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Comparative analysis of *brassica rapa* var. *cymosa* grown in hydroponic condition, lunar maria, and lunar highland regolith simulants: biochemical profiling and effects on *drosophila melanogaster* as an in vivo model

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ABSTRACT

Background: Sustainable crop cultivation in extraterrestrial environments is essential for future space missions. Lunar regolith simulants may influence plant biochemical properties and safety, yet their effects on plant quality and downstream biological responses remain underexplored.

Methods: Brassica rapa var. cymosa was cultivated under hydroponic conditions and in two lunar regolith simulants (lunar maria and lunar highland). Polyphenol profiles, antioxidant capacity, chlorophyll, and carotenoid contents were analyzed. *Drosophila melanogaster* (Oregon-K) flies were fed media supplemented with five concentrations of each plant sample and a control. DNA damage in larval brain neuroblasts was assessed via comet assay. Adult fly behavior, longevity, and prolificacy were evaluated.

Results: Lunar maria sample showed significantly higher levels of neochlorogenic acid, ferulic acid, and p-coumaric acid, along with increased total phenolic content and chlorophyll a compared to hydroponic and lunar highland samples. Antioxidant capacity, assessed using ferric reducing antioxidant power (FRAP), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, was higher in lunar-grown plants than in hydroponic ones, whereas oxygen radical absorbance capacity (ORAC) showed no significant difference. DNA damage was increased across all larval groups but was lowest in the lunar maria group at moderate concentrations. Despite early genotoxic stress, adult flies developed normally, with comparable longevity and reproductive output. Notably, flies fed lunar maria samples exhibited enhanced climbing ability.

Conclusion: Cultivation in lunar regolith simulants alters plant biochemistry and induces early DNA damage in drosophila larvae without impairing adult health or behavior. The lunar maria simulant, in particular, promotes beneficial phytochemical traits and organismal resilience. These findings underscore both the challenges and potential of lunar agriculture, emphasizing the critical role of substrate choice in developing sustainable space farming systems.

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Abbreviations

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	2,2-diphenyl-1-picrylhydrazyl
ORAC	oxygen radical absorbance capacity
FRAP	ferric reducing antioxidant power
TPC	total phenolic content
BLSS	bioregenerative life support systems
SOLE	Space Orbital Life Enhancement
ASI	Italian Space Agency.
LHS	lunar highlands regolith simulant
LMS	lunar mare regolith simulant
MGS	Martian global regolith simulant
CEA	confined-environment agriculture
EC	electrical conductivity
HPLC-MS/MS	High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry
DW	dry weight
FW	fresh weight
RH	relative humidity
LMP agarose	low-melting-point agarose
NMP agarose	normal-melting-point agarose
EDTA	Ethylenediaminetetraacetic acid
PBS	Phosphate-Buffered Saline
DAPI	4′,6-Diamidino-2-phenylindole
SD	standard deviation
CI	confidence interval
BH	Benjamini-Hochberg
FDR	false discovery rate
ISRU	in-situ resource utilization
NER	nucleotide excision repair
HR	homologous recombination
BER	base excision repair
MMR	mismatch repair
DDR	DNA damage response
AU	Arbitrary Unit

1. Introduction

The concept of cultivating plants in extraterrestrial environments has evolved from science fiction into a well-established field of scientific inquiry. Increasing interest in lunar and planetary agriculture has led to an extensive body of research focused on plant physiological responses, substrate challenges, and adaptive cultivation strategies under space-like conditions (Davies et al., 2003; Paul et al., 2022; Wamelink et al., 2014; Peyrusson, 2021; Kasiviswanathan et al., 2022; Zhao et al., 2024; Poulet et al., 2022). In parallel, efforts have investigated the physical and chemical properties of the lunar surface to assess its potential for supporting plant growth (Crawford et al., 2012; Ohtake et al., 2009). Studying plant cultivation in space not only advances fundamental questions in astrobiology, such as the potential for life beyond Earth and understanding of terrestrial organisms' physiological adaptation to space conditions, but also is crucial for the development of bio-regenerative life support systems (BLSS) essential for supporting long-term human habitation on the Moon or Mars (Crawford et al., 2012; LG Duri et al., 2022; Mitchell, 1994; Barker et al., 2020; Kordyum and Hasenstein, 2021). On the other hand, as space agencies and private stakeholders approach long-term lunar missions, developing autonomous in situ food production systems is essential for future space infrastructure (Ch et al., 1996; Farges et al., 2008; Sanders and Kleinhenz, 2022). Importantly, this research also informs Earth-based solutions addressing food insecurity and sustainability challenges (International Space Exploration Coordination Group 2018).

Lunar regolith, which almost completely covers the Moon's surface, consists of rock fragments, mineral particles, volcanic and impact glasses, and unique "agglutinates" found only on the Moon (Noble, 2009). Its highly reactive minerals, lack of organic matter, fine and cohesive particles, and variable density restrict aeration, water retention, and root penetration, posing challenges for plant growth. Yet, its abundance of elements such as calcium, aluminum, magnesium, and iron, together with modifiable properties and the potential to reduce reliance on imported media, make lunar regolith a promising substrate for off-world agriculture (Fackrell et al., 2024; Heiken et al., 1991; Venugopal et al., 2020).

Lunar regolith simulants are synthetic terrestrial materials created to mimic the chemical, mechanical, mineralogical, and particle size properties of actual lunar regolith (Zheng et al., 2009; McKay et al., 1994). As reviewed by Duri et al. (LG Duri et al., 2022) there are currently eleven Lunar Mare simulants, six Lunar Highland simulants, and four Lunar Dust simulants, each with distinct mineral, physicochemical, physical, and hydrological properties. They can serve as substrates for crop cultivation and agriculture, and while technically challenging, they enable innovation in agricultural techniques for both space and Earth applications (Davies et al., 2003; Poulet et al., 2022; LG Duri et al., 2022). While simulants enable testing of cultivation strategies, the success of extraterrestrial agriculture ultimately depends on selecting crops that are physiologically adaptable to suboptimal substrates while also delivering high nutritional and functional value to support human health in space.

Brassica rapa (*B. rapa*) L. subsp. *sylvestris* var. *esculenta,* or turnip top, is a crop from Brassicaceae family and *Brassica rapa* species. *B. rapa* var. *cymosa* is valued for its high nutritional content, including vitamins A, C, and K; essential minerals such as calcium, potassium, selenium, and iron; dietary fiber; and diverse bioactive compounds, notably flavonoids (isorhamnetin, kaempferol, and quercetin glycosides), glucosinolates, indole alkaloids, and other phenolic derivatives (Conversa et al., 2016; Mithen et al., 2003; Giamoustaris and Mithen, 1996; Cartea and Velasco, 2008; Connolly et al., 2021; Llorach et al., 2003; Rosa, 1997; Jahangir et al., 2009; Romani et al., 2006; Schonhof et al., 2004; Cartea et al., 2011). Compared to other Brassicaceae, *B. rapa* varieties also produce a broader spectrum of isothiocyanates and nitriles, enhancing their functional potential under stress conditions (Jahangir et al., 2009; Valette et al., 2003; Miyazawa et al., 2005). These phytochemical features, combined with the crop's safety as a vegetable-type *Brassica* (free from harmful glucosinolate degradation products found in oilseed *Brassicas*) (Cartea and Velasco, 2008; Rosa et al., 1996), indicate higher nutritional density, antioxidant capacity, and human safety per unit biomass — critical advantages for space agriculture where resource efficiency and crew health are priorities.

Several agronomic and physiological traits highlight the potential of *B. rapa* var. *cymosa* as a space crop. Its short growth cycle (~35 days from sowing to harvest, depending on cultivar) enables rapid turnover in controlled-environment agriculture (Cartea et al., 2011; Morone Fortunato and Damato, 2000; Johnson et al., 2022). The edibility of multiple plant parts (leaves, flowers, roots) maximizes biomass use and reduces waste (Connolly et al., 2021; Cartea et al., 2011; Dixon, 2017; Podsędek, 2007). The species also tolerates abiotic stresses such as variable light, drought, and suboptimal soils (Mohan et al., 2025; Dela Cruz et al., 2022), directly relevant to extraterrestrial cultivation. Stress resilience extends postharvest, as vitamin C, carotenoids, and flavonols remain stable across systems, with storage even enhancing flavonol content and antioxidant capacity under wounding or chilling stress (Conversa et al., 2016). Together, these traits support its use in both routine cultivation and emergency scenarios, including rapid food supply or supplementation with antioxidants and micronutrients to mitigate oxidative stress and radiation during missions (Smith and Zwart, 2008; Wheeler, 2017; Schuerger et al.).

Beyond crop-level traits, animal and clinical studies support the health-promoting potential of *B. rapa* species. In mice, *B. rapa* polysaccharides reduced inflammation, reinforced gut integrity, and modulated microbiota (Du et al., 2024). In diabetic sand rats, aqueous extracts of *B. rapa* var. *rapifera* improved glucose regulation and reduced

oxidative stress (Berdja et al., 2016). A randomized, placebo-controlled human trial showed that *B. rapa* ethanol extract, enhanced lipid metabolism in overweight subjects (Jeon et al., 2013), while Tibetan turnip (*B. rapa* L.) improved hypoxia tolerance through greater antioxidant capacity and oxygen utilization (Chu et al., 2017).

