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Research Article

Standardization of packaging material, storage durations and chemical treatments on vase life of soft shield fern *Polystichum squarrosum* (D. Don)

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Abstract
Polystichum species, native to warm-temperate and montane-tropical environments (including some alpine regions), are terrestrial or rock-dwelling ferns. These plants exhibit dark green, leather-like, and lustrous fronds, which are highly valued in floral arrangements due to their aesthetic elegance and longevity. *Polystichum squarrosum* is not being cultivated commercially but collected from forests for its use as cut green in the florist industry. Hence the collected material should be handled carefully to utilize every frond. Therefore, the present study aimed to develop suitable post-harvest handling procedures for this valued cut green. A Completely Randomized Design (CRD) Factorial layout with 19 treatment modules (M_0 to M_{18}) was employed. Treatment modules included packaging material as poly mesh net bags and packing materials as corrugated fiberboard boxes , two storage durations (3 and 6 days) and chemical treatments [glycerol (4%) and Benzyl adenine (25mg L^{-1})]. Results revealed that spraying cut fronds with 4% glycerol, wrapping them in cellophane sheets and storing them for three days (M_9) resulted in significant improvements in appearance, weight change, vase life and water uptake. Fronds from Season-II (March-April 2020) exhibited excellent performance over Season-I (August-September 2019). The interaction between M_9 and Season-II further enhanced the postharvest parameters. The combination of glycerol treatment and cellophane wrapping for three-day storage proved most effective in preserving the quality of cut fronds.

Keywords: Benzyl adenine, Glycerol, Packaging, *Polystichum squarrosum,* Vase life, storage

INTRODUCTION

Polystichum is a diverse genus of 500 species in Dryopteridaceae family, commonly found in the temperate and subtropical regions (He and Zhang, 2022). In North-Western Himalayan regions it occurs at altitudes of 1900-2400 above msl (Saggoo and Kaur, 2018). There are 50 species under the genus Polystichum reported from India (Jangi *et al*., 2021). *Polystichum squarrosum* is being commercially utilized in floriculture industry as a cut green. It is well-known for its medicinal properties, including antibacterial effects, and for treating pyloric diseases (Singh, 1999). *P. squarrosum* (2n = 82) is reported from various North-Western Himalayan regions viz. Nainital, Mussoorie, Shimla, Matiana, Dalhousie, Manali, Solan, Barog and Chamoli (Saggoo and Kaur, 2018). The fronds have dark green, leathery and shiny foliage valued in floriculture for their elegance and long lasting nature (Yadav *et al*., 2018). The leaves have commercial importance and are extensively used to decorate marriages, pandals and temples. They are commonly sold by the name 'Pahadi patti' in florist shops (Yadav *et al*., 2018). Cut greens impart freshness and background contrasting colour and variety to the floral designs; hence, they constitute an important component of floricultural industry largely used as fillers in bouquets and flower arrangements. They also play a major role in enhancing the national GDP (Pacifici *et al*., 2007, Reid and Jiang, 2012, Abou-Dahab *et al*., 2013).

Antitranspirants reduce water loss from plant leaves by reducing stomata size and number (Goreta *et al*., 2007). They fall into three categories: film-forming types like glycerol, reflecting materials that bounce off radiation from leaf surfaces and stomatal closing agents such as ($MgCO_3$) which impact leaf tissue metabolism (Kumar *et al*., 2023; Warmund *et al*., 2021; Lv *et al*., 2020). Antitranspirants have different modes of action (Kociecka *et al*., 2023; Chaves *et al*., 2009) and their formulations prevent excess water loss without reducing CO_2 uptake. They can be applied in the foliar form by reducing transpiration in three different ways (Mphande *et al*., 2020). The first set of techniques involves the application of reflective materials through spraying to reduce the absorption of radiant energy (Mphande *et al*., 2020). The second approach entails the utilization of antitranspirants, which are sprayed onto foliage and form transparent films which effectively obstruct the outward movement of water vapor from the leaf surface (Moroni *et al*., 2020). An additional mechanism employed by antitranspirants involves the inhibition of stomata opening, consequently reducing the water loss from the leaf (Sow and Ranjan, 2021).

