

Research Article

Malays. j. med. biol. res.



Efficacy of Physiologically Active Anti-Transpirants on Excised Leaves of Potted Plants

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ABSTRACT

Pot plant production in the greenhouse is most of the time under high relative humidity and frequent irrigation. While, during shipping and retailing plants may be exposed to high temperature and infrequent irrigation. These unfavorable conditions often cause water loss, desiccation of plants and short shelf life. So as to reduce the deteriorating effect of water loss, application of anti-transpirants is one of the integral options to implement. In this study, we evaluated several physiologically active stomata closing compounds. *Spathiphyllum sweet checo* and *Calathea rufibarba* were sprayed with different active compound formulations. Weight loss of leaves as gram of water per gram of initial leaf weight and gram of water per centimeter square leaf area and stomatal conductance were measured. On both plant species most physiologically active compound treatments reduce weight loss and stomatal conductance significantly. 1.5 mM Absciscic acid (ABA), 5 mM Salicylic acid (SA) and 200 μ M Sodium nitroprusside (SNP) on *Spathiphyllum sweet checo* and 1.5 mM ABA, 200 μ M SNP and 200 μ M Pyrabactin on *Calathea rufibarba* were the three most effective active compound treatments. Significant positive correlation were found between weight loss and stomatal conductance in both plant species ($R=0.888$, $P<0.05$), ($R=0.811$, $P<0.05$) for *Spathiphyllum* and *Calathea* respectively. The relative efficacy of different active compound treatments and the effects of leaf weight and leaf area on water loss were also inseparable in both plant species.

Key words: Leaf shine, Absciscic acid, Salicylic Acid, Sodium nitroprusside and Pyrabactin

8/18/2015

Source of Support: Netherlands Fellowship Program (NFP), No Conflict of Interest: Declared

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INTRODUCTION

Pot plant production in the greenhouse is most of the time under high relative humidity and frequent irrigation. While, during shipping and retailing plants may be exposed to high temperature and infrequent watering. These unfavorable post production environments can cause rapid substrate drying, desiccation of plants and acceleration of senescence and then short shelf life (Waterland et al., 2010). Water loss is the main post production problem that deteriorate the quality of potted plants and make them unsalable (Van Iersel et al., 2009). For example, in common potted foliage plants *Monstera deliciosa* and *Philodendron sp* the amount of water loss during shipping and retailing was much higher than their growing environment leading to premature leaf wilting and death (Nerman and Emad 2011).

The principal cause of water loss from plants is because of transpiration through the stomatal opening. The opening of stomata allows gas exchange between the plant and its environment. In the course of stomatal opening CO_2 can enter into the plant and water can be lost as a vapor. Hence, to avoid desiccation and eventual senescence, it is vital to have a right balance between carbon gain and water loss through stomatal movements (Atwell et al. 1999). A stoma (plural stomata) is a microscopic pore on the surface (epidermis) of land plants. It is surrounded by a pair of specialized epidermal cells called guard cells, which act as a turgor-driven valve that open and close its pores in response to a given environmental conditions. The opening and closing mechanism of stomata depends upon the turgor pressure in the guard cells. When the guard cells are turgid, the stoma opens, and when the guard cells lose water, stoma closes. Stomatal closure can be induced

by many factors, such as osmotic stress, darkness, high CO₂ concentration and mechanical stresses (Kearns and Assmann, 1993). Naturally, plants react to water deficit by closing stomata mainly mediated by Absciscic acid (ABA). During water stress ABA triggers the closure of stomata, consequently a reduction in water loss by transpiration (Christmann et al., 2005). In the course of ABA-induced stomatal closure, first ABA binds to its receptors at the surface of the plasma membrane of the guard cells. The receptors in the guard cells can then activate several interconnecting pathways that converge to produce: Cytosolic PH rise and transfer of Ca²⁺ from the vacuole and intercellular spaces to the cytosols of the guard cells. The changes in cytosol pH and transfer of Ca²⁺ can stimulate the loss of negatively-charged ions (anions), especially NO₃⁻ and Cl⁻ from the guard cells. The loss of these anions can lead to the depolarization of guard cells membrane. The depolarization of the plasma membrane can trigger the loss of K⁺ from guard cells. The loss of these solutes in the cytosol then reduces the osmotic pressure of the cell and thus turgor (Geiger et al., 2011; Nilsson and Assmann, 2007).

