Original Research Article

INDUCTION OF SENESCENCE AND FOLIAR ABSCISSION IN APPLE TREES WITH THE USE OF ABSCISIC ACID

ABSTRACT

Aims: Evaluate the effects of different concentrations of abscisic acid (ABA) on adult plants of productive age of 'Daiane' and 'Fuji Suprema', to follow the ecophysiological effects and their efficiency in inducing leaf senescence, sprouting and productivity, in the Midwestern region of the state of Santa Catarina. **Study design:** The experiment was arranged in a randomized block design with six replications. **Place and Duration of Study:** The experiment was carried out in the municipality of Caçador, Brazil (latitude 26º50'07" S, longitude 50º58'32" W, altitude 969 meters), during the growing seasons of 2013/2014 and 2014/2015. **Methodology:** The apple fruit tree were submitted to the following treatments: 1) Control (without treatment), 2) abscisic acid 750 mg L^{-1}, 3) abscisic acid 750 + 750 mg L^{-1}, 4) abscisic acid 1500 mg L^{-1} and 5) abscisic acid 1500 + 1500 mg L^{-1}. ABA applications in the first year were carried out on May 2, 2013 in treatments 2, 3, 4, and 5, and after 11 days (05/13/13) the plants of treatments 3 and 5 were sprayed again. In the second year, ABA applications were carried out on May 6 and 15, 2014, following the same application methodology described for the first year. As the source of abscisic acid (ABA) the commercial product ProTone® (20% i.a.) was used, without the use of adjuvant. The exogenous application of ABA was effective in promoting the senescence and leaf fall of the 'Daiane' and 'Fuji Suprema' cultivars. In the 2013/2014 season, the concentration of 750 mg L^{-1} was enough to induce 100% leaf fall in 'Daiane' plants. However, a difference was observed between the concentrations of ABA used. There was a difference between concentrations and number of applications. Lower concentrations were efficient and deflated as much as the higher concentration. The application of ABA induces the abscission of apple leaves in early autumn in 'Daiane' and 'Fuji Suprema' apple plants. The ABA does not affect shoots of axillary buds and anticipates the beginning of shoots.

Keywords: Malus domestica Borkh, leaf abscission, dormancy.

1. INTRODUCTION

The apple is the second most produced fruit in the world, and in the year 2017, were produced 83.1 million tons, being surpassed only by the banana. China holds the first position in the world ranking, with about 41.4 million tons, equivalent to 49.8% of the total production. In Brazil, the production was around 1.3 million tons [1], mainly produced in the southern region of the country (regions with the highest winter cold). The main cultivars produced belong to the Gala and Fuji groups [2].

With the reduction of temperatures from autumn, leaves of plants originating in temperate regions such as apple trees senescence due to the activation of several enzymes from the hydrolases group, resulting in degradation of proteins, carbohydrates and nucleic acids, which are translocated from the leaves to branches, trunks and roots through the phloem. On the other hand, during the rest period, the low temperatures are responsible for the induction of the degradation of the starch in soluble sugars. This increase in the levels of soluble sugars, proteins and amino acids, is cited in the literature as an important mechanism of resistance to cold [3].

Leaf senescence is influenced by several internal and external factors or controlled by a number of biological and non-biological factors, such as changes in source-sink interaction and hormonal balance, water, light and nutrient supply, or induction by pathogens and pests [4,5]. During the process of leaf senescence of deciduous plants, nitrogen in the leaves is generally translocated to the storage tissues, and after remobilization to a new growth in the following spring [5,6].

In climatic conditions suitable for growth and development in the Southern hemisphere, senescence and leaf fall occur naturally in early autumn, when there is a reduction of photoperiod and ambient temperature [7]. In the colder regions of Southern Brazil, leaf senescence generally occurs at the beginning of May, when there is a reduction in temperature, and it is ideal that all leaves fall to the end of this month as a way to optimize the control of foliar diseases and induce plant dormancy [8].

