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Post-production Growth and Quality of *Echeveria agavoides* and *Echeveria elegans* Treated with Artificial Coloring

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***Keywords*— Quality control, Succulent plants, Value add, Vegetative growth.**

***Abstract*— *This study aimed to evaluate the growth and commercial quality of two species of Echeveria plants after the surface of their leaves were treated with artificial dyes. The goal of the experiment was to show that the three main products and processes used for artificial coloring of potted plants by Brazilian nurseries, subject to inspection and quality control for trade purposes, are harmless to the plants and do not impair their growth or market quality. The study is justified by the need to offer safe and scientifically guaranteed indicators for consumers who are concerned about the effects of interventions on plants. Three experiments, with three treatments each, were carried out simultaneously, according to the most frequent artificial coloring products and processes in Brazil. The samples were collected directly from the producers that supply them to the market and composed of young plants, conducted in 11 cm diameter pot and filled with identical commercial substrates for each producer. The plants were kept in the laboratory for 90 days and exposed to lighting of 1,000 lux in alternating cycles of 12 hours light (from 6:00 a.m. to 6:00 p.m.) and 12 hours of dark (from 6:01 p.m. to 5:59 a.m.). The temperature was kept at 22ºC with a tolerance of ± 1ºC and the relative humidity was at 60± 5%. Conclusively, all experiments showed that the interventions were harmless to the plants, and that the new leaves continue to be produced with all the natural characteristics of the species.*

I. INTRODUCTION

Artificial coloring processes in ornamental cut or potted natural flowers and plants have been gaining ground in international markets over the last decades, mainly in the Netherlands, South Korea [1] and India [2]. These processes include interventions, treatments or other techniques to apply artificial coloring to flowers and plants *in natura*, performed indoors or outdoors with mostly nonindustrial, non-toxic, water-based, biodegradable dyes, and that may be enhanced with glitter or other substances. The main goal of these processes is to create innovative, distinguished and attractive features in the goods placed on the consumer market, which always welcomes novelties [3], and, thus, to add value to various products in the floral industry [4]. Over the last two decades, there has been a considerable rise in consumer interest in color variation in succulents both through genetic or phenotypic diversity [5], [1] and natural and artificial coloring processes [6].

Artificial coloring of flowers and ornamental plants can be obtained through systemic alteration, that result of physiological processes where the plants or their cut parts internally absorb a pigment, dye or chemical product mixed into the water used to hydrate them [2], [7], [8], [9]. Another category of techniques makes superficial color changes by external application of dyes or pigments either by painting or spraying them onto the surface of flowers, leaves or any other part of the plants [10]. In both the international and Brazilian markets, the main ornamental plants that customarily receive this type of treatment are cacti, succulents in general, and sansevierias, among other species.

Succulents are a large ornamental group made up of over 50 botanical families, 459 genera and 22 thousand species [11]. The noteworthy consumer interest in these plants is due to several factors, such as the ease of cultivation and maintenance in domestic environments, low level of care requirements, good adaptability to both indoor and outdoor environments [12], [13], diversity of colors, attractive plant architecture, and good aesthetic results in compositions and arrangements with other species [14], [15],[16], [17], [18].

The flowers and plants marketed by the Veiling Holambra Cooperative, which include the succulents featured in this study, are subject to prior registration and periodic quality control, in accordance with internal regulations. Artificially colored products have their own forms that must be filled out by nurseries to report on processes, products and doses, as well as information about exclusions and restrictions when it comes to using unauthorized substances, such as automotive paint and inorganic glitter considered harmful or inadequate for use in ornamental plant coloring .

This is the context for this research aimed at evaluating the growth and quality of *Echeveria agavoides* and *E. elegans* plants after they were colored by applying external and superficial products related to three different processes. The goal of the experiment was to prove the initial hypothesis that the artificial coloring products and processes used by nurseries on potted plants, which are subject to inspection and sales controls, are harmless to plants, do not harm their growth or development and help to increase the supply of new sought-after goods with high added value that are in line with the main consumer trends in international flower markets [10].

II. MATERIAL AND METHODS

The experiments was conducted on the premises of the Veiling Holambra Cooperative's Test Center (Santo Antônio de Posse, São Paulo, Brazil), located at a latitude of 22º36'22″ south, longitude of 46º55'10″ west, and elevation of 695 meters above the mean sea level (MSL).

