**Journal of Applied Botany and Food Quality 98, 41 - 52 (2025), DOI:10.5073/JABFQ.2025.098.005**

[1]Laboratory of Botany and Valorization of Plant and Fungal Resources, Faculty of Sciences of Rabat, Mohammed V University of Rabat, Morocco
[2]Microbiology and Molecular Biology Team, Center of Plant and Microbial Biotechnologies, Biodiversity and Environment, Faculty of Sciences, Mohammed V University, Rabat, Morocco
[3]Department of Crystallography, Mineralogy and Agricultural Chemistry, Higher Technical School of Agronomic Engineering, University of Seville, Sevilla, Spain
[4]The Center for Research, Technology and Innovation (CITIUS) of the University of Seville, Seville, Spain
[5]Department of Biology, Faculty of Sciences of Tetouan, Abdelmalek Essaadi University, Tetouan, Morocco
[6]Research Unit on Environment and Conservation of Natural Resources, Regional Center of Agronomical Research of Rabat (URECRN), National Institute of Agricultural Research (INRA), Rabat, Morocco
[7]Department of Aromatic and Medicinal Plants, National Institute of Agricultural Research (INRA), Morocco

# Metabolomic analysis of *Aloe vera* (L.) Burm.f. plant response to organic and inorganic fertilization

**Basma Boukour[1*], Farid Rachidi[2], María C. Florido[3], Maria E. Soria Diaz[4], Rocio Valderrama[4], Lamiae Amallah[5], Fatima Bouazza[1], Souad Skalli[1], Ahmed Douaik[6], Chaouki El Faiz[7], Rachida Hassikou[1]**

(Submitted: September 14, 2024; Accepted: March 16, 2025)

## Summary

*Aloe vera* is well known for its medicinal and cosmetic properties and is relatively undemanding in terms of growing conditions. However, the impact of fertilizers on the quality of its bioactive compounds remains underexplored. This study evaluates the effect of three types of fertilizers (one nitrogen-based inorganic fertilizer and two organic fertilizers) on the the metabolomic profiling and pathways of *Aloe vera*. Using the MetaboAnalyst 5.0 platform, 61 metabolites or more and 54 metabolic pathways were identified as being affected by the treatments. The results show that the inorganic treatment significantly increased fatty acids, particularly palmitic and stearic acids, while organic fertilizers (compost and compost-humus) stimulated the production of essential amino acids such as leucine, methionine, and phenylalanine. Additionally, compost-humus enriched 45 metabolic pathways, mainly related to amino acid metabolism, whereas inorganic nitrogen induced 33 pathways, predominantly related to fatty acid biosynthesis. The study reveals that organic fertilizers enhance the enrichment of amino acid metabolism, thus improving *Aloe vera's* medicinal and cosmetic properties. In contrast, inorganic nitrogen optimizes fatty acid production, known for its moisturizing and anti-inflammatory effects. These findings highlight the need for targeted fertilization strategies to maximize specific metabolites for industrial applications.

**Keywords:** *Aloe vera,* fertilizers, targeted metabolomics profiling, metabolomic pathways analysis, GC-MS.

## Introduction

*Aloe vera* (L.) Burm.f. (family Liliaceae) is a perennial herb that is widely distributed in arid and semi-arid regions of the world, such as Africa, India, and other regions with dry climatic conditions. It is a succulent plant recognized for its medicinal properties, relies heavily on its metabolic adaptation to various environmental conditions (Javed et al., 2014). It can grow in almost all types of environmental conditions, but several factors can affect the quality and quantity of a particular constituent (Cristiano et al., 2016).

*Aloe vera* is used in the cosmetics industry, for its soothing and moisturizing effects, such as soaps, moisturizing creams, shampoos lotions, and cleansers (Ijaz et al., 2022; Khaldoune et al., 2024). The gel of *Aloe vera* quickens the skin's regrowth process after injury (Marzanna and Dziedzic, 2019; Khaldoune et al., 2024). The plant has been widely used for its therapeutic properties, including antimicrobial, anti-inflammatory, antidiabetic, and anti-oxidant effects (Eshun et al., 2004; Amallah et al., 2024). *Aloe vera's* pharmaceutical importance is due to its ability to produce a wide range of secondary metabolites, including polysaccharides, phenolic compounds, alkaloids, amino acids, vitamins, and enzymes (Hamman et al., 2008). This plant is also used in the food industry, the antioxidant properties from the skin, flower, and gel help extend the shelf life of foods, prevent spoilage, and reduce lipid oxidation (Marzanna and Dziedzic, 2019; Khaldoune et al., 2024).

The beneficial properties of *Aloe vera* are attributed also to its bioactive compounds with nutritional values (Chowdhury et al., 2021). Palmitic acid and stearic acid present in *Aloe vera* are saturated fatty acids commonly found in various food sources (Ding et al., 2006). Fatty acids play a key role in energy production, membrane formation, and plant defense. Under stress such as drought, membrane lipid modifications activate defense mechanisms against biotic and abiotic stresses, (Lai and Chye, 2021; Ghorbanzadeh et al., 2023). Sugars, amino acids, and organic acids are the major energy source for photosynthesis and respiration in plants (Lee et al., 2012). Aminoacids, as a nitrogen source, promote rapid growth along with an increase in leaf size (Lee et al., 2012), organic acids are generally intermediate respiration and photosynthesis products in higher plants, which are essential for ammonia assimilation and aminoacid synthesis (Zhao et al., 2023).

The bioactive compounds of *Aloe vera* can be influenced by environmental conditions and agricultural practices (Kumar et al., 2017; Chowdhury et al., 2021). Environmental interactions directly affect the quality and quantity of the plant's primary active constituents. Fertilization plays a significant role in crop cultivation systems, including medicinal plants. It is crucial to improving plant growth and increasing the production of bioactive compounds, influencing plant metabolism, impacting development, and stress tolerance (Li et al., 2020; Anjum et al., 2022). Furthermore, it can impact biomass production and influence the biosynthesis and accumulation of physiologically active compounds that contribute to the phytochemical quality of *Aloe vera* (Kováčik and Klejdus, 2014; Cristiano et al., 2016).

Considering the importance of environmental issues, increasing attention is being paid to different fertilizers and the methods of

* Corresponding author

application of organic and inorganic fertilizers (Cristiano et al., 2016). Organic and inorganic fertilizers are commonly used to supply nitrogen to plants. Organic fertilizers are derived from plant or animal residues and provide a slow release of nutrients, whereas inorganic fertilizers are synthetic compounds that supply nutrients in an immediately available form (Saha et al., 2005; Liontakis and Tzouramani, 2016). A large amount of inorganic N fertilizer input can lead to increased plant nitrogen-tolerance and decrease amplitude of yield improvement, which results in significant N loss, energy waste and environment pollution (Ahmed et al., 2017).

Researchers have previously described the kinetics of N for improving crop quality and yield from the perspectives of agronomic traits, photosynthetic physiology, ecological effects, and nutrient absorption and transport (Li et al., 2021; Zhao et al., 2023). Metabolomic strategies can be applied to improve crop function, enhance nutritional quality, and increase grain yield (Zarei et al., 2018; Zhao et al., 2023).

Nitrogen is one of the most important nutrients required for plant growth and is known to influence the biosynthesis of secondary metabolites (Deng et al., 2019). The impact of nitrogen availability on tomato plant's metabolomic profile, observed significant alterations in the levels of several aminoacids and other metabolites, highlighting the influence of nitrogen on plant metabolism (Urbanczyk-Wochniak and Fernie, 2005; Xun et al., 2020). Metabolomics approaches provide valuable insights into plant metabolism, responses to environmental factors alterations of metabolite production (Ghorbanzadeh et al., 2023).

In our previous study, we demonstrated that *Aloe vera* plant fertilized with *Organova* alone or combined with *Humivital* induced positively the morphogical traits and mineral content, compared to inorganic nitrogen (Boukour et al., 2024). *Aloe vera* is known for its relatively easy cultivation requirements and very responsive to nutrient (Barandozi et al., 2011). Additional researches are required to develop tailored fertilization strategies for *Aloe* cultivation to elucidate the alterations that can affect metabolic profile of *Aloe vera*. Our study aims to evaluate these alterations in response to different fertilization regimes including compost, compost-humus, and inorganic nitrogen, using Gas Chromatography-Mass Spectrometry (GC/MS) techniques and metabolic enrichment analysis.

**Fig. 1:** *Aloe vera* plant in the greenhouse.

**Tab. 1:** Physicochemical properties, and textures of soils.

| Parameter | Value | Method | Reference |
|---|---|---|---|
| **pH** | 7.6 | Saturated soil-paste extract | (Rhoades et al., 1989) |
| **EC (mS/cm)** | 1.8 | | |
| **OM (%)** | 1.1 | Chromic acid wet oxidation | |
| **Dry matter (%)** | - | | |
| **N (%)** | 0.1 | Kjeldahl | |
| **$K_2O$ (mg/kg)** | 530.2 | Flame Photometry | |
| **$P_2O_5$ (mg/kg)** | 103.1 | | (Kilmer and Alexander, 1949) |
| **Clay (%)** | 27.0 | Pipette | |
| **Loam (%)** | 32.8 | | |
| **Sand (%)** | 40.2 | | |
| **Texture** | Sandy loam | | |

EC: Electrical conductivity (measured at 25 °C), OM: Organic matter.

## Materials and methods

### Experimental design

The experimental setup was carried out at the National Institute of Agricultural Research of Rabat (INRA), Morocco (33°58'47.3"N 6°51'26.9"W) in a greenhouse. Thirty six samples of *Aloe vera* plant were transplanted into clods of 50% peat, 30% sand and 20% of original soil taken with the plant from Marrakesh region. The *Aloe* plants (suckers) were kindly supplied by the company Best *Aloe* SARL (*Sidi Abbad* – Marrakech, Morocco). *Aloe vera* is propagated using suckers or lateral shoots and rhizome cuttings as planting materials. The plants around 18-month-old, were identical in size, health, and appearance, and reaching 25 to 30 cm in height (Fig. 1).