Growth regulators are recommended to prolong postharvest longevity, benzyl adenine (BA) is one of the growth regulators that delays leaf yellowing and consequently increases leaf longevity (Zhang *et al*., 2023). Spraying of 6- benzylaminopurine (BAP) at concentrations of 37.5–300 mg L^{-1} improved postharvest durability of *Anthurium andreanum* 'Apalai' flower without inducing spathe blueing (Favero *et al*., 2020). The accelerated onset of senescence poses a challenge in exporting cut foliage plant species. External factors like temperature, moisture, gases, radiation and pathogens induce senescence in cut flowers (Raza *et al*., 2022).

Packaging materials and chemical treatments helps extend the life of cut flowers vase (Sharma *et al*., 2010). Packaging plays a pivotal role in the flower trade, significantly impact the quality of cut flowers and foliage to provide quality producs for consumers (Shinde *et al*., 2023). Effective packaging methods are vital for prolonging storage duration and preserving flower quality during transportation (Jang et al., 2021). The key principles underlying successful packaging involve minimizing the rates of transpiration, respiration, and cell division (Khalid *et al*., 2020). Corrugated fiber board boxes stand out among the most suitable packaging materials (Lai *et al*., 2022). Additionally, a wide array of interior packaging materials such as paper, paperboard, transparent films, polyethylene, and other innovative materials are available, necessitating thorough evaluation for their suitability and cost-effectiveness. Packaging should minimize transpiration, respiration, and cell division to maintain long-term cut foliage storage and quality during transportation (Bhattacharjee, 1999). Therefore, a comprehensive comparison of diverse packaging options is essential to ensure the extended freshness of flowers or cut greens for secure transport to their destinations (Sarkar *et al*., 2019). Polystichum is not being cultivated commercially but it is being collected from the forests for its utilization as cut green in florist industry. As a result of indiscriminate collection from forests, it has become an endangered plant species. Hence, the collected material should be handled carefully to utilize every frond. Therefore, suitable postharvest handling procedures for this valued cut green are needed. Hence, the present study aimed to assess the storage materials, storage durations, and chemical treatments that enhance the longevity of *P. squarrosum* cut fronds.

MATERIALS AND METHODS

The present study on vase life of soft shield fern (*P. squarrosum*) was performed at the farm laboratory of the Department of Floriculture and Landscape Architecture, Dr Yashwant Singh Parmar University of Horticulture and Forestry in Nauni, Solan H.P. India, 173230. The study was conducted in two seasons, August - September 2019 (Season-I) and March – April 2020 (Season-II). Fronds from Chail forest, Solan district of Himachal Pradesh, were promptly collected and brought to the laboratory after harvesting. Treatment modules included three packaging material cellophane sheets, poly mesh net bags and corrugated fiberboard boxes (CFB), two storage durations (3 and 6 days) and spraying of glycerol (4 %) and Benzyl Adenine (25mg L^{-1}). The cut fronds of Soft shield fern measuring 80 cm were prepared in the morning (7-8 am) and their fresh weight was recorded simultaneously. Thereafter each cut frond was sprayed with glycerol (4 %) and Benzyl Adenine (25mg L^{-1}) treatment-wise. Then, the treated fronds were stored in different packaging materials (i.e. cellophane sheets, polymesh net bags and corrugated fiberboard boxes) for three and six days at ambient temperature. During the study period, the average temperature, relative humidity, and light intensity were recorded as 24.05 degrees Celsius, 61.38%, and 827.50

Lux in season-I. However, for season-II, the values were slightly different, with a temperature of 19.10 degrees Celsius, relative humidity of 62.39%, and light intensity of 734.82 Lux. The observation of weight loss and appearance of fronds were recorded after storage. Subsequently, the stored fronds and fresh fronds were placed in test tubes containing 70 ml of distilled water to assess their longevity. Freshness and color were graded (A, B, C) with corresponding points of 5, 3 and 1, respectively, based on the Royal Horticulture Society colour chart (RHS) (Voss, 2002).

