Effects of pH and Phosphorus Concentrations on the Chlorophyll Responses of *Salvia chamelaeagnea* (Lamiaceae) Grown in Hydroponics

Kerwin Lefever, Charles P. Laubscher, Patrick A. Ndakidemi and Felix Nchu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67610

**Abstract**

*Salvia chamelaeagnea* (Lamiaceae) is a slow growing water-wise evergreen shrub originating from the western province of South Africa. It is an attractive landscape, and *S. chamelaeagnea* is a medicinal plant. It is important to develop enhanced cultivation protocols that could result in high yield and high-quality medicinal materials. Chlorophyll is a fundamental part of the light-dependent reactions of the photosynthesis process. This chapter investigates the effects of four phosphorus concentrations and three pH levels of supplied irrigated water on the production of chlorophyll A, chlorophyll B, total chlorophyll, leaf colour and the nutrient uptake of *S. chamelaeagnea* grown in hydroponics over an 8-week period at the Cape Peninsula University of Technology. The treatments of pH 4, pH 6 and pH 8 at 31, 90, 150 and 210 ppm of phosphorus were received by 12 groups of plants and were replicated 10 times. The results indicated that at pH 4, P fertilization significantly (*P* < 0.05) induced a higher chlorophyll production of *S. chamelaeagnea* grown in hydroponics compared to other pH treatments (pH 8 and pH 6).

**Keywords:** hydroponics, pH, chlorophyll production, medicinal plants, *Salvia chamelaeagnea*

**1. Introduction**

*Salvia chamelaeagnea* P.J. is a member of the Lamiaceae family. Plant species in this family include many culinary and medicinal herbs like *Salvia officinalis*, *Salvia verbenacea* and *Salvia libanotica*, which have been used for many years against diarrhoea, indigestion, colic, abdominal trouble, influenza, bacterial infections, tuberculosis, cough, cold and many other
ailments [1–3]. Some of these uses date back to medieval times [4]. Many of the Lamiaceae secondary metabolites are of commercial interest to the food industry as sources of natural preservatives, flavourants and antioxidants [2, 5], as well as to the pharmaceutical industry as sources of antioxidants, anti-inflammatories [6], antibacterials and anti-mycobacterials [7].

Salvias are renowned for their variety and their many uses around the home and garden; they have beautiful flowers and attract birds [8]. In its natural habitat, S. chamelaegnea will develop into attractive foliage and flowering landscape plants, with small mid-green egg-shaped leaves and masses of bright blue or white flowers borne at the tops of each stem, which are suitable for the cut flower trade [8–10]. S. chamelaegnea also has value in the medicinal plant trade as it contains the phenolic compounds carnosol, rosmarinic acid and caffeic acid, which exhibits antioxidant and anti-bacterial activities [2, 6, 11].

Unfortunately, very little information has been documented on the cultivation of this species. Cultivation of medicinal plants is gaining traction worldwide; it is seen as a tool for biodiversity conservation, poverty alleviation and cultural preservation [12]. However, good knowledge of plant physiology must be attained in order to develop enhanced cultivation protocols that could result in high yield and high-quality medicinal materials. Effects of nutrients and nutrient ratios on many food and medicinal crop plants, such as soya bean, thyme, wheat cultivars, barley, spinach and pelargoniums, have been studied. In most cases, a positive result in growth is noticed with the addition of some macro-nutrients such as N, P, K, Mg or Ca [13–21]. It is therefore crucial that adequate plant nutrition and soil pH levels are met for any given plant so that the cell’s functioning is not impeded. Chlorophyll is a fundamental part of the light-dependent reactions of the photosynthesis process, capturing light rays from the sun and producing energy-storing ATP molecules that are essential for the functioning of a healthy plant [22, 23]. The effects of poor nutrition, be it through infertile soils or incorrect soil pH level, directly affect the production of chlorophyll molecules resulting in chlorosis of leaves and a reduced photosynthetic rate, thus inhibiting some biological processes and decreasing the general health of the plants [23–25]. There are plausible mechanisms through which the production of chlorophyll could be affected, for example, the pH level of a growing medium affects the uptake of P [26] and the P level influences the nutrient uptake by plants [27]. The relationship between the nutrient P and chlorophyll is not fully understood. According to Nicholls and Dillon [28], there are substantial variations of the published phosphorus-chlorophyll relationship, which they ascribed to variations in sampling and analytical techniques.

This chapter aims to investigate the effects of P and pH on the chlorophyll production, leaf colour and the nutrient uptake of medicinal S. chamelaegnea in hydroponics, in order to determine a fertilizer regime that will promote the development of S. chamelaegnea without degrading soils and leaching nutrients into the water table.

2. Materials and methods

2.1. Experimental process

The experiment took place in the research glasshouse at the Cape Peninsula University of Technology (CPUT), Cape Town campus, South Africa, latitude and longitude S33°55′ 58
E18°25′ 57, from June 2012 to August 2012. Inside the glasshouse was a 40%‐Aluminet shade cloth, raised 2 m above the floor, resulting in light intensities ranging from 10 to 13 Klx, determined by using a Toptronic T630 light meter. The climate was controlled between 16 and 28°C during the day while 10–20°C during the night, with an average relative humidity of 42%.