The soil samples were collected from the surveyed sites, and taken to the laboratory of the Research Unit on the Environment and the Conservation of Natural Resources (URECRN), of the INRA. The soil samples were air-dried, crushed and then sieved. Tab. 1 presents the physical and chemical properties of the soils as well as the corresponding analytical protocols used (Kilmer and Alexander, 1949; Rhoades et al., 1989).

The physico-chemical analysis of the irrigation water was carried out in the laboratory of the URECRN, of the INRA. The frequency of irrigation was once a week. Tab. 2 shows irrigation water tests report.

The experimental setup in the greenhouse was a randomized complete design (RCD), the study period lasted six months between February and October 2019. The average temperature of the greenhouse was 17 °C, with minimal temperature of 10 to 12 °C in January/February and maximal temperature of 20 to 24 °C in July/August. The fertilizers were applied only once at the beginning of the experiment and not reapplied during the study. As shown in (Tab. 3), the dosis of compost (F1) namely ORGANOVA® was 10 g per pot mixed into the soil, this treatment was applied manually in solid form and in a

**Tab. 2:** Physico-chemical analysis of water irrigation.

| pH | EC (mS/cm) | $Na^+$ (mg/l) | $K^+$ (mg/l) | $Ca^{2+}$ (mg/l) | $Mg^{2+}$ (mg/l) | $Cl^-$ (mg/l) | $HCO_3^-$ (mg/l) |
|---|---|---|---|---|---|---|---|
| 7.3 | 2.0 | 10.61 | 0.1 | 7.2 | 3.2 | 20.3 | 5.0 |

EC.: Electrical conductivity (measured at 25 °C)

**Tab. 3:** Characteristics and dosis of the fertilizer treatments; Compost (*Organova*), F1; Compost combined with humus (*Humivital*), F2 ; and ammonitrate, F3

| Fertilizer | pH | OM(%) | OC (%) | N (%) | C/N ratio |
|---|---|---|---|---|---|
| **ORGANOVA®** | - | 30 | 17.44 | 1.5 | 10 |
| **HIMIVITAL** | 8 | 27 | 15.69 | 3 | 43.5 |
| **Ammonium nitrate** | - | - | - | 33 | - |

| Treatment | Dosis of Treatments (kg/ha) ORGANOVA | HUMIVITAL | Amonium nitrate | Dosis of OM and N (kg/ha) OM | N |
|---|---|---|---|---|---|
| **F1** | 15 | - | - | 4,5 | 0.2 |
| **F2** | 15 | 50 | - | 18 | 1.7 |
| **F3** | **-** | **-** | 50 | **-** | 16.5 |

O.C: Organic carbon; %OM: Organic matter.

single application at the beginning of the study as well as the other treatments. The dosis of compost-humus (F2) *Humivital* was applied in combination with ORGANOVA®, which 2 ml of humus in liquid form was added after applying compost in solid form (10 g per pot). The dosis of ammonium nitrate (F3), 0.15 g per pot, was applied in a single dose.

**Metabolite Extraction and Analysis**

Metabolites were extracted from lyophilized *Aloe vera* whole leaves samples, harvested at the end of the expriment, following the method described by Bozzi et al. (2007). Before extraction, the three biological replicates for each treatment were mixed, then separated into two technical replications and then analysed. 100 mg of ground and freeze-dried *Aloe* powder and 1 ml of filtered water were added and mixed with vortex, then switched to the thermo-centrifuger at 60 °C for 30 min and a frequency of 1500 rpm. Samples were centrifuged at 4200 rpm for 15 min. After the extraction process, samples were subsequently filtered through a 0.45 um nylon membrane. For quality control of the data and to improve the accuracy and precision of quantitative analysis, the samples were injected in duplicate (the two technical replications) with and without internal standard IS (20 µg for 100 ppm) of each one and then freeze-dryed. In our case two IS were used: xylitol and myo-inositol (De la Osa et al., 2022). The resulting extracts were stored at -80 °C until derivatization.

**Gas Chromatography-Mass Spectrometry (GC/MS) protocol Derivatisation**

The derivatisation of the samples was done by methoxilation (methoxilamineMeOx), in addition to 20 mg of pyridine for (1 h, 40 °C) then trimethylsilylated with N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) for 1 h at 40 °C; 90 ul and 240 ul, respectively. According to a modified method described by Cerdán-Calero et al. (2012), the tubes must be cooled with liquid nitrogen.

**Analytical instrument**

Samples were analyzed by GC/MS at the Mass Spectrometry Facilities in CITIUS, University of Sevilla. Analyses were performed using a triple quadrupole mass spectrometer coupled to a gas chromatograph instrument TSQ8000 (ThermoScientific) equipped with ZB-5ms column (30m×0.25 mm). The GC parameters for the analysis of TMS derivatives were: carrier gas, helium at a flow rate (constant mode) of 1.1 mL/min; injector temperature, 230 °C; injection, split mode (1:10); injection volume, 1 µL; oven temperature ramp, initial temperature 70 °C, held for 5 min, then heating at 4 °C/min up to 325 °C. The ionisation potential was 70 eV. The total chromatographic time was 74 min. The fixed MS settings for the analysis of volatile compounds were: transfer line temperature, 300 °C; ion source temperature, 250 °C; scan mode, full with mass range m/z 60–650; ionization mode, electron impact at 70 eV; data file format, XcaliburTM Raw Data File (Cerdán-Calero et al., 2012; De la Osa et al., 2022).

**GC–MS metabolite identification and quantification**

For data processing of raw GC–MS data files, metabolites were identified by Automated Mass Spectral Deconvolution and Identification System (AMDIS) and downloaded to NIST$^{TM}$ website software (http://chemdata.nist.gov/mass-spc/amdis/downloads). Compounds were classified into fatty acids, amino acids, organic acids, sugars, sugar alcohols and sugar acids. Then, semi quantitative concentration of compounds was calculated using the formula below based on the known concentration of internal standard (xylitol and myo-inositol, 0.1 mg.mL$^{-1}$) as outlined in Roessner et al. (2000), Roessner et al. (2006) and Mazlan et al. (2019).

**Semi quantitative concentration = ((Area percent of a replicate / Average area percent of standards) * 0.1mg.ml$^{-1}$)/0.1g DM**

**Statistical analysis**

GraphPad PRISM (version 9.0; GraphPad Software, Inc.) was used to do statistical analysis for metabolites profiling. Analysis of variance (ANOVA) was used to test significant differences in metabolite levels among the four treatments. In the case of a significant effect, the Tukey HSD post hoc test was used to compare pairs of treatments. The tests were performed to reveal pairwise differences, with critical *p-value* threshold set at 0.05.

KEGG and MetaboAnalyst (http://www.metaboanalyst.ca/) were employed for metabolite and pathway annotations, as well as metabolite set enrichment analysis. The data, formatted as a comma-separated values (CSV) file, was uploaded to the MetaboAnalyst 5.0 online platform. To evaluate variations across treatments and metabolomics parameters, both Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) were applied.

Analysis tools within MetaboAnalyst 5.0, including PCA, Variable Importance in Projection (VIP), heatmap clustering, and pathway analysis, were utilized, with *Arabidopsis thaliana* serving as the reference pathway database. PCA was particularly used to distinguish metabolic profiles among different treatments. Prior to statistical analysis, data normalization was performed using sum normalization, log transformation, and auto-scaling.

Metabolites identified through GC/MS were mapped to metabolic pathways using MetaboAnalyst 5.0. Enrichment analysis was performed to pathways that were significantly impacted by each treatment. All MetaboAnalyst analyses were based on three data

points: two technical replicates and a third value representing the average of these replicates. Each metabolomic analysis involved two technical replicates for each of the three biological replicates.

## Results

### Multivariate analysis of differentially expressed metabolites

According to results of quantified metabolites by GC/MS, 61 metabolites were significantly affected following the treatment used. The F1, F2, and F3 treatments induced differentially expressed metabolites compared to F0. Significant effect was observed for the most of metabolites, including FA, AA, CA, sugars, sugar alcohols and sugar acids. A positive correlation was observed, especialy among these metabolites (Fig. 2a).

The PCA analysis revealed that the first two principal components; PC1 and PC2 explained 44.1% and 29.5% of the variance respectively, with a total of 73.6%. The first two principal components accounted for the majority of the variance, separating F3 treatment from F1 and F2 treatments. A clear separation between F3 group and the three treatment groups was detected based on PCA; different samples from F1 and F2 groups clustered together and the control group displayed a distinct pattern. The control group formed a distinct cluster, underscoring the metabolic impact of fertilization. PCA revealed distinct clustering of samples according to treatment, indicating significant metabolic shifts in *Aloe vera* (Fig. 4b). The scores plot of different treatments on the two main components (PC1 and PC2) generated separate groups of which three are homogeneous (control F0, compost-humic acid F2 and nitrogen F3) and one heterogeneous (compost F1).

We find that the first two principal components can explain the relationships between the different variables and treatments studied (Fig. 2c, d). Biplots-PCA and heatmap according to the partial least square discriminant analysis of treatment scores and metabolite loadings allowed us to determine the relationship between treatments and metabolites in terms of correlation and the type of metabolites induced by each treatment.