There were 19 treatment modules and details are M_0 = Control (Fresh fronds collected from forest and placed in test tube containing water), M_1 = Fronds packed in Corrugated fiberboard boxes and stored for three days, M_2 = Fronds packed in Corrugated fiberboard boxes and stored for six days, M_3 = Fronds wrapped in cellophane sheets with three days storage, M_4 = Fronds wrapped in cellophane sheets with six days storage duration, M_5 = Poly mesh net bags as packaging material with three days storage, M_6 = Poly mesh net bags as packaging material with six days storage, M_7 = cut fronds sprayed with glycerol (4%) and packed in Corrugated fiberboard boxes and stored for three days, M_8 = fronds sprayed with glycerol (4%) and packed in Corrugated fiberboard boxes and stored for six days, M_9 = fronds sprayed with glycerol and wrapped in cellophane sheets with three days storage, M_{10} = spraying the fronds with glycerol @ 4% and wrapped in cellophane sheets with six days storage, M_{11} = fronds sprayed with glycerol @ 4% and kept in Poly mesh net bags with three days storage, M_{12} = spraying the fronds with glycerol 4% and kept in Poly mesh net bags with six days storage, M_{13} = Fronds sprayed with Benzyl Adenine (25mg L^{-1}) and packed in Corrugated fiberboard boxes with three days storage, M_{14} = Fronds sprayed with Benzyl Adenine (25mg L^{-1}) and packed in Corrugated fiberboard boxes with three six storage, M_{15} = Fronds sprayed with Benzyl Adenine (25mg L^{-1}) and kept in Poly mesh net bags with three days storage, M_{16} = Fronds sprayed with enzyl Adenine (25mg L^{-1}) and kept in Poly mesh net bags with six days storage, M_{17} = Fronds sprayed with Benzyl Adenine @ 25mg L^{-1} and wrapped in cellophane sheets with storage duration three days and M_{18} = BA - Fronds sprayed with Benzyl Adenine @ 25mg L^{-1} and wrapped in cellophane sheets with storage duration six days.

Statistical analysis

By employing a Completely Randomized Design (CRD), the data for quantitative estimation of various physico-chemical attributes were analyzed. All the data recorded was subjected to statistical analysis using MS-Excel and OP-Stat to determine the significance of the results obtained.

RESULTS AND DISCUSSION

Appearance (Freshness and colour)

The data in Table 1 shows that treatment module M_9 i.e. spraying of cut fronds with Glycerol @ 4 % then wrapping in cellophane sheets and 3 days storage, resulted in the best appearance score (4.67) for cut fronds, while module M_2 (i.e. fronds packed in CFB Boxes with 6 days storage) had the lowest score (1.73). Cut fronds from Season-II (S_2) exhibited a better appearance (3.36) than those from Season-I (S_1, 3.23). The interaction of $M_9 \times S_2$ gave the best appearance (4.80), while $M_2 \times S_1$ had the lowest (1.47). The treatment module M_9, involving spraying fronds with 4% glycerol and wrapping them in cellophane sheets for 3 days of storage, improved turgidity and minimized water evaporation, creating a favorable micro-climate for physiological processes. The results were supported by the findings of Punetha and Trivedi (2018) in the rose cultivar 'Naranjo'. Fronds from Season-II outperformed Season-I due to physiological maturity and accumulated food reserves, while Season-I fronds suffered from succulence caused by prolonged rainy conditions. The results were supported from the findings of (Wahba *et al*., 2017) in *Cynara cardunculus* L and (Gololo et al., 2016) in *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides* that seasonal changes affected the quantity of inherent phytochemicals in the plant leaves.

