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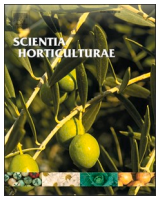

Research Paper

Production of parsley and pennyroyal with an African catfish-based aquaponics partially fed with yellow mealworms - *Tenebrio molitor*

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ABSTRACT

Insects can be used as alternative protein/food sources. Here, a novel aquaponic system based on the African catfish (*Clarias gariepinus*) fed with 30% of yellow mealworms (YM) (*Tenebrio molitor*) as substitute feed to 100% of fish meal (FM) was employed to produce parsley (*Petroselinum crispum*) and pennyroyal (*Mentha pulegium*). The two systems implemented (30YW/70FM and 100FM - control) showed identical water quality parameters. The 30YW/70FM operation led to a reduction by 27% of the carbon footprint, relatively to the 100FM system. Plants cultivated in the 30YW/70FM aquaponic system led to lower plant biomass (↓~75%), as stated by the statistically significant ($p < 0.05$) lower values of "plant height", "foliage diameter", "leaf number", "biggest leaf length" and "root length". Moreover, 100FM parsley showed higher levels of greenness (100%) and health status (97%) than the 30YW/70FM plants (↓50% greenness; ↓20% health status). Likewise, 100FM pennyroyal also showed higher levels of greenness (100%) and health status (100%) than 30YW/70FM pennyroyal (↓56 % greenness; ↓59% health status). Also, even if all plants presented equivalent levels of P, K, Ca and Fe, the plants grown in the 30YW/70FM system showed some Cl-accumulation (>7.0 g Cl.kg^{-1} for parsley and > 4.0 g Cl.kg^{-1} for pennyroyal, in dry weight) in the leaves and Cu and Mn accumulation in the roots (> 0.4 g.kg^{-1} in dry weight). The presence of high concentrations of Cl in *T. molitor*, and consequently in the water and fish faeces, might have caused some abiotic stress and toxicity to plant tissues, reducing plant growth.

1. Introduction

Sustainability of resources relies on circular economy practices. The global population is expected to reach almost 10 billion by 2050 (FAO, 2024) leading to a serious food/protein deficiency crisis. Additionally, climate changes (temperature and sea level rise, pH decrease, soil salinity and changes in ocean productivity) directly impact the production of food and feed (Gomez-Zavaglia et al., 2020; Mirón et al., 2023). As such, to overcome these acute worldwide problems, the use of food production systems based on aquaculture, aquaponics or other polyculture systems, has increased significantly in the last two decades (Thomas et al., 2021). According to the latest report of the Food and Agriculture organization of the United Nations on "The State of World Fisheries and Aquaculture" (FAO, 2024), the production of aquatic animals by aquaculture in 2022 (a total of 130.9 million tons, mostly produced on the Asian continent, ~ 70 %) has already surpassed capture fisheries (92.3 million tons). Moreover, when compared to aquaculture, fish farming through aquaponic/polyculture systems has shown to be even more efficient and productive, due to a more sustainable management of nutrients, water parameters, environmental safety and

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animal welfare (Okomoda et al., 2022; Thomas et al., 2021; Wirza & Nazir, 2021). Aquaculture is constantly affected by climate change and unsought phenomena that frequently lead to fish mortality and decreased productivity (Vasdravanidis et al., 2022). Thus, the continuous monitoring performed in aquaponics can mitigate the abiotic stress factors (e.g. dissolved oxygen, pH, temperature) that generally hinder the productivity of aquaculture systems. Hence, aquaponic systems are the most sustainable systems for producing animal and vegetable goods, answering the fast-growing demand for food products (Okomoda et al., 2022). Aquaponics combines the strengths of aquaculture and hydroponics and addresses the waste production problem by using an integrated pesticide-free biological system. Microorganisms convert aquaculture waste, resulting from fish metabolism (e.g., tilapia, catfish, trout and perch) and feed excess, into biologically available forms of nitrogen (e.g. nitrate) that promote plant growth (Thakur et al., 2023). Nutrient uptake by plants, which act as natural filters, cleans the recirculating water that returns to the fish tanks, efficiently using 95–99 % of the water (Cifuentes-Torres et al., 2021). Nonetheless, as for sustainable aquaculture, aquaponics depends on multiple factors, such as water quality, fish diets, and farmed fish and plants. Farming carnivorous and omnivorous fish depends on the use of fish meals (FM), which are the major source of protein in aquatic feeds, given their balanced essential amino acids, high digestibility, and good palatability (Robaina et al., 2019). However, the sole use of FM as aquafeed is environmentally and economically unsustainable. Therefore, it is imperative to find alternative feed ingredients and protein sources to replace FM.

Yeast (Rimoldi et al., 2020), microalgae (Ansari et al., 2021), and plant-based products (soybean, pea, corn, and wheat) (Bai et al., 2019; Dhanasiri et al., 2020; Wee et al., 2023) have been suggested as possible alternative aquafeed ingredients. Nonetheless, plant-based ingredients are poor in proteins (< 50 %) and have been reported to cause adverse effects to the gut of some fish due to the presence of indigestible materials, diminishing fish growth and welfare (Bai et al., 2019). Thus, the use of insects as a substitute for fishmeal has been considered as the most environmentally and economically sustainable option, given their high contents of crude protein (50 % to 80 % dry weight), fat, and minerals (Hasan et al., 2023). Insects have a very low ecological footprint (Grau et al., 2017). Compared to conventional livestock products, insects require much less arable land, energy, and water and have low greenhouse gas and nitrogen emissions (Grau et al., 2017). Insects have short breeding cycles and high reproduction rates and can be reared on discarded organic by-products. Moreover, as ectotherm organisms, insects present high feed utilization rates and have better conversion efficiency (conversion of food into body weight), presenting much higher yields per hectare than other animal crops (Seyedalmoosavi et al., 2022). Thus, given the authorization granted by the European Union (EU) for the use of certain flies, worms, and crickets (Regulation No 2017/893 – EU Directive), insects are being used for animal feed manufacturing and are readily accepted by various fish species (as they are part of the natural diet of omnivorous and carnivorous fish) (Maulu et al., 2022). Among the eligible insects, the yellow mealworm is considered one of the most promising species (Shafique et al., 2021).