The experiment was laid out in a randomized block design with plants being spaced 30 cm apart and consisted of 12 treatments of four differing nutrient solutions offering a low concentration of P, a balanced concentration of supplementary P, a moderate concentration of supplementary P and a high concentration of supplementary P at three differing pH levels. The control treatment of 31 ppm was chosen due to the nature of fynbos soils being low in available P [29–31].

Hoagland solution, a well-known hydroponic nutrient solution modified by Hershey [32, 33], offering all the necessary macro- and micro-nutrients for healthy plant growth, was used as a base nutrient and supplemented with P.

The plants for the experiment were rooted tip cuttings sourced from healthy mother stock plants at the CPUT Glass House Nursery. The rooted cuttings were gently rinsed in deionized water to remove any rooting media from the root's zone. They were then weighed and planted into 25-cm plastic pots filled with leca clay and placed into a recirculating closed hydroponics system at a spacing of 30 cm, where their heights were recorded (Figure 1).

Figure 1. S. chamelaegnea rooted cuttings exposed to varied combinations of pH and P treatments in hydroponics under greenhouse conditions (Picture: K. Lefever).
The plants were irrigated with the treatments 15 times per day at equal timed intervals for the duration of the experiment. For each treatment, there were 10 plants. The treatments were as follows:

1. Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 4.
   - Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 6.
   - Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 8.

2. Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 4.
   - Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 6.
   - Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 8.

3. Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 4.
   - Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 6.
   - Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 8.

4. Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 4.
   - Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 6.
   - Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 8.

2.2. pH level

The pH levels of the nutrient solutions were monitored using a Martini Instrument PH55 pH probe and were adjusted accordingly using either hydrochloric acid (HCl) to lower the pH or sodium hydroxide (NaOH) to raise the pH.

2.3. Irrigation

The treatments were set to irrigate 15 times daily for a duration of 15 min using a 1350 L/h Boyu submersible pump and a Tedelex analogue timer to regulate irrigation frequencies.

2.4. Data collection

2.4.1. Measurement of leaf colour

Green leaf colour intensity was measured using a hand-held, dual-wavelength SPAD meter (SPAD 502, chlorophyll meter, Minolta Camera Co., Ltd., Japan). Readings were taken from the top three fully developed leaves of each plant. For each treatment, 30 fully developed leaves were used weekly. The SPAD meter stored and automatically averaged the recordings to generate one reading per plant.
2.4.2. Measurement of chlorophyll content in leaves

The extraction of leaf chlorophyll using dimethylsulphoxide (DMSO) was carried out as described in Hiscox and Israelsta [34]. A third of plant leaves from the tip were collected from each plant. About 100 mg of the middle portion of the fresh leaf slices was placed in a 15-mL vial containing 7 mL DMSO and incubated at 4°C for 72 h. After the incubation, the extract was diluted to 10 mL with DMSO. A 3-mL sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E) was used to determine absorbance values at 645 and 663 nm, which were then used in the equation proposed by Arnon [35] to determine the total leaf chlorophyll content against DMSO blank, expressed as mg L⁻¹ as follows: Chl \( a = 12.7D663 - 2.69D645 \), Chl \( b = 22.9D645 - 4.68D663 \) and Total Chl = 20.2D645 + 8.02D663.

2.4.3. Measurement of the levels of macro- and micro-nutrients in dry plant material

The measurements of macronutrients (N, P, K, Ca and Mg) and micronutrients (Cu, Zn, Mn, Fe and B) were determined by ashing a 1 g ground sample in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and putting it in an oven at 50°C for 30 min; 35 mL of deionized water was added, and the extract was filtered through Whatman no. 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA) [36].

2.5. Statistical analysis

Data collected was analysed for statistical significance using the two-way analysis of variance (ANOVA), with the computations being done using the software program STATISTICA. Fisher’s least significance difference (LSD) was used to compare treatment means at \( P \leq 0.05 \) level of significance [37].

3. Results

3.1. Effects of pH and phosphorus concentrations on the chlorophyll content of \textit{S. chamelaeagnea} grown in hydroponics

Treatment significantly \( (P \leq 0.001) \) affected the chlorophyll A, chlorophyll B and total chlorophyll contents of \textit{S. chamelaeagnea} grown hydroponically (Table 1). The chlorophyll A (10.9–12.2), chlorophyll B (3–3.4) and total chlorophyll (13.9–14.7) values of the plants exposed to phosphorus at pH 4 treatments were significantly \( (P \leq 0.001) \) higher compared to the corresponding values at pH 6 (chlorophyll A [8.3–10.3], chlorophyll B [2.2–2.8] and total chlorophyll [10.7–13.4]) and at pH 8—chlorophyll A [3.5–10.17], chlorophyll B [0.91–2.7] and total chlorophyll [4.4–12.9]) treatments (Table 1). Leaf chlorosis of plants grown at pH 8 was observed.
3.2. Effects of pH and phosphorus concentrations on the leaf colour of \( S. \) chamelaeagnea grown in hydroponics

Effects of various P treatments at differed pH levels induced varied colour intensities, ranging from 16 to 31.7 from week 1 to week 8 on the leaf colour of \( S. \) chamelaeagnea \((P \leq 0.001)\) (Table 2, Figure 2). While treatment 1 offering a pH level of 4 at 31 ppm P generally yielded the highest leaf colour values over the 8-week growth period, these values did not differ significantly from that of the other pH 4 treatments receiving supplementary P. Of these treatments receiving supplementary P, the highest results were recorded at pH 4 receiving 210 ppm P closely followed by pH 4 at 90 ppm P and pH 4 at 150 ppm P treatments, respectively.