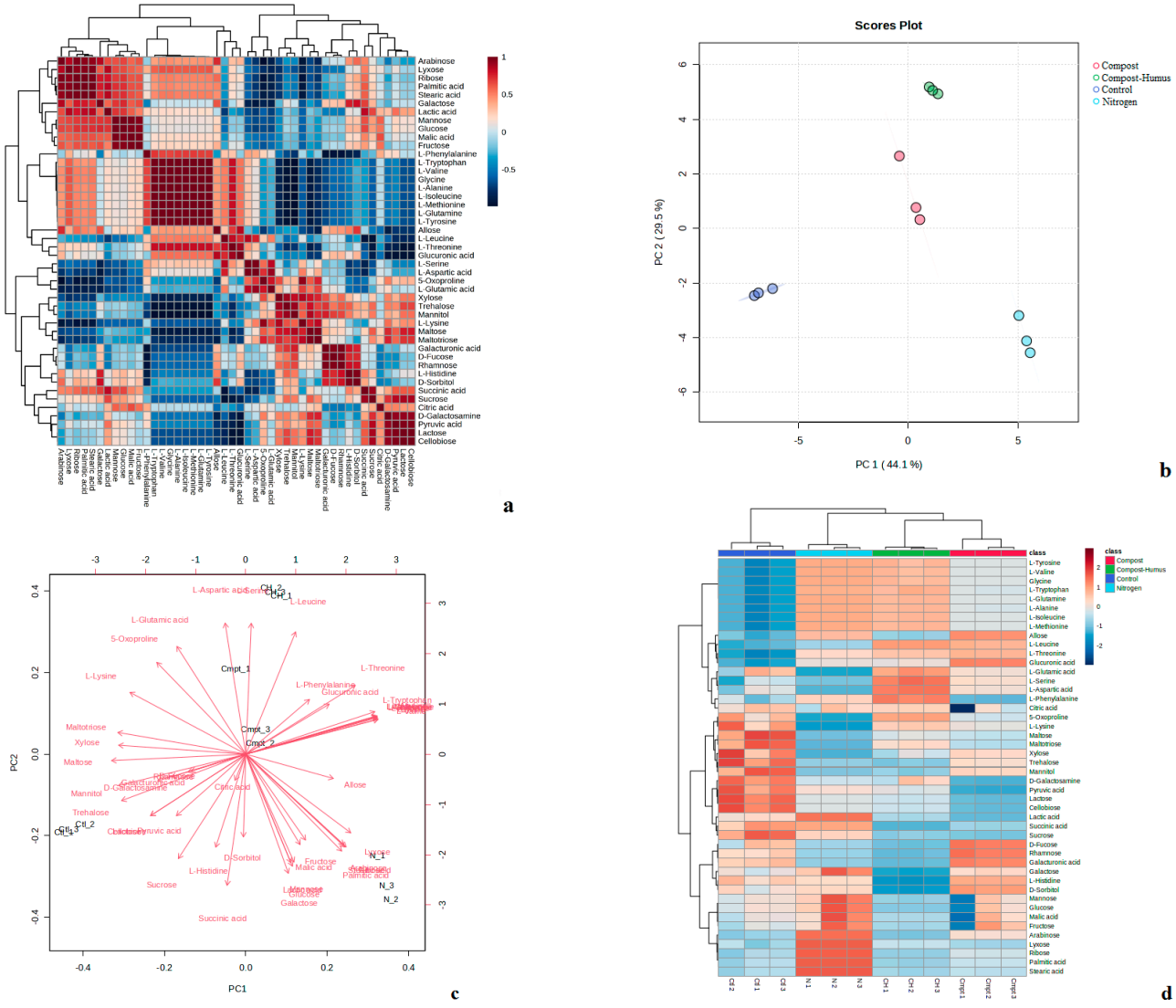


**Fig. 2:** Metabolomic analysis of *Aloe vera* plants treated with different fertilizers using GC/MS approach and analysed using MetaboAnalyst 5.0 online tool. (a) Heatmap of the pearson correlation between all metabolites detected, the colors in the legend on the right represent the significance level of the Pearson correlation between the metabolites of different treatments based on p-value, red is highly significant, blue no significance. (b) Scores plots-PCA of the metabolites detected in all treatments; Ctl, Control (F0); Cmpt, Compost (F1) ; CH, Compost-Humus (F2) ; N, inorganic nitrogen (F3) ; two technical replicates and the mean. (c) Biplots-PCA of scores and loadings, two technical replicates and the mean. (d) Heatmap clustering metabolites according to the partial least square discriminant analysis. Cell colors indicate normalized compound concentrations, with samples in rows, and compounds in columns. The color scale at the right indicates the relative metabolite concentrations with high concentrations in red and low concentrations in blue.

According to (Fig. 4c, d), the control F0 positively induced the following metabolites : D-fucose, rhamnose, galacturonic acid, galactose, L-histidine, D-sorbitol, 5-oxoproline, L-lysine, maltose, maltotriose, xylose, trehalose, mannitol, D-galactosamine, pyruvic acid, lactose, cellobiose, lactic acid, succinic acid, sucrose, mannose, glucose, malic acid, fructose, L-glutamic acid, and citric acid.

F1 treatment positively induced the following metabolites (Fig. 4c, d) : D-fucose, rhamnose, galacturonic acid, galactose, L-histidine, D-sorbitol, L-lysine, xylose, trehalose, mannitol, arabinose, mannose, glucose, malic acid, fructose, allose, L-leucine, L-threonine, glucuronic acid, L-glutamic acid, L-serine, and L-aspartic acid.

F2 treatment positively induced the following metabolites (Fig. 2c, d): 5-oxoproline, L-lysine, maltose, maltotriose, xylose, D-galactosamine, L-tyrosine, L-valine, glycine, L-tryptophan, L-glutamine, L-alanine, L-isoleucine, Lmethionine, L-leucine, L-threonine, L-glutamic acid, L-serine, L-aspartic acid, Lphenylalanine, and citric acid

F3 treatment positively induced the following metabolites (Fig. 2c, d) : galactose, L-histidine, D-sorbitol, pyruvic acid, lactic acid, succinic acid, sucrose, arabinose, lyxose, ribose, palmitic acid, stearic acid, mannose, glucose, malic acid, fructose, L-tyrosine, L-valine, glycine, L-tryptophan, L-glutamine, L-alanine, L-isoleucine, Lmethionine, allose, L-threonine, glucuronic acid, L-phenylalanine, and citric acid.

According to the partial least square discriminant analysis (Fig. 2d), the heatmap showed that F3 treatment highly induced, galactose, lactic acid, arabinose, lyxose, ribose, palmitic acid, stearic acid, mannose, glucose, malic acid, and fructose. F1 treatment strongly induced, D-fucose, rhamnose, galacturonic acid, L-histidine, D-sorbitol, allose, Leucine, L-threonine, and glucuronic acid. F2 treatment highly induced, 5-oxoproline, L-leucine, L-glutamic acid, L-serine, L-aspartic acid, and L-phenylalanine.

**Metabolomic changes with organic and inorganic fertilization**

Based on the VIP index via PLS-DA represented in (Fig. 3), we can see that 25 metabolites were selected as differential variables using VIP values (VIP > 1) that are significantly induced by each fertilizer. The variable with a (VIP > 1) is considered important. F1 treatment induced only an average accumulation of FA (Stearic acid and palmitic acid) and three sugars (Rhamnose, D-fucose and galacturonic acid). However, F1 highly induced the glucuronic acid. F2 treatment induced significantly 4 AA: L-leucine, L-aspartic acid, L-serine, and L-glutamic acid. For F2 treatment, FA were reduced as well as sugars such as ribose, mannose, glucose, rhamnose, and D-fucose and two CA, such as malic acid and galacturonic acid. F3 induced a high accumulation of FA (Stearic acid and palmitic acid), and one sugar which is lyxose. However, F3 induced a reduction of three CA: Lactic acid, succinic acid, malic acid and three sugars (Ribose, mannose, glucose). Finally, F0 induced only sucrose, pyruvic acid, lactose, and cellobiose.

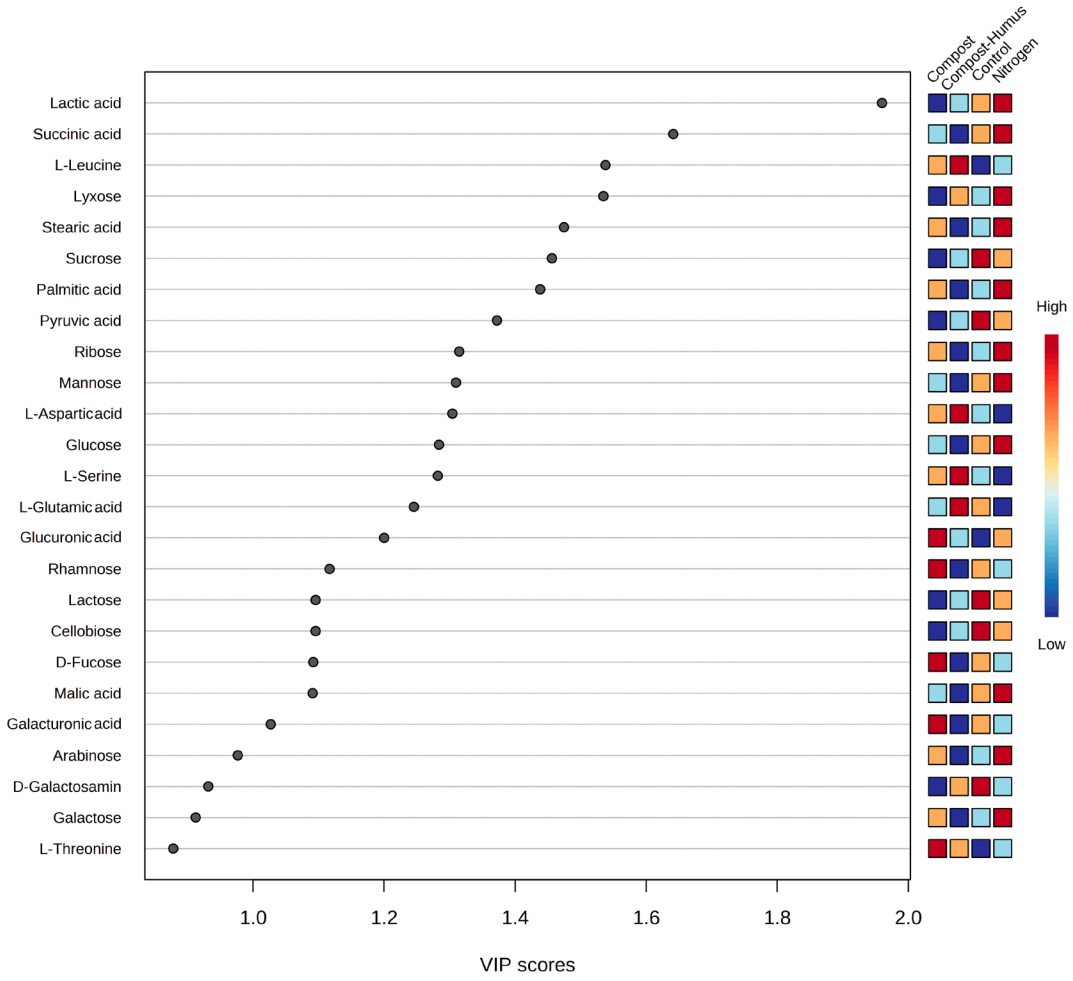


**Fig. 3 :** Metabolomic analysis of *Aloe vera* plants treated with different fertilizers using GC/MS approach and analysed using MetaboAnalyst 5.0 online tool. Key compounds separating the treatements based on variable importance in projection (VIP) in partial least squares discriminant analysis (PLS-DA) analysis. Control (F0); Compost (F1); Compost-Humus (F2); Inorganic Nitrogen (F3).