To reduce water loss and its subsequent negative effect on the qualities of pot plants, it is important to develop techniques that can be engaged to reduce transpiration and preserving postharvest quality for extended time (Łukaszewska and Skutnik, 2003). The major techniques that can be used to reduce transpiration could be the use of anti-transpirant agents. Anti-transpirant agents are compounds or provisions capable of reducing water loss from leaves of plants. These agents can be grouped into three groups (Prakash and Ramachandran, 2000), i) Film-forming types: that can create a physical barrier between the leaf and the surrounding (e.g. glycerol). ii) Reflecting materials: that can reflect the radiation falling on the surface of the leaves to reduce leaf temperature and the light needed for signaling during stomatal opening (e.g. Kaolin), and iii) Physiologically active stomata closing types: such as ABA and NO, ethylene etc. capable of affecting metabolic processes in leaf tissues. All things considered, the interest of this study was the evaluation of physiologically active stomata closing compounds for their anti-transpirant effect on two pot plant species and to compare the extent at which these active compounds control transpiration relative to the successful commercially available film forming anti-transpirant Leaf shine (LS). Demonstrated by many studies, physiologically active compounds instituted to be effective in reducing stomatal apertures in different plant species and different conditions are Absciscic acid (ABA) Aminoethoxyvinylglycine (AVG), Ethylene, 1-methylcyclopropene (1-MCP), Nitrous oxide (NO), Hydrogen peroxide (H₂O₂), Pyrabactin, Salicylic acid (SA) and Methyl Jasmonate (MJ) etc.

MATERIALS AND METHODS

Excised leaves of *Spathiphyllum sweet Checo* and *Calathea rufibarba* were used. Leaves were cut from their mother plant using sharp scissor leaving a 10 cm petiole intact with the leaf and 30 ml of tap water filled in a plastic tube enclosed by a rubber cap was used as a vase solution. Matrix solution (producer unknown) as carrier solution for active compounds, film forming anti-transpirant: Chrysal Leaf shine concentrate solution diluted 1:20 with deionised water (Chrysal international B.V., Naarden, the Netherlands), active compounds: ABA 10% w/v (Valent USA, Walnut Creek, CA, United states of America), Hydrogen peroxide 30 wt. sol. in water, Sodium nitroprusside dehydrate, Salicylic acid sodium salt (2 hydroxy benzoic acid), Methyl jasmonate 95% and (CaCl₂) Calcium chloride dehydrate (all from Sigma-Aldrich Corporation, Steinheim Germany) were used. Spraying was done under the flow hood using a very fine airbrush sprayer powered by pressurized gas. The abaxial and adaxial surfaces of excised leaves were sprayed homogeneously up until the spraying solution started to flood off the leaf surface. After spraying was done, leaves were left to dry outside the flow hood in the laboratory for about two hours. After leaves had been completely dried, they were transferred to the flow hood under continuous light exposure. The Temperature in the flow hood was revolving between 20 to 22 °C and relative humidity was 45 to 50 throughout the 10-day experimental period.

At the start of each experiment, initial leaf weight of each leaf was determined. Once leaves were sprayed and dried, the weight of the leaf + tube + vase solution were measured and recorded as a starting point. In the course of the experimental period weight loss from the system was measured and recorded every 24 hour. At the last day of each experiment leaf area was also measured using LI-3100C Area Meter; before the actual leaf area measurement, the area meter was turned on for 15 minutes and calibrated. Daily water loss from the leaf surface was calculated using a simple formula: **Daily water loss** = (Weight of the leaf + tube + vase solution at day 0, 1, 2...10 - weight of leaf + tube + vase solution at day 1, 2, 3...10). The amount of water transpired from the leaf surface each day was then calculated both in terms of amount of water transpired per grams of initial leaf weight and per centimetre square leaf area using the formula: **Transpiration 1** = ((Weight of the leaf + tube + vase solution at day 0, 1, 2... (n) - weight of leaf + tube + vase solution at day 1, 2, 3... (n) / initial leaf weight)) and **Transpiration 2** = ((Weight of the leaf + tube + vase solution at day 0, 1, 2... (n) - weight of leaf + tube + vase solution at day 1, 2, 3... (n) / leaf area)). Cumulative water loss over time was also calculated by summing up weight loss at a given time points. Stomatal conductance was measured every morning 10:00 to 12:00 hours using SC-1 leaf Porometer for stomatal conductance measurements (Decagon Devices Inc., Pullman, USA).