3.3. Effects of pH and phosphorus concentrations on the uptake of macro-nutrients in \( S. \) chamelaeagnea grown in hydroponics

Macro-nutrient uptake of P, K and Mg was significantly \((P \leq 0.001)\) affected by the treatment (Table 3). There was a noticeable higher tissue P content \((1.07 \pm 0.08\%)\) at pH 8, 150 ppm of P (Table 3). Tissue nitrogen content \((4.41 \pm 0.20\%)\) was significantly higher in plants in treatment \(90 \text{ ppm of P}\) at pH 6. Highest uptake of Ca was recorded at a pH of 8 at 90 ppm of P.

| Treatments               | Chlorophyll A | Chlorophyll B | Total chlorophyll |
|--------------------------|---------------|---------------|-------------------|
| pH 4, P 31 ppm           | 12.242 ± 1.7a | 3.446 ± 0.5a  | 15.684 ± 2.2a     |
| pH 6, P 31 ppm (Control) | 10.384 ± 1.0cd| 2.848 ± 0.3cde| 13.229 ± 1.3cd    |
| pH 8, P 31 ppm           | 10.173 ± 1.1cde| 2.784 ± 0.3ef | 12.954 ± 1.5cde   |
| pH 4, P 90 ppm           | 11.419 ± 0.5ab| 3.233 ± 0.2ab | 14.649 ± 0.6ab    |
| pH 6, P 90 ppm           | 8.348 ± 1.1g  | 2.227 ± 0.3hi | 10.574 ± 1.4g     |
| pH 8, P 90 ppm           | 9.327 ± 1.3ef | 2.600 ± 0.4g  | 11.924 ± 1.7ef    |
| pH 4, P 150 ppm          | 10.929 ± 0.7bc| 3.014 ± 0.3bcd| 13.941 ± 0.9bc    |
| pH 6, P 150 ppm          | 8.463 ± 1.4g  | 2.282 ± 0.4h  | 10.744 ± 1.8g     |
| pH 8, P 150 ppm          | 7.063 ± 0.6h  | 1.988 ± 0.2i  | 9.049 ± 0.7h      |
| pH 4, P 210 ppm          | 10.900 ± 0.7bc| 3.108 ± 0.3bc | 14.005 ± 0.9bc    |
| pH 6, P 210 ppm          | 9.817 ± 1.0de | 2.650 ± 0.3g  | 12.465 ± 1.3de    |
| pH 8, P 210 ppm          | 3.547 ± 0.5i  | 0.910 ± 0.2j  | 4.456 ± 0.7i      |

One-way ANOVA (F-statistic)

|                         | **46.757***   | **43.425***   | **46.388***   |

\*Means followed by same lowercase letters in the same column are not significantly different \((P > 0.05)\) following comparison using Tukey test.

\***represents a statistical significance of \((P \leq 0.001)\) according to Fisher’s least significant difference.

Table 1. The effects of pH and Phosphorus concentrations on the chlorophyll content of \( S. \) chamelaeagnea grown in hydroponics.

3.2. Effects of pH and phosphorus concentrations on the leaf colour of \( S. \) chamelaeagnea grown in hydroponics

Effects of various P treatments at differed pH levels induced varied colour intensities, ranging from 16 to 31.7 from week 1 to week 8 on the leaf colour of \( S. \) chamelaeagnea \((P \leq 0.001)\) (Table 2, Figure 2). While treatment 1 offering a pH level of 4 at 31 ppm P generally yielded the highest leaf colour values over the 8-week growth period, these values did not differ significantly from that of the other pH 4 treatments receiving supplementary P. Of these treatments receiving supplementary P, the highest results were recorded at pH 4 receiving 210 ppm P closely followed by pH 4 at 90 ppm P and pH 4 at 150 ppm P treatments, respectively.

3.3. Effects of pH and phosphorus concentrations on the uptake of macro-nutrients in \( S. \) chamelaeagnea grown in hydroponics

Macro-nutrient uptake of P, K and Mg was significantly \((P \leq 0.001)\) affected by the treatment (Table 3). There was a noticeable higher tissue P content \((1.07 \pm 0.08\%)\) at pH 8, 150 ppm of P (Table 3). Tissue nitrogen content \((4.41 \pm 0.20\%)\) was significantly higher in plants in treatment \(90 \text{ ppm of P}\) at pH 6. Highest uptake of Ca was recorded at a pH of 8 at 90 ppm of P.
The micro-nutrient uptake of Na, Mn, Fe, Cu, Zn and B was significantly ($P \leq 0.001$) affected by the treatments (Table 4). The highest nutrient uptake values of Na (867.67 ± 131.72%) and Zn (46.78 ± 7.31%) were recorded at pH 8, 210 ppm of P treatment. The Fe uptake value (175.00 ± 14.42%) in the treatment at pH 4 of 210 ppm was the highest value. Highest recorded uptake values of Cu were obtained in plants receiving a pH of 4 at 31 ppm of P closely followed by the plants receiving a pH of 4 at 210 ppm.