While nutrient density and bioactive compound content are common across *B. rapa* species, specific traits can vary with growth environment. In this study, *B. rapa* var. *cymosa* was evaluated as a candidate space crop by assessing its nutritional and bioactive responses to space-relevant substrates. Plants were cultivated in hydroponic solution, lunar maria regolith simulant, and lunar highland regolith simulant, and analyzed for chlorophyll, carotenoids, polyphenols, and antioxidant capacity using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), and ferric reducing antioxidant power (FRAP) assays. These analyses revealed how cultivation environment shapes the phytochemical and antioxidant profile of the crop.

To assess the biological relevance of these biochemical differences, we employed Drosophila melanogaster (Oregon K strain) as an in vivo model. This species offers well-characterized genetics, DNA damage response pathways comparable to mammals (Song, 2005), and established use in comet assay–based genotoxicity studies, dietary bioactivity, and environmental toxicology (Sario et al., 2018; Gonçalves and Gaivão, 2024; Ferreira et al., 2019). Comet assay, longevity analysis, locomotor performance (negative geotaxis), and prolificacy tests were used to assess whether substrate-driven phytochemical variations translate into functional outcomes. Together with biochemical profiling, these assays provide an integrated assessment of *B. rapa* var. *cymosa* as a nutritionally valuable and biologically safe crop for future space agriculture.

2. Materials and methods

2.1. Plant cultivation and substrate preparation

2.1.1. Cultivation system of B. rapa var. cymosa

B. rapa var. *cymosa* plants were cultivated under rigorously controlled environmental conditions within the Space Orbital Life Enhancement demonstrator (SOLE) greenhouse, a space-oriented agronomic platform developed by G & A Engineering in collaboration with the Italian Space Agency (ASI). Three distinct root zone environments were employed for cultivation: (1) a standard hydroponic system using Ferrari Farm nutrient solution without any solid substrate (hydroponic sample); (2) an ultra-fine lunar highlands regolith simulant (LHS-1D), serving as both a structural and partially nutrient-retentive medium (lunar highland sample); and (3) a coarser-textured lunar mare regolith simulant (LMS-1), selected for its mineral composition representative of basaltic lunar maria (lunar maria sample). The Controlled Environment Agriculture (CEA) greenhouse operates on a closed-loop water system, meaning that all nutrient solution supplied for irrigation is recovered if not absorbed by the plants and reused in subsequent irrigation cycles, minimizing water consumption and reducing the need for external supplies. The hydroponic system was static, with nutrient solution maintained at a stable volume and refreshed periodically. Seeds in the hydroponic system were placed on mesh supports above the nutrient solution, allowing roots to access the solution without being fully submerged, preventing stress due to oxygen deprivation.

For cultivation on lunar regolith, seeds were directly scattered on the regolith surfaces within the trays, and a water misting system was implemented to maintain adequate hydration. No additional substrate was used, in order to simulate the direct use of in-situ lunar materials and to evaluate the feasibility of cultivation relying solely on extraterrestrial resources. The goal of this approach was to validate the potential of in-situ resource utilization (ISRU) for food production in space, avoiding the introduction of terrestrial materials that would not be available on the Moon.

The use of lunar regolith as the sole substrate allowed for a direct assessment of its mechanical and chemical properties on plant growth, isolating the effects of regolith composition and texture from those of standard terrestrial substrates. While conventional solid substrates could serve as controls, their inclusion would reduce the relevance of the results for ISRU applications. Cultivation under these conditions demonstrates the potential for autonomous, resource-efficient food production in extraterrestrial environments and contributes to the development of protocols for lunar and Martian colonies.

Growth conditions were precisely maintained, with a photoperiod of 16 hours of light and 8 hours of dark, at the light intensity of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ (average measured at 300 mm from the LED array), temperature controlled at 22 ± 1 °C, and relative humidity maintained between 60 and 70 %. Illumination was provided by a programmable LED array equipped with six independently controlled spectral channels. The six spectral channels and their relative intensities are summarized in Supplementary Table 1. Sterility was maintained throughout all experimental phases by using sterile gloves and surgical face masks during plant handling, and by pre-sterilizing all instruments and containers with hydrogen peroxide (H_2O_2).

2.1.2. Substrate and nutrient media preparation

In the standard hydroponic culture, *B. rapa* var. *cymosa* plants were grown directly in Ferrari Farm's commercial hydroponic nutrient solution, KAdriatica IDRON NPK 20–20–20 + ME contains 20.00 % total nitrogen, of which 5.50 % is nitrate, 3.50 % ammoniacal, and 11.00 % ureic. It also contains 20.00 % phosphorus pentoxide (P_2O_5), 20.00 % potassium oxide (K_2O), and ≤2.00 % chlorine (Cl). Micronutrients, all chelated via ethylenediaminetetraacetic acid (EDTA), include iron (Fe, 0.02 %), manganese (Mn, 0.01 %), zinc (Zn, 0.002 %), copper (Cu, 0.002 %), boron (B, 0.01 %), and molybdenum (Mo, 0.001 %). The solution was prepared by dissolving 15 g of fertilizer in 15 L of deionized (DI) water, resulting in a final concentration of 1 g/L, and was continuously monitored for pH and electrical conductivity (EC) to ensure optimal nutrient delivery. This nutrient solution was selected under agreement with ENEA – BioAgricoltura (ENEA-BioA) and has been used in previous CEA trials, including high-value crops such as saffron. Its use ensures methodological consistency, reliable nutrient availability, and comparability with prior studies.

For the regolith-based cultivation systems, two types of lunar regolith simulants were employed, both supplied by Exolith Lab (University of Central Florida, USA), and formulated based on reference standards established by NASA and the Johnson Space Center (JSC). The first, LHS-1D Dust Simulant, is an ultra-fine particulate analog of lunar highlands dust, with a mean particle size of 7 μm, median of 5 μm, and a distribution range between <0.04 and 35 μm. Its mineralogical composition by weight included 74.4 % anorthosite, 24.7 % glass-rich basalt, 0.4 % ilmenite, 0.3 % olivine, and 0.2 % pyroxene. Its bulk oxide chemistry (as determined by XRF) comprised SiO_2 (48.1 %), Al_2O_3 (25.8 %), CaO (18.4 %), Fe_2O_3 (3.7 %), K_2O (0.7 %), MgO (0.3 %), MnO (0.1 %), P_2O_5 (1.0 %), TiO_2 (1.1 %), SO_3 (0.3 %), Cl (0.4 %), and SrO (0.1 %), totaling 99.9 % composition. The second substrate, LMS-1 mare simulant, was used as a general-purpose analog of lunar mare basalts, characterized by a coarser texture with an uncompressed bulk density of 1.56 g/cm^3, a mean particle size of 50 μm, and a range from <0.04 to 300 μm. Its mineralogical profile weight/weight (w/w) included 32.8 % pyroxene, 32.0 % glass-rich basalt, 19.8 % anorthosite, 11.1 % olivine, and 4.3 % ilmenite. Oxide chemistry measured by X-ray fluorescence (XRF). It consisted of SiO_2 (40.2 %), Al_2O_3 (14.0 %), CaO (9.8 %), Fe_2O_3 (13.9 %), K_2O (0.6 %), MgO (12.0 %), MnO (0.3 %), P_2O_5 (1.0 %), TiO_2 (7.3 %), Cl (0.4 %), Cr_2O_3 (0.3 %), NiO (0.2 %), and SrO (0.1 %), amounting to 100.0 % total composition.

For the regolith-based systems, substrate pastes were prepared by gradually hydrating 50 g batches of dry simulant with nutrient solution until a cohesive but unsaturated consistency was reached. Moisture content was adjusted to approximately 30–35 % w/w, producing a paste

capable of retaining structure for root anchorage while minimizing free water release. This hydration process allowed the formation of a semi-solid substrate suitable for plant establishment and nutrient retention under hydroponic-like conditions.

2.1.3. Sowing, growth monitoring, harvest, and sample handling

Sowing was performed by surface-broadcasting *B. rapa* var. *cymosa* seeds directly onto the regolith–nutrient paste or by immersing them in the liquid-phase hydroponic nutrient solution, depending on the system. Transparent, food-grade plastic containers with a depth of 4–6 cm were selected to enable visual monitoring of root development, facilitate morphometric assessments, and ensure sterility. Plants were monitored daily to document germination rates, seedling emergence, the progression of leaf development (including the number and size of true leaves), and root system architecture (elongation and branching). From each sample, ten plants were selected for morphometric analysis. Plant height (from the substrate surface to the apical meristem), root length (along the main taproot axis), cotyledon area, hypocotyl height, and the length, width, and area of all leaves, including bilobed leaves were measured using digital calipers with a precision of ±0.01 mm. Environmental parameters—including light intensity, temperature, humidity, CO_2 concentration, and pH/EC (Electrical Conductivity) of the nutrient solution—were continuously logged and linked to individual plant sample IDs for correlation with morphological traits. Plants were harvested at the vegetative-mature stage, typically 10–15 days after sowing. Harvesting was performed using sterile forceps to prevent contamination.

The plant samples were transported to the laboratory in expanded polystyrene containers with lids, providing thermal insulation, mechanical protection, and minimizing moisture loss. During transport, plant water status was maintained by lightly misting with nutrient solution, avoiding stagnation that could compromise tissue integrity. The tissues were not washed prior to packaging or transport to preserve the leaf surface and retain the residual substrate supporting the root microenvironment, thereby maintaining the physiological state of the seedlings. Upon arrival at the laboratory, samples were stored in vacuum-sealed packages at –20 °C. Prior to lyophilization and extraction, the samples were visually inspected, and any adhering dirt or residues were carefully removed.

