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Effect of anti-transpirants on the transpiration rate and photosynthetic index of sweet orange (*Citrus sinensis* (L.) Osbeck)

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Abstract

Sunburn is a major problem caused by climate change *i.e.* high temperature and direct solar radiation, which leads to significant economic losses in sweet orange yield. So, a field experiment was conducted on ambe bahar crop of 2018 and 2019 in two locations, to study the effect of foliar application of antitranspirants twice in a month from March to June on transpiration rate and photosynthetic index of 'Sathgudi' sweet orange trees. The obtained results showed that, foliar application of antitranspirants was effective to control fruit drop due to sunburn as compared to fruits from trees applied with 1% urea and 2% quick lime. Moreover, foliar application of liquid paraffin @ 2% has decreased rate of transpiration and increased the water use efficiency. Spray of salicylic acid @ 500 ppm reduced stomatal density and cycocel @ 2000 ppm has highest rate of photosynthesis. Therefore, it could be recommended that, spraying antitranspirants twice in summer months had a positive impact on preventing fruit sunburn damage and improved the yield and fruit quality of sweet orange.

Keywords: Anti-transpirants, photosynthetic, *Citrus sinensis* L.

Introduction

Sweet orange (*Citrus sinensis* (L.) Osbeck), an important group of citrus is produced all over the world majorly in China, Brazil, India, Egypt, the European Union and Morocco. Sathgudi, is the choicest variety of sweet orange which is grown in many districts of Andhra Pradesh. The changing climate is impacting weather in many ways with drought and temperature rise which may drastically bring down the production, if other possibilities were not initiated to increase water use efficiency in water stress conditions. Sunburn - a physiological disorder in citrus caused by excess light and high fluctuation densities of solar radiation. It affects the natural defense systems of plants, fruit is discolored and exhibits varying degrees of cell death causing commercial losses of fruits. It is particularly problematic in arid and semi-arid regions and has been attributed to the combination of visible light and high temperatures. Mishra *et al* (2016) [27], reported that, antitranspirants can be used to minimize water loss in times of drought or heat stress. However, the physiological impacts and interactions of these compounds on citrus have not been studied thoroughly.

Antitranspirants are chemical compounds which favour the reduction in rate of transpiration from plant leaves gradually hardening them to stress (Ahmed *et al.* 2014 and El Khawaga, 2013) [1]. They have the chance of conserving irrigation water, aiding plant survival under dry conditions and protecting foliage against fungus, insects, smog, and salt spray. Nearly 95-98% of the water absorbed by the plant is lost in transpiration (Prakash and Ramachandran, 2000 and Gaballah, 2014) [33, 12]. It is a substance involved in increasing drought stress resistance. The effectiveness of any antitranspirant is based on its concentration, the species, the stage of development of the plant and the environmental conditions (Gale and Hagan, 1966) [14]. Al-Moftah and Al-Hamaid (2005) [3] stated that the cost of antitranspirant is one third cost of the pesticide, fast absorption immediate and safe for the environment, no toxic effects on humans, animals and plants.

Materials and methods

This investigations, was conducted at the experimental field of Citrus Research Station, Tirupati, Department of Fruit Science, Dr.Y.S.R. Horticultural University, in Chittoor District (Location-1) and also at farmer's field of Railway Kodur in Kadapa District, (Location-2) of Andhra Pradesh during the year 2018 to 2019. The experiment was conducted in randomized block design with nine treatments, three replications and two trees for each replication.

The experiment involved following ten treatments.

- A₁ Cycocel 1000 ppm
- A₂ Cycocel 2000 ppm
- A₃ Salicylic Acid 500 ppm
- A₄ Salicylic Acid 1000 ppm
- A₅ Kaolin 1%
- A₆ Kaolin 2%
- A₇ Liquid Paraffin 1%
- A₈ Liquid Paraffin 2%
- A₉ Farmers Practice: Urea @ 1% spray followed by Quick lime @ 2% spray at 15 days interval

These treatments are imposed at fortnightly intervals during the dry spell of fruit development. So, the treatments were imposed from March 15th 2019 to June 30th 2019.

Relative water content of leaf (%)

It is a reliable trait, for screening drought tolerance (Rachmilevitch *et al.* 2006). It is defined as the percentage of water present at the time of sampling, relative to the amount of water in a saturated leaf.

