Dataset on the Effects of Anti-Insect Nets of Different Porosity on Mineral and Organic Acids Profile of *Cucurbita pepo* L. Fruits and Leaves

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**Abstract:** The growing interest in healthy foods has driven the agricultural sector towards eco-friendly implementation to manage biotic and abiotic factors in protected environments. In this perspective, anti-insect nets are an effective tool for controlling harmful insect populations concomitantly with reducing chemicals’ interference. However, the low porosity of nets necessary to ensure high exclusion efficiency for a designated insect leads to reduced airflow, impacting the productivity and quality attributes of vegetables. The evidence presented in this dataset pertains to the content of total nitrogen, minerals (i.e., NO\(_3\), K, PO\(_4\), SO\(_4\), Ca, Mg, Cl, and Na), and organic acids (i.e., malate and citrate) of zucchini squash (*Cucurbita pepo* L. cv. Zufolo F1) in leaves and fruits grown with two anti-insect nets with different porosities (Biorete® 50 mesh and Biorete® 50 mesh AirPlus), is and analyzed by the Kjeldahl method and ion chromatography (ICS3000), respectively. Data of total nitrogen concentration, macronutrients, and organic acids provide in-depth information about plants’ physiological response to microclimate changes induced by anti-insect nets.

**Dataset:** [https://zenodo.org/record/4749122#.YJqTLrUzbZR](https://zenodo.org/record/4749122#.YJqTLrUzbZR)

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**Keywords:** zucchini squash; insect-proof screens; protected environment; ion chromatography

1. **Summary**

Agriculture supplies more than half of the food for feeding the world’s rapidly growing population, but less than 50% of the total production is harvested (i.e., yield potential) due to abiotic and biotic factors such as photothermal stress, pathogenic fungi and insects [1–6]. Guaranteeing ideal growing conditions is mandatory, especially in warm Mediterranean areas where the constraints imposed by climate change are causing severe yield and quality losses [1]. Growers and consumer awareness for environmentally sustainable agricultural systems drive the agricultural sector to implement agronomic strategies relying on integrated pest management (IPM). In the past, farmers have used chemical pesticides indiscriminately for pest control with detrimental impacts on the ecosystem [7]. However, the severe limitations on the use of chemical insecticides and the increasing consumer demand for organic and pesticide-free vegetables have pushed growers towards ecologically and economically sustainable alternatives [8]. In this framework, anti-insect nets represent an effective and “green” solution for the containment of harmful insects in protected environments, achieving 90% effectiveness in excluding designated harmful pests [9]. On the other hand, their exclusion performance depends on the weft and warp thread arrangement and, consequently, on the holes’ geometry and structure; hence, this performance decreases when the holes’ size increases [10,11]. However, low porosity...
(percentage of the ratio of open mesh area to total mesh area) causes a high-pressure drop, which reduces airflow with a consequent increase in temperature in the growing environment, which is detrimental for crop growth [4,12,13]. It is well-known that heat stress induces molecular, biochemical, and morphological changes in plants as an adaptive response to adverse conditions [14,15]. Several studies have highlighted the critical role of Ca and K in stress signaling and the regulation of growth and developmental processes [16]. High temperatures affect the photosynthetic process (primary metabolism) [17,18], which is strictly related to leaf macronutrient concentrations such as nitrogen (N), which is the main constituent of proteins involved in the C3 cycle (Calvin–Benson), magnesium (Mg), sulfur (S), and phosphorus (P) [19]. The latter plays a crucial role in cellular processes, stabilizing cell membranes, contributing to the synthesis of energy molecules such as ATP and ADF and nucleic acids [20]. On the other hand, the high consumer demand for fresh vegetables all year round has encouraged growers to expand their production potential in protected environments. In the last decades, zucchini squash (Cucurbita pepo L.) has gained popularity in the European horticultural markets and has become one of the most demanded and consumed vegetables. In the Italian horticultural market, a consumption of about 9 kg per capita is estimated [21], with a greenhouse production of more than 200 tons per year [22]. At present, research has focused mainly on the exclusion efficiency of anti-insect nets, ignoring the impact induced on the inner microclimate of growing environments and thus on the production and quality of Cucurbitaceae. This data descriptor reports a dataset acquired about total nitrogen and ion chromatographic analysis on leaves and fruits of Cucurbita pepo L. to integrate our previous study aimed at assessing the effects of the microclimate induced by two anti-insect nets with different porosities (Biorete® 50 mesh and Biorete® 50 mesh AirPlus; Arrigoni S.P.A, Uggiate Trevano, Como, Italy) on the qualitative–quantitative performance of zucchini squash [1]. Our goal was to investigate how the suboptimal microclimate induced by two anti-insect nets could affect the mineral profile of zucchini leaves and fruits, as well as the content of malate and citrate, which are organic acids crucial for the taste and flavor of food and represent important phytochemicals for biological processes [23].