Although direct gravimetric water content (fresh vs. dry weight) was not measured, plant water status was qualitatively monitored throughout cultivation by recording turgidity, wilting episodes, and rehydration recovery. These parameters were used as indirect indicators of relative tissue hydration across treatments (Jones, 2006; Art Spomer, 1985).

2.2. Chemicals and reagents

For polyphenol profiling, stock solutions were prepared at 1000 µg/mL by dissolving 10 mg of each standard in 10 mL of high-performance liquid chromatography (HPLC)-grade methanol (EMSURE® Reag. pH Eur; Merck, Darmstadt, Germany). Liquid chromatography–mass spectrometry (LC-MS)-grade solvents—methanol (CAS Number 67-56-1) and 2-propanol (CAS Number 67-6-0)—were supplied by Merck (Darmstadt, Germany), while ultrapure water was obtained from a Milli-Q SP water system (Millipore, Bedford, MA, USA). Captiva PTFE 13 mm, 0.45 µm syringeless filters were purchased from Agilent Technologies (Santa Clara, CA, USA). Cyanidin-3-glucoside chloride, delphinidin-3,5-diglucoside chloride, delphinidin-3-galactoside chloride, petunidin-3-glucoside chloride, malvidin-3-galactoside chloride, quercetin-3-glucoside, and kaempferol-3-glucoside were purchased from PhytoLab (Vestenbergsgreuth, Germany). The remaining 31 analytical standards of the 38 phenolic compounds were supplied by Sigma-Aldrich (Milan, Italy). Formic acid (99 %) was obtained from Merck (Darmstadt, Germany), and analytical-grade hydrochloric acid (37 %) was obtained from Carlo Erba Reagents (Milan, Italy). All solvents and solutions were filtered through 0.2 µm polyamide filters from Sartorius Stedim (Göttingen, Germany). Before HPLC analysis, all samples were filtered using Phenex™ RC 4 mm, 0.2 µm syringeless filters (Phenomenex, Castel Maggiore, BO, Italy).

For the ORAC assay, all reagents— AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid)—were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the ABTS, DPPH, FRAP, chlorophyll, and carotenoid content assays, Trolox (≥98.0 %), DPPH• (2,2-diphenyl-1-picrylhydrazyl radical, ≤100.0 %), ABTS•⁺ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt, ≥98.0 %), potassium persulfate ($K_2S_2O_8$, ≥99.0 %), TPTZ (2,4,6-tripyridyl-s-triazine, ≥98.0 %), and iron(III) chloride ($FeCl_3$, ≥99.9 %) were obtained from Sigma-Aldrich (Steinheim, Germany). Ethanol was supplied by Merck (Darmstadt, Germany), formic acid by Panreac (Castellar del Vallés, Barcelona, Spain), and acetonitrile by J.T. Baker (Philipsburg, NJ, USA). Distilled water (Millipore, Bedford, MA, USA) was used for all extractions and analytical procedures. For all assays involving *D. melanogaster*, the materials required for culture medium preparation were obtained from Laborspirit (Santo Antão do Tojal, Portugal). All other reagents and analytical-grade chemicals were sourced from Acros Organics (NJ, USA).

2.3. Plant extraction and sample preparation

For polyphenol profiling and ORAC assays, fresh plant samples were extracted using 70 % ethanol. Each extraction was performed in duplicate, using 1 g of wet tissue with a total volume of 30 mL of ethanolic solution. Samples were incubated for 24 h and subjected to this extraction process three times to maximize compound recovery. The resulting extracts were filtered through filter paper using funnels to separate the liquid phase from the residual plant material. The combined ethanolic extracts were then concentrated using a rotary evaporator (Buchi Rotavapor R-200) under reduced pressure at 40 °C. The dried extract was weighed to determine the extraction yield and subsequently stored in Eppendorf tubes at −20 °C for further analysis.

The remaining plant material was subjected to lyophilization to preserve its biochemical integrity. Samples were initially frozen at −20 °C and then freeze-dried under vacuum in a lyophilizer, allowing water to sublimate at low temperature. Lyophilization was carried out by placing the samples in the lyophilizer at −20 °C and 63 Pa for 24 h. This was followed by an additional 48 h at the same pressure while gradually reaching room temperature, effectively removing water while maintaining tissue structure and biochemical composition. After lyophilization, the dried tissue was milled into a fine powder using a laboratory grinder (Retsch GmbH, Model ZM 200, Retsch-Allee 1–5, 42,781 Haan, Germany), achieving an average particle size of 1 mm. The powdered material was stored in airtight containers at −20 °C to prevent moisture absorption and maintain chemical stability.

For antioxidant assays including FRAP, ABTS, and DPPH, phenolic extracts were prepared from the lyophilized samples as follows: 40 mg of powdered material was mixed with 1.5 mL of a 70:30 vol/volume (v/v) ethanol/distilled water (EtOH/dH_2O) solution. The mixture was vortexed and then agitated at room temperature for 30 min. Subsequently, it was centrifuged at 1500 rpm for 15 min at 4 °C to separate the supernatant from the solid residue. This extraction was repeated three times, and the collected supernatants were pooled into a 5 mL volumetric flask and stored at 4 °C until analysis.

2.4. Polyphenol profiling and antioxidant capacity assays

Identification and quantification of thirty-eight distinct phenolic compounds, pertaining to various chemical classes including phenolic acids, flavonols, flavan-3-ols, flavones, proanthocyanidins, anthocyanins and non-phenolic acids, were performed using HPLC-MS/MS in the different samples. The analysis followed a method previously described (Mustafa et al., 2022).

The antioxidant capacity of the extracts was assessed employing three spectrophotometric assays: FRAP, ABTS, and DPPH, based on the methodologies outlined by Queiroz et al. (Queiroz et al., 2017) and Gouvinhas et al. (Gouvinhas et al., 2014), with adaptations as per Barros et al. (Barros et al., 2024). Additionally, the free radical scavenging activity was quantitatively evaluated through the ORAC assay, conducted in accordance with the protocol of Gillespie et al. (Gillespie et al., 2007) and measured using a Fluostar Omega microplate reader (BMG Labtech, Ortenberg, Germany). All measurements carried out in triplicate.

2.5. Chlorophyll and carotenoid content

For the pigment quantification, 5 mg of each sample was incubated with 80 % acetone for 24 h at 4 °C, followed by centrifugation at 5000 rotations per minute (rpm) for 10 min at 4 °C. Chlorophyll a, chlorophyll *b*, and carotenoids were quantified at wavelengths of 663.2 nm, 646.6 nm, and 470 nm, respectively, using the classical spectrophotometric method with a spectrophotometer (Thermo Electron Corporation, UVG 141,604, Horsham and Loughborough, England). All measurements were performed in triplicate. The pigment content of chlorophyll a, chlorophyll *b*, total chlorophyll ($a + b$), and carotenoids was calculated according to the method described by Lichtenthaler et al. (Lichtenthaler, 1987) using Eqs. (1)–(4). The pigment content was expressed in µg/mL of the pigment extract solution and subsequently converted to µg/g dry weight (DW), considering the initial weight of the leaves (Barros et al., 2024).

$$Chlorophyll\ a\ (Ca) = 12.25 \times A663 - 2.55 \times A646\ [\mu g/mL] \quad (1)$$

$$Chlorophyll\ b\ (Cb) = 20.31 \times A646 - 4.91 \times A663\ [\mu g/mL] \quad (2)$$

$$Total\ chlorophyll\ (a + b) = 17.76 \times A646 + 7.34 \times A663\ [\mu g/mL] \quad (3)$$

$$Carotenoids = (1000 \times A470 - 1.82 \times Ca - 85.02 \times Cb)/198\ [\mu g/mL] \quad (4)$$

2.6. D. melanogaster strain and maintenance conditions

The *D. melanogaster* Oregon-K (OK) strain was used in this study. This strain is efficient in DNA repair mechanisms but exhibits low antioxidant enzyme activity, making it particularly sensitive to oxidative and genotoxic stress and suitable for assessing the physiological impacts of dietary treatments (Gaivão and Comendador, 1996; Rodríguez et al., 2023). Additionally, Oregon-K is more sensitive than other wild-type strains (e.g., Oregon-R) to DNA-damaging agents, enhancing its ability to reveal subtle biochemical or stress-related effects in vivo (GaivÃ£o and Sierra, 2014).

Flies were maintained under controlled laboratory conditions at 24 ± 1 °C with 60 % relative humidity (RH). A standard diet was prepared by heating a mixture to 85–90 °C, consisting of 100 g of granulated sugar, 100 g of baker's yeast, 12 g of agar, 5.4 g of sodium chloride, and 5 ml of propionic acid per 1000 ml of distilled water (dH_2O). The medium was allowed to cool and solidify before use.

Anaesthetization using diethyl ether was applied when handling flies, as needed, to minimize stress and facilitate transfer.

2.7. Preparation of sample media and experimental setup for test on drosophila

Experimental media were prepared by supplementing the standard medium with plant material at concentrations of 0 % (control), 1.25 %, 2.5 %, 5 %, 10 %, and 20 %, based on the fresh weight of the samples prior to lyophilization. This range was guided by the concentrations used by Çeliktaş-Köstekçi et al. (Çeliktaş-Köstekçi and Zemheri-Navruz, 2023), and was expanded to include one lower (1.25 %) and one higher (20 %) concentration, capturing both subtle and pronounced biological effects. The water content of the plant material (~93 %) was determined by a calculation based on the weight of the samples before and after lyophilization. Based on this, 1 g of fresh material corresponded to approximately 0.07 g of dried powder. Accordingly, the media were supplemented with 0 mg or control (reared on standard medium), 4.37 mg, 8.75 mg, 17.5 mg, 35 mg, and 70 mg of dried plant powder for the respective 0 %, 1.25 %, 2.5 %, 5 %, 10 %, and 20 % treatments based on fresh weight.