Table 4.

| Treatments | Wk1          | Wk2          | Wk3          | Wk4          | Wk5          | Wk6          | Wk7          | Wk8          |
|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| pH 4, P 31 ppm | 30.156 ± 3.6ab | 31.667 ± 4.1a | 31.433 ± 3.0a | 30.878 ± 1.3a | 30.189 ± 3.1ab | 30.011 ± 2.2a | 30.178 ± 1.8a | 28.467 ± 1.9ab |
| pH 6, P 31 ppm (Control) | 32.800 ± 2.5a | 31.644 ± 4.0a | 30.533 ± 1.3ab | 29.933 ± 2.0a | 30.122 ± 2.6ab | 28.822 ± 2.6ab | 28.644 ± 1.6bcd | 28.922 ± 1.4a |
| pH 4, P 90 ppm | 30.033 ± 4.5ab | 30.567 ± 3.8ab | 30.078 ± 1.4b | 29.944 ± 2.1a | 29.944 ± 2.1a | 28.933 ± 1.4b | 29.589 ± 1.7abc | 28.278 ± 2.3ab |
| pH 6, P 90 ppm | 31.789 ± 3.6ab | 31.156 ± 4.6ab | 30.578 ± 2.0a | 29.444 ± 1.6ab | 29.444 ± 1.6ab | 27.000 ± 1.4b | 28.067 ± 1.9e | 27.056 ± 1.0bc |
| pH 4, P 150 ppm | 29.411 ± 3.3b | 27.356 ± 3.0b | 22.756 ± 3.0c | 20.067 ± 2.3b | 20.067 ± 2.3b | 15.978 ± 2.8d | 21.756 ± 1.6f | 24.278 ± 2.3e |
| pH 6, P 150 ppm | 29.333 ± 3.5b | 30.944 ± 3.6ab | 29.933 ± 2.1ab | 29.356 ± 2.3b | 29.356 ± 2.3b | 15.978 ± 2.8d | 21.756 ± 2.4cd | 24.278 ± 2.3e |
| pH 8, P 210 ppm | 29.411 ± 3.0b | 22.422 ± 4.9d | 15.978 ± 2.8d | 15.756 ± 3.5c | 15.756 ± 3.5c | 21.756 ± 1.6f | 21.756 ± 2.4cd | 24.278 ± 2.3e |
| pH 4, P 210 ppm | 30.289 ± 3.6ab | 30.044 ± 3.7ab | 31.233 ± 2.7ab | 30.622 ± 1.5a | 29.189 ± 2.6ab | 29.189 ± 2.6ab | 30.178 ± 4.0a | 27.956 ± 1.1ab |
| pH 6, P 210 ppm | 29.333 ± 3.5b | 30.944 ± 3.6ab | 29.933 ± 2.1ab | 29.356 ± 2.3b | 29.356 ± 2.3b | 15.978 ± 2.8d | 21.756 ± 2.4cd | 24.278 ± 2.3e |
| pH 8, P 210 ppm | 31.756 ± 3.9ab | 28.489 ± 3.2abc | 24.922 ± 5.7cd | 16.167 ± 4.9d | 13.900 ± 2.7c | 13.900 ± 2.7c | 18.044 ± 1.6d | 18.567 ± 1.6g |

Two-way ANOVA (F-statistic)

| NS represents no statistical significance, *represents a statistical significance of ($P \leq 0.05$), **represents a statistical significance of ($P \leq 0.01$) and ***represents a statistical significance of ($P \leq 0.001$) according to Fisher's least significant difference.

3.4. Effects of pH and phosphorus concentrations on the uptake of micro-nutrients in S. chamelaeagnea grown in hydroponics

The micro-nutrient uptake of Na, Mn, Fe, Cu, Zn and B was significantly ($P \leq 0.001$) affected by the treatments (Table 4). The highest nutrient uptake values of Na (867.67 ± 131.72%) and Zn (46.78 ± 7.31%) were recorded at pH 8, 210 ppm of P treatment. The Fe uptake value (175.00 ± 14.42%) in the treatment at pH 4 of 210 ppm was the highest value. Highest recorded uptake values of Cu were obtained in plants receiving a pH of 4 at 31 ppm of P closely followed by the plants receiving a pH of 4 at 210 ppm.
Figure 2. Observable variations in the leaf's green colour among plants (S. chamelaeagnea) following exposure to varied combinations of pH and P treatments in hydroponics under greenhouse conditions (Picture: K. Lefever).