$RWC (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$

Stomatal Density (No./mm²)

The leaf lamina was treated with acetic-formalin-alcohol and tangential sections of the leaf blades were studied under DMi8 Leica inverted microscope from Wetzlar, Germany Leica Microsystems. Dilute solution of potassium hydroxide is used to remove hesperidin and 90 per cent alcohol to remove the chlorophyll, then stained with alcoholic safronin and finally cleared in dilute potassium hydroxide. The number of stomata in area of 1 mm² was counted and the average of ten or more areas was recorded. Areas in the vicinity of oil glands, epidermal hairs and large veins were avoided (Reed and Hirano, 1931) [35].

Rate of transpiration (mol of H₂O/cm²/s), stomatal conductance (mmol/m²/s) and rate of photosynthesis (μg CO₂/m²/s)

All these three parameters were measured for three newly formed mature leaves which are fully exposed to Sun from all the four sides of the tree branches. This was done in the morning from 8:00 AM to 10:00 AM using artificial sunlight (1000 μEim m⁻² sec⁻¹) with portable photosynthetic meter with light control (Licor, Model-LI 6400).

Water use efficiency (kg/ha/cm)

The ratio of photosynthesis and rate of transpiration gives the water use efficiency.

Change in leaf dry weight (mg/cm²/h²)

To determine the change in leaf dry weight, 20 leaf discs of 1 cm² diameter with a cork borer from one side of these leaves in the morning were taken and dried in an oven for 24 hours at 70 °C. Discs from other side of the midrib of the same leaves were removed in the evening 10 hours after first removal and were dried as those of morning discs. After ensuring total driage of both sets of leaf discs, their dry weight was recorded. Any increase in weight was recorded. Any increase in weight in evening discs over morning discs was attributed to accumulation of photosynthates synthesized during 10 hours and expressed as mg cm⁻² h⁻²

SPAD chlorophyll meter reading (SCMR) (μmol of chlorophyll per m²)

The SCMR was measured on mature active leaves. SPAD meter of Minolta, NJ, USA (SPAD 502).

Results and Discussion

Physiological Parameters

Relative water content of leaf (%)

The data regarding the relative water content of leaf is presented in table – 1a and there was a significant difference among the treatments.

Mean analysis of relative water content of leaf in both the locations revealed that, plants sprayed with liquid paraffin @ 2% have recorded highest (46.81%) and plants sprayed with cycocel @ 2000 ppm have recorded lowest (32.67%).

Relative water content of leaf is considered as one of the most important parameters used for the assessment of plant water balance, and the modification of water balance is a commonly registered response of plants to various environmental factors Mikiciuk *et al.* (2015) [26]. The reduction rate in relative water content of leaf was proportional to the severity of water stress. Liquid paraffin is a film forming antitranspirant, so it forms thin colourless transparent films which decrease the escape of water vapour from the leaves without affecting the gasses exchange. Kahlel *et al.*, (2015) found that foliar application of liquid paraffin @ 2% concentration significantly increased the relative water content in leaves.

Stomatal density (Stomata per mm²)

There was significant difference among the treatments regarding the stomatal density as shown in table – 1a Mean stomatal density among the treatments in two locations was observed lowest (42.29 per mm²) in plants that were sprayed with salicylic acid @ 500 ppm and highest (341.55 per mm²) in plants that were sprayed with kaolin @ 2%.

Less number of stomata were noticed in trees treated with salicylic acid because the application of salicylates can mitigate the rate of transpiration and prevent the water loss from stomata (Mishra, 2015), because salicylic acid works as a signal transduction for activating the ABA activity which is responsible for stomata closure in plants. But kaolin clay make a colourless film over the leaf surface which reflect the high wave length of solar radiation and retarded high temperature stress and water loss and also enhance the productivity of the plant (Mon, 2013) [29], so stomatal density was high in kaolin treated trees. Jifon and Syvertsen, (2003) [20] reported that 1 to 6% kaolin treatment gives better response in under unfavourable condition which inhibit the rate of transpiration and improve the yield quality.

Rate of transpiration (mol of H₂O /cm²/s)

Rate of transpiration differed significantly among the treatments and the data was shown in table -1a.

Mean analysis for rate of transpiration, among the treatments in two locations was recorded lowest (1.83 mol of H₂O/cm²/s) in plants that were sprayed with liquid paraffin @ 2% while highest (6.37 mol of H₂O/cm²/s) in plants that were sprayed with salicylic acid @ 1000 ppm.