The plant material was added immediately after cooking, while the medium was still liquid and before solidification. For each treatment, 5 mL of medium was dispensed into 15 mL flat-bottom glass tubes, which were sealed with cotton plugs to allow for airflow. For mating, five male and five female flies (0–24 h post-eclosion) were introduced into each tube. After five days of incubation under the aforementioned environmental conditions, adult flies were removed following oviposition. Emerging adult progeny were subsequently transferred to 200 ml culture bottles containing 20 ml of the corresponding medium and maintained under the same temperature and humidity conditions.

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After five days of incubation under the aforementioned environmental conditions, adult flies were removed following oviposition. Emerging adult progeny were subsequently transferred to 200 mL culture bottles containing 20 mL of the corresponding medium and maintained under the same temperature and humidity conditions. To ensure a sufficient number of flies for all subsequent tests, five independent sets of tubes were prepared for each plant and specific concentration.

2.8. Comet assay

The comet assay was performed to evaluate DNA strand breaks, alkali-labile sites, and overall genomic integrity in *D. melanogaster* neuroblasts (Sierra et al., 2014; Collins et al., 2008). Third-instar larvae were collected from each experimental group, and neuroblasts were isolated through dissection. Larvae reared on standard Drosophila medium without *B. rapa* supplementation served as the control group and were processed in the same manner as the experimental groups in the comet assay. The extracted tissues were gently dissociated using a rounded-tip Pasteur pipette. Prior to centrifugation, brains were carefully perforated using fine tweezers while immersed in a drop of Ringer's solution to aid desiccation. Subsequently, 100 µL of the same Ringer's solution was added to the Eppendorf tube containing the tissue. The samples were then centrifuged at 268 × *g* for 5 min.

Following centrifugation, the supernatant was discarded, and 140 µL of 1 % low-melting-point (LMP) agarose (Pronadisa micro& molecular biology, Madrid, Spain) maintained at 37 °C, was added to each microtube. This LMP agarose was mixed together with the centrifuged sample. Two 70 µL drops of the resulting cell-agarose suspension were then placed onto pre-coated microscope slides containing 1 % normal-melting-point (NMP) agarose (SIGMA-ALDRICH, St. Louis, USA). For each experimental condition, three independent biological samples (brains from different third-instar larvae) were selected. From each biological sample, two drops were applied to a single slide, with each slide containing two sections. Each drop was read independently, providing two technical reads per drop, resulting in multiple technical replicates per condition. Each drop was immediately covered with an 18 × 18 mm coverslip and left to solidify at 4 °C for 5 min, after which the coverslips were carefully removed.

Slides were then immersed in a lysis buffer (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris-base, 1 % Triton X-100, pH 10) for 24 h at 4 °C. Following lysis, the slides were transferred to an electrophoresis buffer (0.3 M

NaOH, 1 mM EDTA, pH 12.6) and incubated for 30 min at 4 °C to allow DNA unwinding. Electrophoresis was subsequently carried out at 25 V and 300 mA for 30 min at 4 °C using a standard electrophoresis unit.

After electrophoresis, slides were neutralized by sequential washes with 1 × PBS and distilled water, each for 10 min at 4 °C. DNA was stained with 20 µL of DAPI solution (1 µg/mL) per gel. A total of 100 nuclei per gel were scored visually using a fluorescence microscope (Olympus Bx41, 40x magnification), based on the classification criteria described by Collins et al. (Collins et al., 2008). The scoring was performed blindly to ensure unbiased assessment (Azqueta et al., 2011). Nuclei were classified into five damage categories (0 = no damage to 4 = severe damage), and the cumulative scoring values were converted into arbitrary units (AU) (Collins et al., 2008). The percentage of DNA in the tail was then obtained by dividing this value by 4, as outlined by Azqueta (Azqueta et al., 2011). Importantly, no image analysis software was used.

2.9. Negative geotaxis behavior assay

To assess locomotor performance via negative geotaxis, five male and five female adult *D. melanogaster* from each treatment group were separately introduced into flat-bottomed glass test tubes measuring 20 cm in length and 2.2 cm in internal diameter. Each tube was sealed with a 1 cm-thick cotton plug to prevent escape while allowing airflow.

Flies were allowed to recover at room temperature for 2 h following diethyl ether anesthesia before starting the assay. After recovery and acclimation to the test tubes, flies were ready for the initiation of the negative geotaxis response. The response was triggered by gently tapping the flies to the bottom of the tube. An 8 cm mark from the base served as the target threshold, and climbing behavior was recorded using a video camera for subsequent analysis.

The first three trials were considered adaptation sessions and excluded from data analysis. For each tube, the average time for the first fly to reach the 8 cm mark, and the time at which the last fly crossed the 8 cm mark (i.e., when all flies in the tube had surpassed the threshold), were calculated to provide a time-resolved assessment of locomotor performance. The assay was conducted in three independent replicates.

This procedure represents a time-based adaptation of the method described by Ali et al. (Ali et al., 2011), which recorded the number of flies surpassing the 8 cm threshold at a fixed 10 second interval. In contrast, this assay measures the exact time for flies to reach the 8 cm mark, allowing temporal climbing dynamics to be analyzed (de Aquino Silva et al., 2021).

2.10. Prolificacy assessment

To assess prolificacy, 5 ml of experimental media containing 0 %, 1.25 %, 2.5 %, 5 %, 10 %, and 20 % plant material (based on fresh weight) were prepared by adding plant material to the standard medium. Activated charcoal powder (0.5 % of the total medium weight) was also incorporated to improve visibility, making it easier to count the eggs. The medium was dispensed into the lids of 50-ml Falcon tubes (diameter 4.5 cm) to create a flat surface suitable for egg deposition and counting. The lids were allowed to solidify at room temperature.

For mating, five male and five female *D. melanogaster* (0–24 h post-eclosion) were introduced into each tube. Small ventilation holes were pierced in the walls of the tubes using a heated needle to ensure airflow for the flies. After 5 days, the adult flies were removed, and the number of eggs and larvae were recorded. The number of hatched adults was documented after 10 days. Viability rates were determined for each developmental stage: egg-to-larva, larva-to-adult, and egg-to-adult. All tests were performed in three independent replicates

2.11. Longevity assay

From the first batch of hatched adults, 30 males and 30 females were collected from each sample and transferred to 200 ml glass bottles containing 20 ml of standard medium. The assay was performed in three independent biological replicates. The flies were maintained at 24 ± 1 °C with 60 % relative humidity (RH). Every 7 days, the number of decreased flies was recorded, and the surviving flies were transferred to fresh 200 ml glass bottles with 20 ml of new standard medium without plant supplementation. This procedure was repeated until all flies had died.

2.12. Data evaluation and statistical analysis

Plant morphological traits, including cotyledon area, hypocotyl height, leaf length, width, and area, as well as bilobed leaf characteristics, were analyzed. Additionally, plant biochemical profiles were assessed, including total phenolic content (TPC), polyphenol composition, chlorophyll and carotenoid contents, and antioxidant activities (DPPH, ABTS, ORAC, and FRAP). DNA damage was evaluated using the comet assay, with outcomes expressed both as arbitrary units (AU) and as the percentage of DNA in the tail; statistical comparisons, however, were performed exclusively on the AU values. All parameters were statistically analyzed using one-way ANOVA followed by Tukey's post-hoc multiple comparison test. The use of parametric tests was justified based on the assumption of approximate normality, which was considered acceptable given the limited sample size. Results are presented as mean ± standard deviation (SD) from three independent replicates.

The data from the negative geotaxis test were presented as box plots, displaying the median, interquartile range (IQR), minimum, maximum, and mean values. Prolificacy was evaluated based on the number of hatched adults and egg-to-adult viability. Additionally, the egg and larval count, were recorded and analyzed, along with embryonic viability (egg-to larvae viability), and larval (larva-to-adult) viability. Data were presented as mean ± SD. For both negative geotaxis test and comparisons of hatched adults, egg count, larval count, embryonic viability, larval viability and egg-to-adult viability as measures of prolificacy, the normality of the data was assessed using the Shapiro-Wilk test. Since the data violated the assumption of normality, the Kruskal-Wallis test was used for statistical analysis. For pairwise comparisons, Dunn's Test with Benjamini-Hochberg (BH) adjustment was applied to control the false discovery rate (FDR).

To evaluate lifespan and longevity differences among Drosophila groups, mean, median plus 95 % of Confidence Interval (CI), minimum, maximum longevity (days), as well as survival probability were analyzed using the Kaplan–Meier method survival analysis. Statistical significance among groups was assessed using the Log-rank (Mantel–Cox) test to compare survival distributions. To account for multiple group comparisons, p-values from the Log-rank test, and, p-values were adjusted with the Benjamini–Hochberg (BH) procedure to control the FDR for multiple comparisons.