All plants were kept in controlled environmental conditions for 90 days, from September 10 to December 10, 2021, totaling 13 uninterrupted weeks of observation. They were exposed to lighting of 1,000 lux in alternating cycles of 12 hours light (from 6:00 a.m. to 6:00 p.m.) and 12 hours of dark (from 6:01 p.m. to 5:59 a.m.). The temperature was kept at 22ºC with a tolerance of ± 1ºC and the relative humidity was at 60 ± 5% [17]. The pots were watered once a week, which may have varied depending on sporadic needs recommended through manual monitoring of substrate moisture levels.

The artificial plant dyes analyzed came from three different processes prevalent in the Brazilian succulent market. The samples were collected directly from the producers that supply them to the market and composed of young plants, conducted in 11 cm diameter pot and filled with identical commercial substrates for each producer.

The experimental design used was entirely randomized, with 3 treatments (blue dye, pink dye and control), per experiment totaling 18 samples per experiment in a 6 x 3 scheme totaling 54 pots of *Echeveria* sp, split by 36 *E. agavoides* and 18 *E. elegans*. All samples were selected according to size and commercial quality in line with the superior standard defined by the Veiling Holambra Cooperative [19].

Table 1. Experiments and treatments applied by specie of Echeveria

Specie / Cultivar	*E. agavoides* Red Tips			*E. agavoides* Miranda			*E. elegans* Rose		
Experiment	Treatment			Treatment			Treatment		
	Blue	Rose	Control	Blue	Rose	Control	Blue	Rose	Control
A	6	6	6						
B				6	6	6			
C							6	6	6
Total	18			18			18		

The growth of *Echeveria agavoides* and *E. elegans* were evaluated by periodically me asuring the height (cm) and diameter (cm) of the plants [14],[15],[16],[17], during thirteen consecutive weeks. Height was defined as the distance, in centimeters, from the base of the plant to the apex of the tallest leaf [13]. Throughout the term of the experiment, the number of new leaves sprouted by both the artificially colored plants and the control plants was also counted.

From the commercial quality point of view, the samples were visually observed and evaluated taking into consideration a hedonic scale of values [16], [6] following the regular criteria adopted by Veiling Holambra Cooperative Test Center: changes in leaf shape, plant stand traits, leaf coloration and tonality, pest damage and disease damage. Each of these indicators made up 20% of the final classification (Table 2).

Table 2. Hedonic scale of values for visual qualitative evaluation of Echeveria sp. Plants

Criterion	Weighed value in the final grade (%)
Change in leaf shape	20
Plant stand traits	20
Coloring and leaf tone	20
Pest Damage	20
Damage from disease	20
Total	100
Evaluation	
Unsatisfactory	≤40%
Satisfactory	41% a 79%
Excellent	≥ 80%

Source: Veiling Holambra Cooperative, Test Center, 2022.

In the evaluation of the experiment data, the statistical analysis tool ANOVA of linear regression was used for analysis of variance between samples[13], [20], whose data were entered in a Microsoft Excel® spreadsheet editor. Means were evaluated by applying F-tests for one variable, with a significance level (α) of 5%, where:

F = variation between sample group means / variation within samples

F > F-critical = equality between means rejected

F< F-critical = equality between means accepted

p-value = probability of significance, and

P-value > α = initial hypothesis of equality between the means accepte

III. RESULTS AND DISCUSSION

The three experiments conducted showed the absence of statistically significant differences between colored products and controls for all treatments employed (Tables 3 to 8).

These results did not require the Tukey test for the evaluation of variance between the groups of samples analyzed [21], since the comparative results between the F and Critical Factor (F-critical) values and the p-values allowed the acceptance of the initial hypothesis of equality both among treatments and controls [22]. The analysis of the growth indicators shows, in general, growth rates which are typical of xerophytes [13].

The process used in experiment A (MK), with *E. agavoides* Red Tips, was developed especially for flowers and plants by Dutch company Make-Upz Flowers & Plants®. The products are registered in the Netherlands and meet the requirements set by the Rijksinstituut voor volksgezondheid en Mileu (Dutch National Institute for Public Health and the Environment) and the European regulations on the matter (Regulation number 1907/2006, 2015/830/EU). The dyes are not considered dangerous to human health nor to the colored plants themselves, such that they have been assessed as being harmless to the environment and to ecosystems. They do not meet the criteria for being classified as PBT (Persistent, Bioaccumulative and Toxic) or vPvB (very Persistent and very Bioaccumulative) substances [23].