However, in mild autumn or winter regions or in years with atypical winters, the processes of maturation and fall of the leaves of the apple trees are delayed, and may often not occur, and it is often necessary to adopt some technique that promotes leaf fall to promote sprouting and flowering in the next cycle. Manual or early defoliation by an artificial defoliant are alternatives that can be used to mitigate the effects caused by the irregularity of the climate. Manual defoliation is commonly performed in nurseries, which is necessary for better handling of seedlings, however, this technique is quite expensive [9]. The chemical defoliation can also be ~~used,~~used; however, it has been limited only to the use of 10% urea or 1% copper sulphate, two to three months before the onset of sprouting [7]. Recently commercial product ProTone® has been developed, which contains in its formulation 10% or 20% synthetic abscisic acid (ABA). This product, when is applied at the end of the cycle, causes the uniform fall of leaves, a process that would naturally occur when induced by the incidence of low temperatures in plants [10].

The exogenous application of ABA is a common practice among fruit growers with the aim of improving grape coloration [11], increasing water stress tolerance in vegetables [12], promoting somatic embryogenesis in tissue culture in several species [13] and to stimulate leaf and fruit abscission [14]. Because of its defoliant action, the exogenous use of ABA may be an effective alternative to promote the senescence of apple leaves in cases where leaf abscission is uneven and prolonged [15].

The objective of this work was to study the effect of the application of different concentrations of ABA on the induction of senescence and defoliation in adult plants of 'Daiane' and 'Fuji Suprema' apple trees.

2. MATERIAL AND METHODS

The study was conducted in an experimental orchard in the Midwestern region of Santa Catarina, in the municipality of Caçador at an altitude of 969 meters above sea level (26º50'07 "S, 50º58'32" W), in seasons of 2013/2014 and 2014/2015. As plant material, 12-year old 'Daiane' apple trees were harvested on the M7, with planting density of 1,250 plants.ha^{-1} and 'Fuji Suprema', 8 years old, on Marubakaido with M9 intergraft, with planting density of 2,500 plants.ha^{-1}, both conducted in the central leader system and managed according to the recommendations of the apple production system [7], and management practices in the integrated system of apple production [16]. The accumulation of chill during the winter period (April to September), according to the Modified North Carolina model, was 940 and 884 chill units, for the cycles 2013/2014 and 2014 / 2015, respectively [17].

The 'Fuji Suprema' cultivar is a natural mutation of 'Fuji', whose main characteristic is the purple coloration of the fruit epidermis, observed early in its development, which later develops into a uniform red coloration of the epidermis. Ja 'Daiane' is a Brazilian cultivar, with resistance to leaf spot of glomerella and good characteristics of the fruits. Both cultivars present a long cycle, characterized by the maintenance of the leaves until late autumn, a factor that negatively affects sprouting and bud quality in the following spring. the 'Fuji Suprema' cultivar and 'Fuji' clones correspond to approximately 30% of the Brazilian apples production, whereas 'Daiane' has low expressiveness [2].

The experimental design was a randomized block design, consisting of five treatments with six replicates, the experimental unit consisting of one plant, totaling 30 plants per cultivar. The apple plants were submitted to the following treatments: 1) Control (untreated plants), 2) ABA 750 mg L^{-1}, 3) ABA 750 + 750 mg L^{-1}, 4) ABA 1500 mg L^{-1} and 5) ABA 1500 + 1500 mg L^{-1}. ABA applications in season 2013/2014 were carried out on May 2, 2013 in treatments 2, 3, 4, and 5, and after 11 days (05/13/13) the plants of treatments 3 and 5 were sprayed again. In the season 2014/2015, ABA applications were carried out on May 6 and 15, 2014, following the same application methodology described for the 2013/2014 season. It is worth noting, that were considered independent experiments for each cultivar studied apple tree. As the source of ABA the commercial product ProTone® (20% i.a.) was used, without the use of adjuvant.