2. Data Description

The data release is stored on Zenodo (https://zenodo.org/record/4749122#.YJqTLrUzbZR, accessed on 12 May 2021). The dataset has two spreadsheets named “leaves” and “fruits”, each corresponding to zucchini leaves and fruits, respectively. Both worksheets have the same data layout, distributed in thirteen columns (from letters A to M) and ten rows. Specifically, the first column (A) indicates the used treatments: “No-net” (i.e., control without anti-insect net; from A2 to A4 columns); “50 mesh AP” (i.e., treatment with Biorete® 50 mesh AirPlus; from A5 to A7 columns); “50 mesh”, (i.e., treatment with Biorete® 50 mesh; from A8 to A10 columns). The second column (B) reports the number of replicates for each treatment. The third column (C) reports the total nitrogen content determined by the Kjeldahl method, expressed as g kg⁻¹ dw. From column D to column M, the concentrations of NO₃, K, PO₄, SO₄, Ca, Mg, Cl, Na, malate, and citrate, respectively, were determined by ion chromatography and expressed as g kg⁻¹ dw. The data from our dataset were subjected to statistical analysis and are reported in Tables 1 and 2.
Table 1. Total nitrogen, minerals, and organic acids of zucchini squash leaves grown in a protected environment with anti-insect nets. All data are expressed as the mean ± standard error, \( n = 3 \).

| Treatments | Total N (g kg\(^{-1}\) dw) | \(\text{NO}_3\) (g kg\(^{-1}\) dw) | K (g kg\(^{-1}\) dw) | \(\text{PO}_4\) (g kg\(^{-1}\) dw) | \(\text{SO}_4\) (g kg\(^{-1}\) dw) | Ca (g kg\(^{-1}\) dw) | Mg (g kg\(^{-1}\) dw) | Cl (g kg\(^{-1}\) dw) | Na (g kg\(^{-1}\) dw) | Malate (g kg\(^{-1}\) dw) | Citrate (g kg\(^{-1}\) dw) |
|------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| No-net     | 22.40 ± 0.39 \(^a\)         | 0.12 ± 0.01                 | 31.68 ± 1.12                 | 2.82 ± 0.43 \(^b\)         | 4.49 ± 0.30 \(^a\)         | 17.50 ± 0.67 \(^a\)         | 4.84 ± 0.32 \(^a\)         | 15.53 ± 0.53 \(^a\)         | 0.27 ± 0.03 \(^c\)         | 21.30 ± 0.99 \(^b\)        | 12.31 ± 1.03 \(^a\)       |
| 50 mesh AP | 17.45 ± 0.52 \(^c\)         | 0.21 ± 0.04                 | 29.71 ± 1.71                 | 9.72 ± 0.51 \(^a\)         | 1.74 ± 0.14 \(^b\)         | 14.96 ± 0.53 \(^b\)         | 5.12 ± 0.09 \(^a\)         | 4.33 ± 0.19 \(^b\)         | 1.84 ± 0.08 \(^a\)         | 31.70 ± 1.21 \(^a\)        | 9.12 ± 0.32 \(^b\)        |
| 50 mesh    | 19.93 ± 0.91 \(^b\)         | 0.23 ± 0.04                 | 27.07 ± 0.65                 | 9.66 ± 0.76 \(^a\)         | 2.62 ± 0.31 \(^b\)         | 10.47 ± 0.50 \(^c\)         | 4.11 ± 0.05 \(^b\)         | 2.91 ± 0.28 \(^c\)         | 1.20 ± 0.04 \(^b\)         | 24.41 ± 1.45 \(^b\)        | 8.18 ± 0.85 \(^b\)        |
| Significance | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         |

Significance ** ns ns ns ns *** ns ** ns *** ns

ns, *, **, *** non-significant or significant at \( p \leq 0.05, 0.01, \) and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan’s multiple-range test \( (p = 0.05) \).