The design of the experiment was CRD with three replications (leaves as a replication), and data analysis was done using SAS version 9.1. To test differences in weight loss and stomatal conductance one way ANOVA, followed by a Tukey mean separation test was carried out. Correlation analysis was also done to show relationships between different parameters.

RESULT AND DISCUSSION

For *Spathiphyllum sweet checho*, statistically, the amount of water transpired significantly differed between treatments ($p < 0.05$). LS 1:20 was significantly different with all treatments except ABA 1.5 mM, ABA 3 mM, SA 5 mM, SNP 200 µM, SNP 400 µM, H₂O₂ 2 mM, H₂O₂ 4 mM and Pyrabactin 400 µM. Whereas, the best active compound treatment, ABA 1.5 mM was only significantly better than CaCl₂ 100 mM, MJ 100 µM, water, CaCl₂ 50 mM and matrix. The other best active compound

treatments; SA 5 mM, SNP 200 μ M, H₂O₂ 4 mM and SNP 400 μ M were also significantly different with MJ 100 μ M, water, CaCl₂ 50 mM and matrix. Most best performing active compound treatments were insignificantly different with each other but significantly different with the inferior treatments. Unlike *Spathiphyllum*, Leaf shine was less effective than active compound treatments on *Calathea*. There were only significant differences for LS 1:20 with SA 10mM and CaCl₂ 100 mM and ABA 1.5mM with SA 10mM and CaCl₂ 100mM. On the other hand, similar to *Spathiphyllum* the difference in water loss between most active compound treatments was insignificant. Nevertheless, water loss in leaves treated with LS 1:20 remained lower than the other treatments and from active compound treatments ABA 1.5mM and SA 10mM were the most and least effective treatments respectively. None of the active compound treatments bested the efficiency of LS 1:20 and some active compound treatments even caused a higher amount of water loss than the least effective control treatment in the trial (table 1).

Table 1: Total weight loss at the 10th day on *Spathiphyllum* sweet checo and *Calathea* rufibarba expressed as (g/g) of initial leaf weight. Values are based on the three leaves per treatment. Significant differences are denoted by different lower case letters (a,b)

No	Treatments	Weight loss (g/g)	
		<i>Spathiphyllum sweet checo</i>	<i>Calathea rufibarba</i>
1	Water	1.742(e)	0.937(ab)
2	Matrix	1.759(e)	0.909(ab)
3	Leaf shine 1:20	0.587(a)	0.525(a)
4	Matrix + ABA 1.5 mM	0.981(ab)	0.545(a)
5	Matrix + ABA 3 mM	1.092(abcd)	0.719(ab)
6	Matrix + Pyrabactin 200 mM	1.224 (bcde)	0.701(ab)
7	Matrix + Pyrabactin 400 μ M	1.101(abcd)	0.839(ab)
8	Matrix + SNP 200 μ M	1.019(abc)	0.679(ab)
9	Matrix + SNP 400 μ M	1.073(abc)	0.703(ab)
10	Matrix + H ₂ O ₂ 2 mM	1.153(abcd)	0.758(ab)
11	Matrix + H ₂ O ₂ 4 mM	1.028(abc)	0.891(ab)
12	Matrix + CaCl ₂ 50 mM	1.743(e)	0.925(ab)
13	Matrix + CaCl ₂ 100 mM	1.603(cde)	1.258(b)
14	Matrix + SA 5 mM	1.006(abc)	0.790(ab)
15	Matrix + SA 10 mM	1.320(bcde)	1.315(b)
16	Matrix + H ₂ O ₂ 2 mM +SA 10 mM	1.223(bcde)	0.992(ab)
17	Matrix + H ₂ O ₂ 4 mM +SA 10 mM	1.215(bcde)	0.995(ab)
18	Matrix + MJ 100 μ M	1.700(de)	1.024(ab)
19	Matrix + MJ 200 μ M	1.384(bcde)	0.909(ab)
	Mean	1.261	0.864
	Sig	*	*
	CV (%)	15.7	24.8

Statistically, significant differences were also observed between treatments on cumulative stomatal conductance readings for both plant species (table.2). Analogous to weight loss stomatal conductance was higher for *Spathiphyllum* than *Calathea*. The correlation analysis showed a significant positive correlation between weight loss and stomatal conductance ($R=0.888$, $P<0.05$) for *Spathiphyllum* and ($R=0.811$, $P<0.05$) for *Calathea*. The relationship between weight loss and stomatal conductance was more potent on *Spathiphyllum* than *Calathea*.