The influence of ABA was evaluated on the percentage of leaf fall, axillary bud break buds percentage, phenological stages (dates), yield per plant (fruits $plant^{-1}$ and kg $plant^{-1}$) and productive efficiency (fruits cm^{-2} and kg cm^{-2}). The percentage of leaf fall was calculated by the number of leaves remaining in each branch, for each evaluation date, in relation to the initial number of leaves: defoliation (%) = 100 - (NFD / NFI * 100), where NFD = number of leaves at the evaluation date and NFI = number of leaves initial. Before plant sprouting, five and ten branches were identified in the ABA treated plants in the 2013/2014 and 2014/2015 harvests, respectively, for the counting of the total number of buds and the number of buds sprouted and flowered, to obtain the sprouting and flowering percentage of the buds. For the evaluation of the phenological stages, 20 fruiting structures per plant were previously identified, stratified in long branches and spurs, totaling 120 structures per treatment. The evaluations started when the buds were in the green tip (C3) stage, and the phenological stages were evaluated twice a week until full bloom (F2), as described initially by Fleckinger [18] and modified by Francescatto [19]. The production (kg $plant^{-1}$ and fruits $plant^{-1}$) was evaluated by counting and weighing the fruits of each plant; and the mean fresh fruit mass (g) was obtained from the relation between the total mass and the total number of fruits harvested per plant. The productive efficiency (kg cm^{-2} and fruits cm^{-2}) was obtained by the relation between production (kg $plant^{-1}$ and fruits $plant^{-1}$) and cross-sectional area of the trunk.

The data obtained with the percentage of leaf drop were submitted to analysis of variance and for the significant variables the analysis of contrasts was performed. The percentages of sprouts were submitted to analysis of variance and, if significant statistical difference was detected, the means were compared by the Scott-Knott test at 5% of probability, and the data were transformed into arc sen $(x / 100)^{1/2}$. For the phenological evaluations that presented significant statistical difference, the averages were compared through the Scott-Knott test at 5% probability. The contrasts analyzes were performed with the help of Sisvar® [20] software version 5.6 and the others using the software Assistat® [21] version 7.7 beta.

3.1 RESULTS AND DISCUSSION

The exogenous application of ABA was effective in promoting the senescence and leaf fall of the 'Daiane' and 'Fuji Suprema' cultivars. In the season 2013/2014, the concentration of 750 mg L^{-1} has been enough to induce 100% leaf drop in plants of 'Daiane'. However, a difference was observed between the concentrations of ABA used. Lower concentrations were effective when compared to larger or control concentrations (Figure 1).

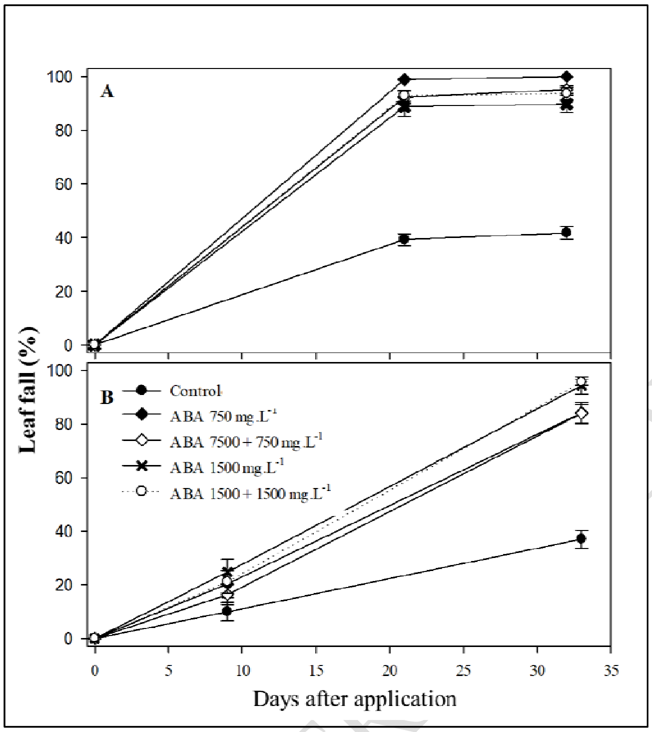

Figure 1. Percentage of leaf fall in 'Daiane' apple plants submitted to different treatments with ABA. A) Autumn 2013; B) Autumn 2014. Caçador-SC, Brazil, 2019.

For 'Fuji Suprema', the highest percentage of leaf fall was observed in the highest concentrations for season 2013/2014. There was a difference between concentrations and number of applications. Lower concentrations were efficient and defoliated as much as the higher concentration (Figure 2).