As shown in (Fig. 4a, b, c and d), the results of ANOVA two-ways showed that organic and inorganic fertilizers significantly affected fatty acid, amino acids and carboxylic acids of *Aloe vera*. F3 treatment led to significant increases in (C16:0) and (C18:0) $p<0.05$ (Fig. 4a), the mean value of C16:0 showed significant increase with 86.3% for F3 treatment (31.38 $\mu$g/g) of dry matter compared to F0 (16.84 µg/g), and the mean value of (C18:0) increased with 92.94 % for F3 treatment (40.89 µg/g) of dry matter compared to F0 (21.2 µg/g). For F1 and F2 treatments, both FA were reduced. C16:0 measured (5.59 and 7.54 µg/g) of dry matter respectively, and C18:0 measured (6.59 and 11.31 µg/g), respectively.

As shown in (Fig. 4b, c), F2 treatment increased significatively the percentage of amino acids $p<0.05$, the mean value of phenylalanine, leucine, serine, and aspartic acid increased with 123.6%, 122.86%, 74.76% and 37.83% respectively, compared to F0. F2 treatment led to increased levels of various amino acids, such as alanine, valine, leucine, isoleucine, glycine, serine, threonine, methionine, phenylalanine, glutamine, tyrosine, and tryptophan. F1 and F3 treatments showed different effects, with either a reduction or absence of amino acids.

For samples treated with F1, only threonine was increased, certain amino acids were reduced compared to F0, including leucine, serine, proline, aspartic acid, glutamic acid, histidine, and lysine. For F1 treatment, some amino acids were not detected, such as alanine, valine, isoleucine, glycine, methionine, phenylalanine, glutamine, tyrosine, and tryptophan. Regarding F3 treatment, none of the amino acids was detected.

As shown in (Fig. 4d), the results indicated that none of investigated CA showed a significant increase in the treatments, compared to F0. Most of them were reduced with the treatments. Malic acid is the most abundant carboxylic acid in the *Aloe vera* samples, followed by citric acid, succinic acid, lactic acid, and pyruvic acid.

As shown in (Fig. 5a, b and c), the results of ANOVA two-ways showed that organic and inorganic fertilizer significantly affected sugars, sugar alcohols and sugar acids of *Aloe vera*. The concentrations of various sugars were reduced in all treatments compared to F0, except lyxose and allose. It can be seen that F3 induced the synthesis of lyxose and allose was induced by F1 (Fig. 5a, b). In addition to the reduction in sugar concentrations, other compounds such as sugar alcohols (mannitol and D-sorbitol, as well as galacturonic acid and D-galactosamine) were found to be decreased in the three treatments compared to F0. However, glucoronic acid was significatively increased by F1 and F2, with (8.8 µg/g and 2.82 µg/g respectively) compared to F0 (Fig. 5c).

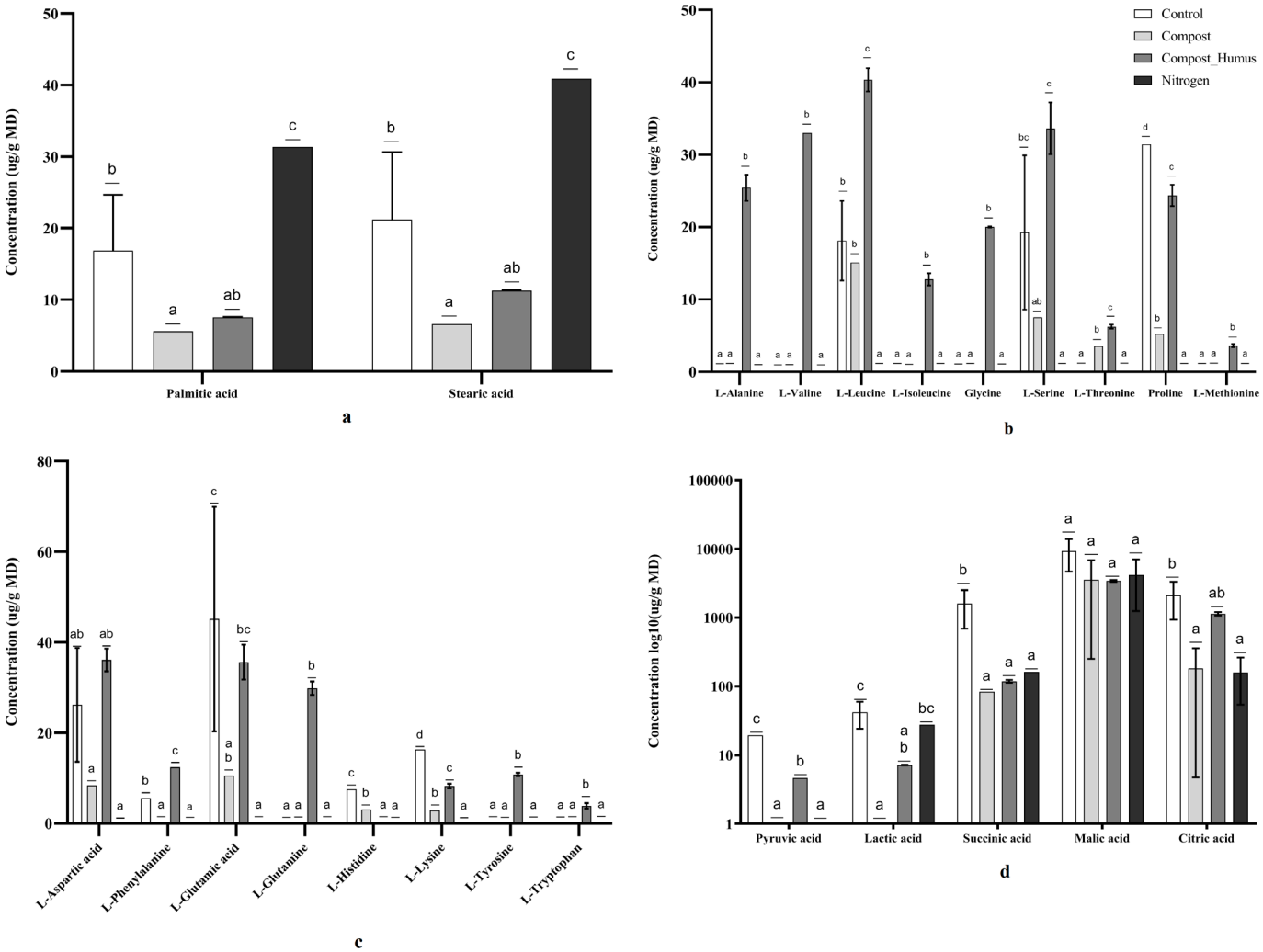


**Fig. 4:** The effect of organic and inorganic fertilizers on fatty acid (a), amino acids (b) and (c), and carboxylic acids (d) of *Aloe vera*. Mean values and standard deviation (± SD) were plotted as analyzed by Tukey test ($P \leq 0.05$); the same lowercase letters indicate no significant differences of the means between treatments.