To further investigate the relationships between plant biochemical parameters and *D. melanogaster* outcomes, linear mixed-effects models were fitted using the lme4 package in R, with p-values for fixed effects obtained via the lmerTest package (Bates et al., 2015; Kuznetsova et al., 2017). For each measured outcome—including AU of DNA damage in neuroblasts, number of hatched adults, egg-to-adult viability, median climbing time, and mean lifespan (longevity)—the corresponding biochemical parameter was included as a fixed effect, while plant identity and concentration were treated as random effects to account for variation between plants and across doses. Effect sizes and p-values were reported for all fixed effects, and all p-values were subsequently adjusted using the Benjamini–Hochberg (BH) procedure to control FDR.

For all analyses, a p-value < 0.05 was considered statistically significant. All statistical analyses were performed using RStudio (version 2023.12.1 + 402).

3. Results

3.1. Morphological characterization of B. rapa var. cymosa under different growth substrates

To assess the influence of substrate composition on seedling development, morphological traits of *B. rapa* var. *cymosa* grown under hydroponic conditions, in lunar maria regolith simulant, and in lunar highland regolith simulant were compared. Measurements included cotyledon area, hypocotyl height, leaf width and length, bilobed leaf dimensions, and corresponding leaf and bilobed leaf areas (Table 1). Seedling growth progression was additionally documented through photographs taken at 3, 9, 12, 15, and 18 days after sowing (DAS) (Supplementary Figure 1).

Quantitative analyses revealed that cotyledon area and bilobed leaf parameters did not differ significantly among groups. In contrast, hypocotyl height was significantly greater in hydroponic seedlings (102.82 ± 8.52 mm) compared with both regolith treatments (78.75 ± 10.24 mm for lunar maria; 74.39 ± 8.96 mm for lunar highland; $P < 0.05$). Leaf width was significantly larger in lunar highland seedings (14.31 ± 2.29 mm) than in lunar maria (10.68 ± 2.04 mm), while hydroponic seedlings showed intermediate values (11.57 ± 1.73 mm). Similarly, leaf length and total leaf area were highest in the lunar highland treatment (20.41 ± 3.74 mm and 288.36 ± 53.78 mm^2, respectively), significantly exceeding values for the hydroponic and lunar maria groups.

Qualitative assessment of plant water status revealed differences among cultivation systems. Hydroponic plants maintained uniform turgidity throughout growth, with only a single transient wilting episode. Seedlings grown in lunar maria regolith simulant remained hydrated but exhibited uneven moisture distribution due to persistent surface water accumulation, which occasionally causing lodging. In contrast, seedings grown in lunar highland regolith simulant showed more frequent wilting episodes and reduced height uniformity, consistent with the substrate's lower water retention capacity. These observations indicated a relative hydration gradient of Hydroponic ≥ Lunar Maria > Lunar Highland, consistent with prior studies showing slightly higher tissue water content in hydroponically grown leafy vegetables compared to solid substrates (Chamoli et al., 2024; Lei and Engeseth, 2021)

Overall, these results suggest that substrate type influences specific aspects of seedling morphology, with hydroponic conditions promoting greater hypocotyl elongation, while lunar highland regolith supports the development of larger leaf blades despite less stable water availability.

3.2. Polyphenol profiling, antioxidant capacity, chlorophyll and carotenoid content

To evaluate the influence of growth conditions on bioactive compounds in *B. rapa* var. *cymosa*, polyphenol profiling was performed using HPLC-MS/MS, which allows identification and quantification of multiple phenolic compounds across chemical classes, including phenolic acids, flavonols, flavan-3-ols, flavones, proanthocyanidins, anthocyanins, and non-phenolic acids. Results are presented in Fig. 1 and Supplementary Table 2. Levels of Gallic acid, Chlorogenic acid, and Kaempferol-3-glucoside showed minor, non-significant variation among treatments. In contrast, Neochlorogenic acid, Ferulic acid, and p-Coumaric acid were significantly higher in plants grown under lunar maria conditions compared with the other groups. 4-Hydroxybenzoic acid was significantly elevated in lunar highland plants, whereas its levels were negligible in other samples. Total phenolic content (TPC) was significantly greater in the lunar maria treatment, followed by the lunar highland and hydroponic groups (Table 2).

Antioxidant capacity was assessed using four complementary assays: FRAP, ABTS, DPPH, and ORAC Table 2. In the FRAP and DPPH assays, the lunar highland sample exhibited the highest activity, followed by the lunar maria, while the hydroponic sample showed significantly lower values. In the ABTS assay, antioxidant capacity was greatest in the lunar maria group, followed by the lunar highland, with hydroponics again showing the lowest activity. In contrast, the ORAC assay detected no significant differences among treatments (one-way ANOVA, $P = 0.655$).

To assess how different growth environments influence the photosynthetic capacity, chlorophyll a, chlorophyll *b*, total chlorophyll ($a + b$), and carotenoids were quantified (Table 2). Chlorophyll a content was significantly highest in the lunar maria sample, followed by the lunar highland sample, with the hydroponic sample showing significantly lower levels. Chlorophyll b content, however, was greatest in the lunar highland sample, intermediate in hydroponics, and lowest in the lunar maria treatment. Total chlorophyll ($a + b$) content did not differ significantly between the lunar samples but was significantly lower in hydroponics. Carotenoid content displayed a different pattern, with the lunar maria sample having the highest levels, followed by the hydroponics, and the lunar highland sample showing the lowest.

Collectively, these findings demonstrate that the growth environment exerts strong effects on both biochemical and physiological traits in *B. rapa* var. *cymosa.* Polyphenol accumulation patterns varied across treatments, with certain compounds enhanced under lunar maria or highland conditions, while others remained stable. Antioxidant capacity was also strongly influenced, particularly in the FRAP, DPPH, and ABTS assays, whereas ORAC values were unaffected. Moreover, photosynthetic pigment levels, including chlorophylls and carotenoids, differed significantly among growth substrates, reflecting environment-dependent modulation of plant physiological status.

3.3. Comet assay

To determine whether different growth substrates influence genomic stability, DNA damage in neuroblasts from third-instar larvae was assessed using the comet assay. For each condition, 100 cells per gel were visually scored on a scale from 0 to 4, and the extent of DNA damage was quantified as arbitrary AU and as the percentage of DNA in the tail. Results are presented in Fig. 2 and Supplementary Table 3.

Table 1
Morphometric traits of *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant.

Plants	Cotyledon area (mm^2)	Hypocotyl height (mm)	Leaf width (mm)	Width of bilobed leaf(mm)	Leaf length (mm)	Length of bilobed leaf(mm)	Leaf area (mm^2)	Bilobed leaf area (mm^2)
Hydroponic	38.12 ± 9.54^{a}	102.82 ± 8.52^{a}	11.57 ± 1.73^{ab}	14.06 ± 2.23^{a}	17.31 ± 2.24^{a}	11.03 ± 2.19^{a}	195.33 ± 37.97^{a}	151.35 ± 49.93^{a}
Lunar Maria	35.51 ± 6.73^{a}	78.75 ± 10.24^{b}	10.68 ± 2.04^{a}	12.49 ± 1.91^{a}	16.32 ± 2.21^{a}	9.42 ± 1.95^{a}	172.68 ± 35.44^{a}	120.65 ± 39.46^{a}
Lunar Highland	34.25 ± 12.25^{a}	74.39 ± 8.96^{b}	14.31 ± 2.29^{b}	11.91 ± 2.26^{a}	20.41 ± 3.74^{b}	10.13 ± 2.18^{a}	288.36 ± 53.78^{b}	125.39 ± 46.51^{a}

Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. Significant differences were determined using one-way ANOVA followed by Tukey's multiple range test. Samples sharing the same letter are not significantly different, whereas samples with different letters are significantly different ($P < 0.05$). Data are presented as Mean ± SD.

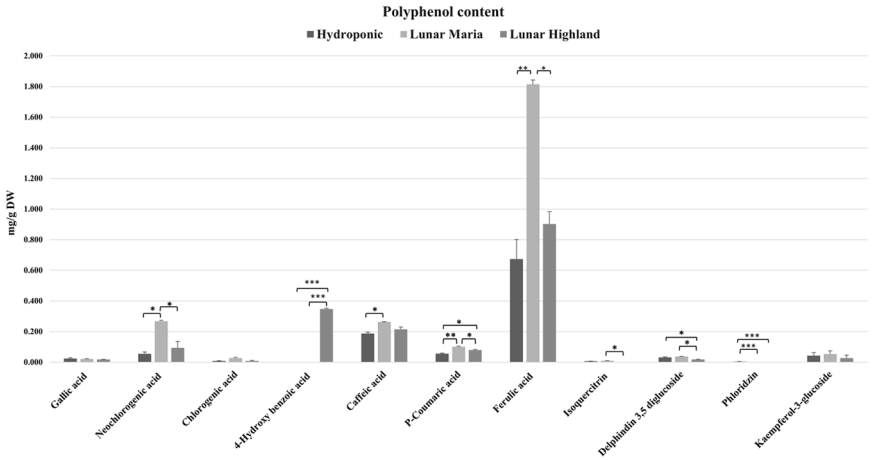

Fig. 1. Polyphenol profiling of *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant. P-values were obtained from analysis of variance (one-way ANOVA) and a multiple range test (Tukey's test). *: $P < 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$. Data are reported as Mean ± SD.

Table 2
Total polyphenol content, antioxidant capacity assay results, chlorophyll profiles, and carotenoid levels in *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant.