Mohawesh *et al.* (2014) reported that, the mode of action of film-forming antitranspirants is based on the premise that transpiration can be suppressed by forming an impervious boundary over the stomata, inhibiting diffusion of water vapor. It would be expected that by increasing the

concentration of such film, the transpiration rate would be lowered if a uniform coverage of the leaves is attained. He also reported that, under well-watered conditions the average transpiration remained around 0.70 mmol/m/s and 0.82 mmol/m/s for 'Washington Navel' and 'Red Blood', respectively, in all antitranspirant treatments. Davies and

Kozłowski (1974) found that environmental conditions as well as species differences had a profound effect on the efficiency of epidermal coatings used to suppress transpiration and these findings were similar to those of Kuganathan and Palaniappan (1980) [23].

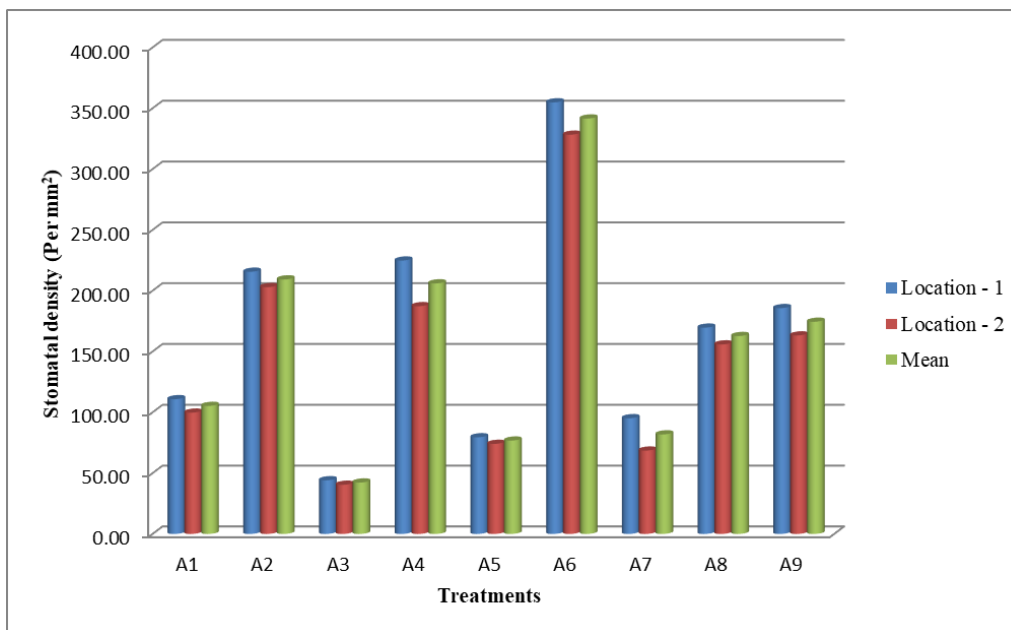


Fig 1: Effect of anti transpirants on stomatal density of leaf (per mm²) in sweet orange cv. Sathgudi

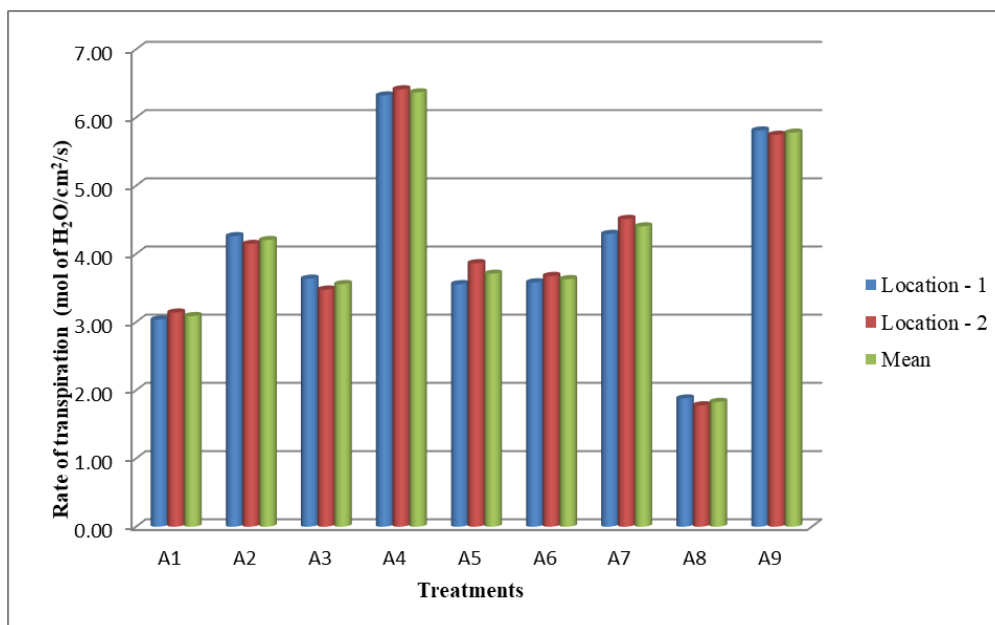


Fig 2: Effect of anti transpirants on rate of transpiration (mol of H₂O/cm²/s) in sweet orange cv. Sathgudi