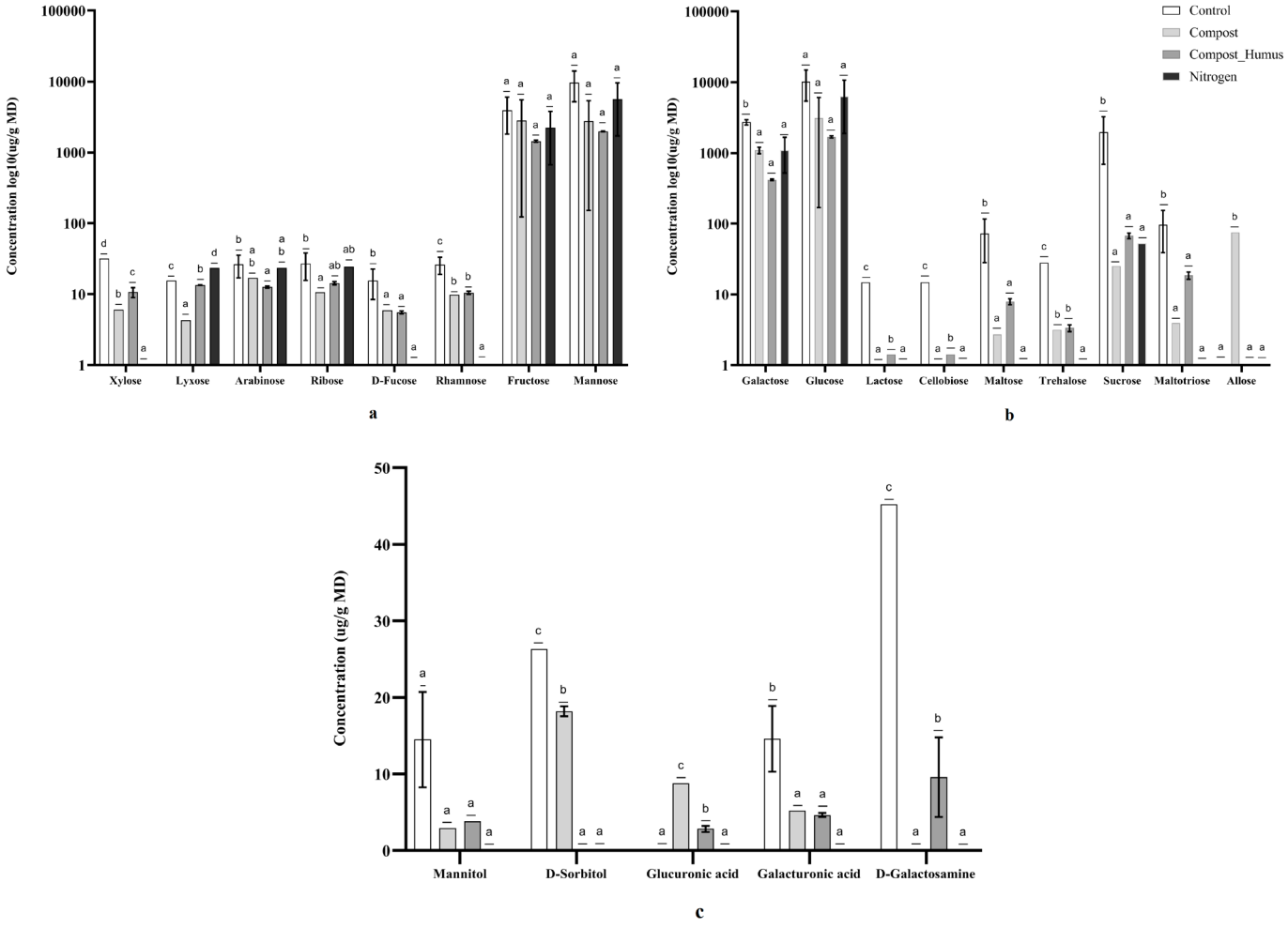


**Fig. 5:** The effect of organic and inorganic fertilizer on sugars (a), (b), sugar alcohols and sugar acids (c) of *Aloe vera*. Mean values and standard deviation (±SD) were plotted as analyzed by Tukey test ($P \leq 0.05$); the same lowercase letters indicate no significant differences of the means between treatments.

**KEGG Classification and Enrichment Analysis**

Metabolic pathways of enrichment analysis using metaboanalyst revealed 54 pathways induced by the treatments. In (Tab. 4), pathways significantly induced by each fertilizer are represented, metabolic pathways (ns) are not significantly affected. F1 treatment enriched 19 pathway compared to F0: 1 (FAp), 6 (AAp), 1 (CAp), 7 sugars, and 4 sugar alcohols and sugar acids. F2 treatment enriched 45 pathways compared to F0: 3 fatty acid pathways (FAp), 19 aminoacids pathways (AAp), 2 carboxylic acids pathways (CA), 7 carbohydrates pathways (Chp), and 14 sugar alcohols ans sugar acids. F3 enriched 33 pathways compared to F0: 7 (FAp), 13 (AAp), 2 (CAp), 5 (Chp), and 6 sugar alcohols and sugar acids pathways.

F1 treatment induced 1 FAp : BM, 6 AAp : AAGM, GSTM, AatRNAB, VLIB, VLID and LysD. 7 ChP : ASNSM, FMM, GM, GlB, PGI, PPP, and SSM. 1 CAp: PyM. 4 other pathways : SM, PrM, IPM, and AAM.

F2 treatment induced 3 FAp : SLM, P-CoAB, and BM. 19 AAp : AAGM, AB, GSTM, C5-BDAM, CAM, AatRNAB, ß-AM, CysMetM, GluM, HM, VLIB, VLID, PhM, PhTyrTryB, LysB, NM, TyrM, TPPAB, and TryM. 7 Chp ASNSM, FMM, GM, GlB, GGg, PGI, and SSM. 2 CAP: TCA cycle, and PyM. 14 other pathways such as, SM, PrM, PuM, PyrM, IPM, PSS, NNM, ThM, TBB, SeM, PhPB, AAM, CFPO, and GDM.

F3 induced 7 FAp: BUFA, FAB, FAD, FAE, SM, BM, and CSWB. 13 AAP: AAGM, APM, AB, GSTM, CAM, AatRNAB, ß-AM, CysMetM, GluM, VLIB, VLID, LysB, and NM. 5 ChP: GM, GlB, GGg, PPP, and SSM. 2 CAP: TCA cycle and PyM. 6 other pathways such as, SM, PrM, NNM, PrChM, CFPO, and GDM.

F2 and F1 showed strong effects on PGI and GSTM, with significant *p-values*. F1, strongly affected ASNSM and F2 on AAGM, P-CoAB, BM, CAM, SM and CFPO. F3 strongly affected pathways such as VLIB, GIB. F2 and F1 showed minimal effects on them.

## Discussion

**Pathways induced by organic and inorganic fertilizers**

Distinct metabolic responses observed across the different treatments highlight the differential impacts of organic and inorganic fertilizers on *Aloe vera*. Metabolites are related to crop yield and have been used to predict plant phenotypes and quality (Zarei et al., 2018; Zhao et al., 2023). Primary and secondary metabolites are the intermediate or ultimate products of complex networks of biochemical pathways involved in plant metabolism (Sung et al., 2015). These networks are highly interconnected, influencing various aspects of nutrient metabolism through complex interactions (Sung et al., 2015). Pathways induced by the organic and inorganic fertilizers are shown in (Tab. 4), certain pathways were activated by a single treatment are specifically induced, and pathways that were highly induced are shown in (Fig. 6).

**Tab. 4:** Metabolomic pathways induced by F1, F2, and F3 on *Aloe vera* plants.

| | | | Control vs compost humus (45) | | Control vs compost (19) | | Control vs Nitrogen (33) | |
|---|---|---|---|---|---|---|---|---|
| | Metabolomic pathways | Total Cmpd | Hits | Raw p | Hits | Raw p | Hits | Raw p |
| Lipid pathway (8) | Biosynthesis of unsaturated fatty acids (BUFA) | 22 | 2 | NS | 2 | NS | 2 | 0.00011652 |
| | Fatty acid biosynthesis (FAB) | 56 | 2 | NS | 2 | NS | 2 | 0.00011652 |
| | Fatty acid degradation (FAD) | 37 | 1 | NS | 1 | NS | 1 | 0.00014443 |
| | Fatty acid elongation (FE) | 23 | 1 | NS | 1 | NS | 1 | 0.00014443 |
| | Sphingolipid metabolism (SLM) | 17 | 1 | 0.0049436 | 1 | NS | 1 | 0.015944 |
| | Pantothenate and CoA biosynthesis (P-CoAB) | 23 | 2 | 1.32E-05 | 1 | NS | 1 | NS |
| | Butanoate metabolism (BM) | 17 | 3 | 3.45E-05 | 3 | 0.0027461 | 3 | 0.018468 |
| | Cutin, suberine and wax biosynthesis (CSWB) | 18 | 1 | NS | 1 | NS | 1 | 0.00014443 |
| Amino acid pathway (21) | Alanine, aspartate and glutamate metabolism (AAGM) | 22 | 6 | 4.92E-05 | 4 | 0.0019366 | 4 | 0.015024 |
| | Arginine and proline metabolism (APM) | 34 | 1 | NS | 1 | NS | 1 | 0.015498 |
| | Arginine biosynthesis (AB) | 18 | 3 | 1.03E-05 | 2 | NS | 2 | 0.012311 |
| | Glycine, serine and threonine metabolism (GSTM) | 33 | 6 | 1.24E-05 | 4 | 1.47E-05 | 3 | 0.0098564 |
| | C5-Branched dibasic acid metabolism (C5-BDAM) | 6 | 1 | 0.0054268 | 1 | NS | 1 | NS |
| | Cyanoamino acid metabolism (CAM) | 29 | 4 | 8.9419E-06 | 3 | NS | 3 | 0.0098713 |
| | Aminoacyl-tRNA biosynthesis (AatRNAB) | 46 | 16 | 0.000069787 | 8 | 0.00001136 | 7 | 0.0064037 |
| | Beta-Alanine metabolism (ß-AM) | 18 | 1 | 0.003511 | 1 | NS | 1 | 0.00876 |
| | Cysteine and methionine metabolism (CysMetM) | 46 | 4 | 4.8811E-06 | 3 | NS | 3 | 0.0098564 |
| | Glutathione metabolism (GluM) | 26 | 6 | 0.00016899 | 2 | NS | 2 | 0.010374 |
| | Histidine metabolism (HisM) | 15 | 1 | 0.0027706 | 1 | NS | 1 | NS |
| | Valine, leucine and isoleucine biosynthesis (VLIB) | 22 | 5 | 0.000057436 | 3 | 0.000024212 | 2 | 9.8663E-06 |
| | Valine, leucine and isoleucine degradation (VLID) | 37 | 3 | 0.00010201 | 1 | 0.0001649 | 1 | 0.00014676 |
| | Phenylalanine metabolism (PhM) | 11 | 1 | 0.0015808 | 1 | NS | 1 | NS |
| | Phenylalanine, tyrosine and tryptophan biosynthesis* | 22 | 3 | 0.0002852 | 1 | NS | 1 | NS |
| | Lysine biosynthesis (LysB) | 9 | 2 | 0.0072668 | 1 | NS | 2 | 0.004136 |
| | Lysine degradation (LysD) | 18 | 1 | NS | 1 | 0.046141 | 1 | NS |
| | Nitrogen metabolism (NM) | 12 | 2 | 0.000092417 | 1 | NS | 1 | 0.015498 |
| | Tyrosine metabolism (TyrM) | 16 | 2 | 1.65E-05 | 1 | NS | 1 | NS |
| | Tropane, piperidine and pyridine alkaloid biosynthesis** | 8 | 1 | 0.0015808 | 1 | NS | 1 | NS |
| | Tryptophan metabolism (TryM) | 28 | 1 | 0.00022497 | NI | NI | NI | NI |
| Sugars pathway (8) | Amino sugar and nucleotide sugar metabolism (ASNSM) | 50 | 3 | 0.00010769 | 3 | 2.17E-05 | 2 | NS |
| | Fructose and mannose metabolism (FMM) | 20 | 1 | 0.0027703 | 1 | 0.0064096 | 1 | NS |
| | Galactose metabolism (GM) | 27 | 4 | 3.73E-05 | 4 | 0.0013721 | 4 | 0.0057745 |
| | Glucosinolate biosynthesis (GlB) | 65 | 6 | 0.00017683 | 2 | 0.00021361 | 2 | 9.23E-06 |
| | Glycolysis / Gluconeogenesis (GGg) | 26 | 3 | 3.12E-05 | 3 | NS | 3 | 0.038293 |
| | Pentose and glucuronate interconversions (PGI) | 16 | 2 | 4.98E-05 | 2 | 3.45E-05 | 1 | NS |
| | Pentose phosphate pathway (PPP) | 19 | 1 | NS | 1 | 0.03976 | 1 | 0.00030091 |
| | Starch and sucrose metabolism (SSM) | 22 | 2 | 7.08E-05 | 2 | 0.00035884 | 2 | 0.002469 |
| CA.P | Citrate cycle (TCA cycle) | 20 | 3 | 0.00011272 | 3 | NS | 3 | 0.046204 |
| | Pyruvate metabolism (PyM) | 22 | 2 | 0.000014647 | 2 | 0.0014602 | 2 | 0.0019571 |
| Others pathway (15) | Sulfur metabolism (SM) | 15 | 2 | 1.42E-05 | 2 | 0.0009074 | 2 | 0.022331 |
| | Propanoate metabolism (PrM) | 20 | 1 | 0.00083592 | 1 | 0.0031612 | 1 | 0.032809 |
| | Purine metabolism (PuM) | 63 | 1 | 0.00021199 | NI | NI | NI | NI |
| | Pyrimidine metabolism (PyrM) | 38 | 1 | 0.00021199 | NI | NI | NI | NI |
| | Inositol phosphate metabolism (IPM) | 28 | 2 | 0.00027973 | 2 | 0.00031358 | 1 | NS |
| | Phosphatidylinositol signaling system (PSS) | 26 | 1 | 0.030146 | 1 | NS | 1 | NS |
| | Nicotinate and nicotinamide metabolism (NNM) | 13 | 1 | 0.003511 | 1 | NS | 1 | 0.00876 |
| | Thiamine metabolism (ThM) | 22 | 2 | 0.000013399 | 1 | NS | 1 | NS |
| | Porphyrin and chlorophyll metabolism (PrChM) | 48 | 1 | NS | 1 | NS | 1 | 0.015498 |
| | Terpenoid backbone biosynthesis (TBB) | 30 | 1 | 0.0054268 | 1 | NS | 1 | NS |
| | Selenocompound metabolism (SeM) | 13 | 1 | 0.00020595 | NI | NI | NI | NI |
| | Phenylpropanoid biosynthesis (PhPB) | 46 | 1 | 0.0015808 | 1 | NS | 1 | NS |
| | Ascorbate and aldarate metabolism (AAM) | 18 | 2 | 0.00027973 | 2 | 0.00031358 | 1 | NS |
| | Carbon fixation in photosynthetic organisms (CFPO) | 21 | 3 | 1.2228E-06 | 2 | NS | 2 | 0.0041352 |
| | Glyoxylate and dicarboxylate metabolism (GDM) | 29 | 6 | 0.000070126 | 4 | NS | 4 | 0.025121 |