Chlorophyll content (mg/g)

From the Table 1, treatment modules significantly affected the chlorophyll content of cut fronds. Maximum chlorophyll content (0.65 mg/g) occurred in M17 in which Benzyl adenine (BA) @ 25mg L^{-1} was sprayed on cut fronds and then fronds wrapped in cellophane sheets with 3-day storage which was at par with the treatment module M_{18}. Minimum chlorophyll content (0.30 mg/g) was in M_2 (i.e. fronds stored in CFB Boxes with 6-day storage duration). Season-II exhibited higher chlorophyll content (0.45 mg/g) than Season-I (0.42 mg/g). $M_{17} \times S_2$ interaction showed maximum chlorophyll content (0.66 mg/g) while $M_2 \times S_1$ had minimum (0.29 mg/g). M_{17} (BA spraying with cellophane wrapping) in 3-day storage was found most effective in maintaining high chlorophyll content in both seasons. The maximum chlorophyll content in cut fronds treated with BA (25 mg L^{-1}) and wrapped in cellophane sheets for three days is attributed to the slowdown of senescence and chlorophyll degradation by application of Benzyl adenine due to the factor that Benzyl adenine application reduces the yellowing of leaves (Favero *et al*., 2020). Packaging provides a barrier against air flow and minimizes transpiration losses, creating a favorable micro-climate for frond freshness (Lufu *et al*., 2020). Glycerol (4%) application also delays chlorophyll degra-

Table. 1. Effect of post-harvest treatment modules on seasonal appearance (freshness & colour), chlorophyll content and fresh weight of cut fronds of *Polystichum squarrosum*

Treatment modules	Appearance (Freshness & colour)			Chlorophyll content (mg/g)			Fresh weight (g)		
	Season-I (S_1)	Season-II (S_2)	Mean	Season-I (S_1)	Season-II (S_2)	Mean	Season-I (S_1)	Season-II (S_2)	Mean
M_0	3.53	3.60	3.57	0.38	0.46	0.42	29.85	29.99	29.92
M_1	2.47	2.60	2.53	0.31	0.33	0.32	29.80	30.04	29.92
M_2	1.47	2.00	1.73	0.29	0.32	0.30	29.89	29.95	29.92
M_3	4.07	4.40	4.23	0.57	0.58	0.57	29.87	29.95	29.91
M_4	3.53	3.60	3.57	0.40	0.45	0.42	29.90	29.90	29.90
M_5	3.67	3.80	3.73	0.43	0.46	0.45	29.89	30.00	29.95
M_6	2.17	2.20	2.18	0.32	0.35	0.34	29.50	29.98	29.74
M_7	3.60	3.60	3.60	0.44	0.46	0.45	29.94	29.97	29.96
M_8	2.40	2.40	2.40	0.35	0.37	0.36	29.87	29.99	29.93
M_9	4.53	4.80	4.67	0.61	0.63	0.62	29.64	29.96	29.80
M_{10}	3.60	3.73	3.67	0.45	0.48	0.47	29.39	29.82	29.61
M_{11}	4.07	4.20	4.13	0.42	0.44	0.43	29.37	29.75	29.56
M_{12}	3.07	3.13	3.10	0.35	0.36	0.35	29.96	29.94	29.95
M_{13}	3.20	3.20	3.20	0.38	0.38	0.38	29.83	29.82	29.82
M_{14}	2.20	2.53	2.37	0.32	0.34	0.33	29.94	30.01	29.98
M_{15}	3.33	3.40	3.37	0.40	0.42	0.41	29.78	29.95	29.87
M_{16}	2.17	2.20	2.18	0.32	0.34	0.33	29.74	29.86	29.80
M_{17}	4.33	4.40	4.37	0.65	0.66	0.65	29.86	29.27	29.57
M_{18}	3.87	4.07	3.97	0.63	0.64	0.64	29.98	29.87	29.92
Mean	3.23	3.36		0.42	0.45		29.79	29.90	
$CD_{0.05}$	Modules		0.15	Modules		0.02	Modules		NS
	Seasons		0.05	Seasons		0.01	Seasons		NS
	Modules × Seasons		0.21	Modules × Seasons		0.03	Modules × Seasons		NS

Season-I- August-September (2019); Season-II- March – April (2020)

dation and enhance freshness (Nguyen *et al*., 2021).

