Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

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PUBLISHED BY

Stimulatory effects of an exogenously applied seaweed extract on the morphological and physiological growth and yield in juvenile *Amaryllis belladonna* L. bulbs

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Amaryllis belladonna L. is a hysteranthous bulbous species indigenous to the Cape Floristic Region of South Africa. The species' attractiveness, adaptability, and low-maintenance needs have drawn international interest to its desirable uses in ornamental and landscape applications constrained by the observably slow rate of natural multiplication to reach flowering. A 24-week study was performed to determine the stimulatory effects of a seaweed extract, Kelpak®, on the morphological and physiological responses of *A. belladonna* bulbs cultivated under greenhouse conditions. Juvenile bulbs from five successive age groups were used to evaluate the consistency of observed responses. Treatments consisted of a 0% untreated control and three Kelpak® concentration dilutions at 0.2%, 0.4%, and 1% (v/v) administered to five age groups of *A. belladonna* bulbs as a monthly soil drench. The results showed that even at low concentrations, Kelpak® treatments improved the phyto-stimulatory responses of both the bulb aerial and, more substantially, the below-ground storage organs in a concentration-dependent manner. While treatments enhanced the morpho-physiological responses, the consistency of bulb age differed. Higher morphological yields were associated with older bulbs; however, bulbs of *A. belladonna* in years 1 and 2 were deemed the most receptive in circumference, weight coefficients, and chlorophyll content. However, to maximize the efficacy and proliferation rate of the species in a reduced timeframe, a 1% Kelpak® dilution applied at an early developmental stage within the first two years is most beneficial and a priority to elicit rapid, uniform, and healthy bulb growth and development.

Keywords: Amaryllidaceae, cultivation, juvenile bulb, Naked Lady, phytohormones, seaweed biostimulant

ARTICLE HISTORY
Submitted: July 04, 2023
Accepted: March 31, 2024

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DOI: 10.21608/ejbo.2024.220974.2395

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INTRODUCTION

Amaryllis belladonna L. (Amaryllidaceae) more commonly known as the "Belladonna Lily", "March Lily" or "Naked Lady", is an endemic drought-tolerant ornamental bulbous geophyte from the Cape Floristic Region (CFR) of South Africa (Manning et al. 2002; Duncan et al. 2020). As a representative of only two species within the genus, it has become a popular plant that has migrated and naturalised in several Mediterranean climatic areas worldwide (Adams 2001; Duncan 2004; Duncan et al. 2020). The advent of pink-scented, trumpet-shaped flowers on a single inflorescence from late summer to early autumn signifies the bulb's hysteranthous nature and a species characteristic. The expansion of winter leaf growth subsequently follows this. As the seasonal transition into spring intensifies, bulb resources are preserved by the withering of leaves and the persistence of summer dormancy (Manning et al., 2002; Duncan, 2010; Duncan et al., 2020). The attractiveness, versatility, and low-maintenance requirements of this perennial bulbous species have drawn attention to their valuable and desirable uses in a variety of cultivated floricultural, ornamental, and landscape applications (Reinten et al. 2011; Wilmot and Laubscher 2019; Gul et al. 2020; Darras 2021).

Amaryllis belladonna is, however, severely constrained by the observably slow rate of natural multiplication as seedlings, juvenile bulbs (seedlings termed as juvenile bulbs after the first year of seed cultivation), and the division of offsets typically requires several years to attain a critical size competent for reproductive flowering (Theron and de Hertogh 2001; Duncan 2010). In addition, the recalcitrant seeds germinate almost instantaneously and do not survive desiccation; therefore, the seeds need to take advantage of the approaching autumn or winter rains to establish themselves adequately before the upcoming adversities of the summer dormancy period. (Duncan 2004; Duncan 2010; Colville 2017). Currently, the species is propagated primarily by conventional methods, which are the most affordable and simplest options from collected recalcitrant seeds and offsets (also referred to as daughter bulbs or bulblets) (Adams 2001; Duncan 2010). *In vitro* tissue culture has been tested (De Bruyn et al., 1992; Veeraballi et al., 2017), yet costly in comparison (Zhang et al., 2013). As a result, the timely entry of adequate and economically viable plant material into the horticultural distribution network is hindered. According to Kharrazi et al. (2017), Anderson (2019) and Li et al. (2023), the hampering juvenile period (lifecycle stages following embryogenesis) of vegetative growth essential in producing a marketable and viable flowering bulb within the commercial propagation framework of geophytes, is of concern. There is a continuing prevailing interest in advocating precision cultivation practices to expand the paucity of information and alleviate the challenges of South African indigenous plant species that have shown potential for commercialisation (Reinten et al. 2011; Darras 2021). Given the foregoing, the practical expansion of *A. belladonna's* cultivation inefficiencies in reducing the truncated lifecycles in a cost-effective, sustainable, and time-saving manner, and enhancing their performance in various untapped ornamental and commercial product lines within the horticultural distribution chain, is warranted (Le Nard & de Hertogh, 2002; Anderson, 2006; Darras, 2020).

Alternative and innovative cultivation techniques to support sustainable crop production are paramount, particularly given the insurmountable pressures and peripheral effects of climate change, energy crises, worldwide population growth, the availability of and viability of agricultural land, food security, and pest and pathogen resistance (Khan et al. 2009; Wang and Frei 2011; Arioli et al. 2015; Kamenetsky Goldstein 2019; Del Buono 2021). The 'green technology' of naturally based seaweed bioproducts has gained momentum in supporting workable efforts to alleviate these stresses and meet consumer needs (Craigie 2011; Sharma et al. 2014; Ali et al. 2021a) that were once dominated by the availability of a range of harsh synthetic agrochemicals (Tilman et al. 2002). Whole seaweed extracts consist of a plethora of mainly organic substances applied in low quantities that effectively interact with plant and soil systems in enhancing plant phenotypes, microbial restructuring, nutrient acquisition, pathway regulation, quality products, and abiotic stress tolerance (Calvo et al. 2014; Colla and Rouphael 2015; Ali et al. 2021a; Nasr et al., 2024). Moreover, they are at the forefront of the latest trends and sustainable advances in facilitating an inexpensive, environmentally friendly, and safe set of agricultural inputs as part of an integrated system approach for crop production (Caradonia et al. 2019; Souza et al. 2019). As an indication of their consistently proven desirability, effectiveness and relevance, comprehensive research activities of the profitable and experimental products developed from seaweed species (in containerized and field trials) have been extensively investigated (Papenfus et al. 2013; Sharma et al. 2014; Colla and Rouphael 2015; Ali et al. 2021a; Kisvarga et al. 2022). Furthermore, the ongoing exploration, frequency, mechanisms and modes of action, and profound effects towards the sustainable productivity of a variety of ornamental (Kisvarga et al. 2022) and, more prominently, edible food crops have been appraised (Khan et al. 2009; Colla and Rouphael 2015; Caradonia et al. 2019).

Kelpak® is a commercial organic seaweed extract (SWE) formulated from a liquid derived from the commonly used, fast-growing giant brown kelp, *Ecklonia maxima* (Osbeck) Papenfuss (Phaeophyceae) that has undergone a patented cold cellular burst process of extraction (Troell et al. 2006; Stirk et al. 2020). This process has excluded chemicals, dehydration, cooling, and heating while emphasizing pressure differentials to breach the cell walls in releasing the natural biostimulant (Troell et al. 2006). The extract contains trace levels of macro- and microelements in addition to auxins (11 mg/L), cytokinins (0.031 mg/L), alginates, amino acids, mannitol, and neutral sugars (Stirk et al., 2014; Lötze & Hoffman, 2016). The presence of an increased phytohormone proportional ratio (auxin-to-cytokinin) is ascribed to the several plant-promoting synergistic effects in crops, including but not limited to root system expansion, plant development, nutrient translocation and absorption efficiency, and plant stress tolerance to abiotic influences (Colla and Rouphael 2015; Lötze and Hoffman 2016; Kisvarga et al. 2022). Several scientific studies have demonstrated the beneficial effects of Kelpak® applications (Van Staden et al., 1995; Basak, 2008; Papenfus et al., 2013; Makhaye et al., 2021). Additionally, Kelpak®-based research has produced encouraging findings of commercially well-known and valuable indigenous South African species *Erica verticillata* Bergius (Adams et al. 2019) and *Eucomis autumnalis* (Mill.) Chitt (Aremu et al. 2016).

Considering the horticultural prospects of the *A. belladonna* species and the vast agronomic capabilities of seaweed extracts, no information or comprehensive studies have been conducted on the practice of utilizing these bio-stimulators as a potentially viable, alternative technology and advanced cultivation strategy to augment the early development, juvenile timeframe, and ornamental quality of the bulb. Therefore, the best methods conducive to large-scale propagation and cultivation in accelerating the prolonged juvenile stage by reducing the generation time of this slow-growing indigenous bulb remain to be determined. This study aimed to elucidate the stimulatory effects of exogenous applications of selected concentration dilutions of a seaweed extract, Kelpak®, on the morphological and physiological responses in juvenile *A. belladonna* bulbs cultivated under greenhouse conditions. Furthermore, as it takes the bulb several years to reach flowering maturity, an additional objective was set to evaluate the consistency of any observed responses across five successive age groups. This research anticipates enhancing the knowledge and efficiency of facilitating the species' production processes and life cycle assessment (LCA), as well as providing a proposed continuous long-term, practical, and sustainable horticulture propagation and/or cultivation practice for enhancing the production of geophytes with specialized economic value in the conservation, floriculture, and ornamental sectors.

