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Post-harvesting longevity of bird of paradise (*Strelitzia spp.*) treated with carnauba wax

Jean Carlo Baudraz de Paula[1]*, Isadora Bonfante Rosalem[1], Walter Aparecido Ribeiro Júnior[1], Gabriel Danilo Shimizu[1], Ricardo Tadeu de Faria[1], Camilla de Andrade Pacheco[2], Admilton Gonçalves de Oliveira Junior[1]

[1]State University of Londrina, Londrina, Brazil
[2]Fund for Citrus Protection-Fundecitrus, Araraquara, Brazil
*Corresponding author, e-mail: jc_baudraz@live.com

Abstract

The Strelitzias are tropical plants that point out in the market, frequently used in landscaping. The leaves are used for the support and padding, improving the apparency of flower arrangements. This work assessed the effect of different concentrations of a commercial product based on carnauba wax on the post-harvest longevity of Strelitzia juncea and Strelitzia reginae leaves, aiming to improve the post-harvest life of these leaves. The experiment was performed in a completely randomized experimental design, with five treatments and six repetitions, one leaf per repetition for each species. The concentrations used were: 0% (control), 2%, 20%, 40%, and 100%. The experiment assessed the leaf mass loss (%), the mass loss index, and the leaves' visual quality using a score criterion. The work included anatomical analyses of the stomatal structures by scanning microscopy. Mass loss displayed a linear behavior in both species, with mass loss during the time. S. reginae displayed lower mass loss as increasing the wax concentration. High dosages did not reduce the mass loss. In S. juncea, this variable displayed no differences among the dosages. Carnauba wax at concentrations between 20 and 40% effectively preserved the commercial quality of S. reginae and S.juncea leaves until the 24th day. Further studies are required to obtain the best dosage for these species. Wax deposition on the stomatal structures might have influenced the mass loss of both species' leaves.

Keywords: floriculture, ornamental plants, *Strelitzia juncea, Strelitzia reginae*

Introduction

Tropical flowers have woken up interest, especially in the international market. Brazil is a prime provider of this material due to its biodiversity and favorable ecosystems. Tropical plants stand out due to the colors' and forms' diversity, resistance to transport, post-harvest durability, and promising acceptance from the external market (Machado Neto et al., 2013; Oliveira Filho et al., 2014). Among the tropical species, Strelitzias, best known as "bird of paradise" or "crane flower" (Vieira et al., 2012), are average-sized herbaceous plants. Their inflorescences have decorative appeal for their vibrant colors, solid, and coriaceous leaves (Lorenzi & Souza, 2001), displaying a particular relevance in ornamentation.

The cutting leaves are used for the support and padding, improving the appearance of flower arrangements. Leaves are chosen according to their size, form, and movement that they will provide. Arrangements may mix more than one leaf each (Lobo-Guerrero, 2009).

One of the main problems in producing cut flowers is the loss of desirable attributes, such as color, brightness, and durability, due to their high perishability (Spricigo et al., 2010). According to Ibraflor (2012), two of the three most severe sector hampers associate with the post-harvest stage: the lack or low use of post-harvest technologies, and the deficient storage. Besides these problems that have been described so far, all floriculture sectors have difficulties in accessing the consumers to the most brand new products.

Among the techniques to preserve the post-harvest, there is the use of polysaccharide polymers, proteins, and lipids. Among them, the amid, chitosan, and carnauba wax (Baldwin et al., 2011). Besides these compounds, other ones have been incorporated to formulate films and biodegradable coatings, such as the grape seeds extracts, ginger, green tea, among others (Li

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et al., 2014).

Among the materials that are used, waxes reduce water loss by being hydrophobic. Wax use aims to reduce mass loss, besides reducing the respiration rate. Wax also gives more shine, improves the visual aspect, and extends the post-harvest life (Pereira et al., 2014).

Carnauba wax derives from the extraction and processing of the wax dust of the carnauba plant (*Copernicia prunifera* (Miller) H. E. Moor), typical of the North-eastern Brazilian region (Junior & Marques, 2009). The commercial product based on this wax has a broad utility to preserve fruits, extending their shelf life (Barman et al., 2011; Machado et al., 2012). Even if in a lower proportion, carnauba wax has been used in ornamental plants to extend the post-harvest life (Dias & Castro, 2009; Mattos et al., 2017).

