number of 2.28 ± 0.63 cocoons per broodstock and 23.28 ± 7.24 hatchlings per broodstock (Table 2). In contrast, the corresponding values in the pool model were 1.02 ± 0.44 cocoons per broodstock and 11.56 ± 6.49 hatchlings per broodstock, respectively.

In groups using used peat, both the hybrid model and the pool model exhibited markedly lower numbers of cocoons and hatchlings per broodstock (Table 2). In some groups with used peat, no cocoon formation or hatchling production was observed.

Cocoon weight values in the hybrid model groups using new peat ranged between 0.63 ± 0.25 g and 0.82 ± 0.29 g. Lower cocoon weight values were recorded in the pool model and in groups using used peat (Table 2).

As illustrated in, the mean numbers of cocoons and hatchlings per broodstock are comparatively presented

Table 1: Protocol parameters applied in the hybrid and pool models.

Protocol	Water change frequency	Cocoon collection interval	Peat type
A	Once a week	Once every 2 weeks	Used/New
B	Twice a week	Once every 2 weeks	Used/New
C	Every 10 days	Once every 3 weeks	Used/New
D	Once every 2 weeks	Once every 4 weeks	Used/New

according to peat substrate type and applied production protocols.

Studies on the laboratory-based production of medicinal leeches have largely relied on classical rearing approaches centered on individual care under fixed temperature and humidity parameters. However, in these systems, the reproductive process is generally conducted under limited environmental control and it has been reported that environmental fluctuations and inter-individual interactions (particularly in multi-individual pool systems) play a decisive role in reproductive performance. Indeed, reproductive success in *Hirudo* species has been shown to be closely associated with environmental factors such as temperature, humidity and overall environmental stability and even minor variations in these parameters can significantly affect cocooning periods and hatchling yield (Petrauskienė *et al.*, 2011).

Nevertheless, classical production approaches are reported to involve substantial limitations in terms of both productivity and sustainability, mainly due to high stocking density, restricted individual monitoring and stress-related adverse effects (Ceylan *et al.*, 2015).

The hybrid model developed within the scope of this study was designed by restructuring the classical pool system in order to overcome the aforementioned limitations and it offers a semi-modular production approach that allows broodstock leeches to be monitored in separate compartments. Through this system, cocooning frequency and hatchling output could be recorded in a more regular and systematic manner, while key parameters directly influencing the reproductive process (such as ambient temperature, humidity and substrate moisture) could be managed in a more stable and controlled way (Petrauskienė *et al.*, 2011; Ceylan *et al.*, 2015).

Moreover, compared with conventional pool systems, the hybrid model reduced inter-individual interactions and provided a lower-stress rearing environment, thereby contributing to the maintenance of individual reproductive behaviors under conditions closer to their natural course (Ceylan *et al.*, 2015).

The quantitative findings clearly support these structural advantages. Under Protocol A of the hybrid model, a total of 16 cocoons and 163 hatchlings were obtained from only 7 broodstock leeches, corresponding to a mean

Table 2: Comparison of cocoon productivity and morphological characteristics in medicinal leeches according to peat type and cultivation protocols (Mean $\pm$ SD).