Samples	TPC (mg/g DW)	FRAP (μmol Trolox/g)	ABTS (μmol Trolox/g)	DPPH (μmol Trolox/g)	ORAC (μmol Trolox/g)	Chlorophyll a (μg/g DW)	Chlorophyll b (μg/g DW)	Total chlorophyll (*a* + *b*) (μg/g DW)	Carotenoids (μg/g DW)
Hydroponic	1.09 ± 0.18^{c}	0.08 ± 0.00^{c}	1.75 ± 0.12^{c}	0.16 ± 0.04^{c}	0.34 ± 0.08^{a}	1.21 ± 0.05^{c}	0.77 ± 0.07^{b}	1.95 ± 0.06^{b}	0.24 ± 0.04^{b}
Lunar Maria	2.60 ± 0.07^{a}	1.01 ± 0.01^{b}	3.29 ± 0.08^{a}	0.24 ± 0.03^{b}	0.31 ± 0.05^{a}	1.66 ± 0.05^{a}	0.70 ± 0.01^{c}	2.42 ± 0.06^{a}	0.31 ± 0.04^{a}
Lunar Highland	1.70 ± 0.17^{b}	1.23 ± 0.01^{a}	2.26 ± 0.28^{b}	0.25 ± 0.00^{a}	0.30 ± 0.05^{a}	1.40 ± 0.03^{b}	1.06 ± 0.08^{a}	2.43 ± 0.02^{a}	0.22 ± 0.01^{c}

TPC: Total Polyphenol Content, FRAP: Ferric Reducing Antioxidant Power, ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay, DPPH: 2,2-Diphenyl-1-picrylhydrazyl assay, ORAC: Oxygen Radical Absorbance Capacity, DW: Dry Weight. Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. Significant differences were determined using one-way ANOVA followed by Tukey's multiple range test. Samples sharing the same letter are not significantly different, whereas samples with different letters are significantly different ($P < 0.05$). Data are presented as Mean ± SD.

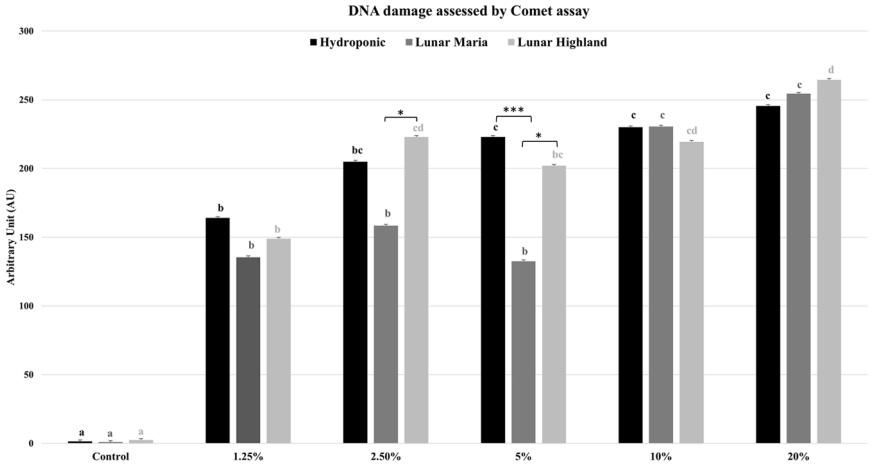

Fig. 2. Comet assay results in larval brain neuroblasts of *D. melanogaster* exposed to *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant. Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. P-values were obtained from analysis of variance (one-way ANOVA) and a multiple range test (Tukey's test). Asterisks show significant differences among same concentrations of different samples (*: $P < 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$) and letters show significant levels among different concentrations within each sample (Concentrations labeled with the same letter are not significantly different; those with different letters are significantly different, $P < 0.05$.). Data are reported as Mean ± SD.

Analysis across different concentrations within each sample revealed that all treatment groups exhibited significantly higher DNA damage compared to the control (larvae reared on standard medium). A dose-dependent increase in DNA damage was observed, with higher concentrations generally causing more severe damage. When comparing different samples at the same concentration, larvae fed on lunar maria samples displayed significantly lower DNA damage at 2.5 % and 5 % compared with both the hydroponic and lunar highland samples. No significant differences were found among samples at other concentrations (Fig. 2 and Supplementary Table 3). The severity of DNA damage ranged from level 0 (no detectable damage) to level 2 (moderate damage), as detailed in Supplementary Table 3.

These results suggest that growth substrates differentially influence DNA integrity, with lunar maria conditions conferring relatively greater genomic protection than hydroponic or lunar highland substrates at certain concentrations.

3.4. Negative geotaxis behavior assay

To evaluate the potential effects of *B. rapa* var. *cymosa* cultivated under different growth conditions on locomotor performance, a negative geotaxis assay in *Drosophila melanogaster* was conducted. In this assay, climbing ability was assessed as the time (mean, median, interquartile range, minimum, and maximum) required for flies to ascend 8 cm in a glass tube. Data are summarized in Fig. 3, while sex-specific analyses are presented in Supplementary Figure 2.

At a concentration of 5 %, flies exposed to the lunar maria group displayed a significantly shorter climbing time (3.37 ± 1.9 s) compared to both the hydroponic group (7.56 ± 3.16 s) and the lunar highland group (7.25 ± 2.97 s). Similarly, at 10 % concentration, the lunar maria group again demonstrated a faster climbing time (4.8 ± 2.65 s) relative to the hydroponic group (7.4 ± 3.4 s). Although the lunar maria group also showed a trend toward faster climbing compared to the lunar highland group (7 ± 2.86 s) at this concentration, the difference did not reach statistical significance (Fig. 3 and Supplementary Figure 2). In all experimental groups, 100 % of flies successfully crossed the 8 cm threshold.

When comparing performance across concentrations within each treatment group, no significant differences were observed, indicating that concentration did not alter climbing time within a given growth condition (Fig. 3). This trend remained consistent when analyzed by sex. Although males tended to climb faster than females across several concentrations, these differences were not statistically significant (Supplementary Figure 2). Overall, these results suggest that *B. rapa* var. *cymosa* cultivated under lunar maria regolith simulant improves climbing performance in *D. melanogaster* at certain concentrations.

3.5. Prolificacy

To evaluate the effects of *B. rapa* var. *cymosa* grown under different conditions on reproductive output, prolificacy was assessed by analyzing of the number of eggs, larvae, hatched adults, egg-to-adult, embryonic (egg-to-larvae), and larval (larva-to-adult) viability were also calculated. Figs. 4 and 5, along with Supplementary Table 4, summarize the results.

At the 1.25 % concentration, the lunar maria sample produced a significantly higher number of hatched adults (83.4 ± 1.74 flies) compared to lunar highland sample (61.6 ± 6.37 flies), while the difference from the hydroponic sample (71.2 ± 1.93 flies) was not statistically significant. At 20 % concentration, the lunar highland sample showed a significantly higher number of hatched adults (93.8 ± 1.93 flies) compared to the hydroponic sample (75.2 ± 1.3 flies), while no significant difference was found with lunar maria sample (82.6 ± 2.33 flies) (Fig. 4). Moreover, the egg-to-adult viability rate showed a slight increase from the control (0 %) up to the 1.25 %, 2.5 %, and 5 % concentrations, followed by a decline at 10 % and 20 % for all samples. However, these differences were not statistically significant after adjustment for multiple comparisons (Fig. 5).

Detailed values for all prolificacy parameters are presented in Supplementary Table 4. Although some variation was observed among samples, differences in egg count, larval count, embryonic viability, and larval viability were not statistically significant (all adjusted $P > 0.05$). Overall, these results suggest that *B. rapa* var. *cymosa* grown under specific substrates can influence the reproductive output of *D. melanogaster* at certain concentrations.

3.6. Longevity

To evaluate the impact of *B. rapa* var. *cymosa* cultivated on different substrates on adult lifespan, longevity of *D. melanogaster* was measured. Mean, Median, Minimum, and Maximum days of Longevity estimated by Kaplan–Meier Survival Analysis are reported in Table 3. Minimum

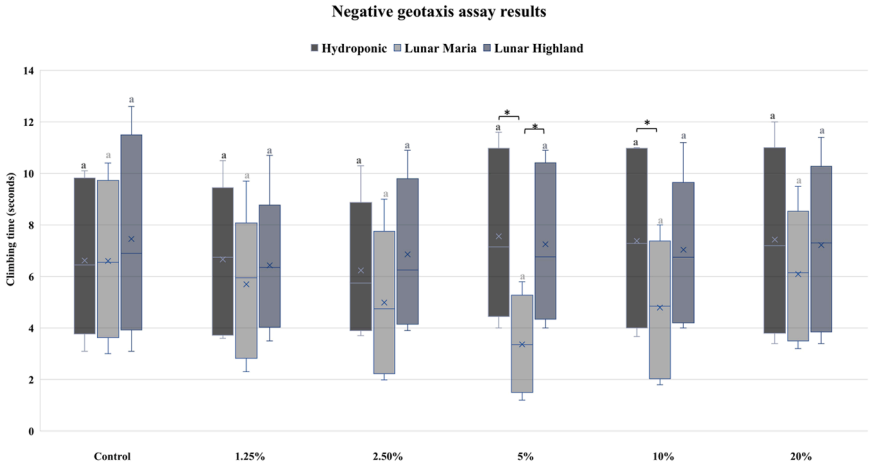

Fig. 3. Negative geotaxis assay results showing the climbing time of *D. melanogaster* exposed to *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant. Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. P-values were obtained using the Kruskal-Wallis test with post hoc Dunn's test and Benjamini-Hochberg correction. Asterisks show significant differences among same concentrations of different samples (*: $P < 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$) and letters show significant levels among different concentrations within each sample (Concentrations labeled with the same letter are not significantly different; those with different letters are significantly different, $P < 0.05$.). Data are presented as median, interquartile range, minimum, and maximum values; the multiplication symbol (×) indicates the Mean.