Weight of fronds after storage/weight change (%)

The data in Table 2 shows that treatment modules significantly affected the weight change (%) of cut fronds. M_9 (i.e. glycerol spraying, cellophane wrapping and 3-day storage) showed the minimum weight change (3.33%), superior to all other treatments. M_2 (i.e. fronds stored in Corrugated Fiber Board boxes and stored for 6-days) exhibited the maximum weight change (35.66%). Minimum weight change of cut fronds (15.35%) was found in Season-II over Season-I (20.59%). In interaction M_9 × S_2 (glycerol spraying, cellophane wrapping and 3-day storage in Season-II) had the minimum weight change (2.80%) whereas, in M_2 × S_1 (fronds stored in Corrugated Fiber Board boxes, 6-day storage in Season-II) had the maximum weight change (40.04%). The minimum weight change in cut fronds treated with glycerol (4%) and wrapped in cellophane sheets for three days is due to low transpiration loss and maintained relative humidity during storage. During the season-II, the low temperatures significantly contributed to minimal physiological weight loss. This is because water loss is reduced in colder conditions, positively impacting the physiological weight of the cut fronds. (Pranuthi et al., 2018) Similar results of low physiological weight loss were found in rose cv. 'Kiro' under low temperature conditions. Moreover, the maximum weight loss observed during prolonged storage can be attributed to the process of respiration or the breakdown of complex compounds into simple compounds with low molecular weight (Anggraeni, 2021). Senescence processes in cut stems lead to consuming stored food in jujube fruit (Yang et al., 2021).

Vase life (days)

The data in Table 2 indicate that treatment modules and seasons significantly influenced the vase life of cut fronds. The maximum vase life (19.14 days) was observed in M_9 (glycerol spraying, cellophane wrapping and 3-day storage), while the minimum (7.87 days) was in M_2 (CFB boxes, 6-day storage) which was at par with M_6 (8.47 days). Longer vase life (13.33 days) was observed in Season-II as compared to that in Season-I (10.19 days). The M_9 × S_2 interaction showed significantly maximum vase life (21.14 days) in cut fronds with glycerol spraying and cellophane wrapping for three days in Season-II, while M_2 × S_1 had the minimum (6.88 days) in Season-I.

The glycerol treatment might have affected stomata conductivity, reducing water loss and maintaining turgidity, thus prolonging vase life. Cellophane wrapping create a favorable atmosphere, slowing respiration, transpiration, and cell division processes (Sarkar *et al*., 2019). However, due to physiological changes during storage, rapid senescence occurred after a specific period in *Nephrolepis exaltata* (Abou-Dahab *et al*., 2013).

Water uptake (ml)

Table. 2 Effect of treatment modules on weight change (%), vase life (days) and water uptake/consumed (ml) *by Polystichum squarrosum*

