International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 15-11-2020; Accepted: 28-11-2020: Published: 12-12-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 449-454

Trehalose foliage application influences growth and physiological attributes of holy basil (*Ocimum tenuiflorum* L.)

Yawar Habib[1], M Masroor A Khan[2], Nausheen Khanam[3], Abid Ali Ansari[4], M Naeem[5*]
[1, 2, 3, 5] Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[4] Department of Biology, Faculty of Science, University of Tabuk, Tabuk, Saudi Arabia

Abstract
Trehalose (α-D-Glucopyranosyl-1, 1-α-D-glucopyranoside; Molecular formula: $C_{12}H_{22}O_{11}$) is a non-reducing disaccharide formed through a 1-1 alpha bond linking two glucose moieties. Trehalose (Tre), a non-reducing disaccharide of glucose and is considered as one of the most effective in stress protectant in plants. Keeping this fact in mind, it was hypothesized whether foliar application of Tre can affect the secondary metabolites particularly, phenol contents and other physiological and biochemical parameters in *Ocimum tenuiflorum* L. (Holy basil). This study was performed using a pot experiment with a simple randomized design. Plant's secondary metabolites are of immense value in the pharmaceutical, perfume, cosmetic and other industries. Trehalose has been demonstrated as a plant growth regulator, plays a pivotal role in growth and various metabolic processes of plants. The present study is aimed to elucidate the effects of Tre through foliar application on *O. tenuiflorum*. Various concentrations of Tre *viz*. 0, 5, 10, 20, 40 mg L^{-1} were applied on the plants five times in crop duration with an interval of one week. Biochemical parameters were studied spectrophotometrically. Chlorophyll fluorescence or photochemical quenching was determined using Junior PAM chlorophyll fluorometer. The effect of leaf-applied Tre was found significant for all growth and physiological attributes of the studied plant. Of the tested concentrations, Tre at 20 mg L^{-1} has proved the optimum concentration for overall performance of the medicinal herb.

Keywords: trehalose $C_{12}H_{22}O_{11}$, holy basil, *Ocimum tenuiflorum,* phenol content

Introduction
Ocimum tenuiflorum L. (Holy basil), is an aromatic herb prominent throughout the world for its healing and other medicinal properties. Plant's secondary metabolites are of immense value in the pharmaceutical, perfume, cosmetic and other industries. Trehalose is a sugar which was first derived from insect cocoons. Trehalose (Tre) (α-D-glucopyranosyl [1-1]-α-D glucopyranose), a nonreducing disaccharide of glucose (Figure 1), has no free aldehyde groups and is very stable at a wide range of pH or temperature (Crowe and Crowe, 1982; Crowe, *et al*. 1996; Lefort *et al*. 2007) [10, 11, 28]. It has been already established that the Arabidopsis Trehalose-6-P synthase AtTPS1 gene is a regulator of glucose, abscisic acid, and stress signaling (Avonce *et al*, 2004) [7]. Being a signaling and antioxidant molecule, Tre has been shown to stabilise biological structures and macromolecules (proteins and membrane lipids) in different organisms during dehydration (Crowe *et al*. 1996; Iturriaga *et al*. 2009; Luo *et al*. 2010; Fernandez *et al*. 2010) [11, 22, 33, 15]. In plants, Tre is present at markedly lower concentrations (close to detection level) but plays a pivotal role as an osmoprotectant and has an indispensable role in metabolic processes associated with various abiotic stress tolerance (Räsänen *et al*. 2004, Bae *et al*. 2005, Aghdasi *et al*. 2008; Duman *et al*. 2010; Luo *et al*. 2010; Lunn *et al*. 2014; Matthew *et al*. 2020) [44, 8, 2, 12, 33, 32, 34]. Various researchers have attempted to escalate the accumulation of Tre in plants through genetic engineering in both model and crop plants using genes of bacterial and yeast origin (Pellny *et al*. 2004; Wang *et al*. 2005) [43]. Additionally, various researchers reported that Tre is readily taken up by the plants through leaves and root surfaces when applied exogenously (Smith and Smith 1973; Luo *et al*. 2010) [50, 33]. Also Tre plays role in preventing the damage of biological structures during desiccation as a signaling molecule (Paul *et al*. 2018; Sadak *et al*. 2019; Abdallah *et al*. 2020) [41, 46, 1].