MATERIALS AND METHODS

Experimental location

A 24-week study was conducted in the Cape Peninsula University of Technology's Horticultural Sciences research greenhouse facility in Bellville, Cape Town, South Africa (33°55'45" S, 18°38'31" E) from mid-April 2021 to mid-October 2021. The ventilated greenhouse facility with clear polycarbonate rooftop sheeting and a thermostatically automated system (Envirowatch, Envirowatch Solutions, South Africa) ensured a controlled and monitored environment under natural light conditions. Temperature set ranges fluctuated between 18°C–26°C during the day and 10°C–18°C at night, with an average relative humidity (RH) of 60%. The daylight photoperiod coincided with the prevailing conditions between early autumn and late spring (9–12 hours). The photosynthetic photon flux density (PPFD) recorded a daily average of 420 µmol/m^2/s, with the optimal light conditions logged at 1020 µmol/m^2/s. The average soil substrate temperature was 14/20°C (min/max).

Plant material and preparation

Dormant, juvenile *A. belladonna* bulbs from five successive growing seasons (delineated from one to five years of age), primarily propagated from seed, were sourced from Assegaaibosch Farm on the Agulhas Plain, Western Cape, South Africa in early April 2021 (i.e., mid-autumn). The well-developed bulbs were selectively and sustainably harvested using standard cultural practices, as (Duncan 2010) described, and graded according to categorical age criteria to ensure homogeneity within samples. The bulbs were rinsed of extraneous matter, stripped of senescent leaves, and their desiccated contractile roots removed. Thereafter, the bulbs were immersed for 5 minutes in a 0.1% biocidal solution (Sporekill™, ICA International Chemicals (Pty) Ltd., Stellenbosch, South Africa) (active ingredient: didecyldimethylammonium chloride) removed and adequately air-dried. Before replanting and experimental treatments, the bulbs were conditioned for one week and stored in sealed, breathable crates at a constant ambient temperature of 21°C (± 2°C) and RH of 50-70% in a darkened room.

Experimental design and set-up

The experimental design comprised four levels of a 0% untreated control and three concentration dilutions at 0.2%, 0.4%, and 1% (v/v) of Kelpak® administered to *A. belladonna* bulbs from five consecutive growing seasons (years one through five). Bulbs within each of the five age categories were randomly assigned to each Kelpak® treatment group (Table 1). The bulbs were planted in plastic growing trays (15 × 23 × 7.5 cm, with a 5 L volume) using an inert growing medium consisting of pre-rinsed (to remove any impurities and other extraneous materials) silica sand (grade 6/17 Consol®) and fine river sand at a ratio of 1:1 (v/v). The trays were supplemented with the remaining media, ensuring each bulb's neck was covered at the surface. The growing trays were labeled and placed on galvanized steel mesh tables (2 × 0.85 m) to obtain a flat, uniform surface height that warranted adequate air and temperature circulation. The bulbs were planted in a randomised block design (RBD) with 50 bulbs per Kelpak® treatment (ten sample replicates per age category from years one through five) (Table 1).

Kelpak® treatments

The SWE (Kelpak®, Kelp Products (Pty) Ltd., Simons Town, South Africa) treatment applications were prepared by diluting the liquid concentrate with reverse osmosis (RO) water to obtain three selected dilutions at 0.2%, 0.4%, and 1% (v/v). As inherent South African fynbos species exhibit the need for reduced nutritional requirements (Duncan, 2010), lower dilutions of the recommended manufacturer's dosage were applied. The concentration dilutions were prepared on each treatment day and manually administered as an equally distributed soil drench during the active vegetative growing season, first at planting (0 weeks) and subsequently at 4-week intervals in weeks 4, 8, 12, and 16, while the control was supplemented with RO water (no SWE was applied). Manually irrigated soil drench with municipal tap water was systematically applied weekly to maintain the moisture levels in all bulb treatments between the five SWE applications.

Table 1. Overview of the randomised experimental setup with duplicate growing trays containing five varying bulb ages placed on four selected Kelpak® concentration (%) treatment tables

Table	1 (control)	2	3	4
Growing tray 1	Y2 + 0% Kelpak® (c)	Y1 + 0.2% Kelpak®	Y4 + 0.4% Kelpak®	Y3 + 1% Kelpak®
Growing tray 2	Y5 + 0% Kelpak® (c)	Y4 + 0.2% Kelpak®	Y2 + 0.4% Kelpak®	Y5 + 1% Kelpak®
Growing tray 3	Y3 + 0% Kelpak® (c)	Y2 + 0.2% Kelpak®	Y5 + 0.4% Kelpak®	Y1 + 1% Kelpak®
Growing tray 4	Y1 + 0% Kelpak® (c)	Y5 + 0.2% Kelpak®	Y3 + 0.4% Kelpak®	Y2 + 1% Kelpak®
Growing tray 5	Y4 + 0% Kelpak® (c)	Y3 + 0.2% Kelpak®	Y1 + 0.4% Kelpak®	Y4 + 1% Kelpak®

(c) = control; (Y1–Y5) = Bulb age from year 1 to year 5.

Since soil drenching is associated with enhanced absorption of various compounds (Sarkar et al. 2007) and continuous assimilation of plant crops over an extended period (Parkunan et al. 2011), this treatment was recommended. Moreover, because the bulbs were dormant at the onset of experimentation, the delivery of a foliar spray was invariably constrained.

Data collection

Determination of vegetative morphological plant growth parameters: Morphological data were collected and captured before, during, and post-harvest as indicators of new growth and development using an electronic laboratory scale (Radwag® PS 4500.R2, Radwag Waagen, Hilden, Germany) with a 0.001g legibility, standard metric retractable metal tape measure (Stanley Power Lock®, Builders Warehouse, South Africa) and a soft cloth tape measure (Empisal EMT-001, Builders Warehouse, South Africa) with a corresponding 300×19 mm and 450×25 mm respective readability.Preliminary pre-plant measurements of initial fresh bulb circumference and weight were measured to ensure sample homogeneity. Twenty weeks after planting, morphological leaf parameters of the number of leaves, leaf length, and width produced by each bulb were recorded. The leaf numbers were quantified manually, whilst the longest established leaf was measured from its base (where it emerged from the bulb) to its apex, and the broadest point of the same leaf was used to determine the leaf width. In addition, the leaf area was determined using the Montgomery equation (ME) as described by Yu et al. (2020) and Shi et al. (2021).

$$A_{leaf} = \alpha \times L_{leaf} \times W_{leaf}$$

where

A_{leaf} = leaf area; L_{leaf} = leaf length; W_{leaf} = width; α = Montgomery parameter

At 24 weeks, on completion of the treatment period, once natural leaf senescence had occurred, whole bulbs were harvested, rinsed, and air-dried overnight. Thereafter, accumulated post-harvest measurements of fresh bulb circumference, weight, number of roots, and root length were recorded. The root count was obtained manually, whilst the root length was determined as the span from the bulb basal plate to the apex of the longest root. Moreover, the bulb circumference coefficient (the ratio of the fresh bulb circumference at harvest and the initial circumference) and weight coefficient (the ratio of the fresh bulb weight at harvest and the initial weight) were calculated.

$$\text{Circumference coefficient} = \frac{C2}{C1}$$

$$\text{Weight coefficient} = \frac{W2}{W1}$$

where

C1 = initial fresh bulb circumference
C2 = harvest fresh bulb circumference
W1 = initial fresh bulb weight
W2 = harvest fresh bulb weight

Determination of leaf chlorophyll content: Chlorophyll content (mg/m^2) in the leaf primordia was monitored as a measure of chlorophyll production described by Gitelson et al. (1999) using a portable modulated chlorophyll content meter (CCM-300, Opti-Sciences Inc., Hudson, USA). The absorption instrument was calibrated and clipped to three different positions along the leaf blade (top, middle, and bottom) of the 2nd developed outer basal leaf of each sample bulb in a non-destructive manner, and the average relative mean value was recorded. Data readings were logged between 10 a.m. and 2 p.m. at 20 weeks of the experiment, with ten analytical replicate samples performed for each treatment combination.

$$\bar{X} = \frac{(R1+R2+R3)}{3}$$

where

$\bar{X}$ = mean value
R = chlorophyll content reading

Statistical analysis

The morphological and physiological data were computed and statistically analysed using the Minitab analysis software (Minitab 17.0, Minitab LLC, Pennsylvania State University, USA). Experimental results were subjected to a two-way analysis of variance (ANOVA) for factors: Kelpak® concentration dilutions (4 levels) and bulb age (5 levels) and presented as mean values with predicted standard errors (S.E). Tukey's least significant difference (LSD) was used to determine the main effects and interactions at a $p \leq 0.05$ significance level. Mean values that do not share a letter(s) are significantly different at an α level.