Table 2: A 10-day cumulative stomatal conductance of *Spathiphyllum* sweet checo and *Calathea* rufibarba ($\text{mmol/m}^2/\text{s}$). Values are based on the three leaves per treatment. Significant differences are denoted by different lower case letters (a,b)

No	Treatments	Stomatal conductance ($\text{mmol/m}^2/\text{s}$)	
		<i>Spathiphyllum sweet checo</i>	<i>Calathea rufibarba</i>
1	Water	13.900 (i)	4.567(f)
2	Matrix	20.600(j)	3.033(cde)
3	Leaf shine 1:20	0.200(a)	0.000(a)
4	Matrix + ABA 1.5 mM	1.067(ab)	0.000(a)
5	Matrix + ABA 3 mM	2.933(abc)	0.067(a)
6	Matrix + Pyrabactin 200 mM	4.433(cdef)	0.700(a)
7	Matrix + Pyrabactin 400 μ M	3.867(bcde)	0.300(a)
8	Matrix + SNP 200 μ M	1.667(abc)	0.133(a)
9	Matrix + SNP 400 μ M	3.467(bcd)	0.267(a)
10	Matrix + H ₂ O ₂ 2 mM	3.500(bcd)	1.233(ab)
11	Matrix + H ₂ O ₂ 4 mM	6.333(defg)	0.833(a)
12	Matrix + CaCl ₂ 50 mM	13.633(i)	3.833(cdef)
13	Matrix + CaCl ₂ 100 mM	12.667(hi)	2.900(cd)

14	Matrix + SA 5 mM	1.900(abc)	0.500(a)
15	Matrix + SA 10 mM	7.533(fg)	5.300(f)
16	Matrix + H ₂ O ₂ 2 mM +SA 10 mM	6.733(efg)	4.467(ef)
17	Matrix + H ₂ O ₂ 4 mM +SA 10 mM	9.500(gh)	2.533(bc)
18	Matrix + MJ 100 µM	10.733(hi)	4.300(def)
19	Matrix + MJ 200 µM	4.667(cdef)	2.500(bc)
	Mean	6.868	1.971
	Sig	*	*
	CV (%)	15.2	24.3

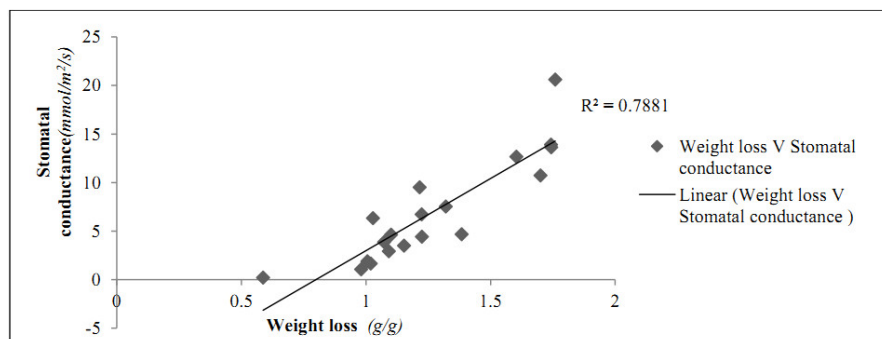


Figure 1: Graph showing the relationship of weight loss (g/g) and stomatal conductance (mmol/m²/s) on *Spathiphyllum sweet checo*, X axis showing weight loss of all treatments and Y axis represents stomatal conductance of all treatments. Values are averages of the three leaves per treatment.