*(PhTyrTryB) ; **(TPPAB)

**The compost (F1) treatment**

Metabolomic changes indicated that F1 treatment induced an average accumulation of FA and positively three sugars, compared to control F0. For F1 treatment, most of amino acids were reduced except for threonine, and significant increase was observed for sugars especially for allose and glucuronic acid. F1 treatment specificly induced lysine degradation. According to (Fig. 6), F1 highly induced the following pathways: Aminoacyl-tRNA biosynthesis, Glycine, serine and threonine metabolism, Pentose and glucuronate interconversions, Valine, leucine and isoleucine biosynthesis, Valine, leucine and isoleucine degradation, Ascorbate and aldarate metabolism, Inositol phosphate metabolism, Glycolysis/Gluconeogenesis, Pyruvate metabolism, Starch and sucrose metabolism, Propanoate metabolism, Butanoate metabolismn. Sugar are essential for plant development and affects every stage of the life cycle of the plant. It controls growth and development of stressed plants, by interacting with the plant hormones. Lower sugar accumulation can lead to weak root system, while higher concentration promotes drought tolerance and behave as a signal molecule in root growth of rice (SAIA et al., 2019; GHORBANZADEH et al., 2023). The highest concentration of vermicompost tea altered the production and accumulation of terpenoids, phenolic compounds, fatty acids, and alkanes, with a negative correlation observed between vermicompost levels and fatty acid accumulation (SOUFFRONT et al., 2022). Vermicompost tea application improved growth parameters in plant tissues by improving physical structures and influenced secondary metabolite production. Increased concentrations of monoterpenes, diterpenes and hydrocarbons from epicuticular waxes could explain enhanced pest resistance (SOUFFRONT et al., 2022).

Organic fertilizers, such as compost, provide several benefits when applied alone or in combination with synthetic chemical fertilizers or other organic amendments (ROTHÉ et al., 2019). It was reported that organic fertilizers, when applied once at planting, exhibit significant potential compared to mineral fertilizers applied at regular intervals (SADDHE et al., 2021; GHORBANZADEH et al., 2023).

**The compost-humus (F2) treatment**

F2 treatment increased amino acids in *Aloe vera* leaves with very low *p-values*. This fertilizer showed the highest induction of amino acids especially phenylalanine, leucine, aspartic acid, serine, and glutamic acid. Significant increases in essential amino acids such as

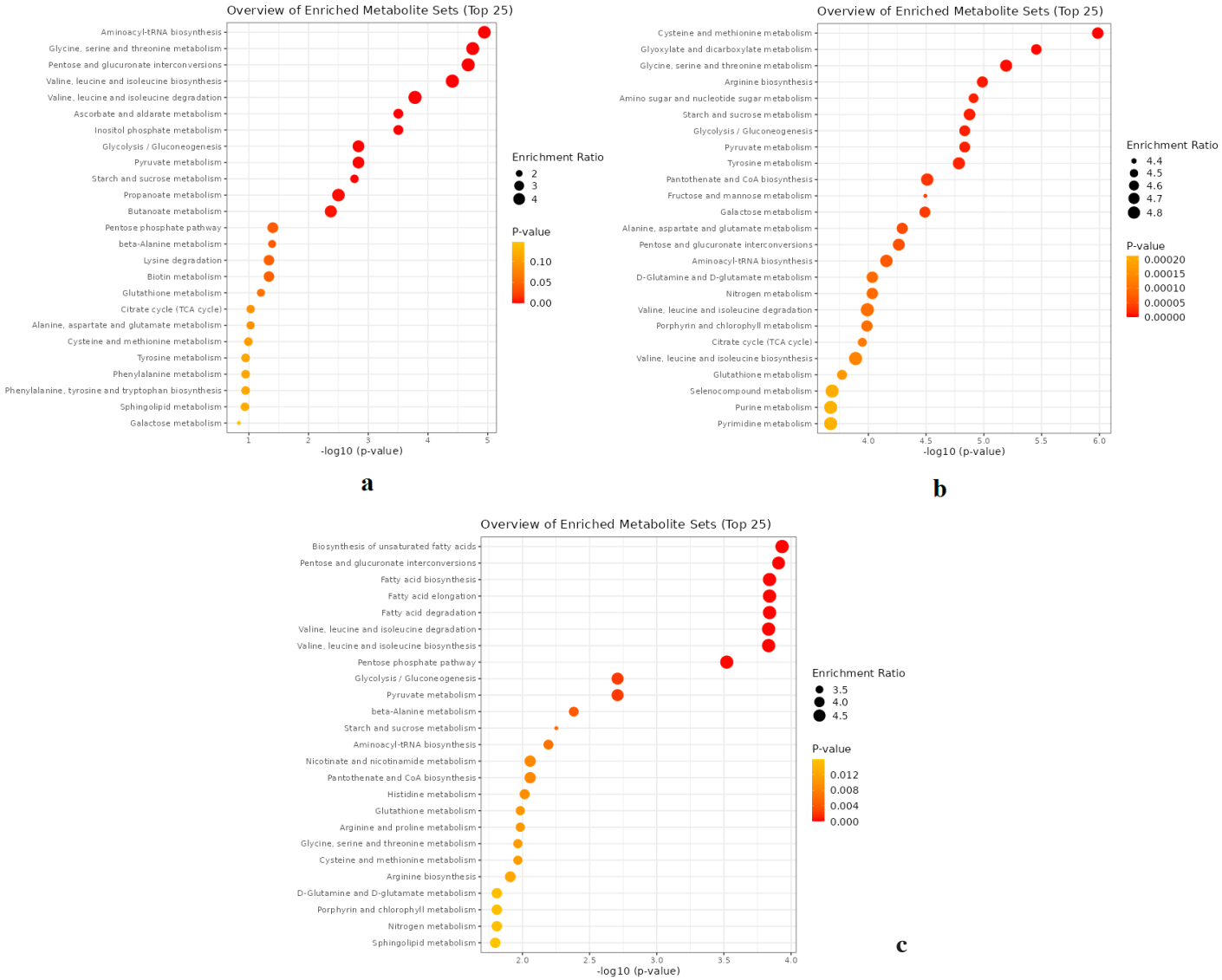


**Fig. 6 :** Overview of enriched metabolites sets (top 25). Metabolic enrichment pathway analysis in three comparative groups, of metabolomic analysis of *Aloe vera* plants; compared with the treatments F0, F1 (a), F2 (b) and F3 (c) using GC–MS approach and analysed using MetaboAnalyst 5.0 online tool.

phenylalanine and leucine were observed. F2 specifically induced, P-CoAB; C5-BDAM; HisM; PhM; PhTyrTryB; TyrM; TPPAB; Trym; PuM; PyrM; PSS; ThM; TBB; SeM; and PhPB. According to (Fig. 6), F2 highly induced: Cysteine and methionine metabolism, Glyoxylate and dicarboxylate metabolism, Glycine, serine and threonine metabolism, Arginine biosynthesis, Amino sugar and nucleotide sugar metabolism, Starch and sucrose metabolism, Glycolysis/ Gluconeogenesis, Pyruvate metabolism, and Tyrosine metabolism. A similar increase in amino acids content was reported in leaves of pak choi (*Brassica rapa*) and maize (*Zea mays*) plants in response to compost application. Frequent application of municipal solid waste compost highly enhanced total amino acids in lettuce, beets and carrot (Vinci et al., 2018; Abbey et al., 2021). The increase in amino acids by frequent municipal solid waste compost application is important for the improvement of biological processes and functions in plants (Yao et al., 2020; Abbey et al., 2021). Amino acids are key N reserves for biological carriers involved in internal transport networks between plant organs (Abbey et al., 2021). Amino acid and protein synthesis may increase carbohydrate production for plant growth (Bentley et al., 2022). Organic fertilizers in solid and liquid form can be used successfully in plant growth in organic agriculture (Tavali and Ok, 2022). Compost improves soil fertility by enriching soil microbial communities, amino acids and soil nutrients. Soil enrichment properties may contribute to the high amino acids content in the plants (Bustamante et al., 2019; Abbey et al., 2021). However, Tavali and Ok (2022) suggested that vermicompost applications combined with liquid form are more effective than applications in solid form alone.