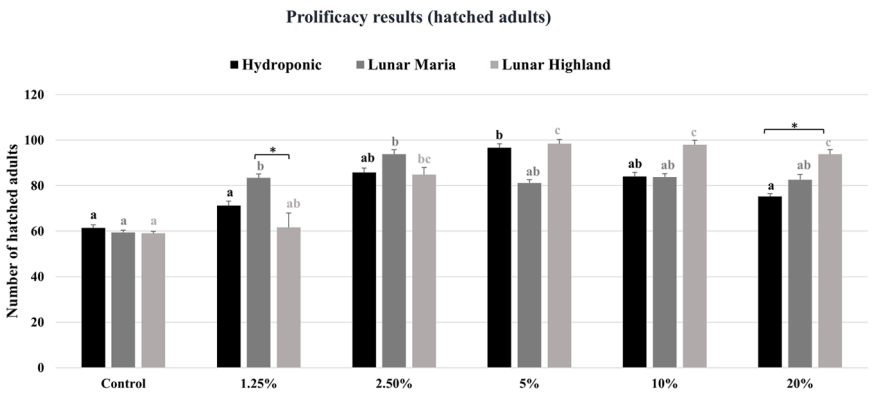

Fig. 4. Prolificacy assay showing the number of hatched adult *D. melanogaster* from sample cultures containing varying concentrations of *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant. Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. P-values were obtained using the Kruskal-Wallis test with post hoc Dunn's test and Benjamini-Hochberg correction. Asterisks show significant differences among same concentrations of different samples (*: $P < 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$) and letters show significant levels among different concentrations within each sample (Concentrations labeled with the same letter are not significantly different; those with different letters are significantly different, $P < 0.05$.). Data are reported as Mean ± SD.

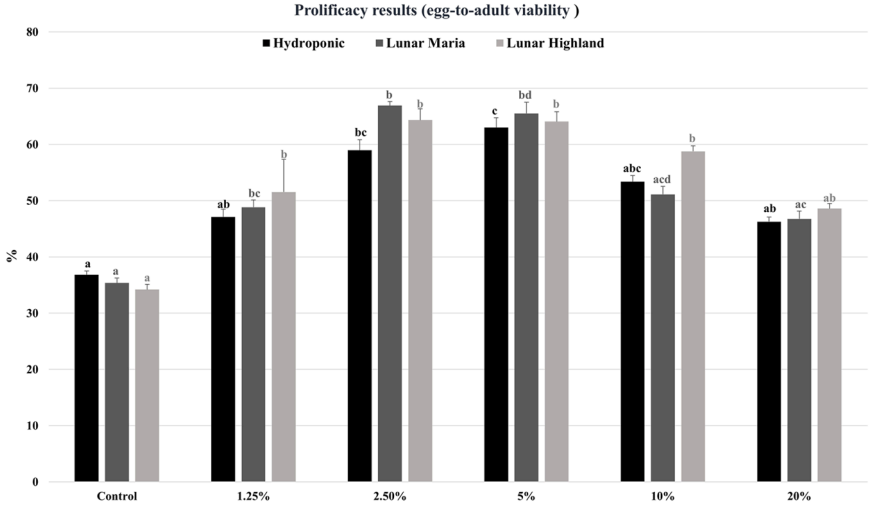

Fig. 5. Prolificacy assay showing Egg-to-adult Viability of *D. melanogaster* from sample cultures containing varying concentrations of *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant. Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant. P-values were obtained using the Kruskal-Wallis test with post hoc Dunn's test and Benjamini-Hochberg correction. Differences among same concentrations of different samples did not reach statistical significance. Letters show significant levels among different concentrations within each sample (Concentrations labeled with the same letter are not significantly different; those with different letters are significantly different, $P < 0.05$.). Differences among same concentrations of different sample were not statistically significant. Data are reported as Mean ± SD.

longevity ranged from 7 to 21 days among all samples, while maximum longevity ranged from 84 to 98 days. Median longevity days ranged from 42 to 56 days. In order to compare longevity among all samples, pairwise Log-Rank test (Mantel–Cox) was conducted and the results are reported in Supplementary Table 5. At 2.5 % concentration, hydroponic samples showed significantly lower longevity compared to lunar maria (Adjusted $P = 0.03$) and lunar highland (Adjusted $P = 0.024$), while no significant differences were observed at other concentrations. Within-sample comparisons of longevity across concentrations revealed that, in the lunar highland sample, longevity was significantly greater at 2.5 % concentration compared to 1.25 %, whereas hydroponic and lunar maria samples did not differ across concentrations (Supplementary Table 5).

Kaplan–Meier survival curves illustrating the survival probabilities of *D. melanogaster* adults over time—both between samples at the same concentrations and within sample—are presented in Supplementary Figures 3 and 4, respectively.

These results suggest that the substrate on which *B. rapa* var. *cymosa* is grown can influence adult longevity in *D. melanogaster*, with hydroponic samples exhibiting reduced lifespan at specific concentrations compared to lunar maria and highland substrates.

3.7. Correlation between plant biochemical parameters and D. melanogaster outcomes

To examine the relationships between plant biochemical parameters and *D. melanogaster* outcomes, linear mixed-effects models were applied. The analysis revealed that climbing ability was significantly associated with the content of several phenolic compounds. Specifically, higher levels of Neochlorogenic acid ($\beta = -0.11$, SE = 0.02, adjusted $P = 0.009$), Chlorogenic acid ($\beta = -1.15$, SE = 0.23, adjusted $P = 0.008$), and Ferulic acid ($\beta = -0.02$, SE = 0.004, adjusted $P = 0.009$) were

Table 3
Estimated Mean, Median, Minimum, and Maximum Longevity Values from Kaplan–Meier Survival Analysis of *D. melanogaster* Exposed to Various Concentrations of *B. rapa* var. *cymosa* cultivated under hydroponic condition, lunar maria regolith simulant, and lunar highland regolith simulant.

Samples	n	Mean Longevity (days)	95 % CI of Mean	Median Longevity (days)	95 % CI of Median	Maximum Longevity (days)	Minimum Longevity (days)
Hydroponic							
Control	60	48.47	[45.70, 63.87]	42	[42, 42]	98	14
1.25 %	60	47.819	[45.89, 65.04]	49	[49, 56]	98	7
2.5 %	60	44.8	[42.43, 57.88]	42	[42, 42]	98	21
5 %	60	52.15	[49.10, 69.07]	56	[49, 56]	98	7
10 %	60	50.092	[46.99, 67.21]	49	[49, 56]	98	7
20 %	60	47.027	[44.23, 62.47]	45.5	[42, 49]	98	7
Lunar Maria							
Control	60	49	[46.44, 63.14]	49	[49, 49]	98	7
1.25 %	60	46.256	[43.89, 59.33]	49	[49, 49]	84	7
2.5 %	60	51.392	[48.60, 66.82]	49	[49, 56]	98	7
5 %	60	48.442	[45.51, 64.63]	49	[49, 49]	98	7
10 %	60	50.517	[47.49, 67.23]	49	[42, 49]	98	14
20 %	60	48.342	[45.93, 61.63]	49	[49, 49]	98	7
Lunar Highland							
Control	60	48.1	[45.35, 63.31]	49	[49, 49]	91	14
1.25 %	60	45.208	[42.02, 62.82]	49	[42, 49]	98	7
2.5 %	60	53.492	[51.04, 67.06]	56	[49, (63)]	84	7
5 %	60	53.317	[50.08, 71.21]	56	[49, 56]	98	7
10 %	60	48.767	[45.70, 65.69]	49	[42, 56]	91	14
20 %	60	49.875	[77.01, 65.75]	49	[49, 56]	98	7

CI: Confidence Interval, Hydroponic: *B. rapa* var. *cymosa* grown in hydroponic condition, Lunar Maria: *B. rapa* var. *cymosa* grown in regolith simulant of lunar mare regions, Lunar Highland: *B. rapa* var. *cymosa* grown in lunar highland regolith simulant.

correlated with faster climbing, indicated by shorter times (fewer seconds) to reach the target height. Associations between other biochemical parameters and *D. melanogaster* outcomes are reported in Supplementary Table 6.

These results suggest that *D. melanogaster* outcomes can be strongly correlated with the biochemical composition of *B. rapa* var. *cymosa* grown in different substrates.

4. Discussion

This study evaluated *B. rapa* var. *cymosa* cultivated in three different conditions: hydroponic, and two lunar regolith simulants—lunar highland and lunar maria. Assessments included morphological traits, biochemical properties such as antioxidant capacity, polyphenol profiling, chlorophyll and carotenoid contents, as well as in vivo effects on *D. melanogaster* using genotoxicity, behavioral, prolificacy, and longevity assays.

Substrate type influenced both seedling morphology and biochemical composition. Hydroponic seedlings showed the greatest hypocotyl elongation, lunar highland seedlings had wider and longer leaves, and lunar maria seedlings displayed intermediate leaf growth with less uniform hydration. Lunar-grown plants, particularly from the maria simulant, had higher levels of several polyphenols (e.g., neochlorogenic acid, ferulic acid, p-coumaric acid) and higher antioxidant activity (FRAP, DPPH, ABTS) compared to hydroponic plants. Chlorophyll levels were generally higher in lunar-grown samples, while carotenoids peaked in lunar maria plants. These differences are likely driven by the distinct physicochemical properties of the substrates—such as water retention, porosity, and nutrient availability—which shape how plants allocate resources and trigger secondary metabolic responses under suboptimal growth conditions.