Among the various MAPs, *Ocimum tenuiflorum* L. syn. *Ocimum sanctum* (Holy basil), belonging to family Lamiaceae, is an aromatic plant famous throughout the globe for its healing and other medicinal properties. It is native of eastern world tropics and is considered sacred and is worshipped in a sanctorum of its own in traditional Hindu rituals, sacred groves, and households throughout the subcontinent. Different species of the plant bears various secondary metabolites *viz*. linalool, linalyl, geraniol, citral, camphor, eugenol, methyleugenol, methyl chavicol, methyl cinnamate, thymol, safrol, taxol, urosolic acid etc. (Kelm *et al*. 2000; Uma Devi 2000; Shishodia *et al*. 2003; Jaggi *et al*. 2003) [25, 51, 48, 23]. These metabolites are of immense value in the pharmaceutical, perfume, cosmetic and other industries as well. These metabolites also have properties including anticancer, antifungal, anti-inflammatory, antioxidant, analgesic, diuretic, digestive, carminative (Gupta *et al*. 2002; Singh *et al*. 2010; Pattanayak *et al*. 2010; Clinical and Laboratory Standards Institute 2012; Yamani *et al*. 2016) [19, 45, 39, 9, 53] and are also recommended for the treatment of malaria, bronchitis, diarrhoea, dysentery (Singh *et al*. 2010; Pattanayak *et al*. 2010; Mishra and Mishra 2011) [45, 39, 35].

Since there is a severe gap in the demand and supply of *Ocimum tenuiflorum* L. and its by-products in the global market, putting a heavy strain on the existing resources, it is the need of the hour to enhance the production of this medicinal herb in the present reducing fertile land cover, to meet the increasing demands in world markets. Therefore, large scale cultivation of this plant on scientific lines is the sole dependable alternative. Bearing in mind the importance of holy basil and the encouraging effects of Tre, a hypothesis was designed aiming to understand whether application of Tre through leaf could improve the growth, physiological and biochemical attributes of this useful medicinal plant. To test the proposed hypothesis, a pot experiment was conducted on the plant.

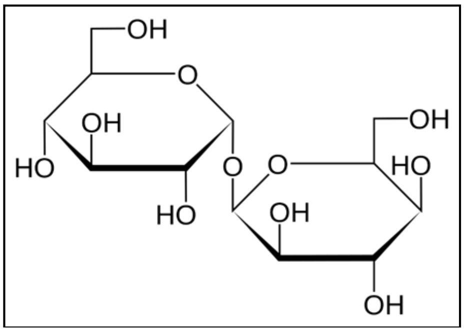

Fig 1: Structure of Trehalose [*α-D-Glucopyranosyl-α-D-glucopyranoside*]

Materials and Methods

A simple randomized design pot experiment was conducted on holy basil plant in the Department of Botany, Aligarh Muslim University, Aligarh during Rabi (winter) season of 2018-2019. Seeds were procured from the Department of Applied Biological Sciences, Jordan University of Science & Technology, Irbid, Jordan. Ten seeds were sown in each earthen pot containing a mixture of 6 kg of soil and 2 kg of farmyard manure. One healthy plant was maintained in each pot. There were four replicates for each treatment. The objective of this experiment was to study the effect of foliar sprays of five levels of trehalose (Tre) on the plant, *viz*, 0, 5, 10, 20, 40 mg L^{-1}. Five foliar sprays of each concentration of Tre were applied at a gap of one week from 75 days after sowing (DAS). Trehalose for the treatment was purchased from SRL Pvt. Ltd., India. The effect of Tre was evaluated in terms of growth parameters (plant fresh and dry weights), physiological and biochemical parameters (total chlorophyll content, total carotenoids content, chlorophyll florescence (fv/fm), nitrate reductase (NR) and carbonic anhydrase (CA) activities and total phenol content and yield). Sampling of plants was carried out at 115 DAS. Standard agricultural practices were adopted during the entire crop duration.

Measurement of growth parameters

The fresh and oven dried (80°C for 48 h) weight of plant of each treated plant and control was determined separately using an electronic balance. The contents of total chlorophyll and carotenoids were estimated in the fresh leaves according to the method of Lichtenthaler and Buschmann (2001) [30]. The chlorophyll fluorescence parameters such as PSII efficiency (Fv/Fm), non-photochemical quenching (NPQ), photochemical quenching (qP), and electron transport rate (ETR) were measured in fully expanded leaves in the daytime using a portable saturation-pulse fluorometer PAM-2000 (Walz, Effeltrich, Germany). Activity of nitrate reductase (NR) was estimated in fresh leaves by using the method developed by Jaworski (1971) [24]. The activity of CA was measured in fresh leaves selected randomly, using the method described by Dwivedi and Randhawa (1974) [14]. Total phenolics content was determined using the method of Ainsworth and Gillespie (2007) [3].