Table 3. Variation of plant height (cm) in treatments of the experiment A.

Experiment A Variable	Treatment A (MK)		
	Pink	Blue	Control
Height (cm)			
Home	4.6	4.4	4.4
Final	5.2	5.0	5.0
Average weekly growth (AWG) (cm)	0.0350	0.036	0.0442
AWG Variance	0.0008	0.0009	0.0012
F-critical	3.2849		
F	0.3295		
p-value	0.7216		

Table 4. Variation of plant diameter (cm) in treatments of the experiment A.

Experiment A Variable	Treatment A (MK)		
	Pink	Blue	Control
Diameter (cm)			
Home	10.9	11,0	10,4
Final	11.8	11,4	10,8
Average weekly growth (AWG)(cm)	0.0433	0,0433	0,0500
Variance	0.0017	0,0034	0,0013
F-critical	3.2849		
F	0.0829		
p-value	0.9206		

The experiment B (RA), performed with *E. agavoides* Miranda plants, was the application of commercial dye based on modified acrylic resin mixed with active and inert pigments, surfactants, coalescent, thickeners, microbicides and other additives, diluted in water, offered by several companies and commercial brands in Brazil. These products are registered and controlled in the country according to health and environmental rules and standards.

Table 5: Variation of plant height (cm) in treatments of experiment B.

Experiment B Variable	Treatment B (RA)		
	Pink	Blue	Control
Height (cm)			
Home	4.60	5.63	5.72
Final	5.46	6.45	6.34
Average weekly growth (AWG) (cm)	0.07	0.07	0.05
Variance	0.0029	0.0016	0.0021
F-critical	3.2849		
F	0.6924		
p-value	0,5075		

Table 6: Variation of plant diameter in treatments of the experiment B.

Experiment B Variable	Treatment B (RA)		
	Pink	Blue	Control
Diameter (cm)			
Home	11.28	12.82	12.28
Final	12.39	13.88	13.09
Average weekly growth (AWG) (cm)	0.0925	0.0900	0.0683
Variance (AWG)	0.0083	0.0036	0.0004
F-critical	3.2849		
F	0.5197		
p-value	0.5995		

Finally, the process used in experiment C (PD), with *E. elegans* Rose, was the Paint & Draw® spray paint developed and marketed in Brazil by the Floral Atlanta company [24], specially made for coloring flowers and natural plants, among other decorative items. Each process evaluated considered artificially colored samples using the main coloring options available on the market, which happen to be pink and blue dyed plants.

Table 7. Variation of plant height (cm) in treatments of experiment C.

Experiment C Variable	Treatment C (PD)		
	Pink	Blue	Control
Height (cm)			
Home	5.04	5.40	5.32
Final	5.48	5.83	5.91
Average weekly growth (AWG) (cm)	0.0383	0.0358	0.0483
Variance (AWG)	0.0007	0.0009	0.0005
F-critical	3.2849		
F	0.7590		
p-value	0.4761		

Table 8. Variation of plant diameter (cm) in treatments of experiment C.

Experiment C Variable	Treatment C (PD)		
	Pink	Blue	Control
Diameter (cm)			
Home	11.72	11.50	12.28
Final	12.67	12.27	13.11
Average weekly growth (AWG) (cm)	0.0792	0.0692	0.0633
Variance (AWG)	0.0054	0.0063	0.0005
F-critical	3.2849		
F	0.1897		
p-value	0.8281		

In addition to these dimensional criteria, the growth of new leaves by the colored plants and the respective controls was also evaluated, and the results showed that there were no statistically significant differences between the different treatments (Tables 9, 10 and 11).

Table 9. Variation in the number of new leaves in treatments of the experiment A.

Experiment A Variable	Treatment A (MK)		
	Pink	Blue	Control
Number of new leaves			
Average	2.6715	3.1339	2.8415
Variance	2.6140	3.8923	1.8696
F-critical	3.2595		
F	0.2546		
p-value	0.7766		

Table 10. Variation in the number of new leaves in treatments of the experiment B.

Experiment B Variable	Treatment B (RA)		
	Pink	Blue	Control
Number of new leaves			
Average	2.3277	2.3915	2.3985
Variance	1.5342	2.1713	2.0218
F-critical	3.2596		
F	0.0104		
p-value	0.9897		

Table 11. Variation in the number of new leaves in treatments of the experiment C.