Knowing the use of carnauba wax as a conservative agent and aiming to extend the post-harvest durability of the vegetal products, this work aimed to assess different concentrations of commercial products based on carnauba wax on the post-harvest preservation of leaves of *Strelitzia juncea* and *Strelitzia reginae*.

Material and Methods

Local description

The experiment was performed at the plant science/ agronomy department of the State University of Londrina (UEL). The experiment used the species *Strelitzia reginae* and *Strelitzia juncea.*

Plant material and harvesting

The researchers harvested the samples from plants belonging to the Agriculture Science Center (UEL) in the morning (8 to 9 AM). The leaves were from the plant's base (inferior third) and were harvested at the juvenile stage. The leaves from the species *Strelitzia reginae* and *Strelitzia juncea* were 40, 23 cm long, and 10 and 3.5 cm wide. After harvesting, the material with physical, physiological, or phytopathogenic defects was dismissed.

Experiment performance

Leaves were previously washed by tap water, cut on a beveled edge with a scissor inside a plastic container (29x29x30cm) filled with drinking water, obtaining an approximatively 12 cm long stem. The preserving solution was the commercial product based on carnauba wax and vegetal resin (Aruá BR 12%), diluted for each treatment in one liter of distilled water. After this, the leaves were dipped for 14 seconds in solutions with different wax concentrations: 0 (control), 2, 20, 40, and 100%. The control received no wax, being dipped just in distilled water.

After the treatments, the leaves were stored in 200 ml glass containers containing distilled water. The leaves remained in these containers during the entire experiment. Five ml of 3% sodium hypochlorite were used as a bactericide agent. The preserving solution was renewed twice a week. The storage room was preserved at room temperature: 25±3°C, 60% of mean relative humidity, and 2000/lux luminosity.

Quality and hydric status of the leaves

Assessments during 26 storage days analyzed the quality and hydric status attributes of the leaves.

Every two days, leaves were assessed as to their attributes of color, brightness, and turgidity. This assessment followed the score criteria adopted by Vieira et al. (2013). The author considered the leaves as excellent (score 4): turgid leaves, bright, and with a good coloration; good (score 3): leaves display yellowness, wilted and some loss of brightness; regular (score 2): Leaves display drying at their extremities, wilted, loss of color and brightness; bad (score 1): drying is visible by the loss of brightness and turgidity, wilted, and loss of color; discharge (score 0): floppy leaves, without turgidity and brightness, complete drying or darkening (Figure 1).

Figure 1. Score classification criterion to assess the post-harvest longevity of the *Strelitzia reginae* leaves.

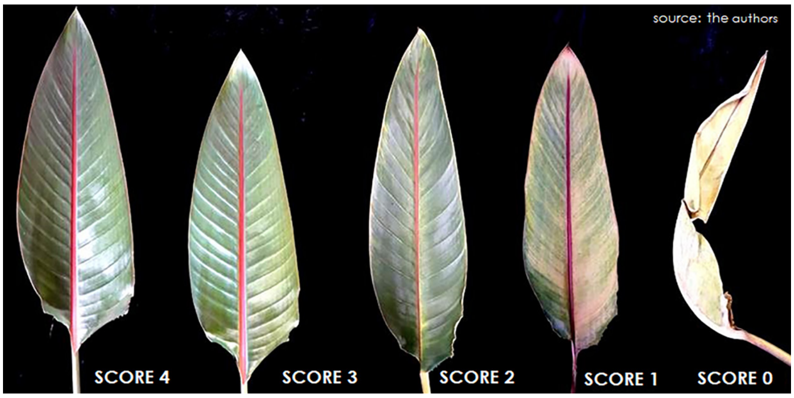

Figure 2. Score classification criterion to assess the post-harvest longevity of the *Strelitzia juncea* leaves.

Besides, every three days, the fresh mass was measured by a semi-analytical scale with ±0,01g precision to assess the water loss of the leaf tissues. The results were converted into mass loss index (MLI) by the formula:

$$\mathrm{IPM} = \sum \frac{\left(100 - \frac{M_i}{Min} \times 100\right)}{t_i} \quad (1)$$

Where:
MLI: mass loss index
M_i: Mass at the i-th time;
Min: Initial mass;
T_i: i-th time;

Anatomical analyses

At the end of the experiment, leaf disks were sampled and forwarded to the electron microscopy and microanalysis laboratory (LMEM) of the State University of Londrina (UEL) to be observed under the scanning microscope.