Cultivation model protocol		Pool model ^y		Hybrid model ^x		
		Protocol A ¹	Protocol A ¹ ✓	Protocol B ²	Protocol C ³	Protocol D ⁴
New peat	Total cocoon count (pcs)	126	16	9	14	1
	Total offspring count (pcs)	1423	163	57	122	0
	Cocoons per broodstock (avg.)	1.02 $\pm$ 0.44	2.28 $\pm$ 0.63*	1.28 $\pm$ 0.54	2.00 $\pm$ 0.57	0.14 $\pm$ 0.38
	Offspring per broodstock (avg.)	11.56 $\pm$ 6.49	23.28 $\pm$ 7.24*	8.14 $\pm$ 4.94	17.42 $\pm$ 6.60	0.00 $\pm$ 0.00
	Average cocoon weight (g)	0.88 $\pm$ 0.23	0.63 $\pm$ 0.25	0.63 $\pm$ 0.27	0.82 $\pm$ 0.29	0.07 $\pm$ 0.27
	Average cocoon length (mm)	23.21 $\pm$ 2.15	29.01 $\pm$ 6.07	18.96 $\pm$ 2.53	20.50 $\pm$ 3.31	15.60 $\pm$ 1.53
	Average cocoon width (mm)	14.46 $\pm$ 1.86	12.23 $\pm$ 2.02	11.53 $\pm$ 0.38	12.56 $\pm$ 1.32	12.60 $\pm$ 1.58
Used peat	Total cocoon count (pcs)	11	12	3	0	1
	Total offspring count (pcs)	30	77	32	0	0
	Cocoons per broodstock (avg.)	0.08 $\pm$ 0.27	1.71 $\pm$ 0.54	0.42 $\pm$ 0.54	0.00 $\pm$ 0.00	0.14 $\pm$ 0.38
	Offspring per broodstock (avg.)	0.24 $\pm$ 1.20	11.00 $\pm$ 4.57	4.57 $\pm$ 3.35	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
	Average cocoon weight (g)	0.09 $\pm$ 0.05	0.54 $\pm$ 0.25	0.68 $\pm$ 0.27	0.00 $\pm$ 0.00	0.08 $\pm$ 0.27
	Average cocoon length (mm)	13.23 $\pm$ 4.22	16.85 $\pm$ 3.53	19.93 $\pm$ 3.03	0.00 $\pm$ 0.00	13.70 $\pm$ 2.41
	Average cocoon width (mm)	10.11 $\pm$ 1.58	10.86 $\pm$ 1.91	11.96 $\pm$ 1.11	0.00 $\pm$ 0.00	12.90 $\pm$ 2.36

Values are presented as mean $\pm$ standard deviation (SD).

Between-group comparisons were performed using the Kruskal-Wallis H test.

The asterisk (*) indicates that Hybrid Model Protocol A exhibited statistically significantly higher values compared with the other protocols ($p = 0.003$).

^x HYBRID MODEL groups consisted of 7 broodstock leeches each (2-7 g).

^y POOL MODEL consisted of 123 broodstock leeches (2-7 g).

Note: Definitions of new and used peat substrates are described in the Methodology section.

✓ Indicates the most productive protocol based on cocoon and offspring yield.

¹ Water changed weekly; cocoons collected every 2 weeks (Standard Protocol).

² Water changed twice weekly; cocoons collected every 2 weeks.

³ Water changed every 10 days; cocoons collected every 3 weeks.

⁴ Water changed biweekly; cocoons collected every 4 weeks.

of 2.28 cocoons and 23.28 hatchlings per broodstock. In contrast, under Protocol A of the classical pool model, 163 cocoons and 1423 hatchlings were obtained from 126 broodstock individuals, resulting in mean values of 1.02 cocoons and 11.56 hatchlings per broodstock, respectively.

The statistically significant differences detected between the two models with respect to both cocoon and hatchling numbers ($p < 0.01$) indicate that the hybrid model provides approximately two-fold higher reproductive efficiency compared with the conventional pool system (Table 2). Furthermore, the absence of broodstock mortality throughout the experimental period in the hybrid model represents an important finding supporting the system's capacity to maintain low stress levels and environmental stability.

The biological basis of the enhanced reproductive performance observed in the hybrid model can be explained by the reduction of stress and physical or microbial disturbances during the critical stages of the reproductive process. In hirudiniform leeches, a foamy secretion released around the clitellum during cocoon deposition gradually hardens to form a "spongy outer layer." It has been reported that inter-individual contact during the formation of this layer may interfere with proper cocoon enclosure, which can be associated with cocoon deformation and defects in embryonic development. Indeed, increased stocking density has been shown to elevate cocoon deformation rates and in some deformed cocoons, dehydration may accompany the process, preventing successful hatchling development (Ceylan *et al.*, 2023).

In this context, the provision of individual isolation and the limitation of inter-individual interactions in the hybrid model constitute a fundamental biological mechanism that may enhance hatchling yield by reducing both mechanical disturbances and stress responses.

In addition, reproductive success has been shown to be highly sensitive to environmental stability. Under conditions involving controlled temperature (approximately 25-26/27°C), strict regulation of humidity, adequate ventilation and the use of an appropriate peat-soil substrate, increased hatchling yield and reduced rates of defective cocoons and mortality have been reported. Furthermore, a slightly acidic environment (pH 5.0-6.5) and high humidity levels (70-85%) have been demonstrated to be favorable for embryonic development and successful hatchling emergence (Aminov *et al.*, 2025).