The yellow mealworm (*Tenebrio molitor* (L.), family Tenebrionidae, order Coleoptera) is a widely distributed beetle easy to breed, feed and rear on low-nutritive plants, converting food waste and agricultural by-products into high-quality biomass (Shafique et al., 2021). Larvae of *T. molitor* are rich in protein (~60 % dry weight), unsaturated fat (~35 % dw), essential amino acids, minerals (e.g.: iron and zinc), and vitamins (eg: vitamin B12) (Oliveira et al., 2024), beyond presenting high digestibility and abundant functional substances such as chitin, antimicrobial peptides, and anti-freezing proteins. Yellow mealworm whole larvae have been used as partial or total replacement of FM in the diet of several aquatic species reared in aquaculture, such as, the African catfish (*Clarias gariepinus*) (Gebremichael et al., 2023); Atlantic salmon (*Salmo salar*) (Habte-Tsion et al., 2024); European sea bass (*Dicentrarchus labrax*) (Henry et al., 2018); gilthead seabream (*Sparus aurata*) (Bousdras et al., 2022); ide (*Leuciscus idus*) (Homska et al., 2022); Nile tilapia (*Oreochromis niloticus*) (Anany et al., 2023); rainbow trout (*Oncorhynchus mykiss*) (Bruno et al., 2023); and red seabream (*Pargus major*) (Ido et al., 2019). These studies reported that at dietary inclusion levels up to 30 % (dw), most fish showed good development and growth.

Hence, to meet the goals of "The 2030 Agenda for Sustainable Development" of the United Nations (UN, 2015) regarding the management of water resources, end of hunger, and development of sustainable communities, a pioneering aquaponics system to produce parsley and pennyroyal based on an omnivorous fish (African catfish), partially feed (30 %) with yellow mealworm, has been developed and is here described. The African catfish (*Clarias gariepinus*) is one of the most farmed fish in Africa and around the world (Adeshina & Abdel-Tawwab, 2020) and is an omnivorous fish with fast growth, high-quality flesh and great economic value. Given its tolerance to environmental stress, *C. gariepinus* can be cultivated in densely stocked waters and has been shown to adapt well to aquaponic systems (Endut et al., 2010; Oladimeji et al., 2020; Sebastião et al., 2024) as well as to diets partially composed (up to 50 %) by yellow mealworm larvae (Gebremichael et al., 2023). Parsley (*Petroselinum crispum*) and pennyroyal (*Mentha pulegium*) are angiosperms of the Apiaceae and Lamiaceae families, respectively. These leafy vegetables are widely consumed around the world and are well known for their contents in fibre, vitamins, minerals, and bioactive compounds. Parsley has already been produced in aquaponic systems supplied by regular fish meals (Braglia et al., 2022), but not by insect-based meals. Thus, the aims of this research were to: I) test the dietary inclusion of 30 % mealworm aquafeed (produced locally with almost zero waste), as a partial substitute of fishmeal to breed an omnivorous fish (African catfish); II) implement an aquaponics catfish-based system focused on the development of two edible plants (parsley and pennyroyal); and III) determine the quality levels of the plants produced, by monitoring morphological, physical and chemical parameters.

2. Materials and methods

2.1. Aquaponics system

Animal care and wellbeing were guaranteed in compliance with the European legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU, EU Regulation 2019/1010, Decreto-Lei n 113/2013). Experiments were conducted in a greenhouse at the Laboratory of Integrated Multitrophic Systems of the Polytechnic Institute of Leiria, Portugal (latitude 39°44′37'' N, longitude 8°48′25'' W, 33 m above sea level) from March to June 2021, as approved by the committee for animal welfare of the Polytechnic Institute of Leiria (2017/43,583).

The aquaponics workflow was composed of two independent gravity-based parallel recirculating operations (Figure S1.A). Each set-up (Figure S1.B) was composed of a fish-rearing tank, a drum filter coupled to a sedimentation tank (for solids) followed by a biofilter to convert ammonia to nitrates (25 × 12 mm plastic bioballs, Xingfeng, MBBR carrier, China), a Deep-Water Culture (DWC) hydroponic tank (with eight 150 × 60 × 4 cm polystyrene foam rafts) for plant production and a sump tank for water recirculation at a maximum flow rate of 10,000 $L.h^{-1}$. Water recirculation was also promoted by the force of gravity, with water levels in the tank, filters and cultures controlled by the height of a 90 mm pipe interconnecting the components. Water losses were controlled with buoyancy levels and were within expected ranges (< 10 %) (Love et al., 2015). Set-up and dimensions were (Table S1): fish tank, 3000 L; biological filter, 300 L; DWC unit, 2250 L; sump, 500 L; truncated-conical sedimentation filter, 150 L; total water volume in the system, 6450 L; submersible water pump, 10,000 Lh^{-1}; total area occupied, 150 m^2. No water discharges or extra lighting was applied. System aeration (fish tanks, biofilters and plant cultures) was provided by two piston air compressors. Systems were operated with fish

for some months prior to the experiment to promote biofilter maturation and nutrient build-up and diminish fish stress.

2.2. Air and water parameters

Air temperature and relative humidity were measured hourly (LogTag HAXO-8, NY, USA), while illuminance was measured three times per day of ~16 h photoperiods (noon, 2 h after sunrise and 2 hours before sunset) with a PCE-174 Datalogging light meter (PCE Instruments, Meschede, Germany) (Table S1). Water temperature, pH, electrical conductivity (EC, $mS.cm^{-1}$), total dissolved solids (TDS, $mg.L^{-1}$), dissolved oxygen (DO; $mg.L^{-1}$) and oxidation–reduction potentials (ORP, mV) were measured daily (Multiparameter probe, Edge HI2030/HI763100, Hanna Instruments, Italy). Fish tank and DWC water parameters were measured weekly including ammonia nitrogen, nitrite, nitrate, phosphate, iron, and potassium concentrations (in $mg.L^{-1}$), 12 hours after fish feeding and wastewater processing by the biofilter. Ammonia nitrogen, nitrite and nitrate were determined according to ISO 7150–1:1984 (EPA, 2002), SMEWW 4500-NO_2^-B (EPA, 2002) and Brucine methods (Baird & Bridgewater, 2018) respectively. Dissolved phosphorus (phosphate) was determined according to the SMEWW 4500P – ascorbic acid method (EPA, 2002). Iron and potassium were determined by flame atomic absorption spectroscopy (SpectrAA55B; Varian Inc., Palo Alto, USA), according to the SMEWW3111B method (EPA, 2002) with iron (248.3 nm) (Heraeus, Hanua, Germany) and potassium (766.5 nm) (Agilent, Santa Clara, CA, USA) hollow cathode lamps, respectively. Solutions were prepared with Ultrapure Milli-Q water (Type 1).