| Treatments   | N (%)       | P (%)       | K (%)       | Ca (%)      | Mg (%)      |
|--------------|-------------|-------------|-------------|-------------|-------------|
| pH 4, P 31 ppm | 4.18 ± 0.29bcd | 0.64 ± 0.06g | 4.23 ± 0.29g | 1.13 ± 0.07a | 0.28 ± 0.01h |
| pH 6, P 31 ppm | 4.24 ± 0.55abc | 0.73 ± 0.06f | 4.41 ± 0.23fg | 1.12 ± 0.10a | 0.36 ± 0.03e |
| pH 8, P 31 ppm | 4.19 ± 0.18abcd | 0.62 ± 0.08g | 4.47 ± 0.26efg | 1.10 ± 0.11ab | 0.43 ± 0.04c |
| pH 4, P 90 ppm | 4.27 ± 0.26abc | 0.77 ± 0.08ef | 4.64 ± 0.36cdef | 1.10 ± 0.10ab | 0.31 ± 0.02g |
| pH 6, P 90 ppm | 4.41 ± 0.20a  | 0.82 ± 0.08cde | 4.53 ± 0.13defg | 1.08 ± 0.07ab | 0.38 ± 0.03d |
| pH 8, P 90 ppm | 4.20 ± 0.12abcd | 0.80 ± 0.07def | 4.45 ± 0.24efg | 1.14 ± 0.05a | 0.48 ± 0.03b |
| pH 4, P 150 ppm | 4.37 ± 0.19ab | 0.82 ± 0.04cde | 4.79 ± 0.58cd | 1.07 ± 0.06abc | 0.32 ± 0.03fg |
| pH 6, P 150 ppm | 4.09 ± 0.22cd | 0.88 ± 0.07bc | 4.87 ± 0.19c  | 1.01 ± 0.07cd | 0.36 ± 0.02de |
| pH 8, P 150 ppm | 4.00 ± 0.08de | 1.07 ± 0.08a  | 6.29 ± 0.39a  | 0.77 ± 0.03e | 0.55 ± 0.02a |
| pH 4, P 210 ppm | 4.13 ± 0.18cde | 0.84 ± 0.06bc | 4.73 ± 0.32cde | 1.05 ± 0.06bc | 0.31 ± 0.02g |
| pH 6, P 210 ppm | 4.05 ± 0.18cde | 0.87 ± 0.08bcd | 4.23 ± 0.35g   | 0.97 ± 0.06d | 0.35 ± 0.02ef |
| pH 8, P 210 ppm | 3.77 ± 0.16e  | 0.91 ± 0.13b  | 5.71 ± 0.38b   | 0.46 ± 0.03f | 0.48 ± 0.03b |
| One-way ANOVA (F-statistic) | | | | | |
| N.S.        | 4.35***     | 21.34***    | 31.67***     | 68.64***    | 89.74***    |

NS represents no statistical significance, 
*represents a statistical significance of ($P \leq 0.05$), 
**represents a statistical significance of ($P \leq 0.01$) and 
***represents a statistical significance of ($P \leq 0.001$) according to Fisher's least significant difference.

Table 3. The effects of pH and Phosphorus concentrations on the uptake of macro-nutrients in S. chamelaeagnea grown in hydroponics.
In this chapter, the significantly \((P \leq 0.001)\) higher chlorophyll values recorded in the treatments at a pH of 4 with supplementary P show that phosphorous fertilization under an acidic condition of chlorophyll production by *S. chamelaeagnea* will largely increase in hydroponic production. Also, high leaf colour intensity values were recorded in treatments with a pH of 4 compared to that of treatments with a higher pH of 6 or 8. On the other hand, it seems that a higher P concentration had a minimal effect on leaf colour intensity. It is worth noting that