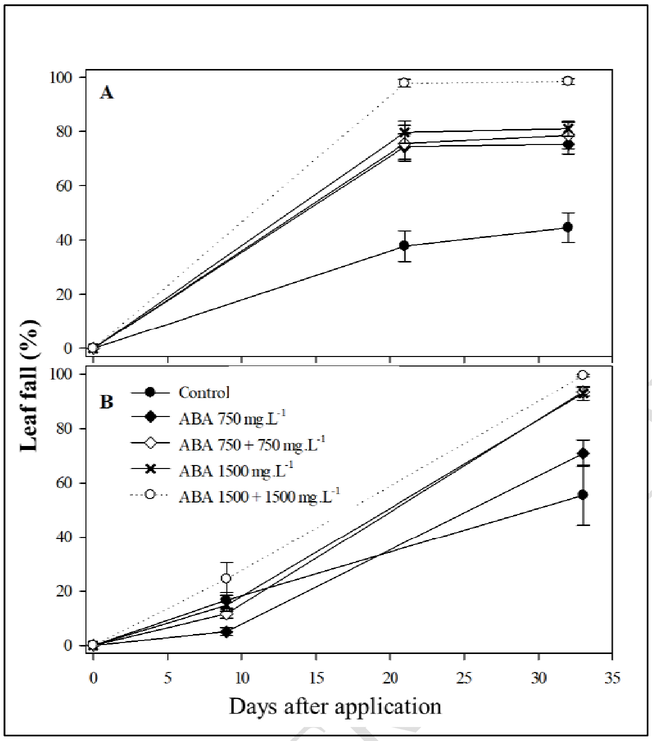

Figure 2. Percentage of leaf fall in 'Fuji Suprema' apple plants submitted to different treatments with ABA. A) Autumn 2013; B) Autumn 2014. Caçador-SC, Brazil, 2019.

In the first season, the highest percentages of defoliation were reached at 21 days after treatments (DAT). In the second season, unlike the first, the leaf fall period was prolonged, reaching the highest percentages at 33 DAT. All ABA treatments promoted senescence and leaf fall, while control plants remained leafy until late fall. Only with the first frosts occurred in June did the leaves fall (Figure 1 and 2).

According to Zhao et al [22], ABA plays a crucial role in plant survival under water stress conditions, promoting stomata closure, growth inhibition, bud dormancy and foliar senescence. In the case of water stress situations, the accumulation of abscisic acid occurs, reducing stomatal conductance, due to its stomata being closed for longer

[23]. Therefore, a possible accumulation of ABA due to water stress may have been the reason for the most pronounced defoliation in all treatments with ABA in the first season, agreeing with the results found by Gomez-Cadenas et al [24] and Meyer et al [25].

In Chile, in the Maule region, Yuri [26] tested the use of ABA and etefon as defoliants in apple trees. In ABA treatments, leaf fall occurs more readily. The treated plants require less hours of cold for flowering, whereas the control plants accumulate less hours of cold (HF) and require a higher sum of HF to start budding. Also from the Maule region, Yuri [27] found that plants treated with ABA and etefon reached 50% of the leaf fall about 10 and 18 days after the applications, respectively.

The shoots of axillary buds in cv. Daiane in the 2013/2014 crop were superior in the control treatments, ABA 750 mg L^{-1} and ABA 1500 mg L^{-1}, but in the 2014/2015 season, treatment no differed between them. Only in the evaluation of the axillary flower buds, the control was superior to the other ABA treatments (Table 1).

Table 1. Evaluations of axillary buds of long branches 'Daiane' apple trees submitted to different concentrations of abscisic acid (ABA) in the 2013/2014 and 2014/2015 seasons, Caçador-SC, Brazil, 2019.

Treatments	Evaluations of axillary buds (%)		
	2013/2014[1]	**2014/2015[2]**	
	GAB[3]	**GAB**	**GAF[4]**
T1. Control	43,4 a	41,2 ns	21,9 a
T2. ABA 750 mg L^{-1}	44,9 a	38,6	9,7 b
T3. ABA 750 + 750 mg L^{-1}	35,5 b	44,8	12,1 b
T4. ABA 1500 mg L^{-1}	55,2 a	46,1	3,8 c
T5. ABA 1500 + 1500 mg L^{-1}	36,4 b	39,3	9,9 b
CV (%)	45,1	47,4	139,5

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (P=.05). * ns: not significant (P>.05). [1]In the 2013/2014 harvest, five branches were marked per plant, totaling 30 branches per treatment. [2]In the 2014/2015 harvest ten branches were marked per plant, totaling 60 branches per treatment. [3]GAB: Sprouting Axillary Gems. [4]GAF: Axillary Gems with Flower.