2.3. Insects, fish, and plants

Yellow mealworms (*Tenebrio molitor*) were obtained from local breeders and reared at the Laboratory of Integrated Multitrophic Systems of the Polytechnic Institute of Leiria, with cereal leftovers from Moagem Leiriense (Cereais do Lis, Leiria, Portugal), contributing to the circularity of the approach. Larvae were fed with wheat bran/grains composed of carbohydrates (23 %), protein (15 %), fat (4 %), minerals (2 %), and fibre (40 %). Mealworms were reared continuously from January to June 2021, at room temperature (~23 ºC) and humidity (~75 %). Mealworm larvae were grown in 8 recycled polypropylene lidded containers (50 × 34 × 19 cm, 80 g), filled to approximately 2.5 cm in height with wheat bran (4250 cm^3, ~1.5 kg) per container. A total of 3.0 kg of mealworm larvae were used during the 8-week experiment. Larvae were harvested after 2 weeks of growth (~3 cm; ~0.15 g), sieved, and fed to the fish within 24 hours. Live mealworms are composed of 20 % of protein, 13 % fat, 2 % fibre, and 62 % moisture in fresh weight (and of 53 % of protein, 28 % fat, 6 % fibre, and 5 % moisture in dry weight) (Anusha & Negi, 2023; Hasnan et al., 2023; Mariod, 2020; Stull et al., 2019).

African catfish (*Clarias gariepinus*) specimens were obtained from Fleuren & Nooijen BV (The Netherlands). Each tank contained 7 fish (3 males and 4 females) varying in length 77.57 ± 6.53 cm (body weight: 3.64 ± 0.36 kg), at a stocking density of 8.5 $kg.m^{-3}$. Fish were fed twice a day at adequate levels of 0.5 % (w/w) feed per body weight per day, as described previously (Sebastião et al., 2024) (125 g/day), with two approximately isonitrogenous (~35 %) and isoenergetic (~12 kcal. $kg^{-1}day^{-1}$) experimental diets (Table 1), for 8 weeks: I) control diet (fish tank 1 – 100FM) composed by 100 % of commercial fish feed (Aquasoja, Tilapia grower 2, São João de Ovar, Portugal, made of 37.9 % protein (of animal and vegetable mixed origin), 10.2 % fat, 8.8 % ash, 4.0 % fibres, 1.6 % calcium, 1.4 % phosphorous and 0.2 % sodium, supplemented with vitamins), and II) *T. molitor* test diet (fish tank 2 – 30YW/70FM) composed of commercial fish feed (70 %FM) and mealworm feed (30 %YW) (Table 1).

Seeds of parsley (*Petroselinum crispum*) and pennyroyal (*Mentha pulegium*) were obtained from Flora Lusitana (Cantanhede, Portugal). Each production was produced for a full life cycle from April to June 2021. Plant sowings were performed with universal substrate (EcoGrow Naturals) in polystyrene reused boxes (35 cm × 40 cm × 80 cm), watered with rainwater. Seedlings were randomly chosen, harvested from the nursery, washed in clean water (roots only) and transplanted into the hydroponic tank (DWC) into plastic hydroponic pots (5 cm in diameter) filled with lightweight expanded clay aggregate (LECA©). Each aquaponics system (30YM/70FM and 100 FM – control) had 34 specimens of each plant (parsley and pennyroyal) (i.e., 30YM/70FM aquaponics system – 17 parsley plants and 17 pennyroyal plants; 100FM aquaponics system – 17 parsley plants and 17 pennyroyal plants). Harvesting was performed after 42 days.

2.4. Morphological measurements

Plant growth was measured during six consecutive weeks (from April to June 2021) in terms of plant height (cm); foliage diameter (cm); leaf number (> 2 cm); and the biggest leaf length (cm). Measurements were performed with a measuring tape (± 1 mm). After the growth period, plants were inspected in terms of colour (leaf greenness: low/high) and health status (weak/strong), as performed previously for lambs' lettuce and arugula (Sebastião et al., 2024). "*Low greenness*" was attributed to plants with discoloured/pale leaves, while "*high greenness*" was attributed to plants presenting medium or high levels of greenness. In turn, a "*weak health status*" was assigned to symptomatic plants showing typical signs of disease, such as yellowing or loss of stiffness, whereas a "*strong health status*" would relate to asymptomatic plants, with no signs of disease. At the end of the experiment, plants were harvested and weighted with and without roots by using an analytical scale (± 0.1 mg) (Precisa Gravimetrics 262SMA-FR, Switzerland).

2.5. Mineral profile

Micro-Energy Dispersive X-Ray Fluorescence (μ-EDXRF) was used to detect the mineral profile of the fish meal (FM), of the yellow mealworm larvae of *T. molitor* and of the two plant species (parsley and pennyroyal) produced in the two aquaponic systems subjected to the two fish feeding treatments (100 FM and 30YW/70FM). Elemental evaluation was performed with a μ-EDXRF equipment operating with a 50 kV X-ray tube and a graphene window detector (Bruker S1 TITAN, Massachusetts, USA). Samples were prepared as described in previous work (Correia et al., 2024; Silva et al., 2025). Plants were manually crushed to powder, compacted into small pellets and submitted to the X-ray beam. Each sample was prepared in triplicate. Standard materials were used for calibration.

2.6. Carbon footprints

Carbon footprints were calculated by making use of the "My

Table 1
- Composition and carbon footprints of the 100FM and 30YW/70FM fish diets used in the aquaponic systems.