Statistical analysis

The data were analyzed statistically according to factorial randomized design using SPSS-22 statistical software (SPSS Inc., Chicago, IL, USA). One-way ANOVA (Analysis of variance) was applied to test differences among the treatments. The least significant difference (LSD) was computed for the significant data at $P < 0.05$. Standard error of replicates were also presented in the Tables.

Results

The effect of foliar application of various concentrations of Tre on the growth and physiological and biochemical attributes of holy basil. Among various treatments, the effect of Tre-20 mg L^{-1} was found significant for all the attributes studied.

Growth characters

The effect of foliar application of Tre resulted in the enhancement of shoot fresh weight as compared to Control. Out of various treatments application of Tre-20 mg L^{-1} significantly increased the fresh weight by 45.6% over the water sprayed Control (Table 1).

Table 1: Effect of foliar application of different concentrations of trehaose on fresh and dry weights of plant of *Ocimum tenuiflorum* L. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$).The data shown are means of four replicates ± SE.

Trehalose concentrations (mg L^{-1})	Plant fresh weight (g)	Plant dry weight (g)
Control (DDW)	17.00 ± 0.49^{e}	5.60± 0.010^{d}
Tre (5)	18.86 ± 0.37^{d}	6.30 ± 0.013^{c}
Tre (10)	22.35 ± 0.32^{b}	7.50 ± 0.012^{b}
Tre (20)	24.75 ± 0.42^{a}	8.35 ± 0.012^{a}
Tre (40)	20.72 ± 0.26^{c}	6.90 ± 0.010^{b}

Physiological and biochemical attributes

Application of Tre-20 mg L^{-1} proved superior to the other treatments (*viz*. 5, 10 and 40 mg L^{-1}) and gave maximum value for total chlorophyll content in the fresh leaves of plant. Control exhibited the lowest response for this parameter. The percent increase in the total chlorophyll content due to applied Tre-20 mg L^{-1} over the Control was 32.0% (Table 2). Application of Tre-20 mg L^{-1} significantly increased the value for total carotenoids content in the fresh leaves of holy basil.

Trehalose at 20 mg L^{-1} resulted in an increase of 33.7% total carotenoids content over the Control (Table 2). Likewise, activities of NR and CA were significantly increased by the foliar application of Tre at 20 mg L^{-1}. This treatment increased NR activity by 13.9% and CA activity by 15.4% over their respective control (Table 2). The maximum value of chlorophyll fluorescence was noted by the application of Tre-20 mg L^{-1} and an increase of 14.1% was recorded over the Control (Table 3).

However, all the treatments were significantly different from each other in their effects. The values of other chlorophyll fluorescence parameters such as qP and ETR were improved by the application of TRIA and a maximum escalation of 10.2% and 20.0% was recorded over the control (Table 3). Application of trehalose decreased non photochemical quenching (NPQ) as compared to the control. Leaf-phenol content and yield were markedly influenced by the application of Tre-20 mg L^{-1}. This treatment significantly enhanced the leaf phenol content and yield by 43.7% and 109.7% as compared to their respective controls (Figures 2 & 3).