Treatment modules	Weight Change (%)			Vase life (days)			Water uptake (ml)		
	Season-I (S_1)	Season-II (S_2)	Mean	Season-I (S_1)	Season-II (S_2)	Mean	Season-I (S_1)	Season-II (S_2)	Mean
M_0	15.82	12.34	14.08	8.25	12.32	10.29	33.00	25.83	29.42
M_1	19.62	11.01	15.32	8.32	10.42	9.37	35.33	26.86	31.10
M_2	40.04	31.28	35.66	6.88	8.85	7.87	24.53	15.90	20.22
M_3	7.31	3.19	5.25	13.57	17.57	15.57	38.33	29.16	33.75
M_4	17.06	10.97	14.02	10.53	13.43	11.98	35.93	26.27	31.10
M_5	15.93	11.98	13.95	10.07	11.78	10.93	39.00	28.87	33.94
M_6	30.43	27.22	28.83	6.97	9.97	8.47	21.60	11.26	16.43
M_7	18.01	11.98	14.99	10.35	14.35	12.35	27.00	18.15	22.58
M_8	29.17	20.67	24.92	8.30	11.29	9.80	24.33	17.09	20.71
M_9	3.86	2.80	3.33	17.14	21.14	19.14	17.80	10.10	13.95
M_{10}	9.72	8.84	9.28	13.23	17.23	15.23	20.47	14.20	17.33
M_{11}	19.57	15.61	17.59	11.34	15.34	13.34	27.27	18.16	22.71
M_{12}	27.29	24.36	25.83	9.53	10.52	10.02	19.07	11.17	15.12
M_{13}	26.46	19.26	22.86	9.61	12.28	10.95	38.60	28.38	33.49
M_{14}	33.69	21.45	27.57	7.59	10.59	9.09	33.87	23.27	28.57
M_{15}	23.99	17.61	20.80	9.55	13.22	11.38	39.93	29.51	34.72
M_{16}	31.40	26.69	29.04	7.45	10.05	8.75	30.40	23.41	26.91
M_{17}	8.45	5.12	6.79	14.17	18.17	16.17	43.73	34.01	38.87
M_{18}	13.33	9.26	11.30	10.83	14.83	12.83	39.73	30.14	34.94
Mean	20.59	15.35		10.19	13.33		31.05	22.20	
$CD_{0.05}$	Modules		1.41	Modules		0.84	Modules		0.05
	Seasons		0.46	Seasons		0.27	Seasons		1.26
	Modules × Seasons		1.99	Modules × Seasons		1.19	Modules × Seasons		1.78

Season-I- Experiment conducted during August-September (2019); Season-II- Experiment conducted during March – April (2020)

The data in Table 2 showed a significant influence of treatment modules and seasons on water uptake by cut fronds. Treatment module M_9 (glycerol spraying, cellophane wrapping, 3-day storage) had the minimum water uptake (13.95 ml/stem), while M_{17} (BA spraying, cellophane wrapping, 3-day storage) had the maximum (38.87 ml/stem) water uptake. Season-II (S_2) exhibited lower water uptake (22.20 ml/stem) compared to Season-I (S_1, 31.05 ml/stem). In the $M_9 \times S_2$ interaction, the minimum water uptake (10.10 ml/stem) occurred (glycerol spraying, cellophane wrapping for three days in Season-II), and the maximum occurred (43.73 ml/stem) in $M_{17} \times S_1$ interaction (BA spraying, cellophane wrapping for three days in Season-I). Glycerol treatment maintained water balance as an osmolyte, promoting normal water absorption (Rida and El-Gedawey, 2022; Revathi *et al*., 2021) while cellophane wrapping created optimal conditions for healthy physiological systems (Punetha and Trivedi, 2018). Higher water uptake in Season-I might be due to increased respiration at higher temperatures (Dusenge *et al*., 2019). Lower water uptake in fronds stored for 6 days could be attributed to decreased water absorption ability, aligning with the findings of Patel *et al*. (2008) in spider lily.

Conclusion

The treatment module M_9 (glycerol spraying, cellophane wrapping, 3-day storage) resulted in the best appearance, maximum chlorophyll content, minimum weight change and longest vase life of cut *Polystichum squarrosum* fronds. Season-II (March-April 2020) fronds performed better than Season-I (August-September 2019). The combination of glycerol treatment and cellophane wrapping for three-day storage proved most effective in preserving the quality of cut fronds.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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