Table 2. Total nitrogen, minerals, and organic acids of zucchini squash fruits grown in a protected environment with anti-insect nets. All data are expressed as the mean ± standard error, \( n = 3 \).

| Treatments | Total N (g kg\(^{-1}\) dw) | \(\text{NO}_3\) (g kg\(^{-1}\) dw) | K (g kg\(^{-1}\) dw) | \(\text{PO}_4\) (g kg\(^{-1}\) dw) | \(\text{SO}_4\) (g kg\(^{-1}\) dw) | Ca (g kg\(^{-1}\) dw) | Mg (g kg\(^{-1}\) dw) | Cl (g kg\(^{-1}\) dw) | Na (g kg\(^{-1}\) dw) | Malate (g kg\(^{-1}\) dw) | Citrate (g kg\(^{-1}\) dw) |
|------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| No-net     | 36.04 ± 0.94 \(^b\)         | 0.20 ± 0.16                 | 44.12 ± 0.14                 | 18.79 ± 0.70                 | 2.51 ± 0.29                 | 4.54 ± 0.14 \(^a\)         | 4.14 ± 0.28                 | 8.69 ± 0.74 \(^a\)         | 2.87 ± 0.16                 | 25.52 ± 1.22 \(^a\)        | 2.43 ± 0.32                 |
| 50 mesh AP | 35.65 ± 1.08 \(^b\)         | 0.19 ± 0.12                 | 41.63 ± 0.91                 | 18.32 ± 1.12                 | 2.40 ± 0.12                 | 2.94 ± 0.21 \(^b\)         | 4.34 ± 0.14                 | 6.43 ± 0.27 \(^b\)         | 3.18 ± 0.17                 | 21.21 ± 0.16 \(^b\)        | 3.19 ± 0.18                 |
| 50 mesh    | 39.71 ± 0.61 \(^a\)         | 0.21 ± 0.05                 | 41.36 ± 1.39                 | 18.62 ± 2.08                 | 2.84 ± 0.46                 | 2.07 ± 0.04 \(^c\)         | 3.61 ± 0.20                 | 5.75 ± 0.10 \(^b\)         | 2.81 ± 0.20                 | 15.37 ± 1.19 \(^c\)        | 2.23 ± 0.34                 |
| Significance | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         | ns                         |

ns, *, **, *** non-significant or significant at \( p \leq 0.05, 0.01, \) and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan’s multiple-range test \( (p = 0.05) \).
3. Methods

3.1. Experimental Design and Plant Samples Collection

The experiment was conducted in 2019 at “Torre Lama” experimental farm of the University of Naples (Bellizzi, Salerno, Italy; latitude 43°31’ N, longitude 14°58’ E, altitude 60 m). Experimental treatments consisted of two 50-mesh anti-insect nets, with different porosity and air permeability (Biorete® 50 mesh and Biorete® 50 mesh AirPlus; Arrigoni S.p.A, Uggiate Trevano, Italy) that covered the sidewalls and ventilation openings of the two tunnels, while the third tunnel was used as an unscreened control. Zucchini seedlings (Cucurbita pepo L. cv. Zufolo F1; Olter, Piacenza, Italy) were transplanted on 1 April 2019, in three single rows with a density of 1 plant m⁻². The experimental trial lasted until 17 July 2019. Marketable fruits (minimum length of 12 cm) were harvested three times a week starting 60 days after transplant (DAT).

At 102 DAT, 30 marketable fruits per treatment were harvested, cut in half, and placed in a ventilated oven at 80 °C until a constant weight was reached (~5 days). The specific time for fruit harvesting (102 DAT) was chosen because the production of zucchini squash fruit was more uniform and representative in all the growing tunnels (screened and unscreened), as also supported by the literature [24,25]. At the end of the experiment (17 July 2019, 107 DAT), 20 fully expanded leaves per plot were harvested and placed in a ventilated oven at 70 °C for 3 days. The dry plant material (leaves and fruits) was ground in a MF10.1 Wiley Laboratory mill, IKA® (Staufen im Breisgau, Baden-Württemberg, Germany) and sieved with an MF0.5 sieve (0.5 mm hole size; IKA®, Staufen im Breisgau, Baden-Württemberg, Germany) for total nitrogen, minerals, and organic acid determination.

3.2. Total Nitrogen Determination

Total nitrogen content in zucchini squash leaves and fruits was determined according to the Kjeldahl method with minor modifications [26].

In detail, one gram of ground dry sample was weighed and mixed in a 250 mL borosilicate glass tube (Ø42 × 300 mm; Velp® Scientifica, Usmate Velate, Monza Brianza, Italy) with 7 mL of 96% sulfuric acid (H₂SO₄; Carlo Erba Reagents Srl., Milan, Italy), and antifoam catalyst (3.5 g K₂SO₄ + 0.1 g CuSO₄ × 5H₂O; Velp® Scientifica, Usmate Velate, Monza Brianza, Italy) and 10 mL of 30% hydrogen peroxide (Carlo Erba Reagents Srl., Milan, Italy). The tubes were placed on a heating digester (DK 20 Heating Digester; Velp® Scientifica, Usmate Velate, Monza Brianza, Italy) for 30 min at 420 °C. As a result of the digestion phase, ammonium sulphate [(NH₄)₂SO₄] was produced:

\[
\text{analyte + H}_2\text{SO}_4 \rightarrow (\text{NH}_4)_2\text{SO}_4 + \text{CO}_2 + \text{SO}_2 + \text{H}_2\text{O} \tag{1}
\]