Fish experimental diets		Protein	Fat	Fibre	Energy	CO_2-eq
100 % Fish Meal (100FM)	FM /100 *g*	37.9 *g*	10.2 *g*	4.0 *g*	251.4 kcal	1.53 kg/kg
	$FM.day^{-1}.kg^{-1}$	1.9 *g*	0.5 *g*	0.2 *g*	12.6 kcal	
30 % *T. molitor*/70 % fish meal (30YW/70FM)	30YW/70FM /100 *g*	32.5 *g*	11.0 *g*	3.4 *g*	235.8 kcal	1.11 kg/kg
	$30YW/70FM.day^{-1}.kg^{-1}$	1.6 *g*	0.6 *g*	0.2 *g*	11.8 kcal	

Emissions" (myemissions.co) and "EX-ACT" (exact.apps.fao.org) online tools, according to the guidelines of the Food and Agriculture Organization of the United Nations - Greenhouse gas emissions from agrifood systems (FAO, 2023), as performed previously (Mendes et al., 2025). Calculations considered the production and transportation (over 160 Km) of fishmeal (FM), composed of animal and plant materials (soy flour, wheat bran, fish residues, vegetable oil and fish oil), at percentages of 100 % and 70 %. A reduced carbon footprint (to account for the 30 % contribution of YM) was considered for the locally produced (< 1 km) yellow meal worms by making use of food waste (wheat bran and residual grains), which are byproducts of wheat milling, (i.e., affected by a reduction of 80 %), also contributing to food waste management, by giving a 2nd life to the product's life cycle.

2.7. Statistical analysis

Statistical analyses were carried out according to the SAMPL guidelines (Statistical Analyses and Methods in the Published Literature) and general principles for reporting statistical methods and results. Quantitative variables were analysed via means and standard deviations. Categorical variables were analysed through frequency analysis, contingency tables, and association measures. Data normality was analysed by the Kolmogorov-Smirnov test. The Mann-Whitney U test was applied to evaluate differences between independent non-normally distributed variables. Hypothesis tests (Kruskal-Wallis and multivariate tests - Pillai's Trace), including Analysis of Variance (ANOVA) were applied to assess cell viability and plant growth and to correlate plant growth with levels of greenness and health status. Statistical analyses were performed with the IBM SPSS Statistics 2019 software v26.

3. Results and discussion

3.1. Aquaponics system performance

Fish and plant productions were carried out in a greenhouse for 8 weeks, with average temperatures and humidity of 23.7 ± 9.9 °C (min 10 °C/max 55 °C) and 64.0 ± 25.4 % (min 10.3 %/max 99.4 %), respectively (Figure S2). Fish accepted the two diets and no fish death occurred during the 8-week trial period. Fish presented normal metabolism, leading to identical amounts of nitrate and of other nutrients essential for plant growth in both systems (30YW/70FM and 100FM). Animal behaviour was considered regular, and fish weight increased slightly in both systems (length, ↑ 8 ± 2 cm; body weight, ↑0.5 ± 0.1 kg) with no significant differences. However, after four weeks, fish fed with the 30 % yellow worm meal and 70 % fish meal (30YW/70FM) did not seek the feed at mealtimes as voraciously as the fish fed with 100 % fish meal (100FM). This behavioural change may have resulted from adapting to the new diet, given its slightly different composition in protein, fat and fibre (Table 1), and/or to different preferences in terms of sensory properties (e.g. taste, odour, hardness) (Assan et al., 2021; Kasumyan, 2019). At the end of the experiment, plant specimens were removed, but the fish remained in the system and returned to the 100 % fish feed meal.

Sunlight illumination was evaluated in terms of illuminance and varied between 2 to 20 $lm.m^{-2}$. Water quality parameters (Table 2) of the two aquaponic systems under the two feeding regimes, i.e., 100FM versus 30YW/70FM were measured in terms of temperature (T, °C), dissolved oxygen (DO, $mg.L^{-1}$), electrical conductivity (EC, $\mu S.cm^{-1}$), total dissolved solids (TDS, $mg.L^{-1}$), oxidation–reduction potentials (ORP, mV) and pH. Water temperatures were identical in both aquaponic systems (100FM and 30YW/70FM) and adequate (100FM – 22.32 ± 1.08 °C and 30YM/70FM – 22.44 ± 1.13 °C) to maintain fish wellbeing (20 °C to 30 °C) (Singh et al., 2017). Mean values of DO were optimal for aquatic life development (Okomoda et al., 2022) and similar among the two systems (5.0 to 7.0 $mg.L^{-1}$), with lower DO values at the two DWCs, as expected, given O_2 consumption by fish, aerobic bacteria and plant roots. High TDS and EC values should be avoided among leafy vegetables (such as parsley and pennyroyal) to prevent salinity stress that would compromise plant growth and tissue quality (Ding et al., 2018; Lobanov et al., 2021). Hence, EC (~860 $\mu S.cm^{-1}$) and TDS (~450 $mg.L^{-1}$) remained adequate and at low levels in both systems. ORP values also remained similar and appropriate (~213 mV) in the optimal range of 200–400 mV, to promote basal bacterial growth, assuring ammonium/nitrite conversions to nitrate. Water pH was similar between the two reservoirs (fish tanks and DWCs) of the same aquaponic system, although slightly lower in the 100FM aquaponic setup (pH 5.3), likely due to higher fish metabolism associated with the commercial feed. Water pH was adequate (100FM, pH 5.6 ± 0.3, 30YM/70FM, pH 5.8 ± 0.6) to avoid nitrogen loss by NH_3 volatilization and to promote nutrient availability to the leafy vegetables under production (Meselmani, 2023).

Table 2
Water quality parameters of the two fish tanks and deep-water cultures (DWC).

		30YW/70FM	100FM
T°C	Fish tank	22.44 ± 1.13	22.32 ± 1.08
	DWC	22.43 ± 1.16	22.36 ± 1.10
DO $mg.L^{-1}$	Fish tank	6.77 ± 1.25	7.00 ± 1.21
	DWC	5.14 ± 1.14	5.64 ± 1.17
EC $\mu S.cm^{-1}$	Fish tank	857.89 ± 59.25	867.33 ± 46.71
	DWC	857.57 ± 58.32	870.57 ± 48.00
TDS $mg.L^{-1}$	Fish tank	449.37 ± 29.56	456.11 ± 22.48
	DWC	449.89 ± 29.41	456.37 ± 22.65
ORP mV	Fish tank	216.67 ± 48.02	211.97 ± 44.40
	DWC	213.50 ± 41.77	211.96 ± 39.18
pH	Fish tank	5.83 ± 0.57	5.63 ± 0.31
	DWC	5.84 ± 0.57	5.63 ± 0.30

Values reported are mean ± standard deviation (n= 68). DO, dissolved oxygen; T, temperature; EC, electrical conductivity; TDS, total dissolved solids; ORP, oxidation–reduction potential; 100FM, aquaponic system fed with commercial fish meal; 30YW/70FM, aquaponic system fed with commercial fish meal at 70 % and *T. molitor* larvae at 30 %.