RESULTS

Effect of Kelpak® treatments and bulb age on morphological bulb growth

Pre-plant bulb circumference and circumference coefficient: Pre-plant bulb circumference showed significant differences ($p \leq 0.05$) when the interaction of Kelpak® treatments and bulb age were evaluated. Age-dependently, older bulbs exhibited the highest pre-plant circumference among treatment combinations (Table 2). In determining the circumferential coefficient, younger bulbs in years 1 and 2 (1.2–1.5) had significantly higher marked responses to treatment interactions, whereas bulbs in years 3, 4, and 5 maintained their circumferential size with a coefficient of approximately 1.0 (Figure. 1, Table 2). The highest coefficient was observed in the 0.2%–year 1 treatment. In arriving at a more synthetic conclusion, the bulb circumference coefficient was evaluated irrespective of Kelpak® treatments and bulb age (Figure 2, Table 2). The findings indicated that the circumference coefficient in Kelpak®-treated bulbs had comparably fewer discernible differences between the 0.2%, 0.4%, and 1% treatments. Nevertheless, the 1% application (1.3) presented the most significant improvement ($p \leq 0.05$) in comparison to the untreated application (1.1) of tap water. Within bulb age, the coefficient decreased significantly with advanced aging, where the greatest coefficient was observed in bulbs from year 1 (1.4). Expansive bulb growth was either maintained or enhanced, culminating in a circumference coefficient of 1.0 or higher within each of the five years.

Table 2. Interactive effects of Kelpak® dilutions and bulb age on the bulb and root characteristics of *A. belladonna* bulbs. Mean values ± standard error (S.E.) in the same column with a different letter(s) are significantly different at $p \leq 0.05$ (*) based on Tukey's least significant difference test; ns = not significant.

Bulb age	Kelpak® treatment	Bulb characteristics				Root characteristics	
		Pre-plant circumference (cm)	Circumference coefficient	Pre-plant fresh weight (g)	Weight coefficient	Number of roots (n)	Root length (cm)
Year 1	0.0% (control)	2.5 ± 0.05 f	1.3 ± 0.04 bcd	0.5 ± 0.02 c	3.2 ± 0.48 e-h	4.9 ± 0.31 bc	6.1 ± 0.38 i
	0.2%	2.4 ± 0.05 f	1.5 ± 0.09 a	0.5 ± 0.01 c	6.7 ± 0.63 ab	5.2 ± 0.25 bc	8.0 ± 0.87 ghi
	0.4%	2.5 ± 0.04 f	1.4 ± 0.04 ab	0.5 ± 0.02 c	6.5 ± 0.66 abc	5.9 ± 0.38 b	7.7 ± 0.54 ghi
	1%	2.5 ± 0.04 f	1.3 ± 0.05 bcd	0.5 ± 0.02 c	6.5 ± 0.67 abc	5.3 ± 0.37 bc	8.7 ± 0.58 ghi
Year 2	0.0% (control)	2.7 ± 0.07 ef	1.2 ± 0.03 b-f	0.7 ± 0.03 c	4.8 ± 0.67 b-e	5.9 ± 0.38 b	7.2 ± 0.44 hi
	0.2%	2.9 ± 0.09 def	1.2 ± 0.06 c-g	0.7 ± 0.03 c	4.0 ± 0.61 d-g	4.5 ± 0.27 bc	6.0 ± 0.62 i
	0.4%	2.8 ± 0.08 def	1.3 ± 0.03 b-e	0.6 ± 0.04 c	6.1 ± 0.38 a-d	5.8 ± 0.36 b	7.6 ± 0.41 ghi
	1%	2.7 ± 0.09 ef	1.3 ± 0.04 abc	0.6 ± 0.04 c	7.2 ± 0.66 a	6.1 ± 0.31 b	9.7 ± 0.62 f-i
Year 3	0.0% (control)	4.0 ± 0.17 d	1.1 ± 0.03 e-h	1.5 ± 0.13 c	2.1 ± 0.30 gh	4.9 ± 0.23 bc	14.3 ± 1.44 d-g
	0.2%	4.0 ± 0.17 de	1.1 ± 0.03 e-h	1.5 ± 0.12 c	3.6 ± 0.33 e-h	4.5 ± 0.22 bc	10.1 ± 0.58 e-i
	0.4%	3.9 ± 0.20 de	1.2 ± 0.06 b-f	1.4 ± 0.12 c	4.5 ± 0.41 c-f	5.2 ± 0.25 bc	10.9 ± 0.82 d-i
	1%	3.9 ± 0.14 de	1.1 ± 0.04 d-h	1.4 ± 0.13 c	4.3 ± 0.36 def	5.3 ± 0.26 bc	13.2 ± 1.16 d-g
Year 4	0.0% (control)	5.3 ± 0.21 c	1.0 ± 0.02 fgh	3.3 ± 0.29 c	2.0 ± 0.15 gh	3.8 ± 0.36 c	16.7 ± 1.94 cde
	0.2%	5.9 ± 0.22 c	1.1 ± 0.01 e-h	3.9 ± 0.40 c	2.5 ± 0.13 fgh	5.3 ± 0.34 bc	15.5 ± 1.33 c-f
	0.4%	5.6 ± 0.35 c	1.1 ± 0.03 d-h	3.6 ± 0.66 c	3.7 ± 0.28 e-h	5.2 ± 0.29 bc	16.7 ± 1.42 cde
	1%	5.9 ± 0.34 c	1.1 ± 0.03 d-h	4.5 ± 0.68 c	2.8 ± 0.15 e-h	6.0 ± 0.49 b	17.1 ± 1.74 cd
Year 5	0.0% (control)	12.7 ± 0.40 a	1.0 ± 0.01 h	34.3 ± 3.30 ab	1.8 ± 0.05 h	9.3 ± 0.47 a	31.4 ± 2.79 a
	0.2%	13.5 ± 0.40 a	1.0 ± 0.01 h	39.7 ± 2.80 a	1.8 ± 0.10 h	9.8 ± 0.39 a	27.9 ± 1.69 a
	0.4%	11.4 ± 0.38 b	1.0 ± 0.02 fgh	28.9 ± 2.38 b	1.9 ± 0.10 gh	8.7 ± 0.65 a	27.1 ± 2.24 ab
	1%	13.5 ± 0.46 a	1.0 ± 0.02 h	38.0 ± 3.53 a	1.7 ± 0.10 h	9.8 ± 0.71 a	21.1 ± 1.34 bc
Two-way ANOVA *F-Statistic*							
Kelpak® treatment		5.06 *	4.78 *	2.70 *	19.38 *	3.71*	1.43 ns
Bulb age		1248.64 *	62.05 *	479.01 *	65.74 *	94.13 *	146.20 *
Kelpak® * Bulb age		3.53 *	2.97 *	2.35 *	4.26 *	2.34 *	3.39 *

Pre-plant bulb fresh weight and weight coefficient: Analysis of variance of the factorial interaction of Kelpak® treatments and bulb age demonstrated significantly different ($p \leq 0.05$) responses in the pre-plant bulb fresh weight. The preliminary evaluation found greater biomass in the oldest bulbs from the onset, whereas in bulbs classified in the first four years of cultivation, no statistically marked differences were observed (Table 2). The weight coefficient in response to treatment combinations showed a statistically significant similarity of improved growth as bulbs were heavier than their initial accumulated weight. The younger bulbs in years 1 and 2 had the highest coefficient when treated with the higher Kelpak® applications of 0.4 and 1%, respectively. In all treatment combinations, the coefficient was greater than the corresponding control within each year. Moreover, as the age of the bulb increased, an overall decline in the weight coefficient was seen, with the 1% Kelpak®–year 5 treatment presenting the lowest overall value (Figure 1, Table 2). As shown in Figure 3, the bulb weight coefficient was further assessed unrelated to the Kelpak® treatments and bulb age for a more thorough analysis. A significant increase in bulbs treated with Kelpak® was found with the optimum weight coefficient observed in the 0.4% and 1% (4.5) treatment applications, markedly 1.6 times greater than the 0% (2.8) use of tap water. The weight coefficient decreased significantly as the comparative bulb age increased. Notably, the response was particularly marked for bulbs in year 1 (5.7) with an approximate three times higher coefficient than year 5 (1.8).

Number of roots: The combination of factors of Kelpak® and bulb age found a significantly pronounced difference in the number of contractile roots initiated at a 95% confidence level. Root numbers were highest in Kelpak®-treated bulbs categorized in year 5, whereas minimal statistically marked variations were observed in bulbs from years 1 through 4 (Figure 1, Table 2). In establishing a systematic representation, the main effects of Kelpak® treatments and bulb age were independently evaluated (Figure 4, Table 2). Kelpak® treatment comparisons promoted the induction of significantly more roots, with the higher concentrations of 0.4% and 1% producing 6.2 and 6.5 roots, respectively. The 0% tap water treatment recorded the lowest root formation (5.8); however, it was statistically negligible compared to the 0.2% (5.9) treatment. The results of bulb age displayed a significantly higher number of newly generated roots in the year 5 cultivated bulbs compared to the bulbs in years 1 through 4, which exhibited statistically similar tendencies.