Samples were fixed in a 2.5% glutaraldehyde solution and 2% formaldehyde in 0.1 M sodium cacodylate buffer. After fixation, the samples were washed three times in 0.1 M sodium cacodylate buffer and then, after-fixed in 1% osmium tetroxide for 30 minutes. After washings, the samples were dehydrated in the following alcoholic series: 30%- 50%- 70%- 90%, and 100% for approximately 15 minutes each. The 100% ethanol washing was repeated three times. After the dehydration process, the samples were dried to the critical point in the BALTEC CPD 030 Critical Point Dryer.

After mounting in stubs, samples were metalized (BALTEC SDC 050 Sputter Coater) and analyzed in a scanning electron microscope Fei Quanta 200, FEI, Netherlands).

Experimental design and statistical analysis

The experiment was performed in a completely randomized experimental design for each species, with five treatments and six repetitions, one leaf per repetition for each species.

A polynomial regression analysis analyzed the effect of the wax concentrations. A *loess* non-parametric regression analyzed the mass loss, the mass loss index, and the scores' qualitative analysis. The parametric analyses were tested as the assumption of errors normality (Shapiro-Wilk) and homogeneity of variances (Levene). All analyses were performed by the R software (R Core Team, 2019).

Results and Discussion

The leaves' physiological quality was assessed until the 26th day after the experiment beginning, as all leaves were scored 2 or 1, being, therefore, unsuitable for commercialization. After harvesting, the leaves stop having the same abundance of the nutrients as planted, increasing the energy reserves consumption's intensity to maintain them alive. As a consequence, the senescence processes are accelerated, hampering their commercialization (Tsegaw et al., 2011). Table 1 displays the scores rating of the species on each day.

The *S. reginae* control treatment displayed acceptable scorings until the 16th experiment day. The leaves treated with 20% and 40% carnauba wax displayed commercial standard scores (score 3) until the 24th day. The *S. juncea* control treatment did no longer display acceptable scorings after the 18th experiment day. The leaves treated with 20% and 40% carnauba wax displayed commercial standard scores (score 3) until the 24th day.

The mass loss index displayed quadratic behavior in *S. reginae* (Figure 3). The 48 to 50% dosage displayed the lowest mass loss. The *S. juncea* species did not display a significant response to the regression models. It was not possible to identify any tendency curve.

Table 1. Average scoring comparison of *Strelitzia* leaves treated with different carnauba wax concentration from the experiment's 12th to the 26th day.

	Strelitzia reginae								
		Time (days)							
		12	14	16	18	20	22	24	26
(%)	0	4*a	4 a	3 b	2 b	2 b	2 b	2 b	1 a
	2	4 a	4 a	4 a	4 a	3 a	3 a	2 b	1 a
	20	4 a	4 a	4 a	4 a	3 a	3 a	3 a	1 a
	40	4 a	4 a	4 a	4 a	3 a	3 a	3 a	1 a
	100	4 a	4 a	3 a	3 a	3 a	2 b	2 b	1 a
	Strelitzia juncea								
		Time (days)							
		12	14	16	18	20	22	24	26
(%)	0	4 a	4 a	3 b	3 b	2 b	2 b	2 b	2 a
	2	4 a	4 a	4 a	4 a	3 a	3 a	2 b	2 a
	20	4 a	4 a	4 a	4 a	3 a	3 a	3 a	2 a
	40	4 a	4 a	3 b	3 b	3 a	3 a	3 a	2 a
	100	4 a	4 a	4 a	3 a	3 a	2 b	2 b	2 a

*Scoring criteria adapted from Vieira et al., 2013.

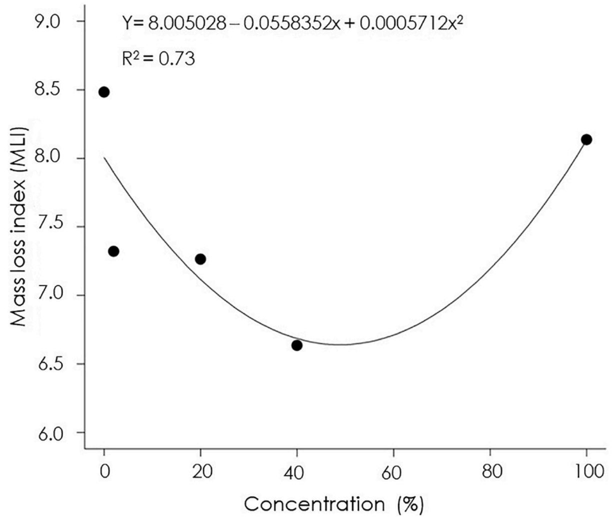

Figure 3. Mass loss index (MLI) of the *S. reginae* leaves as a function of carnauba wax concentration (%).