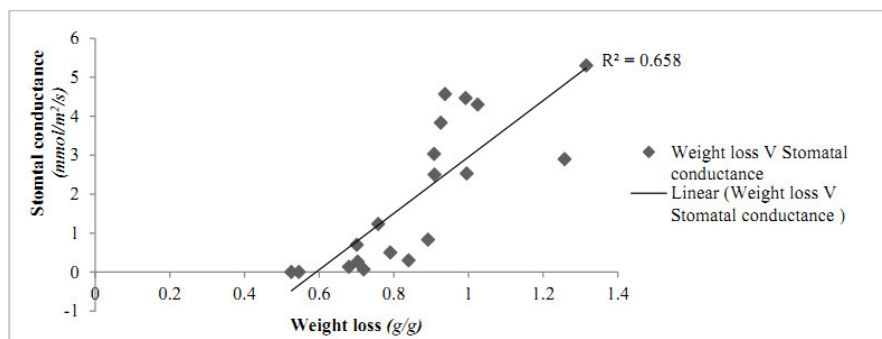


Figure 2: Graph showing the relationship of weight loss (g/g) and stomatal conductance (mmol/m²/s) on *Calathea rufibarba*, X axis showing weight loss of all treatments and Y axis represents stomatal conductance of all treatments. Values are averages of the three leaves per treatment.

As evidenced by the correlation analysis the effects of different treatments on either of the pot plants used were relatively similar ($R=0.637$, $p<0.05$). However, it might be because of the effect of the leaf surface on the performance Leaf shine and because *Calathea* leaves has low stomatal conductance, most treatments inflicted lesser water loss on *Calathea* than *Spathiphyllum*. Also on *Calathea*, it might be because of the delicate nature of the leaves, some active compound treatments also triggered more water loss than the control treatments water and matrix.

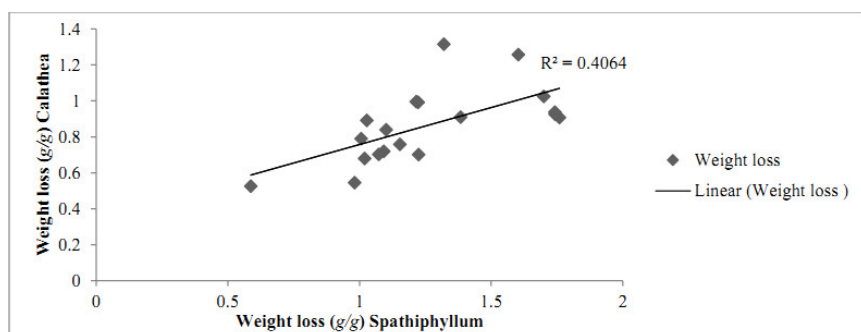


Figure 3: Graph showing the relative effect of different treatments on weight loss (g/g) of *Spathiphyllum sweet checo* and *Calathea rufibarba*. X axis showing the weight loss of all treatments for *Spathiphyllum* and Y axis represents weight loss for *Calathea*. Values are averages of the three leaves per treatment.

The effect of leaf weight and leaf area on transpiration for *Spathiphyllum sweet checo* is shown in the study. The plotted graph confirms their inseparable effect. The correlation analysis also shows a significant positive correlation between (g/g) and (g/cm²) of weight loss ($R=0.985$, $P<0.05$). The same is true for *Calathea rufibarba* and because their effect is similar, for future studies either of the measurements can be used.

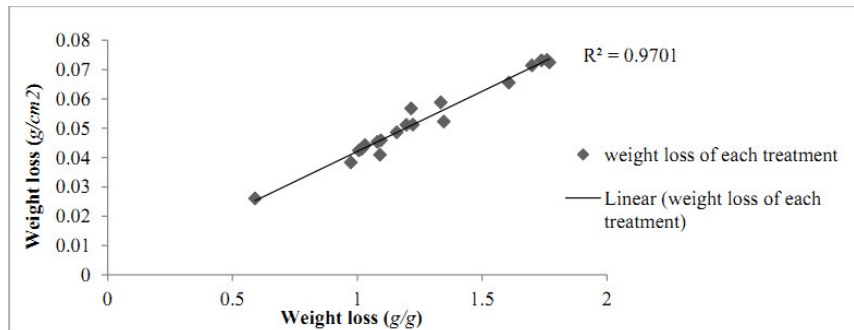


Figure 4: Graph showing the effect of leaf weight and leaf area on transpiration on *Spathiphyllum sweet checo*, X axis showing weight loss of all the 19 treatments expressed as (g/g) of initial leaf weight and Y axis represents weight loss of all the 19 treatments expressed as (g/cm²) of leaf area. Values are averages of the three leaves per treatment.