Table 1a: Effect of antitranspirants on physiological parameters of sweet orange (*Citrus sinensis* L. Osbeck)

Treatments	Relative water content of leaf (%)			Stomatal density (per mm ²)			Rate of transpiration (mol of H ₂ O/cm ² /s)		
	Experimental locations			Experimental locations			Experimental locations		
	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled
A1	36.41	36.43	36.42	110.77	99.74	105.26	3.03	3.14	3.09
A2	31.11	34.23	33.39	215.58	202.94	209.26	4.26	4.14	4.20
A3	37.49	40.79	39.14	44.13	40.45	42.29	3.63	3.47	3.55
A4	32.55	37.64	34.38	224.77	187.26	206.02	6.32	6.41	6.37
A5	32.46	38.45	35.46	79.47	74.07	76.77	3.55	3.86	3.71
A6	37.76	43.11	41.01	354.86	328.24	341.55	3.58	3.67	3.63
A7	44.96	39.77	42.64	95.15	68.53	81.84	4.29	4.51	4.40
A8	50.50	44.26	46.81	169.61	155.82	162.72	1.88	1.78	1.83
A9	41.14	44.14	42.36	185.70	163.22	174.46	5.81	5.74	5.78

SE(m)+	2.68	-	2.03	19.40	10.97	14.34	0.08	0.08	0.07
CD	8.10	NS	6.15	58.66	33.17	43.38	0.24	0.24	0.21
Min	31.11	34.23	33.39	44.13	40.45	42.29	1.88	1.78	1.83
Max	50.50	44.26	46.81	354.86	328.24	341.55	6.32	6.41	6.37

Table 1b: Effect of antitranspirants on physiological parameters of sweet orange (*Citrus sinensis* L. Osbeck)

Treatments	Stomatal conductance (mmol/m ² /s)			Water use efficiency (kg/ha cm)			Leaf temperature(°C)		
	Experimental locations			Experimental locations			Experimental locations		
	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled
A1	0.003	0.003	0.003	9.81	8.85	9.33	34.03	30.63	32.33
A2	0.083	0.084	0.083	8.02	8.28	8.15	33.83	30.26	32.05
A3	0.016	0.016	0.016	8.58	8.88	8.73	33.83	30.23	32.03
A4	0.028	0.027	0.028	4.63	4.39	4.51	32.83	28.80	30.81
A5	0.017	0.017	0.017	8.86	8.33	8.59	31.50	29.30	30.40
A6	0.015	0.016	0.016	8.86	8.84	8.85	32.03	29.46	30.75
A7	0.019	0.018	0.018	6.94	6.33	6.64	32.20	28.60	30.40
A8	0.009	0.008	0.009	15.68	15.30	15.49	33.16	26.06	29.61
A9	0.006	0.005	0.006	3.67	3.89	3.78	31.56	29.13	30.35
SE(m)+	0.000	0.001	0.000	0.46	0.52	0.43	0.39	0.62	0.33
CD	0.001	0.003	0.001	1.41	1.57	1.32	1.19	1.89	1.01
Min	0.003	0.003	0.003	3.67	3.89	3.78	31.50	26.06	29.61
Max	0.083	0.084	0.083	15.68	15.30	15.49	34.03	30.63	32.33

Table 2: Effect of antitranspirants on photosynthetic index of sweet orange (*Citrus sinensis* L. Osbeck)