Root length: Significant variability ($p \leq 0.05$) was observed when the factorial interaction of Kelpak® dilutions and bulb age on the length of bulb roots was evaluated. Newly cultivated and treated bulbs in years 1 and 2 had markedly shorter (6.1–9.7 cm) root lengths compared to the older bulbs in years 3, 4, and 5 (10.1–31.4 cm), with the year 5 bulbs producing the longest roots (Figure 1, Table 2). The results of the root length extension, irrespective of Kelpak® treatments and bulb age, as presented in Figure 5, showed otherwise. Root length was unaffected ($p >$ 0.05) by the dilutions of Kelpak®, and the soil drench applications did not augment this characteristic bulb feature (Figure 5, Table 2). However, the longest roots were observed in the 0% control application (15.2 cm). In contrast, an age-dependent significant variation was seen in the oldest bulbs in year 5, exhibiting the longest roots (26.9 cm) compared to the shortest ones (7.6 cm) observed in the bulbs from the first two years of cultivation.

Number of leaves: Evaluating the interactive effect of Kelpak® treatments and bulb age, a significant difference was found in the number of leaves formed, where the oldest Kelpak® treated bulbs produced the greatest number of leaves (6.6–7.2) in comparison to those treated from the first year of cultivation (2.6–2.7) (Table 3). Analysis of the autonomous results of the main effects showed that the formation of leaves in bulbs treated with Kelpak® significantly increased in a concentration-dependent manner (Figures 6 and 7, Table 3). Compared to the 0% application (3.8), the 1% dosage yielded the most leaves (4.6). Categorically within bulb age, the number of leaves increased significantly, with the oldest bulbs in year 5 producing more leaves (6.9) than their younger counterparts (2.6–4.5).

Leaf length, leaf width, and leaf area: The extension of leaf length in treated bulbs varied significantly ($p \leq$ 0.05) when the factorial combination of Kelpak® treatments and bulb age was examined. Leaf length across treatment interactions ranged from 24.7 to 37.8 cm. Bulbs treated with 0.4% and 1% Kelpak® dilutions typically performed better (Table 3). The interaction of factors significantly enhanced the response of leaf width expansion. Compared to their younger counterparts (years 1 and 2), whose leaves expanded to a maximum width of 0.7 cm, the oldest bulbs in year 5 exhibited the broadest leaf primordia, measuring more than 2 cm when treated with

Kelpak® (Table 3). Considering the leaf area, a significant interaction between Kelpak® treatments and bulb age was observed, with the older treated bulbs in year 5 (73.0–85.8 cm^2) producing a larger surface area in comparison to the younger cultivated bulbs in years 1 and 2 (18.4–23.3 cm^2) (Table 3).

Significant variations were observed in the leaf area when the main effects were evaluated independently (Figures 6 and 8, Table 3). When comparing Kelpak® applications, the lower 0%, 0.2%, and 0.4% concentrations presented smaller numerical values (34.0–38.7 cm^2) than the 1% application (41.4 cm^2). The leaf area decreased significantly as the relative age of the bulbs advanced, with those in years 3, 4, and 5 invariably 1.3–4.0 times greater than those in years 1 and 2, respectively.

Effect of Kelpak® treatments and bulb age on chlorophyll content

Chlorophyll content: At a 95% confidence level, the combined effect of Kelpak® and bulb age on the presence of chlorophyll found in the regenerated leaf blades was significantly enhanced. Compared to the untreated bulbs, applying 1% Kelpak® produced a markedly higher chlorophyll content in the leaves in all five bulb age groups (Table 3). Considering the autonomous monitoring of Kelpak® treatment and bulb age, Kelpak® comparisons significantly enhanced the chlorophyll synthesis in bulb leaves in a concentration-dependent manner (Figure 9; Table 3). The highest Kelpak® dilution application of 1% (407.4 mg/m^2) resulted in an optimal chlorophyll content per unit area, an almost 1.7-fold improvement over the 0% (242.2 mg/m^2) soil drench. Within the progressive aging of the bulbs, the accumulated chlorophyll content in the regenerated leaves significantly decreased. Bulbs in year 1 accumulated the highest concentration (325.6 mg/m^2), which was only marginally greater than the bulbs in year 2 (318.8 mg/m^2). In comparison, the oldest cultivated bulbs in year 5 recorded the lowest chlorophyll value (265.6 mg/m^2) (Figure 9).

Table 3. Interactive effects of Kelpak® dilutions and bulb ages on the leaf characteristics and chlorophyll content of *A. belladonna* bulbs. Mean values ± standard error (S.E.) in the same column with a different letter(s) are significantly different at $p \leq 0.05$ (*) based on Tukey's least significant difference test; ns = not significant.

Bulb age	Kelpak® treatment	Leaf characteristics				
		Number of leaves (n)	Leaf length (cm)	Leaf width (cm)	Leaf Area (cm^2)	Chlorophyll content (mg/m^2)
Year 1	0.0% (control)	2.6 ± 0.16 f	29.2 ±1.99 c-g	0.7 ± 0.03 f	19.7 ± 1.79 de	284.6 ± 23.70 cef
	0.2%	2.6 ± 0.16 f	27.4 ± 1.63 d-g	0.7 ± 0.03 f	18.4 ± 1.35 e	218.6 ± 14.30 ef
	0.4%	2.6 ± 0.16 f	9.4 ± 0.96 c-g	0.7 ± 0.03 f	20.2 ± 1.16 de	405.2 ± 5.95 abc
	1%	2.7 ± 0.15 ef	30.7 ± 1.31 b-g	0.7 ± 0.03 f	19.9 ± 1.18 de	393.8 ± 36.20 a-d
Year 2	0.0% (control)	2.8 ± 0.16 def	27.8 ± 1.72 d-g	0.7 ± 0.03 f	18.4 ± 1.94 e	279.6 ± 26.00 def
	0.2%	3.1 ± 0.18 def	29.6 ± 1.72 c-g	0.8 ± 0.04 ef	22.4 ± 1.94 cde	268.0 ± 26.00 ef
	0.4%	3.1 ± 0.14 def	31.3 ± 1.64 a-f	0.7 ± 0.03 f	21.3 ± 1.57 cde	307.4 ± 24.50 b-e
	1%	3.4 ± 0.22 c-f	31.3 ± 1.38 a-f	0.7 ± 0.05 ef	23.3 ± 2.35 cde	420.2 ± 19.30 ab
Year 3	0.0% (control)	3.2 ± 0.13 c-f	23.9 ± 0.67 g	0.9 ± 0.04 def	20.9 ± 2.26 cde	213.4 ± 19.50 ef
	0.2%	3.9 ± 0.23 cd	27.1 ±1.41 efg	0.9 ± 0.05 def	25.0 ±1.71 cde	210.8 ± 43.60 ef
	0.4%	3.5 ± 0.22 c-f	31.0 ± 1.06 a-f	1.1 ± 0.04 bcd	34.6 ± 2.25 bcd	240.4 ± 12.00 ef
	1%	4.3 ± 0.21 bc	34.4 ± 1.02 a-d	1.0 ± 0.05 cde	34.6 ± 1.98 bcd	397.6 ± 12.60 a-d
Year 4	0.0% (control)	3.6 ± 0.16 c-f	24.7 ± 1.21 fg	1.0 ± 0.05 cde	25.3 ± 2.21 cde	224.6 ± 4.65 ef
	0.2%	3.8 ± 0.20 cde	28.4 ±0.89 c-g	1.2 ± 0.07 bc	36.1 ±1.99 bc	185.6 ± 21.90 f
	0.4%	5.1 ± 0.23 b	33.8 ± 1.27 a-e	1.3 ± 0.07 bc	44.1 ± 3.78 b	246.4 ± 15.50 ef
	1%	5.4 ± 0.16 b	32.5 ± 1.07 a-e	1.3 ± 0.04 b	43.2 ± 2.45 b	429.0 ± 19.10 a
Year 5	0.0% (control)	6.8 ± 0.36 a	37.8 ± 1.69 a	2.2 ± 0.08 a	85.5 ± 6.33 a	208.6 ± 28.00 ef
	0.2%	7.2 ± 0.36 a	33.1 ± 1.24 a-e	2.2 ± 0.13 a	73.32 ± 6.45 a	254.6 ± 25.00 ef
	0.4%	6.6 ± 0.16 a	35.0 ± 1.66 abc	2.1 ± 0.09 a	73.0 ± 4.47 a	250.4 ± 28.00 ef
	1%	7.2 ± 0.33 a	37.2 ± 1.19 ab	2.3 ± 0.07 a	85.8 ± 3.43 a	396.4 ± 22.90 a-d
Two-way ANOVA *F-Statistic*						
Kelpak® treatment		11.49 *	13.03 *	3.58 *	6.38 *	5.86 *
Bulb age		245.48 *	16.89 *	469.41 *	268.01 *	61.32 *
Kelpak® * Bulb age		3.53 *	2.95 *	2.28 *	3.41 *	2.73 *

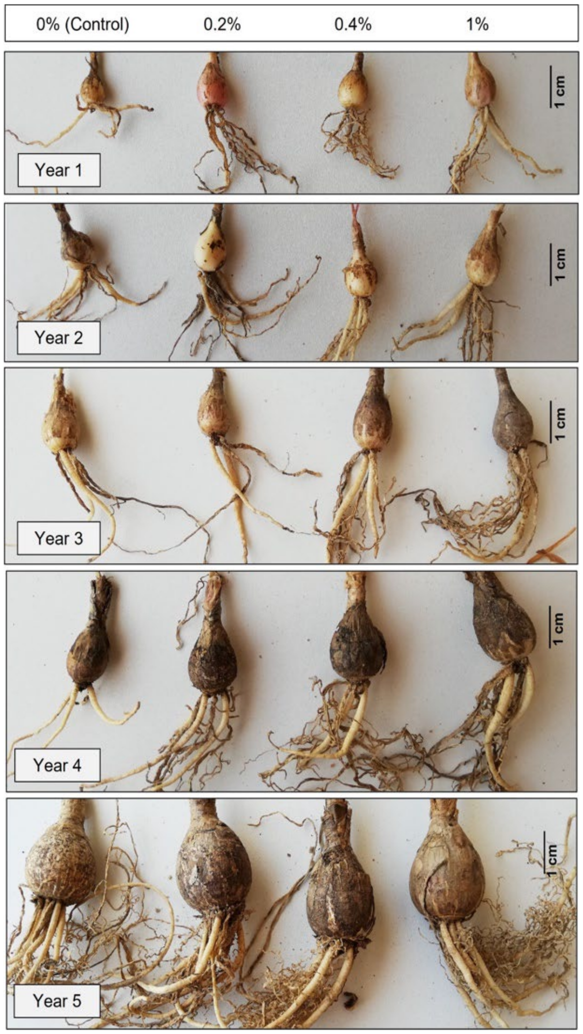

Figure 1. The postharvest visible effects of Kelpak® concentration dilutions on bulb growth and development on a series of five growing seasons (age) of *A. belladonna* bulbs after 24 weeks of treatment (Bar = 1cm). (Photo: C Wilmot).