The mass loss reduction by the application of wax has been abundantly described in the literature. This reduction mainly occurs due to the low steam wax permeability. Water loss reduction decreases the wilting, mainly by slowing down the transpiration and respiration processes, providing a commercial life extension (Calbo et al., 2008).

The film use modifies the internal atmosphere surrounding the product and the gas exchange with the exterior, reducing the plant's respiration rate (Chitarrra & Chitarra, 2005). Gas exchanges mostly occur through the cuticle, lenticels, and the locality where the mother plant's removal was performed. Coats partially obstruct these openings (Paul & Pandey, 2014), have an antioxidant and antimicrobial function, besides forming a barrier against oxygen, carbon dioxide, and UV light (Weiss et al., 2013).

Atmosphere modification results from the respiration activity, O_2 consumption, CO_2 production, occurring in a totally or partially sealed environment (Irtwange, 2006). A modified atmosphere with O_2 concentration decrease reduces the respiration rate, ethylene production, chlorophyll degradation, texture loss, and delays the ripening and senescence of some horticultural products (Guevara et al., 2003).

On the other side, carnauba wax concentrations over 80% in *Strelitzia* sp. leaves did not delay the mass reduction effect and, probably, did not hamper the leaves' respiratory activity reduction. The O_2 and CO_2 balance may explain this event: O_2 concentration must be sufficiently low to delay respiration but higher than the critical concentration to begin anaerobic respiration. These anaerobic conditions at high concentrations of carnauba wax may induce the growth of anaerobic microorganisms. Besides this, CO_2 accumulation at high levels causes physiological disorders (Villadiego et al., 2005) that may have affected and delayed the post-harvest life as observed in the experiment.

Leaves mass loss (%) displayed a linear tendency in both species (Figure 4). At the experiment end, *S. reginae* displayed a higher mass loss percentage than *S. juncea*, being over 25%. This result is probably related to the higher leaves' size and area, and therefore, higher susceptibility to water loss.

Both species reached mass loss proximate to 15% at 18 storage days (Figure 3), when they still were commercially durable.

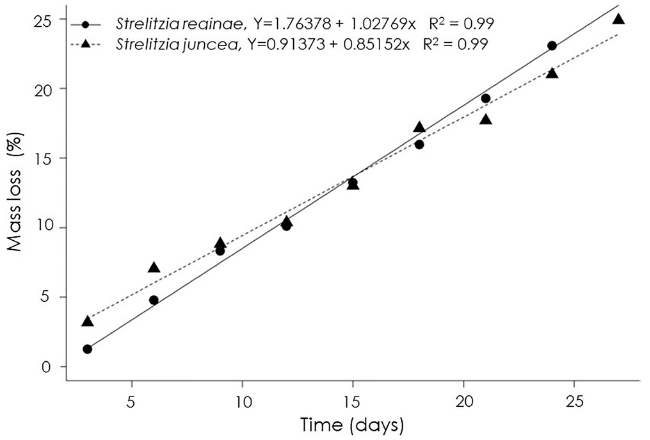

Figure 4. Mass loss (%) of *S. reginae* and *S. juncea* as the storage time.

After the harvest, the fresh mass loss occurred due to the transpiration water loss and the metabolic processes associated with respiration. Several factors influence this loss, such as tissue resistance, water steam diffusion outside the cells, temperature surrounding the material, and the relative humidity. The metabolic processes cause higher membrane disintegration, cellular content loss, and consequently, wilting and turgidity loss, causing a reduction of the post-harvest conservation (Chitarra & Chitarra, 2005; Brackmann et al., 2011).