Treatments	Change in leaf dry weight (mg/cm ² /h ²)			Chlorophyll content (µmol of chlorophyll per m ²)			Rate of Photosynthesis (µg CO ₂ /m ² /s)		
	Experimental locations			Experimental locations			Experimental locations		
	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled	Location - 1	Location - 2	Pooled
A1	0.015	0.013	0.014	69.33	68.23	68.78	29.76	27.80	28.78
A2	0.023	0.026	0.025	69.86	68.43	69.15	34.20	34.33	34.26
A3	0.014	0.016	0.015	63.90	73.60	68.75	31.20	30.86	31.03
A4	0.014	0.020	0.017	65.43	65.63	65.53	29.26	28.20	28.73
A5	0.016	0.016	0.016	68.70	66.80	67.75	31.46	32.20	31.83
A6	0.016	0.013	0.015	70.26	75.26	72.76	31.73	32.50	32.11
A7	0.015	0.014	0.015	69.50	67.16	68.33	29.80	28.53	29.16
A8	0.014	0.013	0.014	67.60	68.73	68.16	29.26	26.96	28.11
A9	0.012	0.013	0.013	69.80	69.36	69.58	21.33	22.36	21.85
SE(m)+	0.002	0.001	0.001	-	-	-	0.99	0.83	0.55
CD	0.005	0.004	0.004	NS	NS	NS	2.99	2.52	1.67
Min	0.012	0.013	0.013	63.90	65.63	65.53	21.33	22.36	21.85
Max	0.023	0.026	0.025	70.26	75.26	72.76	34.20	34.33	34.26

A decrease in rate of transpiration leads to an increased relative water content of the leaves and *vice versa*. Makus (1997) observed that using antitranspirants improved the water use efficiency and reduced leaf transpiration rate by 87-93%.

Stomatal conductance (mmol/m²/s)

Stomatal conductance significantly differed among the treatments and the data regarding this was shown in table -1b. Mean analysis for the two locations was observed highest (0.083 mmol/m²/s) in plants that were sprayed with cycocel @ 2000 ppm and lowest was recorded in plants that were sprayed with cycocel @ 1000 ppm (0.003 mmol/m²/s). Stomatal control is the first and most important step in response to drought, as decrease in stomatal conductance reduces the rate of water loss and slows the rate of water stress development and minimizes its severity (Hanson and Hitz, 1982) [18]. Photosynthesis is strongly affected by water shortage as a decrease in stomatal conductance reduces the CO₂ assimilation (Cornic, 2000) [7]. Antitranspirants film limited stomatal conductance and transpiration. These results are in agreement with McDaniel (1985) [25] who found an increase in the diffusive resistance with antitranspirant applications. Gullo *et al.* (2020) [16, 17] reported that, the light reflected from the kaolin reduced the leaf temperature to an

optimal value for photosynthesis (below 30 °C) and increased the stomatal conductance.

Water use efficiency (kg/ha/cm)

Water use efficiency has shown significant difference among the treatments as given in table -1b. Mean analysis for water use efficiency in two locations was recorded highest in (15.49 kg/ha/cm) plants that were treated with liquid paraffin @ 2% while lowest (4.63 kg/ha/cm) was recorded in farmer's practice of spraying urea @1% spray followed by quick lime @ 2% spray at 15 days interval. Bora and Mathur (1998) [5] reported that, using antitranspirants improved the water use efficiency. It was reported that antitranspirant application on citrus leaves led to improved water use efficiency (Hazarika and Parthasarathy, 2002) [19]. Makus (1997) observed that using antitranspirants improved the water use efficiency.

Leaf temperature (°C)

Significant difference was noticed among the treatments regarding the leaf temperature and the data was presented in table - 2.

Mean analysis for leaf temperature was recorded highest (32.33 °C) in plants sprayed with cycocel @ 1000 ppm which was at par with almost all the treatments while minimum

(29.6°C) was recorded in plants that were sprayed with kaolin @ 1%.

A reflective kaolin spray was found to decrease leaf temperature by increasing leaf reflectance and to reduce transpiration rate more than photosynthesis in many plant species grown at high solar radiation levels (Bergovis *et al.* 2001, Cheng *et al.* 2008 and Peter, 2008) [6, 32]. Urban *et al.* (2017) [38] reported that leaf temperatures can be affected by transpiration rates, with higher temperatures increasing the rate of transpiration to a certain point. This increase in transpiration may have evaporative cooling effects on trees Crawford *et al.* (2012) [8]. Feller (2006) [11] reported that, if transpiration decreases due to stomatal closure, plant temperature can increase, but the plant conserves water. In experiments conducted by Glenn *et al.* (2002) found that kaolin treatments reduced leaf and fruit temperatures of apple by 8°C. Julissa *et al.* (2019) also reported that the increased temperatures in the antitranspirant application indicate that decreases in evaporative cooling through the stomata could have possibly been caused by kaolin treatment, which is supported by Nammah (1979) [30], who found that antitranspirant treatments influenced leaf temperatures. Similar results were also given by Allakhverdiev *et al.* (2008) [12] and Yamamoto *et al.* (2008) [39]. Gullo *et al.* (2020) [16, 17] reported that, the light reflected from the kaolin and calcium carbonate treated trees reduced the leaf temperature to an optimal value for photosynthesis (below 30 °C)

Photosynthetic Index

Change in leaf dry weight (mg/cm/h)

Data pertaining to the change in leaf dry weight was shown in the table - 2 and there was a significant difference among the treatments - 2.