Plant nutrients were measured weekly (Table 3). The ammonia nitrogen (NH_4^+) produced by fish metabolism gradually diminished and was maintained at low and adequate levels for fish production (NH_4^+ < 0.5 $mg.L^{-1}$) (Wongkiew et al., 2019). Likewise, nitrite (NO_2^-) concentrations were also generally low (NO_2^- < 1.0 mg/L), attesting to the conversion efficiency of NH_4^+ and NO_2^- into nitrate (NO_3^-) by nitrifying bacteria. However, given the diet change introduced in the 30YW/70FM aquaponic system, higher values of ammoniacal nitrogen (NH_4^+) were detected on week-0, which were then converted to nitrite (NO_2^-) as seen by the nitrification peak on week-1, followed by reduction and maintenance of both forms of nitrogen at lower values (< 0.5 $mg.L^{-1}$) from week-2 onwards. Regarding nitrate, levels were similar in both aquaponic systems (~300 $mgNO_3^-.L^{-1}$) and provided the needed amounts of nitrate for plant growth (> 100 $mgNO_3^-.L^{-1}$) (Somerville et al., 2014) confirming that in both aquaponic systems (100FM and 30YW/70FM) enough nitrate was generated to guarantee plant development and growth. Phosphate (~60 mg $PO_4^{3-}.L^{-1}$) and potassium (~45 mg $K.L^{-1}$) levels were also adequate for soilless productions (Meselmani, 2023; Naciri et al., 2022; Somerville et al., 2014; Wongkiew et al., 2019). Iron levels were maintained low (< 0.5 mg $Fe.L^{-1}$) (Table 3) and appropriate for aquaponics (Lobanov et al., 2021).

The inclusion of 30 % of yellow mealworm larvae in the feeding regime of the 30YM/70FM aquaponics system led to a lower carbon footprint (1.11 kg CO_2eq/kg), when compared to the 100FM system (1.53 kg CO_2eq/kg) (Table 1). Even if the carbon footprint of both operations is much lower than that of producing meat (that can reach up to 30 kg CO_2eq/kg for beef) (FAO, 2023), the sustainability of the 30YM/70FM system was even higher than that of the 100FM system. In addition to managing natural resources (land, water, nutrients, polyculture production/food of animal and plant origin), the 30YM/70FM system led to a reduction of the carbon footprint by 27 % CO_2 equivalents (CO_2eq), given the use of in-house produced larvae, fed with food

Table 3
– Plants nutrients in the fish tank and DWC water of the two aquaponic systems.

Parameter	week-0	week-1	week-2	week-3	week-4	week-5	week-6	
Ammonium (mg NH_4^+.L^{-1})								
100FM	Fish tank	2.12	0.84	0.29	0.09	0.13	0.12	0.08
	DWC	2.15	0.82	0.29	0.09	0.12	0.12	0.07
30YW/70FM	Fish tank	5.25	2.30	0.41	0.46	0.16	0.15	0.12
	DWC	4.89	2.44	0.33	0.45	0.19	0.14	0.11
Nitrites (mg NO_2^-.L^{-1})								
100FM	Fish tank	0.299	0.225	0.232	0.124	0.155	0.199	0.129
	DWC	0.296	0.240	0.230	0.132	0.158	0.122	0.132
30YW/70FM	Fish tank	0.555	4.744	0.232	0.194	0.167	0.116	0.119
	DWC	0.570	4.884	0.242	0.165	0.166	0.115	0.121
Nitrates (mg NO_3^-.L^{-1})								
100FM	Fish tank	535	338	324	382	360	355	372
	DWC	597	346	318	387	358	359	362
30YW/70FM	Fish tank	400	329	309	333	359	350	350
	DWC	549	311	314	327	367	361	354
Phosphates (mg PO_4^{3-}.L^{-1})								
100FM	Fish tank	73.8	57.6	53.3	58.1	62.0	60.4	56.2
	DWC	72.9	55.5	54.2	58.6	59.5	59.6	56.3
30YW/70FM	Fish tank	78.4	55.4	56.0	61.2	63.2	63.9	56.2
	DWC	79.6	57.6	56.8	61.1	66.5	64.1	55.7
Iron (mg Fe.L^{-1})								
100FM	Fish tank	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	DWC	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
30YW/70FM	Fish tank	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
	DWC	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Potassium (mg K.L^{-1})								
100FM	Fish tank	54.7	41.6	37.7	37.7	34.4	30.6	25.5
	DWC	55.2	44.3	40.1	37.4	33.5	32.3	28.5
30YW/70FM	Fish tank	78.3	44.1	47.5	46.9	44.4	42.9	38.3
	DWC	75.9	51.0	46.2	46.7	44.2	41.9	38.0

100FM, aquaponic system fed with commercial fish meal; 30YW/70FM, aquaponic system fed with commercial fish meal at 70 % and *T. molitor* larvae at 30 %.

waste locally produced by the milling of wheat flour (bran and trace grains). This reduction results from an 80 % decrease in CO_2eq associated to feed production and transportation, since there was no need for fish capture, crop cultivation, expenditure of nutrients, water, gas, electricity or extra greenhouse gas emissions (Purkayastha & Khanal, 2024).

3.2. Production of parsley - Petroselinum crispum

Parsley (*Petroselinum crispum*) was produced (Fig. 1) under the two aquaponic fish feeding regimes (100FM and 30YW/70FM) and monitored during a 6-week period. Morphological measurements were taken in terms of "plant height", "root length", "leaf number", "foliage diameter" and "the biggest leaf length" at the beginning of the experiment (i.e., after transplantation to the DWC culture – week-1) and at week-2, week-4 and week-6. Results are shown in Fig. 2. At the end of the growth period, plants were harvested, weighted and analysed in terms of "greenness" and "health status" (Table 4). The 34 specimens of parsley produced in the 100FM aquaponic system presented maximal levels of greenness (34/34 - 100 %) and health status (33/34 - 97 %) and led to a total fresh weight of 1167.95 g without roots (34.35 ± 16.26 g per plant), with roots weighing 380.94 g (root/shoot biomass ratio of 32.62 %). In turn, the 34 specimens of parsley produced in the 30YW/70FM aquaponic system presented medium levels of "greenness" (17/34 - 50 %) and reasonable "health status" (27/34 - 79 %), also leading to lower amounts of fresh biomass of 299.19 g without roots (8.80 ± 4.32 g per plant), with roots weighing 104.71 g (root/shoot biomass ratio of 35.00 %).