In vivo, DNA damage assessed by comet assay was elevated in all treatment groups compared to controls, but was the lowest in flies exposed to lunar maria-grown plants, particularly at intermediate concentrations. Despite observed DNA damage, flies treated with lunar maria samples exhibited enhanced climbing ability in the negative geotaxis assay, and no significant impairments were observed in reproduction or lifespan.

Importantly, correlation analyses indicated associations between plant phytochemicals and Drosophila outcomes, such as higher levels of neochlorogenic acid, chlorogenic acid, and ferulic acid were associated with faster climbing performance. While suggestive, these correlations cannot be interpreted as direct causation, and the underlying mechanisms remain to be elucidated.

Several studies have explored the biochemical composition and biological activity of different *B. rapa* varieties and subspecies (Baek et al., 2016; Watanabe et al., 2011; da et al., 2024; Yeo et al., 2021). For example, Baek et al. reported considerable variation in carotenoid and chlorophyll content across nine Chinese cabbage cultivars (Baek et al., 2016), while a comparison between orange-colored (*Orange queen*) and normal (*Yuki*) Chinese cabbage highlighted higher carotenoid and phenolic levels with greater antioxidant activity in the orange variety (Watanabe et al., 2011). Broader surveys of *B. rapa* subspecies, including bok choy, wutacai, mizuna, turnip rape, choy sum, and Chinese cabbage, revealed notable differences in polyphenol and glucosinolate content, with specific cultivars exhibiting higher total phenolic or carotenoid levels (da et al., 2024; Yeo et al., 2021).

Despite these extensive analyses in other subspecies, research on *B. rapa* var. *cymosa* remains limited. Existing studies have examined the influence of cultivation system, landrace, and storage on chlorophyll, carotenoid, and antioxidant content, reporting small differences between organic and conventional practices but largely similar antioxidant capacity (Conversa et al., 2016). Additional works have focused on glucosinolate profiling across ecotypes (Barbieri et al., 2008) and the effects of sulfur fertilization and light exposure on chlorophyll and antioxidant activity during storage (Barbieri et al., 2009).

Despite documentation of the nutritional and phytochemical profiles of *B. rapa* subspecies, there is a notable lack of research investigating the impact of extraterrestrial soil analogs on *B. rapa* or indeed any terrestrial plant. Previous studies have explored plant growth on extraterrestrial regolith, revealing both potential and limitations. Leafy vegetables can grow better on Mars and Moon simulants, with organic amendments (Caporale et al., 2022), while Arabidopsis germinates in lunar regolith but shows slow growth and stress morphologies (Paul et al., 2022). Stress responses have also been observed in Italian ryegrass on Martian simulants (Berni et al., 2023), and plants can survive multiple generations on lunar regolith with antioxidant treatments or genetic modifications, though biomass and fertility are reduced (Rodriguez et al., 2025). Molecular and physiological analyses of Vigna radiata highlight further challenges such as nutrient limitation, high alkalinity, and

mechanical constraints (Setiawan et al., 2023).

Moreover, studies in *Arabidopsis thaliana* show that growth on LHS-2 (lunar highlands) and MGS-1 (Martian global) regolith simulants reduces chlorophyll content and increases anthocyanin accumulation and mineral stress responses (Buckner et al., 2025). In another study, *Solanum lycopersicum* ('Inkspot' tomato) grown in lunar simulants exhibits enhanced antioxidant activity and stress tolerance (Lang et al., 2025), and *Lactuca sativa* (lettuce) on Martian and lunar simulants with organic amendments shows improved biomass and modified phytochemical profiles (LG Duri et al., 2022). Collectively, these findings indicate that certain plant varieties can maintain physiological and biochemical function under extraterrestrial substrates, providing a basis for sustainable space agriculture and in-situ resource utilization (ISRU).

The lunar maria samples consistently showed the strongest antioxidant profile, marked by elevated phenolic acids (such as neochlorogenic, ferulic, p-coumaric), chlorophyll *a*, and carotenoids. These compounds act as radical scavengers (Mustafa et al., 2022; Queiroz et al., 2017), stabilize photosynthetic machinery (Gouvinhas et al., 2014; Lichtenthaler, 1987), dissipate excess excitation energy, and suppress reactive oxygen species (ROS)-driven lipid peroxidation (Barros et al., 2024; Gill and Tuteja, 2010). Their accumulation aligns with known Brassica stress responses, suggesting that substrate-induced stress in lunar maria enhances secondary metabolism and activates a coordinated antioxidant defense network (De Pascale et al., 2007; Cartea et al., 2010; Gawlik-Dziki, 2008).

Phytochemical differences among samples corresponded to distinct in vivo effects in *D. melanogaster*. DNA integrity, assessed by comet assay, was reduced in all treatment groups compared to controls, with the lunar maria sample consistently inducing the least DNA damage at intermediate concentrations. This lower genotoxicity may reflect a more favorable antioxidant-to-prooxidant ratio, enabling effective neutralization of ROS and reduced oxidative DNA damage, consistent with the redox-modulating roles of flavonoids and glucosinolates (Cartea et al., 2010; Gill and Tuteja, 2010; Jacob et al., 2011) higher polyphenol levels (zhuang et al., 2021). Despite measurable DNA damage, functional impairments were not evident in adult flies: individuals exposed to 5 % and 10 % lunar maria samples showed enhanced locomotor performance, while prolificacy output remained largely stable. Some variation in lifespan was observed among groups, but overall longevity remained high.

The apparent paradox between DNA damage observed in larval brain neuroblasts and the absence of significant adult impairments can be explained by the activity of efficient DNA repair mechanisms during development, including nucleotide excision repair (NER), homologous recombination (HR), mismatch repair (MMR), and base excision repair (BER) (Sekelsky, 2017; Mota et al., 2019), and the developmental robustness of Drosophila. Transient molecular damage is often corrected during metamorphosis via cell cycle checkpoints and compensatory repair pathways (Sekelsky, 2017; Butterworth et al., 1988; Chong et al., 2018; LaRocque and McVey, 2023; LaRocque et al., 2007; Bergerson et al., 2022; Brodsky et al., 2004). Moderate stress also induced hormetic responses, upregulating antioxidant defenses and mitochondrial function, which could counteract early-life genotoxic effects (Mattson, 2008; Nitti et al., 2022; Franco et al., 2019). Overall, the DNA damage observed likely remained within the capacity of Drosophila's DNA damage response (DDR), allowing core physiological functions to be preserved.

A key strength of this study is its integrative approach, combining detailed biochemical profiling with multiple in vivo endpoints in *Drosophila melanogaster*. This design allows for direct correlations between phytochemical composition and biological outcomes, including genotoxicity, neurobehavioral changes, reproductive fitness, and overall organismal health. The use of *D. melanogaster* is particularly advantageous due to its well-characterized genetics, conserved stress and DNA repair pathways, and short life cycle, which facilitate efficient assessment of plant-derived compounds (Song, 2005; Pandey and Nichols, 2011). Furthermore, this study addresses a novel area by evaluating the biological effects of plants grown in lunar regolith simulants in a live-animal model.

This study has several limitations that should be considered when interpreting the results. Tissue water content was not quantitatively measured, and multi-generational effects were not assessed. The use of a proprietary hydroponic solution may limit comparability, and comet assay scoring, while blinded, retains some observer subjectivity (Collins, 2004). Future studies should include quantitative water measurements, transgenerational analyses, and automated DNA damage scoring for improved accuracy.

5. Conclusion

The cultivation of *B. rapa* var. *cymosa* in hydroponic condition, lunar maria and lunar highland regolith simulants notably altered its biochemical composition and affected biological responses in *D. melanogaster*. Lunar regolith-grown plants—particularly those from the lunar maria simulant—showed enhanced polyphenol and carotenoid levels. While all plant samples caused measurable DNA damage in larval neuroblasts, this effect did not translate into adulthood. Flies showed normal development, longevity, and behavior, with the lunar maria group showing the most favorable outcomes. These results highlight that substrate type can modulate plant composition and biological outcomes, but the responses are complex and likely influenced by multiple interacting factors. Further studies on metabolic profiles, nutrient content, and plant physiological responses under extraterrestrial-like conditions will be important to build a more complete understanding of crop performance and safety for potential space agriculture applications.

Data statement

Data described in the manuscript will be made available upon request pending. For any inquiries regarding the data, requests can be directed to F.M. who is the guarantor of this study, with full access to all data. Email: fatemeh.mansouri@unicam.it.

CRediT authorship contribution statement

Fatemeh Mansouri: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Ana Novo Barros:** Methodology, Investigation, Conceptualization. **Laura Bordoni:** Supervision, Investigation. **Donatella Fedeli:** Validation, Methodology. **Giorgia Pontetti:** Writing – original draft, Resources. **Eugenia Roncolato:** Resources. **Cinzia Mannozzi:** Writing – original draft, Validation, Methodology, Investigation. **Francesca Pompei:** Investigation. **Sauro Vittori:** Resources. **Elena Pettinelli:** Resources. **Natasha Gomes De Miranda:** Investigation. **Isabel Gaivão:** Supervision, Resources, Methodology, Investigation. **Rosita Gabbianelli:** Writing – review & editing, Supervision, Project administration, Funding acquisition, 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

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