DISCUSSION

Several studies have demonstrated the innumerable, all-round benefits of seaweed extracts and their improvement on nutrient signalling, root system enhancement, crop optimisation, yield, and tolerance to abiotic and biotic stresses in plant systems (Calvo et al. 2014; Battacharyya et al. 2015; Ali et al. 2021a). In addition, improved seed germination yield, reduced seed dormancy, seedling establishment, transplant shock, flowering, fruit palatability and quality, increased chlorophyll production and foliage area, delayed senescence, improved storage ability and resistance to pests and pathogens have been reported (Sharma et al. 2014; Li and Mattson 2015; Kapur et al. 2018; Ali et al. 2019).

This study observed the beneficial impact of Kelpak® applications in monitoring bulbs' morphological and physiological characteristics from five consecutive seasons (age) and their interactions over 24 weeks. Even at low concentrations, Kelpak® treatments enhanced the responses in both the bulb aerial and, more substantially, the underground storage organs in a concentration-dependent manner. These findings substantiate those of Robertson-Andersson et al. (2006), Papenfus et al. (2013), and Michalak et al. (2017), who found that the concentration of seaweed extracts affected their efficiency, and in most instances, low concentrations augmented plant developmental characteristics. The low concentrations further support Duncan's (2010) claim that indigenous bulbous species benefit from low Kelpak® application levels. Contractile roots are an important component of geophytes because, upon contraction, the fleshy roots effectively enable them to lower themselves deeply into the earth and provide support during seasons of overwintering and adversity (Halevy 1986; Warrington et al. 2011). Furthermore, root formation improves bulb longevity and survival (Kharrazi et al. 2017). Although root length remained unaffected, the contractile roots increased due to treatment applications. Kelpak® was also found to shorten the root length in *Eucalyptus* species (Van Staden et al., 1995) and increase the root density of *Tagetes erecta* (Crouch and Van Staden 1991). In contrast to these findings, Adams et al. (2019) found that Kelpak® treatments enhanced root length while minimising fresh root weight in *E. verticillata*.

The highly active photosynthetic processes in chlorophyll-rich leaves are evidenced by a plant's robust physiological response of increased chlorophyll concentration, visibly enhanced greenness, and observable delays in leaf senescence (Adams and Langton 2005; Wang et al. 2005). This study found these mechanisms at the higher levels of Kelpak® applications. Furthermore, the increased number of leaves, leaf area, and longer duration of vegetative growth, and thus a greater timeframe of carbohydrate accumulation, as demonstrated by the larger bulb circumference and weight coefficients after treatments, support this. Peng et al. (1991) found that faster vegetative development and higher biomass production are the outcomes of improved photosynthetic effectiveness. Similarly, Byczyńska (2018) found that pre-soaking *E. bicolor* bulbs in a

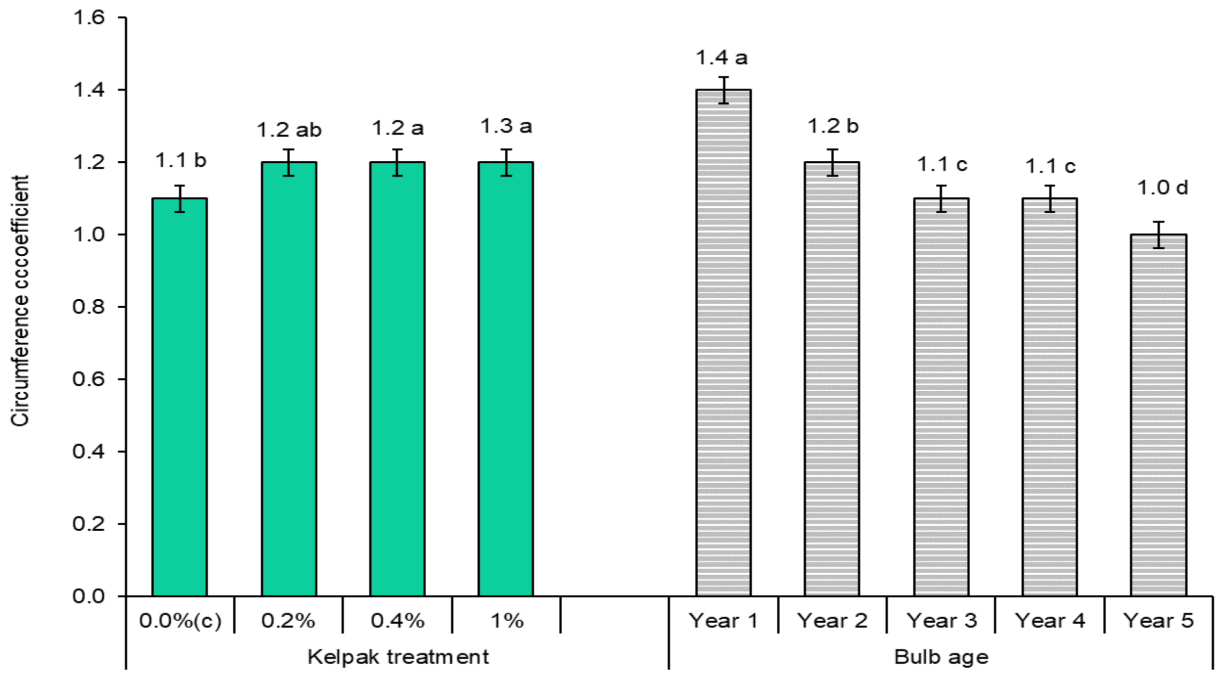

Figure 2. The circumference coefficient analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

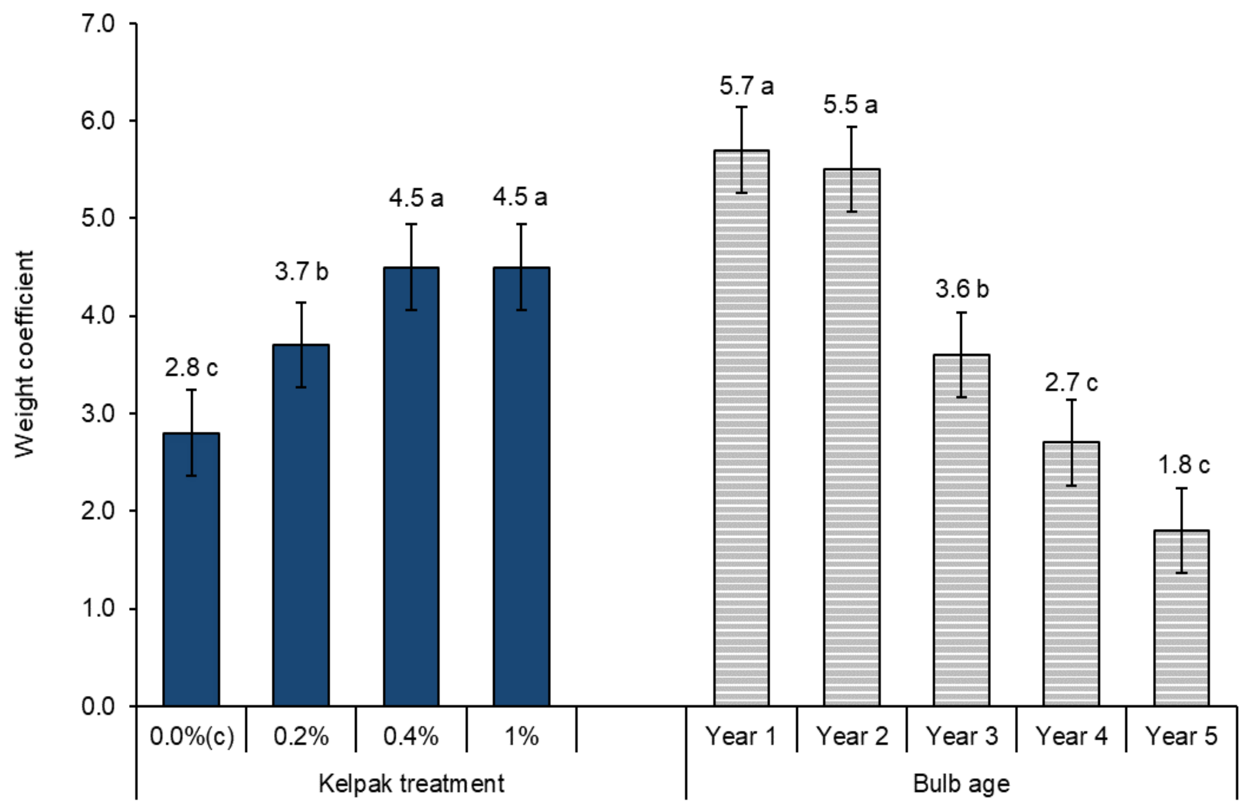

Figure 3. The weight coefficient analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