Plant growth was observed in all specimens submitted to the two aquaponic feeding treatments (Fig. 2). However, from week-2 onwards statistical differences ($p < 0.05$) were detected in terms of "the biggest leaf length", "root length" and "leaf number". At harvest (week-6), parsley specimens grown in the 100FM aquaponic system produced more leaves (> 2 cm, 11.03 ± 3.93 cm), longer aerial parts (44.49 ± 4.92 cm) and longer roots (31.80 ± 7.61 cm), than plants growing in the 30YW/70FM system.

Regarding the average values of "plant height" and "foliage diameter", after applying a MANOVA statistic test (via Pillai's trace) to

Fig. 1. Parsley and pennyroyal specimens produced in the deep-water culture (DWC) of the 100FM aquaponics system.

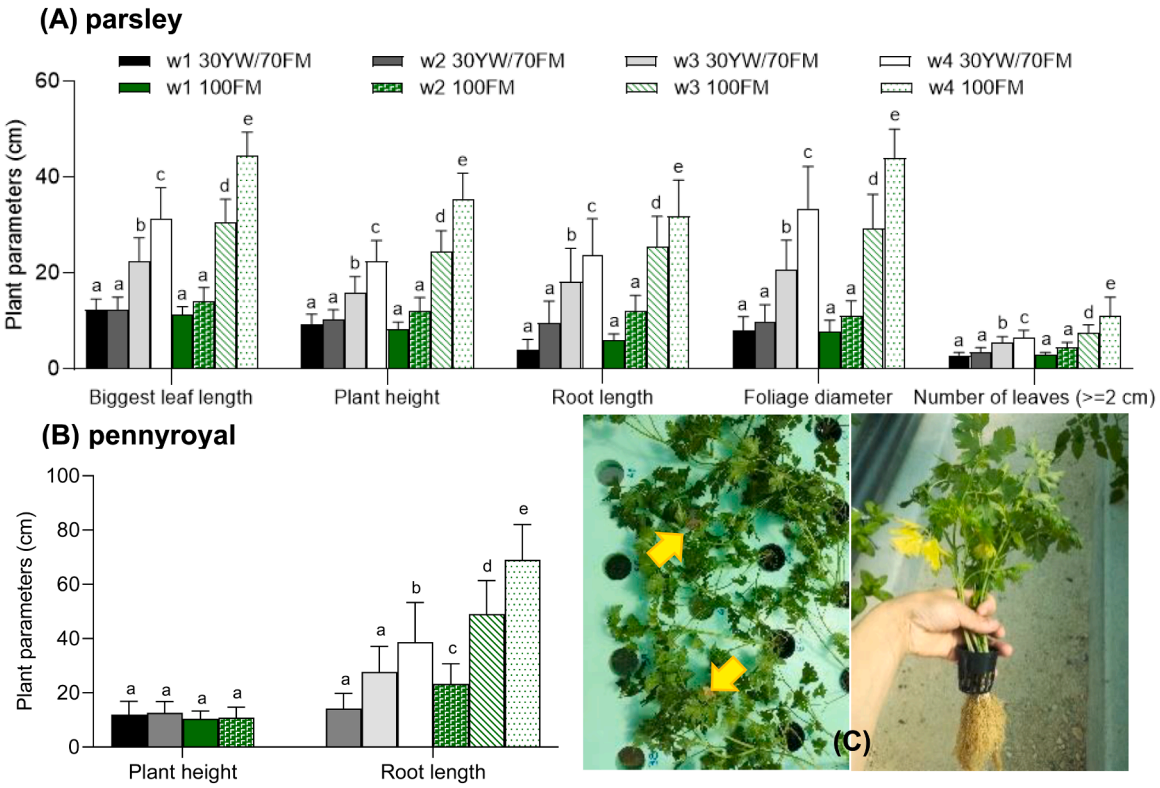

Fig. 2. Plant morphology parameters per week (w). (A) Mean values of plant height; root length; foliage diameter; biggest leaf length and number of leaves (>= 2 cm) for parsley from week 1 (w1) to week 4 (w4) grown under the two feeding systems 30YW/70FM and 100FM (17 replicates for each system). (B) Mean values of plant height and root length for pennyroyal from week 1 (w1) to week 4 (w4) grown under the two feeding systems 30YW/70FM and 100FM (17 replicates for each system). (C) Parsley specimens presenting some discoloring and loss of stiffness. 100FM refers to the aquaponic system fed with commercial fish meal and 30YW/70FM refers to the aquaponic system fed with commercial fish meal at 70 % and *T. molitor* larvae at 30 %. Different letters indicate significant differences ($p < 0.05$).

Table 4
Greenness and health status of parsley and pennyroyal specimens at harvest.

	Greenness		Health status		Total
Fish diet	high	low	strong	weak	
parsley					
30YW/70FM	17	17	27	7	34
100FM	34	0	33	1	34
pennyroyal					
30YW/70FM	15	19	14	20	34
100FM	34	0	34	0	34

100FM, aquaponic system fed with commercial fish meal; 30YW/70FM, aquaponic system fed with commercial fish meal at 70 % and *T. molitor* larvae at 30 %.

analyse the effect of the factor "fish diet", a significant effect on at least one of the measurements ($p < 0.001$) was observed. Four and six weeks after transplantation to the DWCs, average plant height (35.54 ± 5.31 cm in week-6) and average foliage diameters (44.13 ± 5.92 cm in week-6) were also significantly larger (5 % significance) in the 100FM aquaponic system, as checked by the ANOVA tests. In what respected "greenness", significant dependence ($p < 0.001$) with "fish diet" was also observed, given the better results shown by the plants growing in the 100FM feeding regime. Hence, parsley grown under the two fish feeding regimes 100FM and 30YW/70FM presented statistically significant differences in terms of morphology, greenness and health status.

