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

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Additional information is available at the end of the chapter

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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. chamelaeagnea* 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. chamelaeagnea* 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. chamelaeagnea* in hydroponics, in order to determine a fertilizer regime that will promote the development of *S. chamelaeagnea* 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. chamelaeagnea* 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 *S. chamelaeagnea* grown in hydroponics

Treatment significantly ($P \leq 0.001$) affected the chlorophyll A, chlorophyll B and total chlorophyll contents of *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.

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 ± 1.4fg	2.282 ± 0.4h	10.744 ± 1.8fg
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 (<i>F</i> -statistic)	46.757***	43.425***	46.388***

^{a-j}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 ppm of P) at pH 6. Highest uptake of Ca was recorded at a pH of 8 at 90 ppm of P.

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	31.078 ± 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 ± 2.4ab	28.822 ± 2.6ab	28.644 ± 1.6bcd	28.922 ± 1.4a
pH 8, P 31 ppm	30.033 ± 4.5ab	30.567 ± 3.8ab	28.933 ± 1.4b	29.944 ± 2.1a	29.189 ± 1.6ab	30.111 ± 2.4a	29.589 ± 1.7abc	28.278 ± 2.3ab
pH4, P 90 ppm	29.689 ± 3.8ab	29.911 ± 5.3ab	30.867 ± 2.2ab	31.111 ± 1.5a	30.711 ± 2.1ab	29.656 ± 2.4a	30.567 ± 2.1a	28.122 ± 2.0ab
pH 6, P 90 ppm	31.789 ± 3.6ab	31.156 ± 4.6ab	31.078 ± 2.8ab	30.578 ± 2.0a	29.444 ± 1.6ab	27.000 ± 1.4b	28.067 ± 2.2cde	27.056 ± 1.0bc
pH 8, P 90 ppm	29.411 ± 3.3b	27.356 ± 3.0bc	22.756 ± 3.0c	20.067 ± 2.3b	20.278 ± 1.2c	24.489 ± 1.6c	27.000 ± 1.9e	27.800 ± 1.7abc
pH 4, P 150 ppm	30.289 ± 3.6ab	30.044 ± 3.7ab	31.233 ± 2.7ab	30.622 ± 1.5a	29.189 ± 2.6ab	29.989 ± 4.0a	30.178 ± 1.8ab	27.956 ± 1.1ab
pH 6, P 150 ppm	29.333 ± 3.5b	30.944 ± 3.6ab	29.933 ± 2.1ab	29.356 ± 2.7a	28.633 ± 2.3b	28.233 ± 2.2ab	26.456 ± 1.5e	25.100 ± 2.1de
pH 8, P 150 ppm	29.267 ± 3.0b	22.422 ± 4.9d	15.978 ± 2.8d	15.756 ± 3.5c	15.156 ± 2.7d	15.267 ± 3.1e	21.756 ± 1.6f	24.278 ± 2.3e
pH 4, P 210 ppm	30.944 ± 2.8ab	30.456 ± 3.1ab	28.956 ± 2.4b	31.033 ± 2.0a	30.911 ± 3.2a	28.767 ± 2.0ab	29.533 ± 0.7abc	29.278 ± 1.4a
pH 6, P 210 ppm	31.756 ± 3.9ab	28.489 ± 3.2abc	29.478 ± 2.4ab	29.444 ± 1.2a	29.722 ± 1.0ab	28.622 ± 1.7ab	27.756 ± 2.0de	26.278 ± 1.7cd
pH 8, P 210 ppm	31.411 ± 2.6ab	24.922 ± 5.7cd	16.167 ± 3.6d	13.900 ± 2.7c	14.511 ± 1.8d	18.044 ± 1.7d	18.567 ± 1.6g	16.067 ± 1.8f
Two-way ANOVA (F-statistic)	1.013NS	4.333***	45.53***	78.77***	66.25***	38.79***	41.52***	37.33***

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 2. The effects of pH and phosphorus concentrations on the leaf colour of *S. chamelaeagnea* grown in hydroponics.

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 ($867.67 \pm 131.72\%$) and Zn ($46.78 \pm 7.31\%$) were recorded at pH 8, 210 ppm of P treatment. The Fe uptake value ($175.00 \pm 14.42\%$) 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 ± 0.18cd	0.84 ± 0.06bcd	4.73 ± 0.32cde	1.05 ± 0.06bc	0.31 ± 0.02g
pH 6, P 210 ppm	4.05 ± 0.18cd	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)	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.

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 ± 7.48efg	151.56 ± 7.32cde	5.22 ± 1.99a	39.56 ± 2.88bc	37.78 ± 3.63ab
pH 6, P 31 ppm (Control)	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
pH4, 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 ± 2.92ab	34.56 ± 2.51cd
pH 6, P 210 ppm	640.89 ± 36.55cd	90.78 ± 9.38efg	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 (F-statistic)	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*.

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