For 'Fuji Suprema', no difference was observed in the percentage of shoots of axillary buds between treatments with ABA in the first season of evaluation. In the second year, the control treatments, ABA 750 mg L^{-1} and ABA 1500 + 1500 mg L^{-1} presented the highest percentage of shoots (Table 2). The treatments with ABA did not affect the shoots according to the results of Guak et al [28], who using urea and ABA as defoliants in 'Gala' / M26 apple plants, found that urea increased the level of N in the leaves and the reserve in all tissues. On the other hand, the treatments with ABA increased the senescence of the leaves and the mobilization of N, not affecting the shoots. As Kawamata et al [29], ABA induces dormancy and maintains its intensity, however, it has no effect on budding

Table 2. Evaluations of axillary buds of long branches 'Fuji Suprema' apple trees submitted to different concentrations of abscisic acid (ABA) in the 2013/2014 and 2014/2015 seasons, Caçador-SC, Brazil, 2019.

Treatments	Budding axillary buds (%)	
	2013/2014[1]	2014/2015[2]
T1. Control	81,4 [ns]	66,7 a
T2. ABA 750 mg L^{-1}	81,0	68,2 a
T3. ABA 750 + 750 mg L^{-1}	77,0	57,6 b
T4. ABA 1500 mg L^{-1}	73,7	53,3 b
T5. ABA 1500 + 1500 mg L^{-1}	74,4	64,4 a
CV (%)	23,28	28,2

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (*P*=.05). * ns: not significant (*P*>.05). [1]In the 2013/2014 harvest, five branches were marked per plant, totaling 30 branches per treatment. [2]In the 2014/2015 harvest ten branches were marked per plant, totaling 60 branches per treatment.

In the 'Daiane' during the evaluation of the phenological stages in terminal buds it was observed that the onset of budding (C3) was anticipated two days in all treatments with ABA in relation to the control, and full bloom (F2) in one day.

In the evaluation of the phenological stages in spurs, the onset of budding was anticipated in approximately five days in the application of ABA 1500 mg L^{-1} in relation to the control. The ABA treatment 1500 mg L^{-1} presented a significant difference in relation to the other treatments, being the one that most anticipated the beginning of the budding.

Full bloom was anticipated around three days in the treatment ABA 1500 mg L^{-1} in relation to the control. The same treatment presented a significant difference in relation to the others, being the one that anticipated the full bloom (Table 3).

Table 3. Number of days from the application of Abscisic Acid (ABA) until the beginning of Budding (C3) and Full Flowering (F2) in terminal buds of long branches and spurs in cv. Daiane. Caçador-SC, Brazil, 2019.

Treatments	Terminal buds		Spur	
	C3	**F2**	**C3**	**F2**
	------------------- **NDAT** [a,b] -------------------			
T1. Control	144,4 a	159,8 a	146,2 a	159,1 a
T2. ABA 750 mg L^{-1}	143,5 b	158,9 b	145,2 a	158,5 a
T3. ABA 750 + 750 mg L^{-1}	143,1 b	158,4 b	143,8 b	157,1 b
T4. ABA 1500 mg L^{-1}	143,1 b	159,1 b	140,8 c	155,9 c
T5. ABA 1500 + 1500 mg L^{-1}	142,5 b	158,5 b	145,1 a	158,6 a
CV (%)	2,07	0,90	2,43	1,48

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (*P*=.05). [a]NDAT: Number of days after treatment with ABA. [b]Application of the ABA was carried out on 05/06/2014.

In 'Fuji Suprema' the evaluation of the phenological stages, the onset of budding (C3) in terminal buds was delayed by approximately two days in treatment ABA 1500 mg L^{-1} in relation to the others. However, the total flowering did not present a significant difference between treatments. In spurs the onset of sprouting was anticipated in approximately five days in the treatments ABA 750 mg L^{-1} and ABA 750 + 750 mg L^{-1} in relation to the other treatments with ABA. In full bloom there was no difference between treatments (Table 4).

Table 4. Number of days from the application of Abscisic Acid (ABA) until the beginning of Budding (C3) and Full Flowering (F2) in terminal buds of long branches and spurs in cv. Fuji Suprema. Caçador-SC, Brazil, 2019.