After the digestion phase, the tubes with mineralized samples were cooled and then distilled in a UDK 140 distiller (Velp® Scientifica, Usmate Velate, Monza Brianza, Italy) by adding 33% sodium hydroxide (NaOH; Titolchimica, Pontecchio Polesine, Italy) (distillation phase). Under these conditions, the ammonium ion was transformed into ammonia:

\[
(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} \rightarrow \text{Na}_2\text{SO}_4 + 2\text{H}_2\text{O} + 2\text{NH}_3 \tag{2}
\]

Ammonia was trapped in boric acid (H₃BO₃; Honeywell Riedel-de haén, Charlotte, NC, USA) by steam distillation and collected in an Erlenmeyer flask:

\[
\text{NH}_3 + \text{H}_3\text{BO}_3 \rightarrow \text{NH}_4\text{H}_2\text{BO}_3 + \text{H}_3\text{BO}_3 \tag{3}
\]

A methyl red (C₁₅H₁₅N₃O₂) and bromocresol green (C₀₁H₁₄Br₁O₅S) indicator (HACH, Loveland, CO, USA) were added to the distilled solution and titrated with 0.1 N sulfuric acid (Carlo Erba Reagents Srl., Milan, Italy) until the acquisition of reddish color by the solution (pH around 5.0). The titration volume was used to calculate the percentage of total nitrogen that was converted to g of nitrogen per kg of dry weight (dw). Each treatment was analyzed in triplicate.
3.3. Minerals and Organic Acid Determination

In accordance with Rouphael et al. [27], and in order to determine cations (K, Ca, Mg, Na), anions (NO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$, Cl), and organic acids (malate and citrate), 250 mg of finely ground dried leaves and fruits were weighed on a PI-314.1 analytical balance (Denver Instruments, Denver, CO, USA), then placed in centrifuge tubes (Corning®), New York, NY, USA) and mixed with 50 mL of ultra-pure water prepared through an Arium® Advance EDI pure water system (Sartorius, Goettingen, Lower Saxony, Germany). The samples were frozen and thawed in liquid nitrogen three times and immersed in a SW22 shaking water bath (80 °C, 100 rpm, 10 min; Julabo, Seelbach, Baden-Württemberg, Germany) and then centrifuged with an R-10M centrifuge (6000 rpm, 10 min; Remi Elektrotechnik Ltd., Mumbai, Maharashtra, India). The supernatant was filtered with a specific syringe filter (0.45 µm pore size; Whatman International Ltd., Maidstone, Kent, UK), and processed by ion chromatography coupled to an electrical conductivity detector (ICS3000, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), using a sample injection volume of 25 µL. Isocratic separation of cations (K, Ca, Mg, Na) was performed using a 4 × 250 mm analytical column (IonPac CS12A, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) and an electrolytically self-regenerating suppressor (CERS500; 4 mm, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA). The eluent consisted of 25 mM methanesulfonic acid (Sigma Aldrich, Milan, Italy), prepared with ultrapure water. The separation of anions (NO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$, Cl) and organic acids (malate and citrate) were performed in gradient mode using an IonPac® ATC-HC anion trap (9 × 75 mm; Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), an IonPac® AG11-HC guard column (4 × 50 mm; Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), an IonPac® AG11-HC IC column (4 × 50 mm; Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), and a self-regenerating dynamic suppressor (DRS600; 4 mm, Thermo Scientific™ Dionex™, Sunnyvale, CA, USA) using 5 mM–30 mM potassium hydroxide (KOH) with a flow of 1.5 mL min$^{-1}$. All columns were kept at 30 °C. Integration and quantification of minerals and organic acids were performed using z Chromelion™ 6.8 Chromatography Data System (CDS) Software (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA), by comparing the peak areas of samples with those of the standards. Multistandard solutions (anionic and cationic) were prepared as combinations of individual Ion Chromatography certificate standard solutions (Thermo Scientific™ Dionex™, Sunnyvale, CA, USA). Concentrations of anions, cations, and organic acids in leaves and fruits were expressed as g kg$^{-1}$ dw. Each treatment was analyzed in triplicate.

3.4. Statistic

The evidence presented in this dataset was analyzed with IBM SPSS Statistics (SPSS Inc., Chicago, IL, USA) software version 26.0 for Windows 10 (Microsoft Corporation, Redmond, Washington, USA). All data are presented as the mean ± standard error, n = 3. The mean effects of total nitrogen, minerals, and organic acids were subjected to One-way ANalysis Of VAriance (ANOVA). Statistical significance was determined with Duncan’s Multiple Range Test (DRMT) at $p < 0.05$ level.

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