Experiment C Variable	Treatment C (PD)		
	Pink	Blue	Control
Number of new leaves			
Average	1.8762	1.9646	2.4254
Variance	1.3866	1.6756	1.7060
F-critical	3.2594		
F	0.7112		
p-value	0.4978		

From a qualitative point of view, all samples were monitored and visually diagnosed throughout the 13-week term of the experiment. The results of the scores for both the colored samples and the controls, for all treatments, are graphically represented in Figure 1.

In the first ten weeks of the experiments, all the plants showed excellent commercial qualities, compatible with what was expected for plants in the conditions of temperature and luminosity offered [14], [15], [16], [17], [25].

For experiment A, both the colored samples and the control samples obtained maximum quality scores according to parameters assigned for the commercial value of the species, reaching a level of excellence (100%) for all criteria analyzed (Cf. Table 2). (Fig. 1), throughout the duration of the experiment.

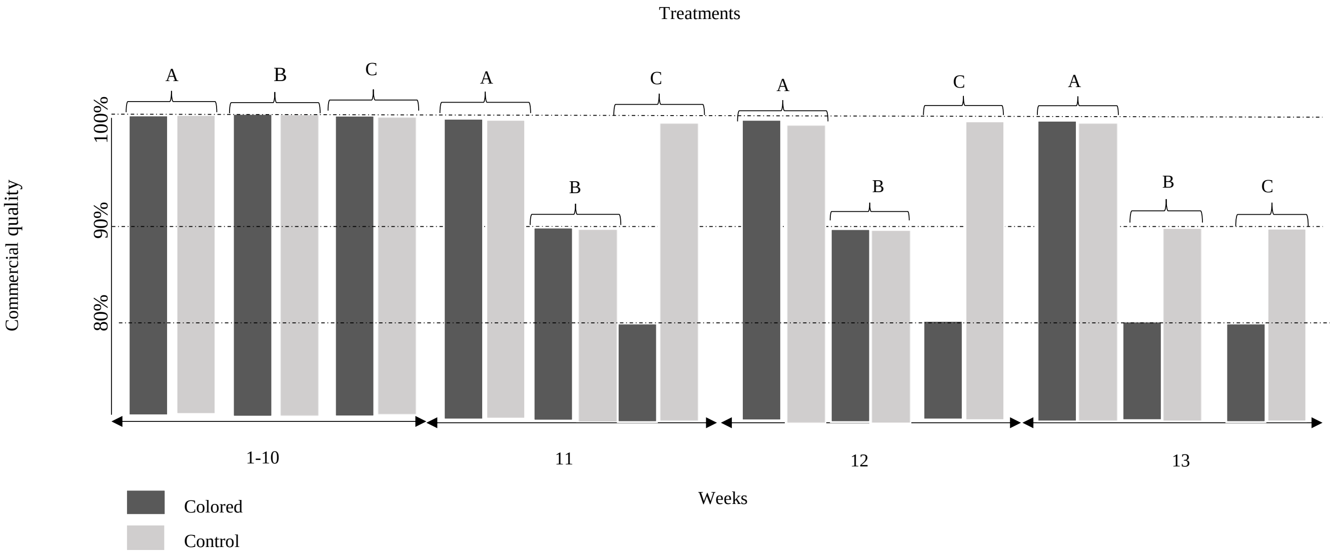

Fig.1. Results of weekly qualitative analyses on control and colored plants, per treatment.

In case of experiment B, a slight decrease in commercial quality was observed for both the control and the colored plants, starting in the 11th week of observation. The reason for the decrease in quality to 90% of the ideal commercial value was due to the appearance of a small number of older yellowish leaves in all the observed treatments, especially along the edges of the lower and more mature leaves. This phenomenon is considered normal for the species in this growth phase [14], [15], [16], [17].

In the case of the colored products, the decrease in commercial value to 80% of the ideal standard was also due to small changes in leaf shapes, starting in the 13th week. None of these occurrences, however, altered the overall quality standard of the plants, which continued to be evaluated as excellent.

Finally, for experiment C, it was observed that the controls maintained a 100% standard of excellence until the 12th week of the experiment, with the level then decreasing to 90% due to the yellowing of some older leaves. As pointed out by Cabaugh, Choi and Nam [14], [15], [16], [17], this occurrence should be considered normal for the growth stage of the plants. For the colored products, the standard decreased to 80% starting in the 11th week of observation, due to minor changes in coloration and leaf shape. As in the cases of the previous nurseries, these leaf modifications did not cause a change in the commercial quality level of the plants observed.