Accordingly, the combination of low inter-individual contact, a stable microclimate and appropriate substrate conditions implemented in the hybrid model contributed to improved cocoon integrity and enhanced embryonic survival, ultimately leading to more successful reproductive outcomes.

These findings do not reflect a phenomenon specific solely to medicinal leech production; rather, they are consistent with the broader aquaculture literature highlighting the effects of environmental stability and stress management on biological productivity in controlled

production systems. Studies conducted on various aquatic organisms have reported that production systems ensuring microenvironmental stability are associated with reduced stress levels, maintenance of metabolic balance and enhanced reproductive or growth performance (Nottanalan *et al.*, 2025; Tanuja *et al.*, 2025). Similarly, modular and controllable production systems have been emphasized for their role in preserving organism health and supporting long-term production sustainability (Kumara *et al.*, 2023).

In this context, the high hatchling yield achieved in the hybrid model can be attributed to the implementation of isolated incubation management, maintenance of controlled temperature and humidity conditions, regulation of water exchange frequency and optimization of cocoon monitoring intervals (Table 1, Fig 1). Accordingly, the hybrid model represents a biologically rational and generalizable controlled production approach that is not limited to medicinal leech cultivation.

Finally, the absence of a statistically significant difference between the two production models in terms of cocoon morphometric parameters ($p > 0.05$) indicates that these morphological characteristics are more closely associated with species-specific biological factors rather than with the production system itself (Xiong *et al.*, 2020). In contrast, the marked reduction in production efficiency observed with the use of used peat substrate highlights the critical role of the substrate's microbiological load and chemical properties in reproductive success. In particular, increased microbial load and waste accumulation in pool systems using used peat have been associated with cocoon deformities and fungal infections (Table 2).

These findings demonstrate that the success of the hybrid model depends not only on structural modifications but also on the quality, hygiene and standardization of the production materials employed (Donahue *et al.*, 2022). In this context, achieving a sustainable and high-yield reproductive cycle requires moving beyond a purely qualitative classification of substrates and taking into account parameters such as microbiological load, pH balance and oxygen capacity during production processes. Several limitations of the present study should also be acknowledged. The hybrid model was implemented using a limited number of broodstock individuals and was evaluated within a single population, with the findings therefore constrained to specific environmental conditions and a relatively short production period. Long-term monitoring, assessment across multiple reproductive cycles and testing of the model under different populations and environmental settings would enhance the generalizability of the results.

Nevertheless, the controlled production approach offered by the hybrid model provides important implications for the conservation of medicinal leeches and for

sustainable commercial production. The high reproductive efficiency observed may reduce the need for harvesting individuals from natural populations, while individual monitoring and low mortality rates support a more ethical and traceable production process. In this respect, the hybrid model can be considered a promising alternative with the potential to balance conservation biology objectives and commercial production goals in medicinal leech cultivation.

CONCLUSION

The hybrid model developed in this study offers a modular approach based on individual monitoring, environmental stability and controlled substrate use as an alternative to conventional pool systems. The findings demonstrate that the hybrid model achieved a statistically significantly higher performance in terms of cocoon and hatchling production. In particular, the combined application of low stocking density, isolated incubation units, stable temperature-humidity conditions and fresh peat substrate emerged as key factors contributing to enhanced production efficiency.

In conclusion, the hybrid model represents a more controlled, sustainable and ethically appropriate alternative to classical pool systems for medicinal leech cultivation. Further evaluation of the model under different population structures and long-term production scenarios constitutes an important area for future research.

Declarations

Author contribution

Alican BİLDEN, Merve KAHRAMAN and Muttalip ÇİÇEK designed the study. Alican BİLDEN, Yasin SARI and Merve KAHRAMAN played a significant role in data collection. Alican BİLDEN and İrfan MARANGOZ contributed to the data analysis. Alican BİLDEN, Muttalip ÇİÇEK and İrfan MARANGOZ were involved in the writing, review and editing of the manuscript.

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Data availability

Data available on request from the authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no competing interests.

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