The leaves' structural analysis was performed on freshly harvested leaves of *Strelitzia juncea* (A e B) and *Strelitzia reginae* (C e D). These leaves served as terms of comparison with the samples submitted to the treatments. The images highlight an arrangement standard of the structures in the leaves' abaxial side, forming runways with stomatal structures (Figure 5). Figure 6 shows the stomatal structure arrangements of fresh leaves of both *Strelitzia* spp. species

The pictures highlighted the deposition of wax layers on the abaxial and adaxial sides of both *Strelitzia* species leaves. The scanning electron microscopy pointed out the covering of the stomatal structures, according to the wax concentration increasing in the solutions. The 100% wax concentrations caused an almost complete covering of the leaf area (Figures 6 and 7). The comparison between the pictures B and D of figure 7 verified the wax deposition due to the natural roughness reduction on the leaf side.

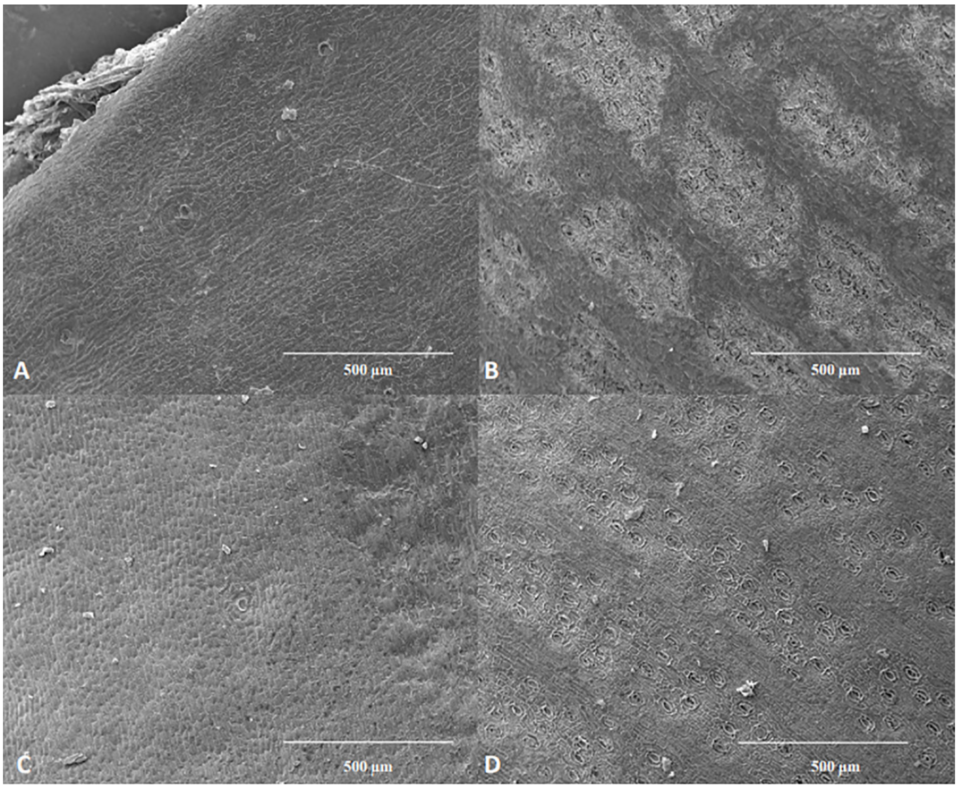

Figure 5. Scanning electron microscopy of fresh *Strelitzia juncea* (A and B) and *Strelitzia reginae* (C and D) leaves. A and C: leaves adaxial side; B and D: abaxial leaves side: 200 x magnification.