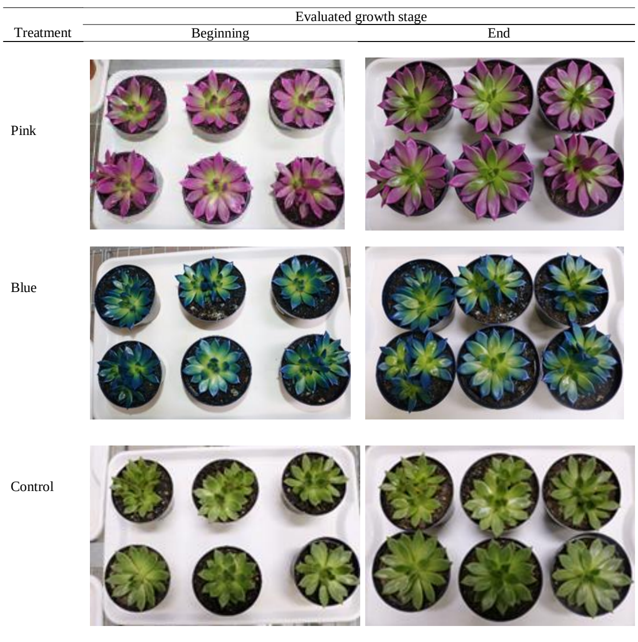

Fig. 2. Photos of the early and late stages of the control and colored plants – Experiment A

Figure 2, 3 and 4 shown sequentially contains photos of the initial and final stages of the set of samples from each treatment, showing the continuity and support of the colored plants and proving the non-differentiation of these in relation to their respective controls.

It is possible to observe that development and sales quality were not compromised by the artificial dyes, since they gradually recovered all their original characteristics once new leaves appeared (Fig.2, Fig.3 and Fig.4)

Fig.3. Photos of the early and late stages of the control and colored plants – Experiment B

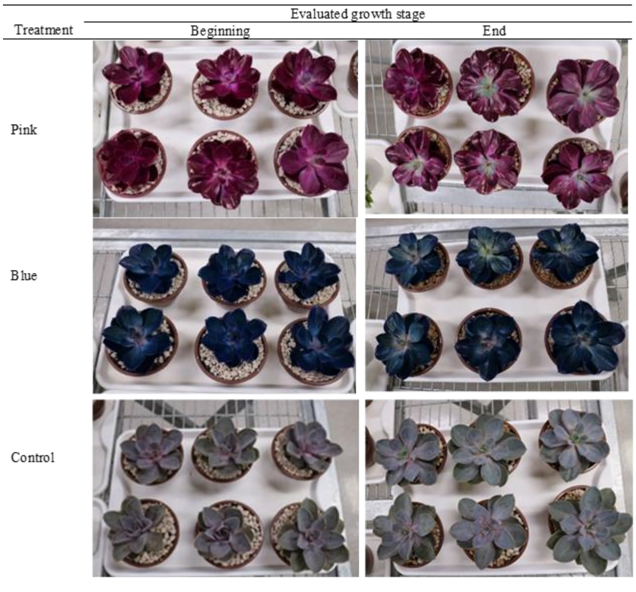

Fig.4. Photos of the early and late stages of the control and colored plants – Experiment C

IV. CONCLUSION

The three different products and processes used for dyeing the *Echeveria* plants analyzed in the experiment were shown to be harmless to the plants, which did not have their growth, development and commercial quality compromised by the artificial coloring.

The experiments carried out bring innovative knowledge to the practices of artificial colorization of live plants, since the topic is still practically unexplored in the scientific literature. Research carried out internationally so far has been limited to the artificial coloring of cut flowers, with no records of research on the surface dyeing of leaves of live ornamental plants, especially in the post-production phase.

The results obtained benefit producers and distributors of flowers and ornamental plants especially in Brazilian market, who have the guarantee and evidence of being able to offer safe, reliable and high value-added goods to the consumers.

The practices of artificial colorization of plants for ornamental purposes are on the rise in the international market. Future research should focus on different products and techniques and their effects on plants of other genera and succulent species, as well as other families usually colored, especially Cactaceae and Asparagaceae.

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