Although most of the previous studies done on physiologically active stomata closing compounds were on epidermal strips wherein the opening and closing of stomata can be monitored on a microscope, our experiment only focused on the practical implications by directly spraying the active compound with its carrier on the leaf surface and evaluating their performance on weight loss and stomatal conductance basis. As hypothesized, on both plant species most active compound treatments marked the substantial reduction of water loss. However, it wasn't possible to get an active compound treatment that can surpass the efficacy of leaf shine, only on *Calathea rufibarba*, an active compound treatment i.e. ABA 1.5mM, as capable as Leaf shine has been discovered. While, on *Spathiphyllum* Leaf shine was superior to the best active compound treatment almost by 40%. We suggest the hairs on *Calathea* leaf surface made it harder for Leaf shine to create a continuous film so as to make a successful physical barrier to obstruct stomatal opening, consequently the best active compound treatment reduced water loss equivalently with leaf shine. On the other hand, the smooth and glossy surface of *Spathiphyllum* might've been conducive to leaf shine. The manufacturer of Leaf shine also acknowledged the ineffectiveness of their product on a hairy leaf surface. Nevertheless, on both plant species most active compound treatments with their effective concentration considerably reduced water loss. As described as the most successful physiologically active anti-transpirant (Acharia and Asmman 2009), ABA was the best active compound inducing stomatal closure in both pot plant species. Similar to our results Berkowitz and Rabin (1988), and Smiljana et al., (2007) found that foliar ABA treated pepper seedling transplants had higher survival rates mainly attributed by a reduced stomatal conductance and increased leaf water potential. ABA's agonist Pyrabactin was also effective. As reported by Puli and Raghavendra (2012) the stomatal closure either by ABA or Pyrabactin might've been reached because of the rise in cytoplasmic pH, reactive oxygen species (ROS) and NO in the guard cells of stomata.

Nitric oxide (NO), as its donor compound SNP and Salicylic acid (SA) were the other successful compounds in either of the plant species. Similar results were also reported using 50μM SNP on excised leaves of wheat (*Triticum aestivum*), *Tradescantia* and *Poncirus trifoliata* (Garcia-Mata and Lamattina 2001; Fan and Liu 2012). Likewise, using SA 1 mM and 10 mM significant reduction of stomatal apertures on epidermal strips of *vicia faba* (Mori et al 2001) and substantial reduction of water loss on excised leaves of *Commelina communis* (Lee 1998) and *Phaseolus vulgaris* (Saavendra 1979) were reported. To achieve their anti-transpirant effect, NO might induced the movement and release of inter-cellular and vacuolar Ca²⁺ into the cytoplasm of the guard cells (Garcia-Mata and Lamattina 2001). While, SA might triggered the generation of H₂O₂ and inhibition of H₂O₂ scavenger catalase (Chen et al., 1993) and as it was reported recently SA might also induced the generation of extracellular super oxide O⁻² and the subsequent increase of cytoplasmic Ca²⁺ (Kawano et al ,1998 ; Kawano and Muto 2000). In contrast, H₂O₂ might also induced stomatal closure by activating Ca²⁺ channels and by inhibiting the uptake of K⁺ through K⁺ channels on the plasma membrane of the guard cells (An et al., 2000).

Although there is strong evidence methyl jasmonate can induce stomatal closure, in our experiments, it might have been because of the concentrations we used it couldn't give us the desired result. Even so, there isn't a study claiming Ca²⁺'s anti-transpirant effect, because Ca²⁺ is the major signaling molecule in the course of stomatal closure we thought it might have some effect and evaluated 50 and 100 mM CaCl₂, but it also couldn't show an anti-transpirant effect. We also thought because SA can inhibit catalase and catalase is H₂O₂ scavenger, the application of 10 mM SA together with 2 and 4 mM H₂O₂ might give us a better result, but it was not as good as anticipated. This might've been because of the concentration of SA we used, unlike 5 mM SA, 10 mM SA alone was relatively substandard.

Even though, it wasn't possible to get an active compound superior to Leaf shine and the appearance of leaves sprayed with Leaf shine were better than the other treatments, given the result of our research it's possible to use active compounds with their appropriate carrier solution as an anti-transpirant agent on pot plants.

ACKNOWLEDGEMENTS

I want to forward my sincere appreciation to Netherlands Fellowship Program (NFP) for funding my study and to my thesis supervisor Professor Dr. Ernst Woltering for his continuous guidance and support throughout the study period. I also want to acknowledge Mr. Sasan Ali for providing me the necessary information about the Porometre and stomatal conductance measurements. I also want to thank Mr. Arjen Van Peppel for his day to day assistance in the course of the study period.

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