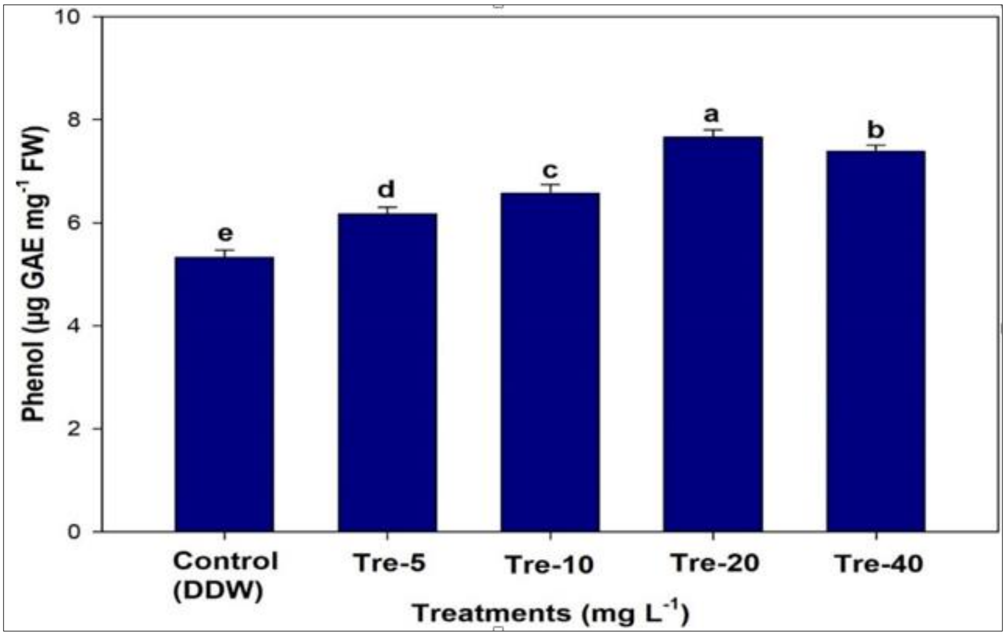

Fig 2: Effect of foliar spray of trehalose on phenol content in the leaves of *Ocimum tenuiflorum* L. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (┬) show ± SE.

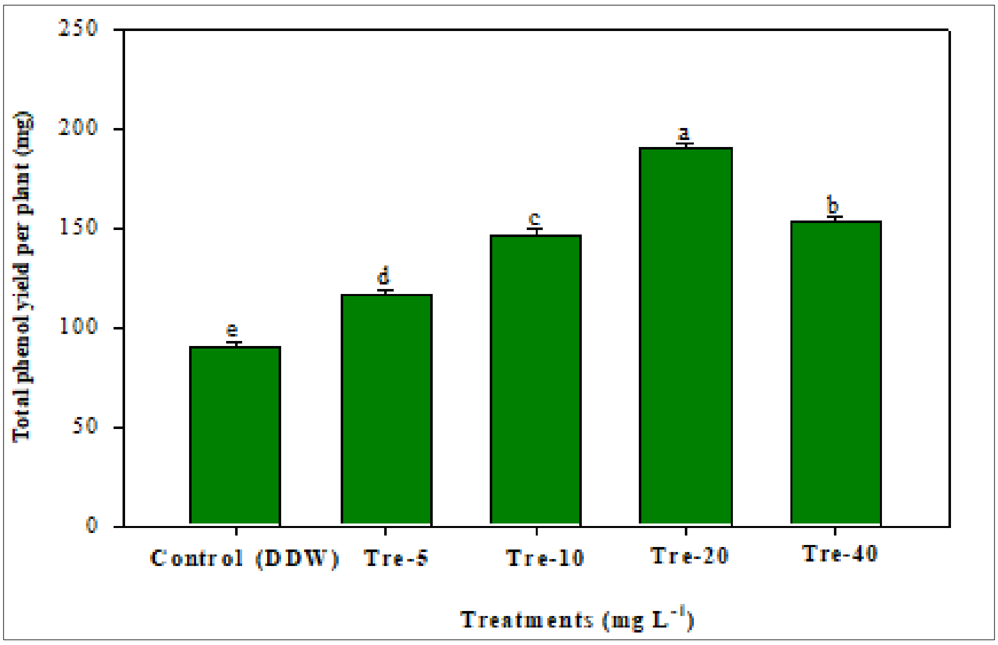

Fig 3: Effect of foliar spray of trehalose on total phenol yield per plant in the leaves of *Ocimum tenuiflorum* L. Means within column followed by the same letter(s) are not significantly different ($p \leq 0.05$). Error bars (┬) show ± SE.

Table 2: Effect of foliar application of different concentrations of trehalose on content of total chlorophyll and carotenoids, activities of nitrate reductase (NR) and carbonic anhydrase (CA) of *Ocimum tenuiflorum* L. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Trehalose concentrations (mg L^{-1})	**Total chlorophyll content (mg g^{-1} FW)**	**Total carotenoids content (mg g^{-1} FW)**	**Nitrate reductase activity (n mol NO_2 g/FW/h)**	**Carbonic anhydrase activity [µmol (CO_2)/kg (FW)/s]**
Control (DDW)	0.977 ± 0.012^d	0.376 ± 0.005^e	281.0 ± 3.23^e	202.3 ± 2.33^d
Tre (5)	1.060 ± 0.015^c	0.421 ± 0.004^d	293.4 ± 3.20^d	216.3 ± 2.40^c
Tre (10)	1.220 ± 0.013^b	0.452 ± 0.003^c	300.7 ± 3.30^b	222.3 ± 2.48^b
Tre (20)	1.290 ± 0.014^a	0.503 ± 0.004^a	320.1 ± 3.27^a	233.4 ± 2.43^a
Tre (40)	1.180 ± 0.011^b	0.471 ± 0.002^b	312.4 ± 3.40^c	226.0 ± 2.53^b

Table 3: Effect of foliar application of different concentrations of trehalose on chlorophyll fluorescence parameters of *Ocimum tenuiflorum* L. Means within a column followed by the same letter(s) are not significantly different ($p \leq 0.05$). The data shown are means of four replicates ± SE.