| Treatments       | Na (mg/kg)  | Mn (mg/kg) | Fe (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | B (mg/kg) |
|------------------|-------------|------------|------------|------------|------------|-----------|
| pH 4, P 31 ppm   | 477.89 ± 36.27fg | 84.67 ± 7.48fg | 151.56 ± 7.32fg | 5.22 ± 1.99a | 39.56 ± 2.88bc | 37.78 ± 3.63ab |
| pH 6, P 31 ppm   | 479.78 ± 57.99fg | 105.89 ± 11.40c | 139.11 ± 10.17def | 2.89 ± 0.60d | 38.00 ± 3.20c | 38.56 ± 3.09a |
| pH 8, P 31 ppm   | 472.89 ± 58.58g | 156.78 ± 9.11a | 137.11 ± 8.25ef | 2.89 ± 0.33d | 37.89 ± 3.44c | 37.33 ± 2.29ab |
| pH 4, P 90 ppm   | 548.44 ± 74.72ef | 84.00 ± 8.19fg | 144.11 ± 10.59def | 4.11 ± 0.60bc | 40.11 ± 4.31bc | 38.67 ± 3.67a |
| pH 6, P 90 ppm   | 505.78 ± 39.02fg | 101.00 ± 4.69cd | 153.33 ± 13.87bcd | 2.56 ± 0.53de | 41.33 ± 6.12bc | 37.44 ± 2.40ab |
| pH 8, P 90 ppm   | 532.56 ± 70.06efg | 150.33 ± 12.56a | 167.33 ± 13.27abc | 3.22 ± 0.44cd | 39.78 ± 6.28bc | 36.56 ± 1.24abc |
| pH 4, P 150 ppm  | 604.22 ± 102.07de | 82.67 ± 9.84g | 168.56 ± 23.51ab | 4.67 ± 1ab | 41.33 ± 6.24bc | 37.67 ± 2.29ab |
| pH 6, P 150 ppm  | 680.33 ± 55.08bc | 94.00 ± 19.68de | 151.00 ± 34.86de | 3.11 ± 1.90d | 38.00 ± 6.75c | 35.22 ± 3.03bcd |
| pH 8, P 150 ppm  | 716.00 ± 117.06b | 131.44 ± 7.32b | 152.78 ± 16.20bcde | 4.33 ± 0.5ab | 39.44 ± 4.48bc | 31.67 ± 3.35e |
| pH 4, P 210 ppm  | 696.78 ± 69.99bc | 82.11 ± 4.43g | 175.00 ± 14.42a, | 5.11 ± 0.60a | 44.00 ± 2.92bc | 34.56 ± 2.51cd |
| pH 6, P 210 ppm  | 640.89 ± 36.55cd | 90.78 ± 9.38fg | 145.11 ± 14.16def | 2.44 ± 0.53de | 43.89 ± 5.69ab | 35.33 ± 2.45bcd |
| pH 8, P 210 ppm  | 867.67 ± 131.72a | 93.44 ± 8.14def | 129.33 ± 21.17f | 1.89 ± 0.60e | 46.78 ± 7.31a | 33.33 ± 2.40de |
| One-way ANOVA    | 22.746*** | 62.30*** | 5.590*** | 11.975*** | 2.573*** | 5.56*** |

NS represents no statistical significance,  
*represents a statistical significance of \((P \leq 0.05)\),  
**represents a statistical significance of \((P \leq 0.01)\) and  
***represents a statistical significance of \((P \leq 0.001)\) according to Fisher's least significant difference.

Table 4. The effects of pH and phosphorus concentrations on the uptake of micro-nutrients in *S. chamelaeagnea* grown in hydroponics.

### 4. Discussions

In this chapter, the significantly \((P \leq 0.001)\) higher chlorophyll values recorded in the treatments at a pH of 4 with supplementary P show that phosphorous fertilization under an acidic condition of chlorophyll production by *S. chamelaeagnea* will largely increase in hydroponic production. Also, high leaf colour intensity values were recorded in treatments with a pH of 4 compared to that of treatments with a higher pH of 6 or 8. On the other hand, it seems that a higher P concentration had a minimal effect on leaf colour intensity. It is worth noting that
studies have shown high correlations between chlorophyll meter readings, that is, the leaf’s green colour intensity and extractable leaf chlorophyll [38]. The effect of P on chlorophyll could be indirect and complex. P fertilization may indirectly influence or hinder the uptake of other nutrients [39], which in turn affects chlorophyll production in plants. Indigenous plants, especially those occurring in the fynbos biome, are expected to be adapted to nutrient-poor and low-pH soils and tend to have low critical levels for most of the nutrients. Therefore, exposing these species to high P concentration may have a minimal effect on plant physiology and can even have detrimental effects on plant growth.

Despite the relatively high nutrient uptake values in plants receiving a nutrient solution with a pH 8, chlorosis of their leaves was apparent during the growth period. This suggests that the uptake of some essential nutrients responsible for chlorophyll development was affected at this pH level, namely the mineral nutrients Cu, B, N and Fe which are directly involved in photosynthesis, respiration, cell division and protein formation [23, 40]. In soil-less media, the affinity of soluble nutrients to negatively charged surfaces and the interactions between charged cations can have a profound effect on nutrient availability and subsequently, the uptake of nutrients by plants. For example, fertilization with phosphorous increases the soil’s nitrogen absorption in young plants of Eucalyptus grandis [39]. Silber [41] argued that a continuous decline of soluble P concentration during fertilization can be explained through two mechanisms, a rapid electrostatic reaction and adsorption of the onto substrate and a slow formation of solid metal-P compounds with Al and Fe under acidic conditions and Ca and Mg under basic-to-neutral conditions. Therefore, the substrate used in hydroponic setups could affect the availability of micro- and macro-nutrients. Shen et al. [42] suggested that the availability of soil P is extremely complex and needs to be systemically evaluated. Previously, Wu et al. [43] showed that under phosphorus stress, no significant changes in chlorophyll A and B, total chlorophyll and carotenoid contents were found, and phosphorus stress generally had no effect on photosynthesis. The highest nutrient uptake values were recorded in nine of the 12 treatments receiving supplementary P, with only Cu and Mn yielding the highest values in treatments receiving no supplementary P. Thus, it is evident that phosphorus treatments had a significant effect on nutrient uptake in S. chamelaeagnea grown hydroponically [44].