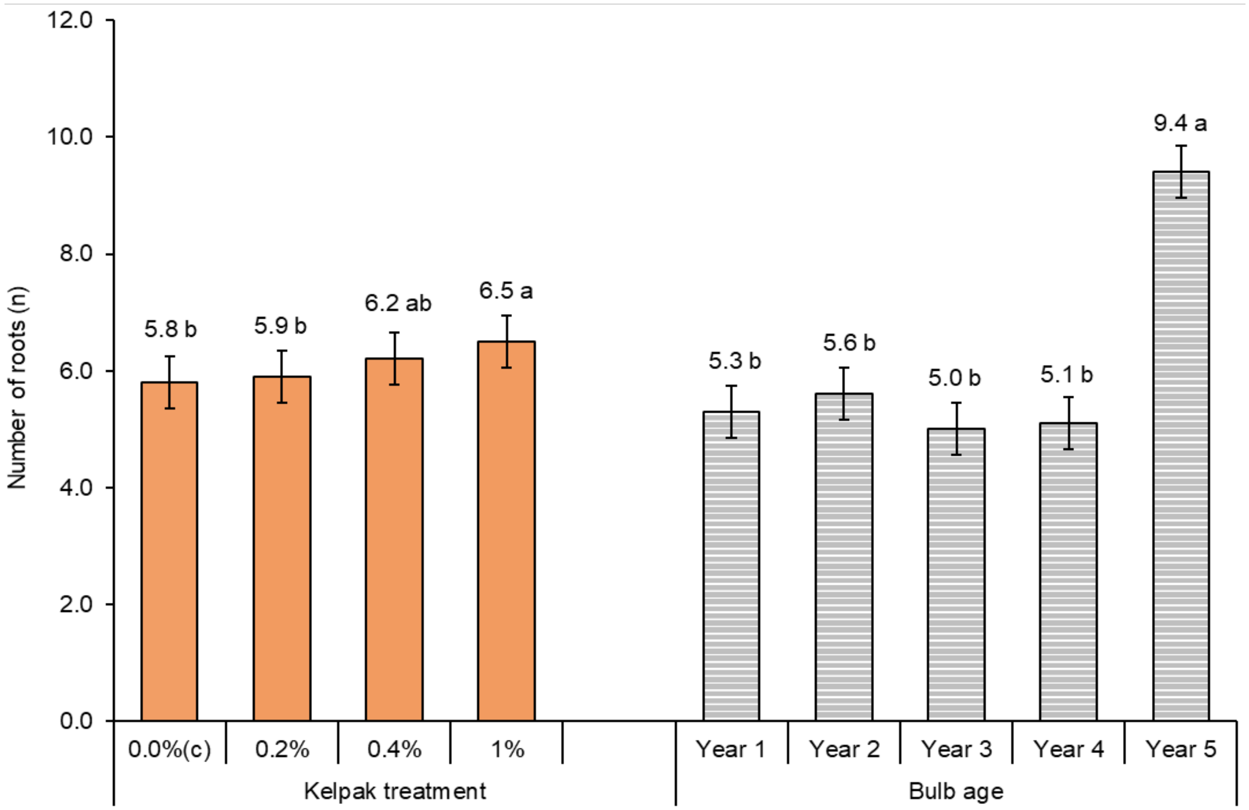

Figure 4. The number of roots analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

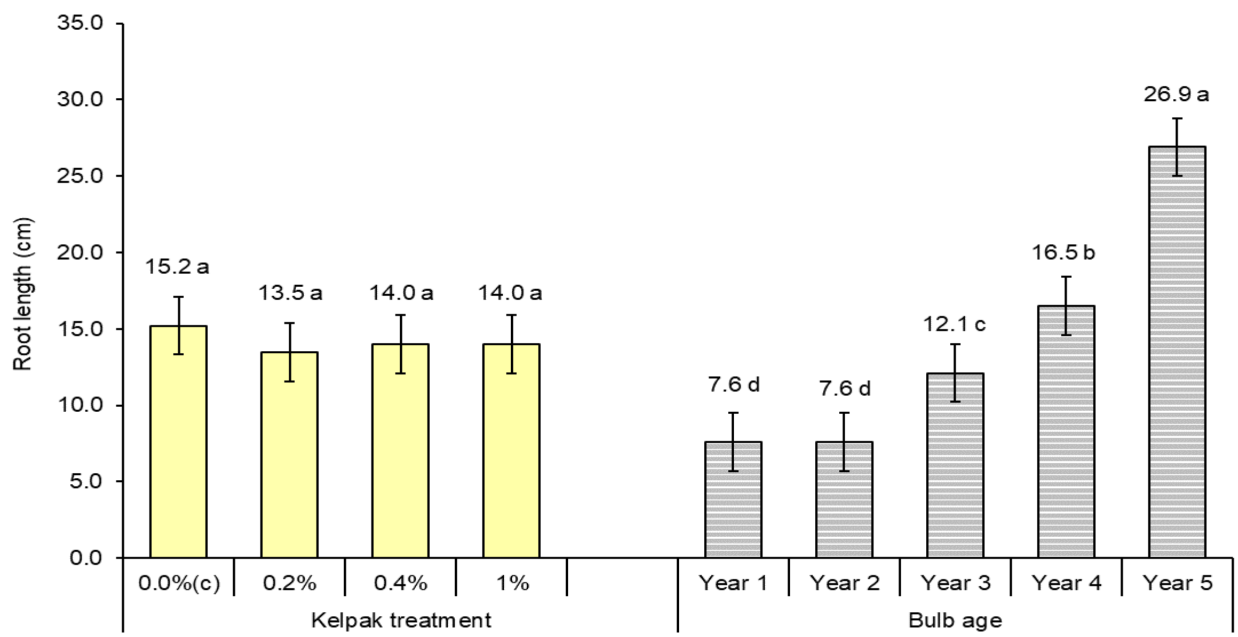

Figure 5. The root length analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

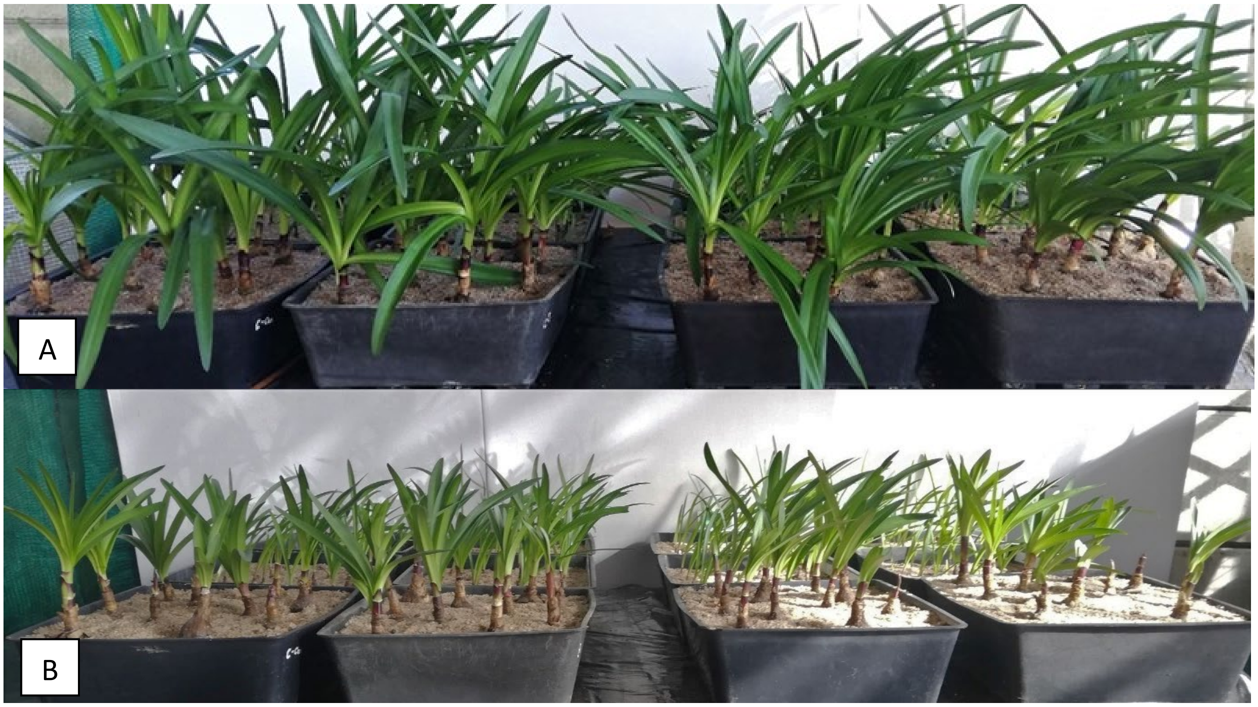

Figure 6. The visible effects of Kelpak® concentration dilutions on leaf expansion and development in a series of five growing seasons (age) of *A. belladonna* bulbs, after 8 weeks (A) and 13 weeks (B). (Photos: C Wilmot)