T. molitor larvae are rich in protein, fat, fibre and sulphur, but poorer than fish meal (FM) in minerals such as potassium and calcium, which are essential macronutrients for plant growth. Parsley specimens' growth in the 30YW/70FM aquaponic system presented lower biomass and some signs of leaf discolouring and loss of stiffness that might be explained either by lower levels of some essential nutrients and/or by toxic levels of certain substances in the DWCs (Fig. 2). Previous studies in aquaculture have already shown that African catfish partially fed with black soldier flies and/or mealworms led to negligible impacts on fish production (Gebremichael et al., 2023). However, if fish reared in aquaculture adapt well to diets partially composed of insect-based meals (Sándor et al., 2022) that might not be the case of plants grown in insect-based aquaponics. Hence, we determined the mineral profile of the fish meal, yellow mealworms and plants produced in the two aquaponic systems 100FM and 30YW/70FM implemented and detected differences, as described in the following section.

3.3. Mineral profiles of aquafeeds and plant biomass

Mineral profiles of the fish meal (FM), yellow mealworms (YW) and parsley produced in the two aquaponic systems 100FM and 30YW/70FM were determined by micro energy dispersive X-ray fluorescence (μ-EDXRF). Table 5 presents the results obtained. Plant metabolism depends on nitrogen, phosphorus, potassium, sulphur, calcium and magnesium as macronutrients (> 0.1 $g.kg^{-1}$ dry weight), as well as on other minerals such as copper, iron, manganese and zinc, as micronutrients (< 0.1 $g.kg^{-1}$ dry weight) (Amtmann & Armengaud, 2009; Maathuis, 2009). Relatively to fish meal, *T. molitor* is richer in chloride and sulphur but poorer in potassium, phosphorous, calcium, iron and manganese (Table 5). Thus, the lower plant biomass obtained in the aquaponic system submitted to the 30YW/70FM feeding treatment could result from the presence of excessive levels of chloride and/or from deficient levels of potassium, phosphorous, calcium, or iron.

Regarding potassium (K), calcium (Ca) and iron (Fe), plants did not suffer from insufficient levels of these minerals, as judged by the equivalent amounts of K, Ca and Fe present in the roots and aerial parts

Table 5
Mineral profiles of the fish meal, *T. molitor* larvae and roots and aerial parts of 30YW/70FM and 100FM parsley and pennyroyal in ppm.

Mineral (ppm)	fish meal (FM)	*T. molitor* larvae (YW)	Parsley*				Pennyroyal*			
			30YW/70FM		100FM		30YW/70FM		100FM	
			aerial parts	roots	aerial parts	roots	aerial parts	roots	aerial parts	roots
P	5345 ± 81	3898 ± 70	3803 ± 73	4643 ± 79	5118 ± 82	7320 ± 96	6424 ± 91	6971 ± 94	8668 ± 103	6893 ± 93
S	1037 ± 29	1472 ± 32	4630 ± 52	4477 ± 50	3424 ± 46	4590 ± 51	5346 ± 55	4745 ± 52	4034 ± 47	4070 ± 48
Cl	424 ± 133	2881 ± 161	7096 ± 215	2155 ± 159	6343 ± 210	3342 ± 176	4125 ± 185	2116 ± 161	1440 ± 150	1875 ± 158
K	11,124 ± 55	5814 ± 41	46,640 ± 118	29,759 ± 92	46,359 ± 117	35,447 ± 102	34,811 ± 102	37,765 ± 106	29,592 ± 92	37,029 ± 103
Ca	1091 ± 15	443 ± 11	8168 ± 37	5342 ± 31	8378 ± 38	6196 ± 33	13,198 ± 45	6662 ± 34	10,017 ± 40	6890 ± 34
Mn	146 ± 10	21 ± 6	698 ± 18	4360 ± 42	134 ± 10	1654 ± 27	434 ± 16	2696 ± 38	194 ± 15	960 ± 24
Fe	203 ± 11	81 ± 8	52 ± 11	639 ± 22	70 ± 10	867 ± 21	81 ± 11	1185 ± 27	115 ± 13	1055 ± 25
Cu	16 ± 2	21 ± 2	17 ± 2	428 ± 6	10 ± 2	284 ± 5	20 ± 2	597 ± 8	19 ± 2	277 ± 6
Zn	101 ± 3	132 ± 3	173 ± 4	1858 ± 13	114 ± 4	1601 ± 12	204 ± 5	1387 ± 13	152 ± 5	776 ± 10

Values reported are mean ± standard deviation. *Average values of 17 specimens of parsley and pennyroyal of each aquaponic system: 30YM/70FM and 100FM. 100FM, aquaponic system fed with commercial fish meal; 30YW/70FM, aquaponic system fed with commercial fish meal at 70 % and *T. molitor* larvae at 30 %.

of both parsley productions (100FM and 30YW/70FM) (Table 5). Conversely, differences were found in the amounts of phosphorous (P), chloride (Cl) and manganese (Mn) in the aerial parts (leaves and stems) and roots of 100FM parsley and 30YW/70FM parsley. In respect to P, even if the P contents were different between the commercial fish meal (~5000 ppm) and *T. molitor* larvae (~4000 ppm), P levels were enough to ensure plant development in both systems, according to the minimal values needed for plant growth (> 2000 ppm, 2.0 g $P.kg^{-1}$ dw) (Maathuis, 2009). In opposition, regarding chloride (Cl), differences were found between the two aquafeeds (fish meal versus yellow mealworm larvae) as well as between the two plant productions of 100FM parsley and 30YW/70FM parsley. The 30YW/70FM parsley production presented higher levels of Cl (Table 5), which related to the higher levels of Cl in the aquafeed used (i.e., 30 % of yellow mealworm *T. molitor*). Optimal Cl levels for plant growth are much lower (~100 ppm Cl, < 0.1 g $Cl.kg^{-1}$ dw) (White & Broadley, 2001) than the 30-fold higher Cl levels detected in insect larvae (~3000 ppm, 3.0 g $Cl.kg^{-1}$ dw). Chloride ions (Cl^-) enter plants through the roots and are mobile within the plant via the xylem and phloem (White & Broadley, 2001). At low Cl^- concentrations, active transport to root cells dominates Cl^- influx but at high saline concentrations passive Cl^- transport also occurs (White & Broadley, 2001). Thus, the presence of high concentrations of Cl in *T. molitor*, and consequently in the water and fish faeces, might have caused some abiotic stress and toxicity to plant tissues, as judged by the Cl levels detected in the aerial parts (~7000 ppm, 7.0 g $Cl.kg^{-1}$ dw) of 30YW/70FM parsley when compared to 100FM parsley (~6000 ppm, 6.0 g $Cl.kg^{-1}$ dw). Accumulation of Cl in Cl-sensitive plants may induce growth decline and chlorotic toxicity due to chlorophyll degradation, as detected in some parsley specimens (Fig. 2) and previously seen in faba beans (Tavakkoli et al., 2010) and barley (Tavakkoli et al., 2011). Thus, Cl-overload might have caused some tissue toxicity that can explain the lower shoot biomass produced by the 30YW/70FM feeding system.