**The nitrogen (F3) treatment**

Metabolic changes induced by F3 resulted in a high accumulation of FA. However, F3 reduced CA, AA and sugars except lyxose. F3 treatment led to significant increase in C16:0 and C18:0, and induced specifically pathways such as BUFA, FAB, FAD, FE, CSWB, APM, and PrChM, with very low *p-values*, indicating a signifant effect on the synthesis and degradation of fatty acids in *Aloe vera*. According to (Fig. 6), F3 highly induced; Biosynthesis of unsaturated fatty acids; Pentose and glucuronate interconversions; Fatty acid biosynthesis; Fatty acid elongation; Fatty acid degradation; Valine, leucine and isoleucine degradation; Valine, leucine and isoleucine biosynthesis; Pentose phosphate pathway; Glycolysis / Gluconeogenesis; Pyruvate metabolism.

Fatty acids are major components of cell membranes, and they also function as precursor molecule to various plant metabolites, including signaling compounds, phytoalexins and cuticular waxes (Souffront et al., 2022). C16 and C18 fatty acids contribute to cuticular wax formation (He et al., 2016). Alterations to epicuticular wax production could further enhance pest resistance. Waxy cuticles serve as the primary plant defense, providing a physical barrier to protect against herbivory (Souffront et al., 2022).

Nitrogen, as a key nutrient, directly or indirectly influences different aspects of plant metabolism, growth, and development, significantly impacting crop quality and yield, as observed in wheat (Zhu et al., 2005; Zhao et al., 2023). Research has indicated that the relative contents of lysine and phenylalanine increase in wheat grains following nitrogen application. Amino acid metabolism, including lysine, tryptophan, tyrosine, phenylalanine, arginine, and proline, is enhanced in nitrogen-fertilized brown rice. These amino acids are associated with flavonoid biosynthesis and are derived from intermediates of the TCA cycle. (Zhen et al., 2016; Desta et al., 2022; Ma et al., 2022). Amino acids significantly decrease when N is limiting (Sung et al., 2015). Glutamate and aspartate levels were recognized as the best indicators of the stresses related to N limitations. N-deficiency tolerance altered metabolites indicated that most of the amino acid content decreased in plant leaves, whereas most phenylpropanoids and organic acids increased (Zhao et al., 2023).

## Conclusions

Fertilization had a significant impact on metabolite profiles, with differentially expressed metabolites influenced by organic and inorganic fertilizers. Nitrogen fertilization enhanced lipid biosynthesis, while compost-humus promoted amino acid metabolism, and compost influenced sugar metabolism. FA levels increased with inorganic nitrogen but decreased with organic fertilizers. AA content was elevated with compost-humus, but only threonine increased with compost. CA, sugars, sugar alcohols, and sugar acids generally decreased under treatment, except for allose and lyxose, which were enhanced by compost and inorganic nitrogen, respectively. Both organic fertilizers positively induced glucuronic acid. Our findings suggest that humic acid namely *Humivital,* supported AA synthesis, while compost promoted glucuronic acid production. However, inorganic nitrogen stimulated lipid metabolism but reduced AA levels, possibly due to nitrogen limitation. These findings highlight the importance of selecting appropriate fertilizers to optimize *Aloe vera* cultivation, with potential applications in improving plant health and productivity.

## References

Abbey, L., Ofoe, R., Gunupuru, L.R., Ijenyo, M., 2021: Variation in frequency of CQA-tested municipal solid waste compost can alter metabolites in vegetables. Food Res. Int. 143, 110225. DOI: 10.1016/j.foodres.2021.110225

Ahmed, M., Rauf, M., Mukhtar, Z., Saeed, N.A., 2017: Excessive use of nitrogenous fertilizers: an unawareness causing serious threats to environment and human health. Environ. Sci. Poll. Res. 24(35), 26983-26987. DOI: 10.1007/s11356-017-0589-7

Amallah, L., Hassikou, R., Boukour, B., Douira, A., Skalli, S., 2024: Promising medicinal plants as a starting point for clinical research into therapies for COVID-19: A literature review. Ethnobot. Res. Appl. 28, 1-30. DOI: 10.32859/era.28.47.1-30

Anjum, S., Bazai, Z.A., Benincasa, C., Rizwan, S., Sajjad, A., 2022: Elemental composition of medicinal plants under changing environmental and edaphic conditions. In: Mahmood, Q. (eds.), Sustainable Plant Nutrition under Contaminated Environments, 135-161. DOI: 10.1007/978-3-030-91499-8_8

Barandozi, F.N., Enferadi, S.T., Naghavi, M.R., Hassani, M.E., Mostofi, Y., Mousavi, A., 2011: Effects of fertilizer on morphological traits in *Aloe vera*. J. Med. Plant. Res. 5(18), 4537-4541.

Bentley, J., Liebrich, P.Y., Farrant, J.M., Mandishonha, M., Reddy, A., Rafudeen, M.S., 2022: Metabolomic analysis of the roots and shoots of tomato seedlings treated with the commercial seaweed-derived biostimulant Afrikelp. S. Afr. J. Bot. 147, 646-651. DOI: 10.1016/j.sajb.2022.02.040

Boukour, B., Florido, M.C., Lama-Muñoz, A., Amallah, L., Bouazza, F., Sehlaoui, H., Douaik, A., El Mekkaoui, A., El Faiz, C., Hassikou, R., 2024: Effect of site conditions and fertilization treatments on morphological traits and mineral content of *Aloe vera* plants. J. Appl. Bot. Food Qual. 97, 36-44. DOI: 10.5073/JABFQ.2024.097.005

Bozzi, A., Perrin, C., Austin, S., Vera, F.A., 2007: Quality and authenticity of commercial aloe vera gel powders. Food Chem. 103(1), 22-30. DOI: 10.1016/j.foodchem.2006.05.061

Bustamante, M.A., Nogués, I., Jones, S., Allison, G.G., 2019: The effect of anaerobic digestate derived composts on the metabolite composition and thermal behaviour of rosemary. Scient. Reports 9(1), 1-15. DOI: 10.1038/s41598-019- 42725-6

Cerdán-Calero, M., Sendra, J.M., Sentandreu, E., 2012: Gas chromato-

graphy coupled to mass spectrometry analysis of volatiles, sugars, organic acids and aminoacids in Valencia Late orange juice and reliability of the Automated Mass Spectral Deconvolution and Identification System for their automatic identification and quantification. J. Chromatogr. A. 1241, 84-95. DOI: 10.1016/j.chroma.2012.04.014

Chowdhury, T., Chowdhury, M.A.H., Qingyue, W., Enyoh, C.E., Wang, W., Khan, M.S.I., 2021: Nutrient uptake and pharmaceutical compounds of *Aloe vera* as influenced by integration of inorganic fertilizer and poultry manure in soil. Heliyon. 7(7). DOI: 10.1016/j.heliyon.2021.e07464

Cristiano, G., Murillo-Amador, B., De Lucia, B., 2016: Propagation techniques and agronomic requirements for the cultivation of *Barbados Aloe (Aloe vera* (L.) Burm. f.) A review. Front. Plant Sci. 7. DOI: 10.3389/fpls.2016.01410

De la Osa, C., Pérez-López, J., Feria, A.B., Baena, G., Marino, D., Coleto, I., Monreal, J.A., 2022: Knock-down of phosphoenolpyruvate carboxylase 3 negatively impacts growth, productivity, and responses to salt stress in sorghum (*Sorghum bicolor* L.). Plant J. 111(1), 231-249. DOI: 10.1111/tpj.15789

Deng, B., Li, Y., Xu, D., Ye, Q., Liu, G., 2019: Nitrogen availability alters flavonoid accumulation in *Cyclocarya paliurus* via the effects on the internal carbon/nitrogen balance. Sci. Rep. 9(1), 2370. DOI: 10.1038/s41598-019-38837-8

Desta, K.T., Hur, O.S., Lee, S., Yoon, H., Shin, M.-J., Yi, J., Lee, Y., Ro, N.Y., Wang, X., Choi, Y.-M., 2022: Origin and seed coat color differently affect the concentrations of metabolites and antioxidant activities in soybean (*Glycine max* (L.) Merrill) seeds. Food Chem. 381, 132249. DOI: 10.1016/j.foodchem.2022.132249

Ding, E.L., Hutfless, S.M., Ding, X., Girotra, S., 2006: Chocolate and prevention of cardiovascular disease: a systematic review. Nutr. Metab. 3(1), 1-12. DOI: 10.1186/1743-7075-3-2

Eshun, K., He, Q., 2004: *Aloe vera*: a valuable ingredient for the food, pharmaceutical and cosmetic industries—a review. Crit. Rev. Food Sci. Nutr. 44(2), 91-96. DOI: 10.1080/10408690490424694

Ghorbanzadeh, Z., Hamid, R., Jacob, F., Zeinalabedini, M., Salekdeh, G.H., Ghaffari, M.R., 2023: Comparative metabolomics of root-tips reveals distinct metabolic pathways conferring drought tolerance in contrasting genotypes of rice. BMC Genomics 24(1), 152. DOI: 10.1186/s12864-023-09246-z

Hamman, J.H., 2008: Composition and applications of *Aloe vera* leaf gel. Mol. 13(8), 1599-1616. DOI: 10.3390/molecules13081599

Ijaz, N., Durrani, A.I., Rubab, S., Bahadur, S., 2022: Formulation and characterization of *Aloe vera* gel and tomato powder containing cream. Acta Ecol. Sin. 42(2), 34-42. DOI: 10.1016/j.chnaes.2021.01.005

Javed, S., 2014: *Aloe vera* gel in food, health products, and cosmetics industry. Stud. Nat. Prod. Chem. 41, 261-285. DOI: 10.1016/B978-0-444-63294-4.00009-7

Khaldoune, K., Fdil, N., AitAli, M., 2024: Exploring *Aloe vera*: A comprehensive review on extraction, chemical composition, biological effects, and its utilization in the synthesis of metallic nanoparticles. Biocatal. Agric. Biotechnol. 103052. DOI: 10.1016/j.bcab.2024.103052

Kilmer, V.J., Alexander, L.T., 1949: Methods of making mechanical analyses of soils. Soil Sci. 68(1), 15-24.