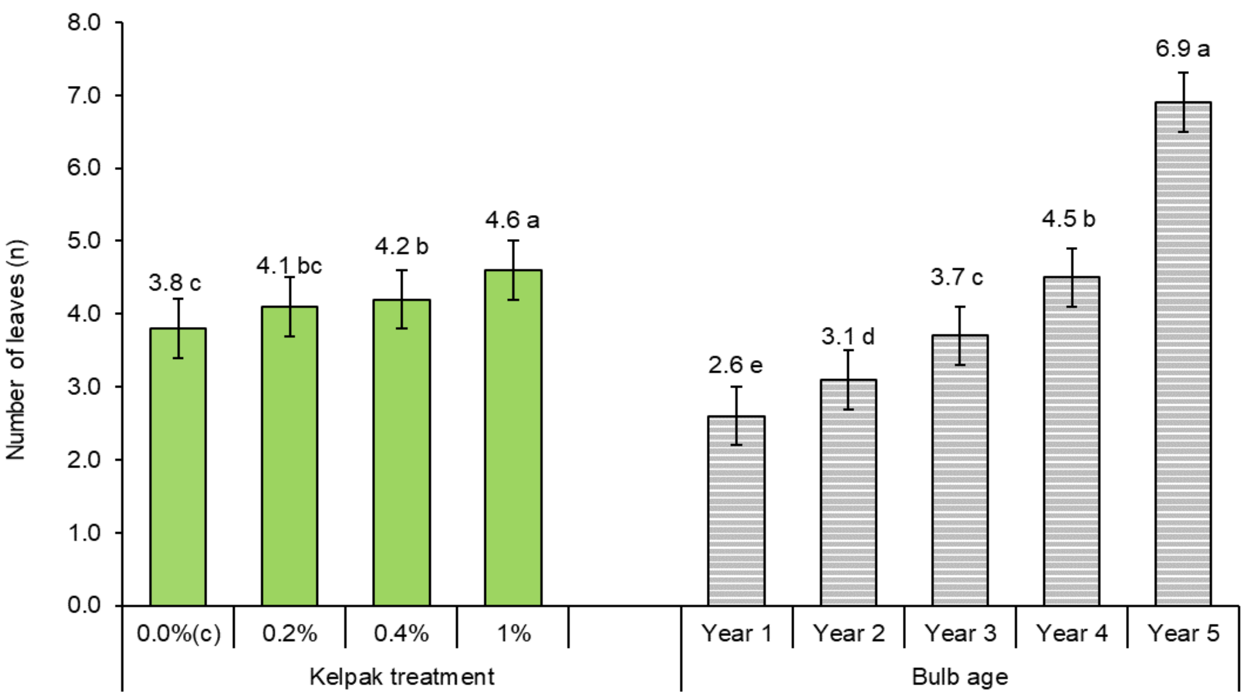

Figure 7. The number of leaves analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

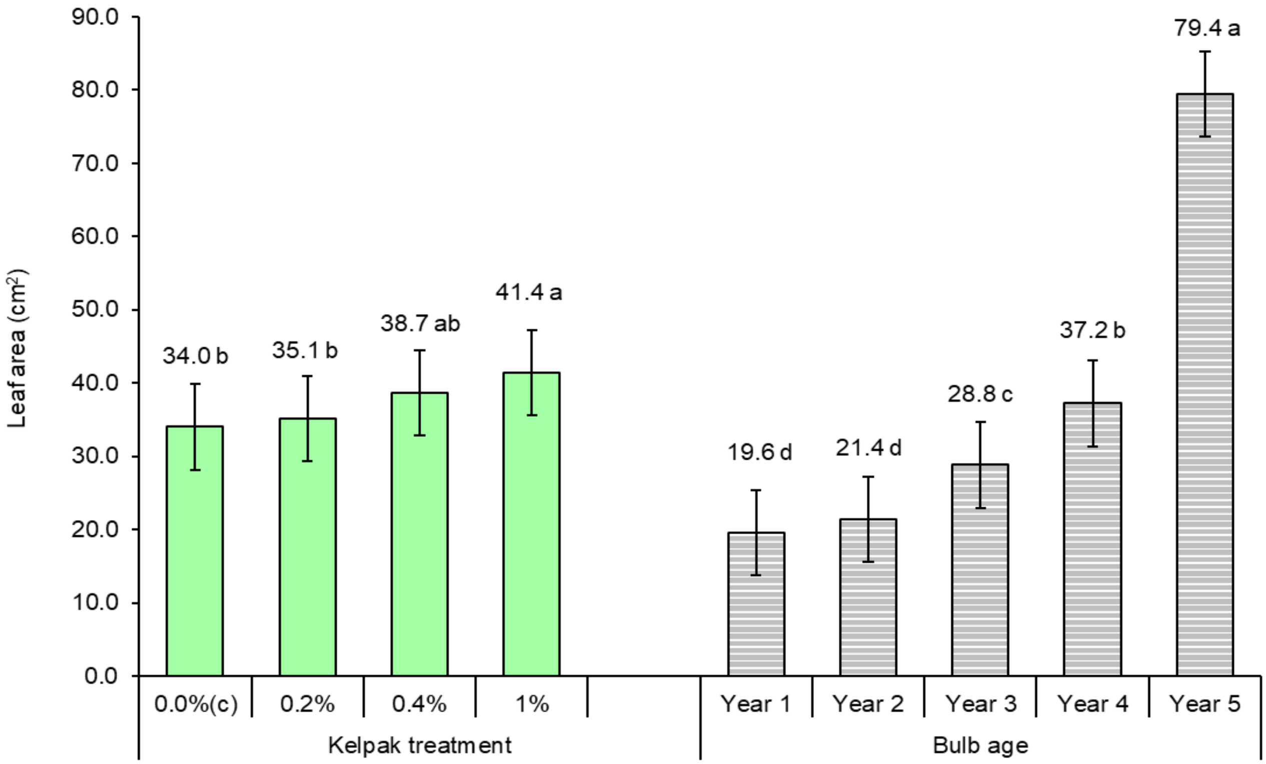

Figure 8. The leaf area analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

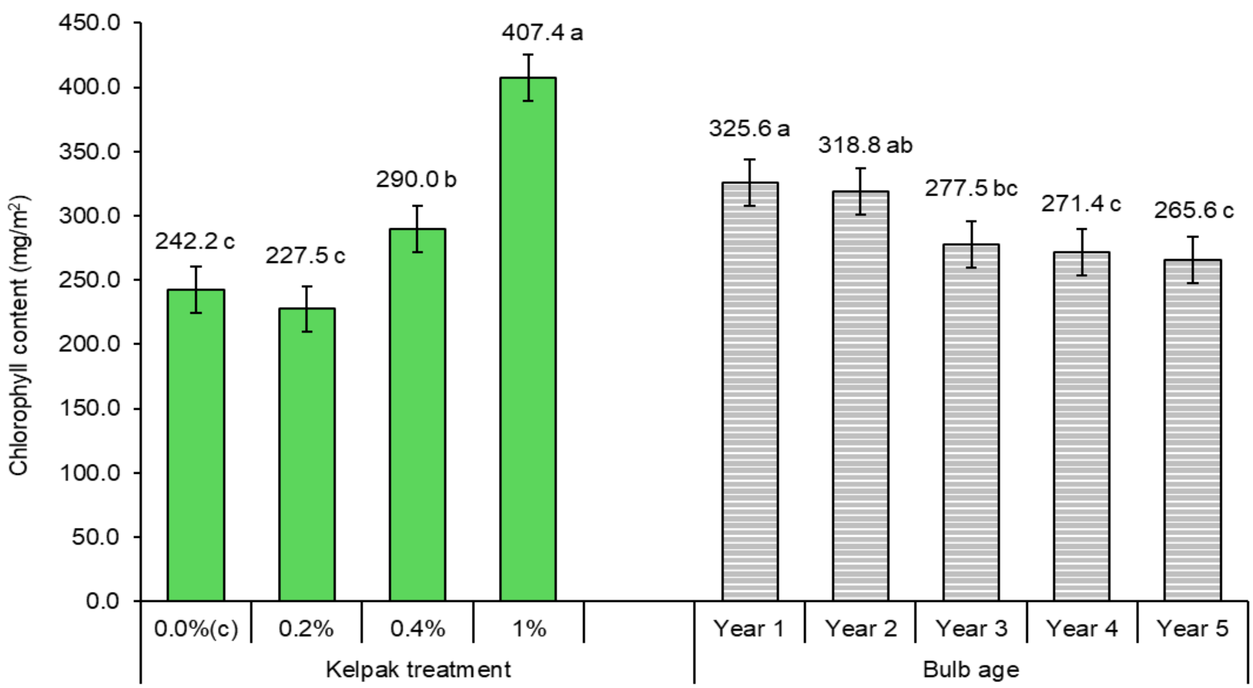

Figure 9. The chlorophyll concentration analysed irrespective of bulb age and irrespective of Kelpak® treatment. Bars represented by mean values followed by different letter(s) are significantly different at $p \leq 0.05$ based on Tukey's least significant difference test.