Mean analysis for change in leaf dry weight among the treatments in two locations was observed highest (0.025mg/cm/h) in plants that were sprayed with cycocel @ 2000 ppm and lowest (0.013 mg/cm/h) was recorded in plants that were sprayed with farmers practice of spraying urea @ 1% spray followed by quick lime @ 2% spray at 15 days interval.

Way back in early sixties, Gale (1961) [13] reported that application of antitranspirants increase dry matter production under both adequate as well as inadequate soil moisture condition. Increase in dry matter production consequent to application of antitranspirants has been reported by Patil and De (1976) [31] and many others while working on different field crops.

Chlorophyll content (µmol of chlorophyll per m²) SPAD Chlorophyll meter reading (SCMR)

Chlorophyll content or SPAD reading was found non significant and the data was revealed in the table 2.

Mean data was in the range of 65.53µmol of chlorophyll per m² to 72.75µmol of chlorophyll per m².

The results suggest that any treatment which has resulted in maintaining higher levels of leaf moisture has led to synthesis of more chlorophyll in the leaves. Salisbury and Ross (1992) [36] have also reported about the necessity of adequate requirement of leaf moisture for proper synthesis of chlorophyll. According to them, slightly more negative plant water potential inhibits the formation of proto-chlorophyll and eventually the chlorophyll synthesis is reduced. The results are similar to those by Mohawesh *et al.* (2014), Save *et al.* (1995) [37] and Garcia and Syvertsen (2006) [15] reporting that a higher concentration of antitranspirants decreases

chlorophyll contents more adversely compared to lower concentrations.

Rate of photosynthesis (µg CO₂/m²/s)

Data pertaining to the rate of photosynthesis was significant among the treatments and the data was presented in table - 2. Mean values of rate of photosynthesis in two locations was recorded highest (34.26 µgCO₂/m²/s) in plants that were treated with cycocel @ 2000 ppm and lowest (21.85 µgCO₂/m²/s) was found in farmers practice of spraying urea @ 1% spray followed by quick lime @ 2% spray at 15 days interval.

A close examination of photosynthetic index in relation to the results pertaining to total chlorophyll and relative water content will reveal that the three parameters are closely related to one another. Higher leaf relative water content leads to higher chlorophyll content and eventually higher dry matter production. Therefore any treatment that maintains an adequate balance of water of leaves is bound to result in higher accumulation of photosynthates. However, during present studies though kaolin was able to synthesize higher levels of chlorophyll owing to higher leaf relative water content yet photosynthetic efficiency was not as high as one would have expected it to be. According to Davenport *et al.* (1969) who reported that, reflecting materials do not cause blockage of stomatal pores when they are applied to upper surface of leaves with stomata exclusively on the lower surface. Application of reflecting material on single surface of leaves is practically impossible, owing to complex crop geometry, while conducting spray, especially of high volume and since stomata serve as portals for both the loss of water vapour and intake of CO₂, an antitranspirant barrier against water loss may affect photosynthesis. Also, they are of the opinion that coating of reflecting material may curtail photosynthesis on overcast days when light is limited. A similar reduction in net photosynthesis as a result of kaolin spray was recorded by Khaled *et al.* (1970) [21] and according to him the level of reduction varied with light intensity being maximum (20-30 per cent) at lowest light intensity. Gullo *et al.* (2020) [16, 17] reported that, the light reflected from the kaolin reduced the leaf temperature to an optimal value for photosynthesis (below 30°C) and increased the stomatal conductance.

Conclusion

Application of antitranspirants on plant physiological parameters (stomatal behavior, stomatal conductance and rate of transpiration) and photosynthetic parameters (chlorophyll content, rate of photosynthesis and change in leaf dry weight) of sweet orange in every fortnightly interval during the dry spell of fruit development has given best results for decreasing the rate of transpiration and increased the water use efficiency which can improve the physiology of plants and increase the productivity of trees by preventing fruit drop caused due to changing climate.

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