In addition, some Cu- and Mn-accumulations (> 0.1 $g.kg^{-1}$ dw) were also detected in the roots of 30YW/70FM parsley. Beyond order functions, Cu and Mn are essential cofactors of various metalloproteins involved in defence mechanisms against oxidative stress (Shabbir et al., 2020). This additional Cu and Mn uptake by plant roots can be related to an extra demand and overexpression of metalloenzymes (e.g.: superoxide dismutase) needed for oxidative stress regulation, which can also indicate that plants were responding to environmental stress. Hence, when developing aquaponic setups depending on insect-based feeding regimes (e.g. yellow mealworm), insect compositions must be prioritized and ion-sensitive species must be chosen carefully, to avoid deficits or excessive amounts of certain minerals.

3.4. Pennyroyal (*Mentha pulegium*) trials

A preliminary trial was also performed with pennyroyal (*Mentha pulegium*) grown under the two aquaponic feeding regimes tested here: 100FM and 30YW/70FM (Fig. 2).

Two morphological variables were evaluated during this period: “plant height” and “root length” at week-1 and week-2 (Fig. 2). Regarding height, two measurements were registered: after transplantation to the DWC (April), and after a 2-week growth period (May). For plant height, no significant differences ($p > 0.01$) were found between the two feeding regimes (100FM and 30YW/70FM), after transplantation to the DWC (no equality of variances by the Levene’s Test) or after two weeks (with equality of variances by the Independent Two-Samples *t* Test). Conversely, regarding average root lengths, when applying the Independent Two-Samples *t* Test (with equality of variances assumed by the Levene’s Test), significant differences ($p < 0.01$) were found between the two fish feeding regimes, which were confirmed in the following 2 weeks.

Mature plants were harvested, weighed and qualitatively analysed in terms of “greenness” and “health status” (Table 4). The 34 specimens of pennyroyal produced in the 100FM aquaponic system presented

maximal levels of greenness (34/34 - 100 %) and health status (34/34 - 100 %), leading to a total fresh weight of 1640.99 g (48.26 ± 29.38 g per plant), with roots weighing 759.95 g (root/shoot biomass ratio of 46.31 %). In turn, the 34 specimens of pennyroyal produced in the 30YW/70FM system presented medium levels of "greenness" (15/34 - 44 %) and "health status" (14/34 - 41 %) (Table 4), and lead to lower amounts of biomass (total of 404.02 g without roots – 11.88 ± 14.41 g per plant), with roots weighing 156.76 g (root/shoot biomass ratio of 38.80 %). Hence, pennyroyal was able to grow in both aquaponic systems, but higher biomass, greenness and health status were achieved by the 100FM system, as observed for parsley.

Concerning minerals, pennyroyal also presented a similar tendency to parsley (Table 5), corroborating the "fish diet" effect. Likewise, P, K, Ca and Fe levels were identical in both types of pennyroyals (100FM and 30YW/70FM) indicating that both aquaponic systems contained enough levels of these plant nutrients. Similarly, 30YW/70FM pennyroyal presented higher levels of Cl than 100FM pennyroyal which relates to the higher levels of Cl found in *T. molitor* larvae, when compared to commercial fish meal (FM). The Cl-accumulation detected in 30YW/70FM pennyroyal, especially in the aerial parts (~4000 ppm, 4.0 g $Cl.kg^{-1}$), along with Cu and Mn root accumulation, can be also indicating that these plants might have also suffered from some environmental stress, leading to minor growth and less levels of greenness and health status.

4. Conclusions

The inclusion of locally produced insects as protein substitutes of commercial aquafeed for aquaponics may be a promising way to overcome food/feed crises and to work towards low carbon footprints and zero-waste practices. However, here, although presenting similar levels of nutrients (nitrate, phosphate, potassium and iron), plants grown under the 30YW/70FM feeding regime (↓27 % CO_2eq carbon footprint, given the use of locally reared yellow mealworms by making use of food waste by-products from wheat milling) led to lower plant biomass (↓74 %), as stated by the lower values of "plant height", "foliage diameter", "leaf number", "biggest leaf length" and "root length", along with lower "greenness" and "health status", pointing towards the occurrence of some abiotic stress. Even if the mineral contents of plant roots and aerial parts were equivalent in terms of P, K, Ca and Fe, some differences regarding Cl-accumulation were detected in the aerial parts of parsley and pennyroyal that could be related to the higher amounts of Cl found in *T. molitor* larvae. Hence, the presence of high concentrations of certain minerals (Cl, Mn and Cu) in the plants produced in the aquaponic system partially fed with *T. molitor* larvae may have led to some cell toxicity. Thus, even if insect-based aquaculture productions are already functioning, further research needs to be carried out to optimize the use of insect-based aquafeeds (flies, worms, and crickets) in aquaponics, to produce ion-sensitive plant species without deficits or excessive amounts of certain minerals, given the high and full circularity of these systems.

Ethical statement

Animal care and wellbeing were guaranteed in compliance with the Legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU, EU Regulation 2019/1010, and Decreto-Lei n 113/2013) and approved by ORBEA - Committee for animal welfare according to the 3 Rs (Replacement, Reduction and Refinement – Practices of animal welfare improvement to optimize aquaculture production according to the Transparency Agreement on Animal Research in Portugal) of the Polytechnic Institute of Leiria.

CRediT authorship contribution statement

Raul Bernardino: Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Judite Vieira:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Daniela C. Vaz:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Ounísia D. Santos:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Vânia S. Ribeiro:** Writing – review & editing, Formal analysis, Data curation. **Cristiana L. Pires:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Luís Cotrim:** Writing – review & editing, Formal analysis, Data curation. **Susana Bernardino:** Writing – review & editing, Methodology, Investigation. **Fernando Sebastião:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114487.

Data availability

Data will be made available on request.

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