5. Conclusions

In conclusion, this chapter gives insight into the unknown cultivation requirements of the leaf’s chlorophyll development of S. chamelaeagnea and shows that the use of a hydroponic nutrient system offering little to no supplementary phosphorus at a pH level of 4 significantly correlated with the chlorophyll development of S. chamelaeagnea grown in hydroponics. Based on the results obtained in the chapter, it is plausible to assume that P has an indirect effect on chlorophyll production in S. chamelaeagnea.

Acknowledgements

This study was funded by Cape Peninsula University of Technology through CPUT Bursary and University Research Funds.
Author details

Kerwin Lefever¹, Charles P. Laubscher¹*, Patrick A. Ndakidemi² and Felix Nchu¹

*Address all correspondence to: laubscherc@cput.ac.za

1 Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, South Africa
2 School of Life Sciences and Bio-engineering, Nelson Mandela Institute of Science and Technology, Arusha, Tanzania

References

[1] Gali-Muhtasib, H., Hilan, C. & Khater, C. 2000. Traditional uses of Salvia libanotica (East Mediterranean sage) and the effects of its essential oils. Journal of Ethnopharmacology. 71(3):513–520.

[2] Kamatou, G.P.P., van Zyl, R.L., Davids, H., van Heerden, F.R., Lourens, A.C.U. & Viljoen, A.M. 2008. Antimalarial and anticancer activities of selected South African Salvia species and isolated compounds from S. radula. South African Journal of Botany. 74:238–243.

[3] Cornara, L., La Rocca, A., Marsili, S. & Mariotti, M.G. 2009. Traditional uses of plants in the Eastern Riviera (Liguria, Italy). Journal of Ethnopharmacology. 125(1):16–30.

[4] Clebsch, B. 2003. The new book of Salvia, 2nd ed. Port Land: Timber Press Inc., pp. 1–80.

[5] Thorsen, M.A. & Hildebrandt, K.S. 2003. Quantitative determination of phenolic diterpenes in rosemary extracts, aspects of accurate quantification. Journal of Chromatography. 995:119–125.

[6] Kamatou, G.P.P., Viljoen, A.M. & Steenkamp, P. 2010. Antioxidant, anti-inflammatory activities and HPLC analysis of South African Salvia species. Food Chemistry. 119(2):684–688.

[7] Kamatou, G.P.P., van Vuuren, S.F., van Heerden, F.R., Seaman, T. & Viljoen A.M. 2007. Antibacterial and antimycobacterial activities of South African Salvia species and isolated compounds from S. chamelaeagnea. South African Journal of Botany. 73(4):552–557.

[8] Western, P. & Claßen-Bockhoff, R. 2006. Bird pollination in South African Salvia species. Flora. 201:396–406.

[9] Pienaar, K. 2000. What flower is that? 2nd ed. Cape Town: Struik, pp. 297–298.

[10] Ulubelen, A. 2003. Cardio active and antibacterial terpenoids from some Salvia species. Phytochemistry. 64:395–399.

[11] Nenadis, N., Zafiropoulou, I. & Tsimidou, M. 2003. Commonly used food antioxidants: a comparative study in dispersed systems. Food Chemistry. 82:403–407.

[12] Wiersum, K.F., Dold, A.P., Husselman, M. & Cocks, M. 2006. Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the amatola...
region, South Africa. In R.J. Bogers (ed.), *Medicinal and aromatic plants*. The Netherlands: Springer, pp. xx–xxx. http://bioculturaldiversity.co.za/publications/CULTIVATION%20OF%20MEDICINAL%20PLANTS%20AS%20A%20TOOL.pdf

[13] Gan, Y., Stulen, I., van Keulen, H. & Kuiper, P.J.C. 2003. Effect of N fertilizer top-dressing at various reproductive stages on growth, N\textsubscript{2} fixation and yield of three Soybean (*Glycine max* (L.) Merr.) genotypes. *Field Crops Research*. 80:147–155.

[14] Khazaie, H.R., Nadjafi, F. & Bannayan, M. 2008. Effect of irrigation frequency and planting density on herbage biomass and oil production of thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*). *Industrial Crops and Products*. 27:315–321.

[15] Ichir, L.L., Ismaili, M. & van Cleemput, O. 2003. Effect of organic and mineral fertilizers on N-use by wheat under different irrigation frequencies. *Competes Rendus Biologies*. 326:391–399.

[16] Maidl, F.X., Panse, A., Dennert, J., Ruser, R. & Fischbeck, G. 1996. Effect of varied N rates and N timings on yield, N uptake and fertilizer N use efficiency of a six-row and a two-row winter barley. *European Journal of Agronomy*. 5:247–257.

[17] Stagnani, F., Bitetto, V.D. & Pisante, M. 2007. Effects of N fertilizers and rates on yield, safety and nutrients in processing spinach genotypes. *Scientia Horticulturae*. 114:225–233.

[18] Manzanares, M., Tenorio, J.L. & Ayerbe, L. 1997. Sowing time, cultivar, plant population and application of N fertilizer on Kenaf in Spain’s central plateau. *Biomass and Bioenergy*. 12(4):263–271.

[19] Liu, X., Ren, G. & Shi, Y. 2011. The effect of organic manure and chemical fertilizer on growth and development of *Stevia rebaudiana* Bertoni. *Energy Procedia*. 5:1200–1204.