Kováčik, J., Klejdus, B., 2014: Induction of phenolic metabolites and physiological changes in chamomile plants in relation to nitrogen nutrition. Food Chem. 142, 334-341. DOI: 10.1016/j.foodchem.2013.07.074

Kumar, S., Yadav, M., Yadav, A., Yadav, J.P., 2017: Impact of spatial and climatic conditions on phytochemical diversity and in vitro antioxidant activity of Indian *Aloe vera* (L.) Burm. f. S. Afr. J. Bot. 111, 50-59. DOI: 10.1016/j.sajb.2017.03.012

Lai, S.H, Chye, M.L., 2021: Plant acyl-CoA-binding proteins their lipid and protein interactors in abiotic and biotic stresses. Cells. 10(5), 1064. DOI: 10.3390/cells10051064

Lee, S., Do, S.G., Kim, S.Y., Kim, J., Jin, Y., Lee, C.H., 2012: Mass spectrometry-based metabolite profiling and antioxidant activity of *Aloe vera* (*Aloe barbadensis* Miller) in different growth stages. J. Agric. Food Chem. 60(45), 11222-11228. DOI: 10.1021/jf3026309

Li, J.P., Zhang, Z., Yao, C.S., Liu, Y., Wang, Z.M., Fang, B.T., Zhang, Y.H., 2021: Improving winter wheat grain yield and water-/nitrogen-use efficiency by optimizing the micro-sprinkling irrigation amount and nitrogen application rate. J. Integr. Agric. 20, 606-621. DOI: 10.1016/S2095-3119(20)63407-4

Li, Y., Dexin Konga, D., Michael, Y.F., HongWu, R.S., 2020: The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80-89. DOI: 10.1016/j.plaphy.2020.01.006

Liontakis, A., Tzouramani, I., 2016: Economic sustainability of organic *aloe vera* farming in Greece under risk and uncertainty. Sustainability. 8(4), 338. DOI: 10.3390/su8040338

Ma, Y., Zhang, S., Wu, Z., Sun, W., 2022: Metabolic Variations in Brown Rice Fertilised with Different Levels of Nitrogen. Foods 2022, 11(21), 3539. DOI: 10.3390/foods11213539

Marzanna, H., Dziedzic, K., 2019: *Aloe vera* (L) webb. Natural Sources of Antioxidants-A Review. Plant Foods Hum. Nutr. 74, 255-265. DOI: 10.1007/s11130-019-00747-5

Mazlan, O., Aizat, W.M., Zuddin, N.S.A., Baharum, S.N., Noor, N.M., 2019: Metabolite profiling of mangosteen seed germination highlights metabolic changes related to carbon utilization and seed protection. Sci. Hortic. 243, 226-234. DOI: 10.1016/j.scienta.2018.08.022

Rhoades, J.D., Manteghi, N.A., Shouse, P.J., Alves, W.J., 1989: Estimating soil salinity from saturated soil-paste electrical conductivity. Soil Sci. Soc. Am. J. 53, 428-433. DOI: 10.2136/sssaj1989.03615995005300020019x

Roessner, U., Patterson, J.H., Forbes, M.G., Fincher, G.B., Langridge, P., Bacic, A., 2006: An investigation of boron toxicity in barley using metabolomics. Plant Physiol. 142, 1087-1101. DOI: 10.1104/pp.106.084053

Roessner, U., Wagner, C., Kopka, J., Trethewey, R.N., Willmitzer, L., 2000: Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry. Plant J. 23, 131-142. DOI: 10.1046/j.1365-313x.2000.00774.x

Rothé, M., Darnaudery, M., Thuriès, L., 2019: Organic fertilizers, green manures and mixtures of the two revealed their potential as substitutes for inorganic fertilizers used in pineapple cropping. Sci. Hortic. 257, 108691. DOI: 10.1016/j.scienta.2019.108691

Saddhe, A.A., Manuka, R., Penna, S., 2021: Plant sugars: Homeostasis and transport under abiotic stress in plants. Physiol. Plant. 171(4), 739-755. DOI: 10.1111/ppl.13283

Saha, R., Palit, S., Ghosh, B., Mittra, B., 2005: Performance of *Aloe vera* as influenced by organic and inorganic sources of fertilizer supplied through fertigation. Acta. Hortic. (676), 171-175. DOI: 10.17660/ActaHortic.2005.676.22

Saia, S., Fragasso, M., De Vita, P., Beleggia, R., 2019: Metabolomics provides valuable insight for the study of durum wheat: a review. J. Agric. Food. Chem. 67(11), 3069-3085. DOI: 10.1021/acs.jafc.8b07097

Souffront, D.K.S., Salazar-Amoretti, D., Jayachandran, K., 2022: Influence of vermicompost tea on secondary metabolite production in tomato crop. Sci. Hortic. 301, 111135. DOI: 10.1016/j.scienta.2022.111135

Sung, J., Lee, S., Lee, Y., Ha, S., Song, B., Kim, T., Krishnan, H.B., 2015: Metabolomic profiling from leaves and roots of tomato (*Solanum lycopersicum* L.) plants grown under nitrogen, phosphorus or potassium-deficient condition. Plant Science 241, 55-64. DOI: 10.1016/j.plantsci.2015.09.027

Tavali, I.E., Ok, H., 2022: Comparison of heat-treated and unheated vermicompost on biological properties of calcareous soil and *Aloe vera* growth under greenhouse conditions in a Mediterranean climate. Agronomy. 12(11), 2649. DOI: 10.3390/agronomy12112649

Urbanczyk-Wochniak, E., Fernie, A.R., 2005: Metabolic profiling reveals altered nitrogen nutrient regimes have diverse effects on the metabolism of hydroponically-grown tomato (*Solanum lycopersicum*) plants. J. Exp. Bot. 56(410), 309-321.

Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Spaccini, R., Piccolo, A., 2018: An alternative to mineral phosphorus fertilizers: The combined effects of *Trichoderma harzianum* and compost on *Zea mays*, as revealed by 1H NMR and GC-MS metabolomics. PLoS ONE, 13(12). DOI: 10.1371/journal.pone.0209664

Xun, Z., Guo, X., Li, Y., Wen, X., Wang, C., Wang, Y., 2020: Quantitative proteomics analysis of tomato growth inhibition by ammonium nitrogen. Plant. Physiol. Biochem. 154, 129-141. DOI: 10.1016/j.plaphy.2020.05.036

Yao, X., Nie, J., Bai, R., Sui, X., 2020: Amino Acid Transporters in Plants: Identification and Function. Plants. 9(8), 972. DOI: 10.3390/plants9080972

Zarei, I., Luna, E., Leach, J.E., McClung, A., Vilchez, S., Koita, O., Ryan, E.P., 2018: Comparative rice bran metabolomics across diverse cultivars and functional rice gene-bran metabolite relationships. Metabolites. 8, 63. DOI: 10.3390/metabo8040063

Zhao, F., Wang, Y., Hu, J., Shi, S., Zhang, H., Wang, Y., Ye, Y., 2023: Metabolite profiling of wheat response to cultivar improvement and nitrogen fertilizer. Metabolites 13(1), 107. DOI: 10.3390/metabo13010107

Zhen, S., Zhou, J., Deng, X., Zhu, G., Cao, H., Wang, Z., Yan, Y., 2016. Metabolite profiling of the response to high-nitrogen fertilizer during grain development of bread wheat (*Triticum aestivum* L.). J. Cereal Sci. 69, 85-94. DOI: 10.1016/j.jcs.2016.02.014

Zhu, X.K., Guo, W.S., Zhou, Z.Q., Feng, C.N., Peng, Y.X., Lin, Q.H., 2005: Effects of nitrogen on N uptake, grain yield and quality of medium-gluten wheat Yangmai 10. Agric. Sci. China 4, 421-428. DOI: 10.1016/j.eja.2023.126919

ORCID
Basma Boukour https://orcid.org/0000-0001-8204-4135
Farid Rachidi https://orcid.org/0000-0002-7047-6691
María C. Florido https://orcid.org/0000-0001-7613-8565
Maria E. Soria Diaz https://orcid.org/0000-0003-1755-6382
Rocio Valderrama https://orcid.org/0009-0003-2684-6637
Lamiae Amallah https://orcid.org/0000-0002-9121-0577
Souad Skalli https://orcid.org/0000-0003-3357-4572
Ahmed Douaik https://orcid.org/0000-0001-7374-4674
Rachida Hassikou https://orcid.org/0000-0002-6025-3371

Address of the corresponding author:
Basma Boukour, Laboratory of Botany and Valorization of Plant and Fungal Resources, Faculty of Sciences of Rabat. Mohammed V University of Rabat. Morocco
E-mail: basma_boukour@um5.ac.ma