Treatments	Terminal buds		Spur	
	C3	F2	C3	F2
	------------------- NDAT [a,b] -------------------			
T1. Control	141,6 b	153,6 [ns]	145,3 a	153,6 [ns]
T2. ABA 750 mg.L^{-1}	140,2 b	152,0	143,3 b	156,6
T3. ABA 750 + 750 mg.L^{-1}	141,1 b	153,6	140,3 b	156,3
T4. ABA 1500 mg.L^{-1}	142,7 a	156,3	147,4 a	158,6
T5. ABA 1500 + 1500 mg.L^{-1}	140,8 b	155,6	148,6 a	0 [c]
CV (%)	2,73	1,33	4,70	1,87

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (*P*=.05). *ns: not significant (P>.05). [a]NDAT: Number of days after treatment with ABA. [b]Application of the ABA was carried out on 05/06/2014. [c]The T5 was not considered in the statistical analysis, because it did not have any flower spur sprouts.

The results indicate that the ABA anticipated the beginning of the shoots and the full flowering. It is suggested that ABA had an effect on bud dormancy, promoting the uniformity of sprouting of apple trees. This fact is of great relevance, since the early sprouting and flowering are subject to frost damage, heavy precipitation and pest attack, can cause direct and indirect losses to the producer, both in the pollination of flowers and in the formation and development of fruits. However, the anticipation of flowering does not correspond to the anticipation of fruit maturation in the same proportion [30].

In the 2013/2014 season, the production (kg plant^{-1} and fruits plant^{-1}) for 'Daiane' was higher in T3 and T5. Plants treated with ABA in concentrations with two applications had higher production although that, in relation to the number of fruits per plant, only the lowest concentration was equal to control (data not shown). In the 2014/2015 season, the plants with the best yield were in the control and ABA 1500 + 1500 mg L^{-1} treatments. There was no difference in fruits per plant between treatments, however, T1, T2, T3 and T5 presented higher fruit mass (data not shown).

For 'Fuji Suprema', in the 2013/2014 season, there was no difference between the treatments of defoliation and control. However, in the 2014/2015 season, production was lower than in the previous year in all treatments, but the average fresh fruit mass (g fruit^{-1}) was higher in treatments T3 and T4, reaching 117g and 116g respectively (data not shown).

The results of production in both cultivars and seasons, do not offer subsidies to affirm that the application of ABA promotes improvement in crop yield, since the plants used in the experiment had never received ABA treatment. It is possible that productivity increases will be more effective after a few years of application of the product, and its effect may be cumulative due to the ABA to promote a better regularization of the vegetative cycle and mobilization of some nutrients from the leaves that senescence to the branches, thus enabling , an increase in the accumulation of nutrients in the wood year after year and that can lead to increased production.

For 'Daiane' there was no significant difference in the productive efficiency in the two seasons evaluated. For 'Fuji Suprema' there was a difference between treatments T1, T3, T4 and T5 in season 2013/2014. However in the second season there was no difference between treatments (data not shown).

The productive efficiency, as well as the production per plant, did not present significant differences indicating that the ABA application may have promoted improvement in this characteristic. Even in the 'Fuji Suprema' in the 2013/2014 season, where the difference between treatments was observed, the applications of higher doses of ABA did not differ from the control. Due to this, it is hypothesized that the increase in productive efficiency should also have a cumulative effect with successive applications of ABA over the years.

4.1 CONCLUSION

Comment [REV A1]: PLEASE INDICATE THE BEST CONECTRATION IN CONCLUSIONS TO INDUCTION OF SENESCENCE AND DEFOLIATION TO EACH VARIETY (DAISAME AND FUJI)

: 1) Control (untreated plants), 2) ABA 750 mg L^{-1}, 3) ABA 750 + 750 mg L^{-1}, 4) ABA 1500 mg L^{-1} and 5) ABA 1500 + 1500 mg L^{-}

The application of ABA induces the abscission of apple leaves in early autumn in 'Daiane' and 'Fuji Suprema' apple plants.

The ABA does not affect shoots of axillary buds and anticipates the beginning of shoots.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [REV A2]: 4 or 5 REFERENCES FROM JEAI

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