SWE before planting improved development, flowering, yield, and increased number of leaves and chlorophyll content upon harvest.

With the advanced aging of bulbs over five consecutive years, the research findings of treatment applications elicited a culmination of stimulatory, neutral, and inhibitory responses. Considering the natural progressive morphological processes modulated by the indicative age of bulbs and resumed growth each season, bulbs from year 5 habitually had the highest values of pre-plant bulb fresh weight and circumference, root length and number, and leaf length, width, and area and number in comparison to their younger counterparts in an age-dependent manner. These findings support work by Halevy (1990) and Langens-Gerrits et al. (2003) that decreased morphological trait values indicate plant age and juvenility. According to De Hertogh & Le Nard (1993) and Kapczyńska (2019), the number of leaves and the relation to plant juvenility are closely linked. Moreover, the duration of the non-flowering juvenile phase to reach a critical bulb size varies by genus, species, and even cultivar (De Hertogh and Le Nard 1993) and is further influenced by the surrounding environmental growth conditions (Du Toit et al., 2001; Khodorova & Boitel-Conti, 2013; Kapczyńska, 2014; Anderson, 2019).

In further examination of the bulb circumference and weight coefficients to Kelpak® treatments, bulbs in years 1 and 2 were the most responsive. As a species with a tunicate bulb, the dry, papery tunic protects the bulb from desiccation by improving water retention and mechanical damage (Al-Tardeh et al., 2008). These membrane-forming exterior scales shield the continuous lamina of interior fleshy scales that are tightly pressed together (Mishra 2005). The coefficient gains found in bulbs from years 1 and 2 suggest that the membranous tissues of the younger juvenile bulbs were significantly more permeable and, therefore, more receptive to treatment applications, which presumably explains the stimulatory effect and synthesis of exogenous Kelpak® and the presence of phytohormones in bulb growth and development. However, the weight coefficient produced results with greater dynamics than the circumference coefficient. Seaweed extracts routinely accelerate and promote the growth, differentiation, and synthesis of new proteins in plant cells (El-Sheekh et al. 2016). Similar stimulatory effects of both the aerial and subterranean organs were observed in four-month-old juvenile bulb seedlings of *E. autumnalis* (Aremu et al. 2016). Furthermore, it was found that active surface areas and bulb sizes of species were enhanced by the compounds isolated from the seaweed *E. maxima* (Aremu et al. 2015). The early establishment of strong genotypes from conceivably unknown genetic backgrounds, uniformity, and increased yields are required to meet bulb grading standards for commercial purposes (Anderson 2006; Barnhoorn 2013). The coefficients may support the quantitative expression of these quality attributes as a selection tool for achieving these outcomes. Further evidence of the stimulatory impact and receptivity of younger bulbs to treatment applications emerged from the findings of increased chlorophyll content in proportion to leaf area. The juvenile bulbs in years 1 and 2 had reduced leaf surface areas and higher chlorophyll levels, which was the exact opposite of the older bulbs, which had higher leaf areas and reduced levels of chlorophyll.

Although the circumference and weight coefficients, as well as chlorophyll content, were significantly lower in the older bulbs of years 3, 4, and 5, they were compensated for by the relatively higher root number, root length, leaf number, leaf length, width, and area, which indicated the time and energy required to regenerate new growth and development after replanting. This is further supported by visual observatio1ns of above-ground leaf development in older bulbs, specifically those in year 5, which were the last to show signs of leaf growth and expansion. According to Stancato et al. (1995) and Khodorova and Boitel-Conti (2013), during the initial stages of recommenced growth, bulbs consume stored nutritional reserves in the form of carbohydrates to support the regenerative growth of roots and shoots, which results in a reduction of size, biomass, and firmness; however, once this is attained, the expansion of bulb organs begin to increase due to photosynthetic activities. The regeneration and accumulation of carbohydrates thus determine the precise sequence of dormancy, development, and flowering (Miller, 1992). Similar delayed growth and subsequent development findings were found in replanted bulbs of *Hippeastrum* hybrids (Stancato et al. 1995; Andrade-Rodríguez et al. 2015) and *Nerine sarniensis* (Warrington et al. 2011). The disruption of bulb growth and the time allotted for rejuvenation support Duncan's (2010) recommendation that the frequent lifting and replanting of *A. belladonna* bulbs be limited to avoid disturbing this slow-growing species. Furthermore, the bulb should be cultivated as a perennial crop like that of *N. sarniensis* (Warrington et al., 2011), with lifting performed after

4–6 years or when the bulbs become overcrowded (Duncan, 2010). According to Ali et al. (2021b), SWE administration enhanced growth and development at all plant stages, including harvest and post-harvest. The finding supports this study's results, demonstrating enhanced growth in all five age groups. In furthering research, it may be beneficial to investigate whether administering potentially higher concentrations of Kelpak® to older bulbs will result in more effective geophilic structural size and vigour outcomes. Kelpak® dilutions of up to 5% in *E. autumnalis* (Aremu et al., 2016) and 10% in three *Eucalyptus* species were efficacious (Van Staden et al., 1995).

The juvenile bulbs, particularly the younger samplings, exhibited improved underground bulbing capabilities and root system architecture following treatment applications. It is, therefore, probable that Kelpak® would not only elicit phyto-stimulatory qualities but also improve the phyto-elicitor activity by inducing responses that would have enabled them to withstand the severe climatic conditions of drought, high temperatures, and salinity during the dormancy period (Battacharyya et al., 2015; Drobek et al., 2019; Stirk et al., 2020; Ali et al., 2021a). Van Staden et al. (1995) and Aremu et al. (2012) advocate investigating propagation and cultivation strategies that promote early plant optimization and performance that potentially afford them a greater chance of survival and adaptation to circumstances within the environment during acclimatization and nursery production. Using seaweed extracts facilitates a simple and affordable multipurpose cultivation technique for commercial and small-scale farmers (Van Staden et al., 1995; Aremu et al., 2016; Makhaye et al., 2021). Moreover, plants respond favourably to treatments as early as 10–14 days after administration (Arioli et al., 2015). Before widespread implementation is adopted, it is essential to assess bulbs' susceptibility to treatment regimes (Warrington et al., 2011). Furthermore, since ornamental bulbs have very diverse behaviours and responses to outside influences, it is crucial to understand their specific growth and physiological developmental cycles (Theron and de Hertogh 2001; Kleyhans 2006; Khodorova and Boitel-Conti 2013; Kamenetsky Goldstein 2019; Kapczyńska and Stodolak 2019). This was evident in the study, in which older bulbs responded differently to treatment applications than their younger counterparts. While the recommendations are commendable, the species responses and the receptive effects of age during cultivation must be evaluated as part of ongoing LCA cultivation efforts to optimise bulb plant material for sustainable commercial planting and production.

CONCLUSION

The current study concluded that exogenous applications of Kelpak® given as a soil drench at various ages significantly improved the morphological and physiological responses in juvenile *A. belladonna* bulbs. In a concentration-dependent manner, the low-dosage treatments increased the phyto-stimulatory responses of both the bulb aerial and, more evidently, the below-ground storage organs. The bulb circumference, weight coefficients and chlorophyll content evaluation identified bulbs in years 1 and 2 as most receptive to treatment applications. Furthermore, this study established the significance of the morpho-physiological responses of the species to Kelpak® treatment and the receptive impact of age during cultivation. A 1% Kelpak® concentration dilution administered during early developmental stages within the first two years is the most advantageous priority for maximising this slow-growing species' proliferation rate. Based on these findings, further investigative research on these aspects is relevant to fully elucidate and broaden the mechanism of action and species receptivity to treatments.

FUNDING

The Research Directorate, Centre for Postgraduate Studies, Consolidated Research Fund (CRF-RJ17), Postgraduate Bursary Fund, and the Department of Horticultural Sciences of the Cape Peninsula University of Technology are gratefully acknowledged for funding and supporting this study.

ACKNOWLEDGEMENTS

The authors would like to thank Assegaaibosch Farm on the Agulhas Plain for their generosity and donation of plant material for this study.

DATA AVAILABILITY STATEMENT

All data are included in the manuscript.

ETHICAL CLEARANCE

This study was approved by the Office of the Chairperson, Research Ethics Committee, Faculty of Applied Sciences, Cape Peninsula University of Technology. Reference no: 205054196/06/2020.

AUTHOR CONTRIBUTIONS

Conceptualisation, C.M.W., M.O.J. and C.P.L.; methodology, C.M.W. and C.P.L.; software, M.O.J. and C.M.W.; validation, C.P.L. and M.O.J.; formal analysis, C.M.W. and M.O.J.; investigation, C.M.W. and C.P.L.; resources, C.M.W., C.P.L.; data curation, M.O.J. and C.M.W.; writing-original draft preparation, C.M.W.; writing, review and editing, C.M.W., M.O.J., and C.P.L.; supervision, M.O.J. and C.P.L.; project administration, C.P.L.; funding acquisition, C.P.L. All authors have read and agreed to the final version of the manuscript.

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