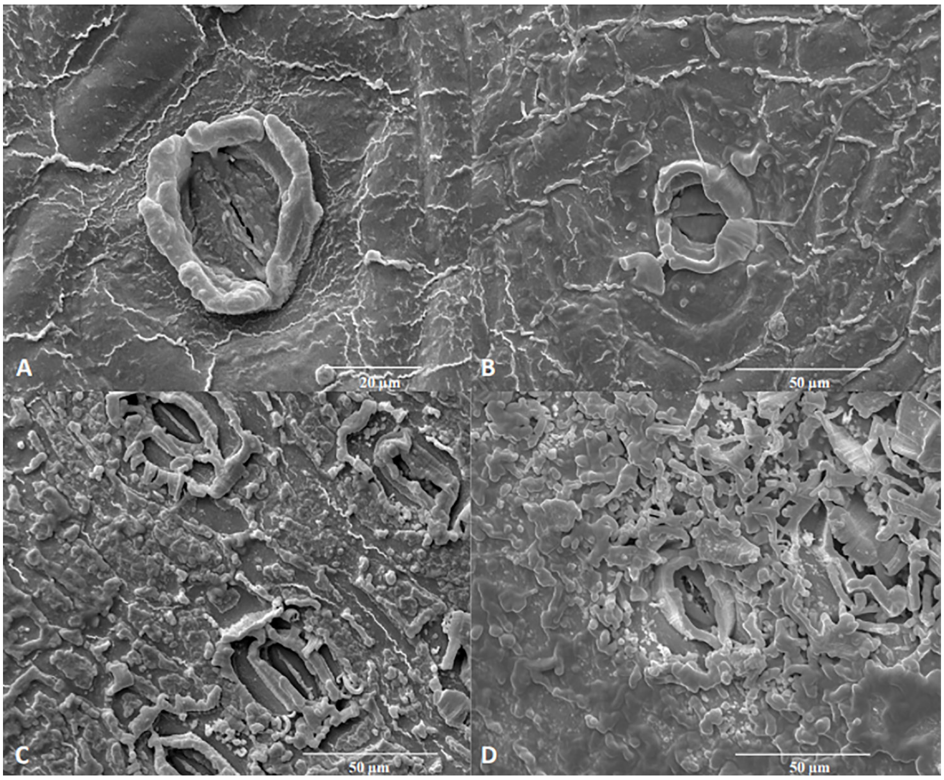

Figure 6. Stomata on the adaxial sides of the species *S. reginae* (A) and *S. juncea* (B), and stomata on the abaxial sides of the species *S. reginae* (C) and *S. juncea* (D). Magnification: 3000 times (A), and 1600 times (B, C, and D).

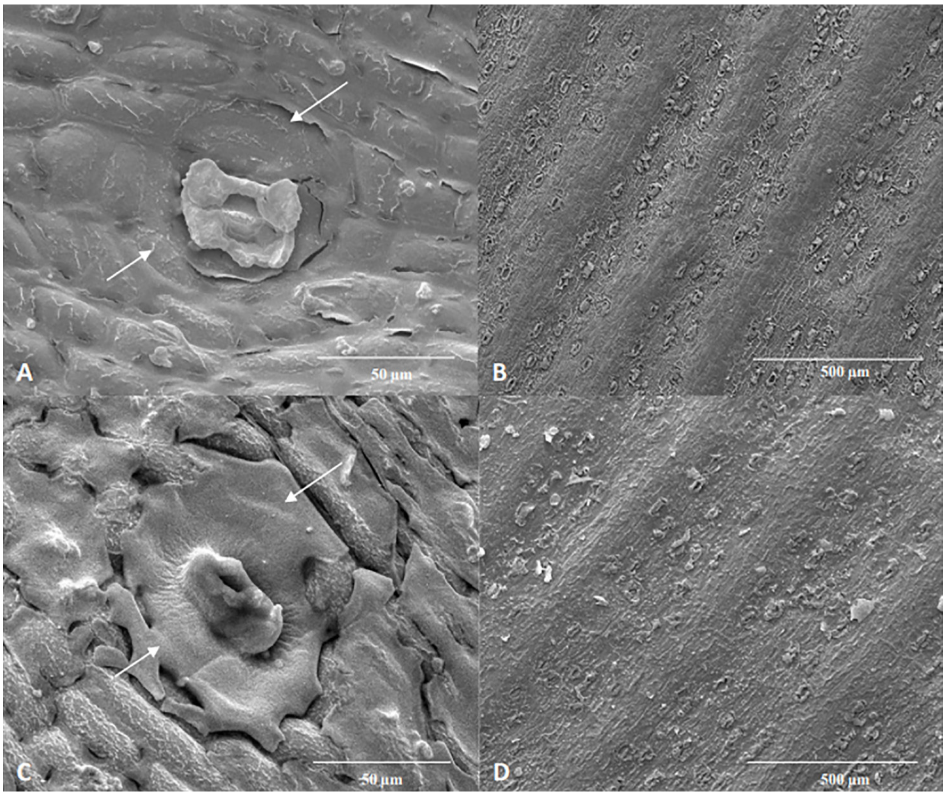

Figure 7. *Strelitzia reginae*. Presence of wax depositions forming plates or layers, covering the stomata and the cuticle structures. A: stoma (adaxial face) of the 20% dosage with wax cover, B: abaxial face with low wax cover, C: Stoma of the 100% dosage, wholly covered with wax., D: abaxial side with intense wax covering. Arrows indicate wax accumulation. Magnification: 1600 times (A and C), and 200 times (B and D).