[20] Ram, M., Ram, D. & Roy, S.K. 2003. Influence of an organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yields in geranium (*Pelargonium graveolens*). *Bioresource Technology*. 87:273–278.

[21] Zhu, Z., Liang, Z., Han, R. & Dong, J. 2009. Growth and saikosaponin production of the medicinal herb *Bupleurum chinense* DC. Under different levels of nitrogen and phosphorus. *Industrial Crops and Products*. 29:96–101.

[22] Glass, A.D.M. 1989. Plant nutrition. An introduction to current concepts. Boston: Jones and Bartlett Publishers, Inc., pp. 30–50, 140–230.

[23] Stern, K. 2006. Introductory plant biology, 10th ed. New York: McGraw-Hill, pp. 81, 193–201.

[24] Leopold, A.C. & Kriedemann, P.E. 1975. Plant growth and development, 2nd ed. USA: McGraw-Hill, pp. 133, 417, 439–442.

[25] Marschner, H. 1995. Mineral nutrition of higher plants. London: Academic Press Limited, pp. 605–609, 641–645.

[26] Hipps, N.A., Davies, M.J., Dodds, P. & Buckley, G.P. The effects of phosphorous nutrition and soil pH on growth of some ancient woodland indicator plants and their interaction with competitor species. *Plant and Soil*. 271:131–141.
[27] Li, Y., Wang, T., Li, J. & Ao, Y. 2010. Effects of phosphorus on celery growth and nutrient uptake under different calcium and magnesium levels in substrate culture. *Horticultural Science*. 37:99–108.

[28] Nicholls, K.H. & Dillon, P.J. 1978. An evaluation of phosphorus-chlorophyll-phytoplankton relationships for lakes. *International Review of Hydrology* 63:141–154.

[29] Hawkins, H.K., Hettasch, H., Mesjasz-Przybylowicz, J., Przybylowicz, W. & Cramer, M.D. 2008. Phosphorus toxicity in the Proteaceae: a problem in post-agricultural lands. *Scientia Horticulturae*. 117:357–365.

[30] Rebelo, A.G. 2001. Proteas. A field guide to the Proteas of Southern Africa. South Africa: Fernwood Press, pp. 180–235.

[31] Witkowski, E.T.F. & Mitchell, D.T. 1987. Variations in soil phosphorus in the Fynbos biome. *South African Journal of Ecology*. 72–76:1100–1200.

[32] Hershey, D.R. 1994. Solution culture hydroponics: history and inexpensive equipment. *American Biology Teacher*. 56:111–118.

[33] Hershey, D.R. 1995. Plant biology science projects. New York: Wiley, pp. 20–41.

[34] Hiscox, J.D. & Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*. 57:1332–1334.

[35] Arnon, D.I. 1949. Copper enzymes in chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 24:1–15.

[36] Giron, H.C. 1973. Comparison between dry ashing and wet digestion in the preparation of plant materials for atomic absorption analysis. *Atomic Absorption Newsletter*. 12:28–29.

[37] Steel, R.G.D. & Torrie, J.H. 1980. Principles and procedures of statistics: a biometrical approach, 2nd edition. New York: McGraw-Hill Inc.

[38] Wood C.W., Reeves, D.W. & Hilmerick, D.G. 1993. Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status, and crop yield: a review. *Proceedings Agronomy Society of New Zealand*. 23:1-9

[39] Graciano C., Goya, J.F., Frangi J.L. & Guiamet, J.J. 2006. Fertilization with phosphorus increases soil nitrogen absorption in young plants of *Eucalyptus grandis*. *Forest Ecology and Management*. 236:202–210.

[40] Molassiotis, A., Tanou, G., Diamantidis, G., Patakas, A. & Therios, I. 2006. Effects of 4-month Fe deficiency exposure on Fe reduction mechanism, photosynthetic gas exchange, chlorophyll fluorescence and antioxidant defense in two peach rootstocks differing in Fe deficiency tolerance. *Journal of Plant Physiology*. 163:176–185.

[41] Silber, A. 2008. Chemical characteristic of soilless media. In: Michael Raviv and J. Heinrich Lieth, editors. Soilless culture: theory and practice. San Diego: Academic Press, 2008, pp. 209–244.
[42] Shen, J., Yuan, L., Zhang, L., Li, H., Bai, Z., Chen, X., Zhang, W. & Zhang, F. 2011. Phosphorus dynamics: From soil to plant. *Plant Physiology*. 156:997–1005.

[43] Wu C., Fan Z. & Wang, Z. 2004. Effect of phosphorus stress on chlorophyll biosynthesis, photosynthesis and biomass partitioning pattern of *Fraxinus mandchurica* seedlings]. *The Journal of Applied Ecology*. 15(6):935–940.

[44] Naeem, M., Khan, M.M.A., Idrees, M. & Aftab, T. 2010. Phosphorus ameliorates crop productivity, photosynthetic efficiency, nitrogen-fixation, activities of the enzymes and content of nutraceuticals of *Lablab purpureus* L. *Scientia Horticulturae*. 126:205–214.