On the other side, Mattos et al. (2017) did not describe wax-covered stomata in their experiments with ornamental ginger. The difference between the present experience results and those described by the authors may be related to the lower carnauba wax concentrations used by the above-quoted authors, as they used 0, 0.75, 1.5, and 3.0% v/m.

Chen & Nussinovitch (2000) related the stomatal cover after the wax application in two citrus varieties and the following weight-loss reduction during the respiratory project. This event suggests that the wax cover may affect the gaseous exchange of these materials.

The comparison between the pictures B and D of figure 8 verified the wax deposition due to the smoothing of the natural roughness reduction on the abaxial leaf side.

The formation of wax layers on the stomata explains the water loss reduction and the leaves' durability increase until a determined storage time.

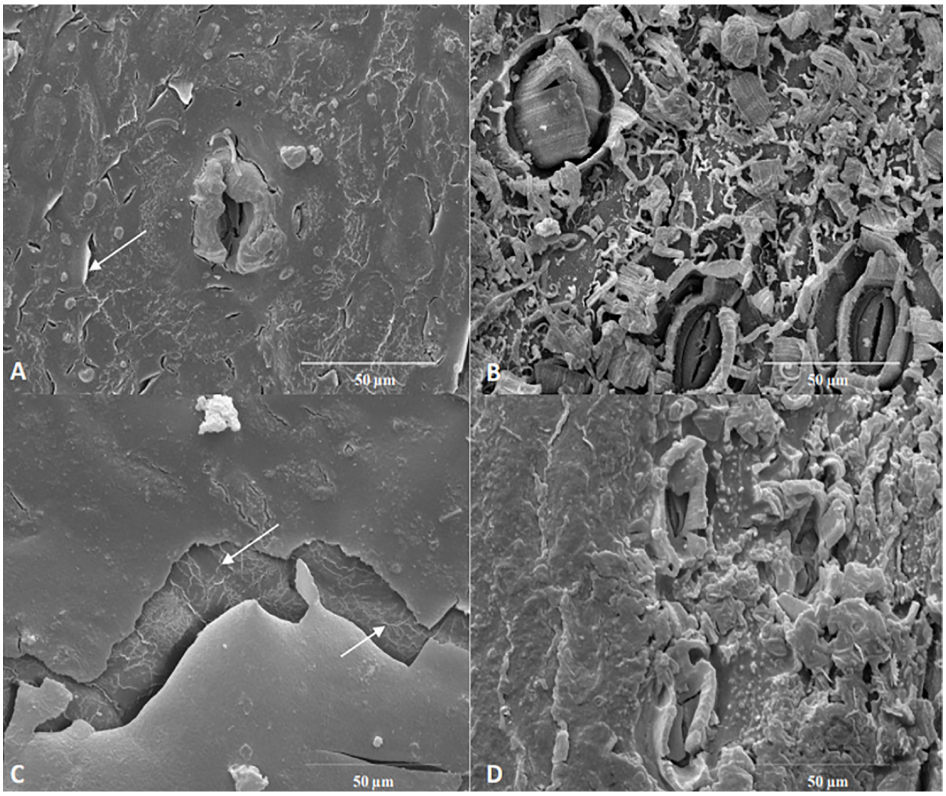

Figure 8. *Strelitzia juncea.* Presence of wax depositions forming plates or layers, covering the stomata and the cuticle structures. A: stoma (adaxial face) of the 20% dosage with reduced wax cover, B: stomatal structures on the abaxial face with low wax cover, C: adaxial face of the 100% dosage, wholly covered with wax plates, D: abaxial side with intense wax covering. The arrows indicate the breaks of the wax layer, identifying its presence 1600 x magnification.

Conclusions

Carnauba wax at concentrations between 20 and 40% effectively preserved *S. reginae* and *S. juncea* leaves' commercial quality until the 24th day. Further studies are required to obtain the best dosage for these species. Wax deposition on the stomatal structures might have influenced the mass loss of both species' leaves.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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