Trehalose concentrations (mg L^{-1})	PSII photosynthetic efficiency (Fv/Fm)	Photochemical quenching (qP)	Non-photochemical quenching (NPQ)	Electron Transport rate (ETR)
Control (DDW)	0.78±0.020^{d}	0.83±0.002^{d}	0.349±0.002^{a}	136.7±1.77^{e}
Tre (5)	0.80±0.015^{d}	0.85±0.002^{c}	0.344±0.004^{b}	140.4±1.43^{d}
Tre (10)	0.84±0.012^{b}	0.86±0.001^{b}	0.340±0.003^{c}	150.5±1.53^{b}
Tre (20)	0.89±0.020^{a}	0.91±0.002^{a}	0.332±0.002^{c}	164.0±1.46^{a}
Tre (40)	0.82±0.023^{c}	0.83±0.001^{d}	0.323±0.003^{d}	155.3±1.36^{c}

Discussion

Plant growth and development is known to be governed by several exogenous and endogenous factors, including sugars. Modern evidence has proved that in plants, sugars such as glucose, sucrose, and fructose are not only playing key role in plant growth but also they affects sugar sensing system that initiates changes in gene expression and then the plant growth (Koch 1996; Rosa *et al*. 2009; Lin *et al*. 2017) [27, 44, 31]. Trehalose has strong effects on growth and development in plants through regulation of carbon metabolism (Lin *et al*. 2017) [31]. It has already been well established that Tre have the potential of promotion of germination and shoot elongation, photosynthesis, secondary metabolites and protein combating stress (Mostofa *et al*. 2015; Ibrahim and Abdellatif 2016; Govind *et al*. 2016; Paul *et al*. 2018) [36, 21, 18, 41]. The results obtained in the present study indicated that a significant improvement in plant growth attributes due to pronounced effects of Tre application. The data presented in Table 1 reveal that Tre-20 mg L^{-1} had considerable positive effect on the fresh and dry weights of the plant. Ali and Ashraf (2011) [6] have reported the Tre-mediated increase in biomass production of maize plant. Further support lands from the studies of Gouffi *et al*. (1999) [17], they reported a significant improvement in the growth of *Sinorhizobium saheli* using exogenous application of Tre. Our studies can be further corroborated with those who observed the positive effect of exogenously-applied Tre on the growth attributes of maize (Zeid 2009) [55], rice (Mostofa *et al*. 2015) [36] and wheat (Ibrahin and Abdellatif 2016) [21].

Trehalose improved photosynthesis by sugar signaling mechanism and through its interaction with sugar signaling pathways that can enhance photosynthetic capacity (Paul *et al*. 2001; Zeid 2009) [40, 55]. Hence, higher values of chlorophyll and carotenoid contents were observed in the Tre-treated plants (Table 2). Moreover, the significant effect of Tre on photosynthetic pigments has been reported by Duman *et al*. (2011) [13] on *Lemmna gibba*, which supports our findings. Khater *et al*. (2018) [26] also stated a marked increase in photosynthetic pigments of *Vigna unguiculata* La. using leaf-applied Tre. Chlorophyll fluorescence is light re-emitted by chlorophyll molecules during return from excited to non-excited states and is effective tool for the detection of PS II reaction centre activity. In case of chlorophyll fluorescence parameters, the balance between light capture and use of photochemical energy was determined. Therefore, their determination was measured as a significant approach for evaluating the health or integrity of the internal apparatus of leaf during photosynthetic process. Chlorophyll florescence parameter like *Fv/Fm* (maximum quantum efficiency of PSII) is well-known for its correlation with photosynthetic efficiency of the leaf (Shangguan, *et al*. 2003) [47]. A significant improvement was noticed in photochemical efficiency of PS II (F_v/F_m), photochemical quenching (qP) and electron transport rate (ETR) in the plant due to the foliar application of Tre (Table 3). However, Tre treatment reduced non-photochemical quenching (NPQ) as compared to the control. Trehalose does not damage photosynthesis reaction centers under natural growth conditions as reported by Gao *et al*. (2013) [16]. Similarly, Pang *et al*. (2017) [38] and Zhao *et al*. (2019) [54] suggested that Tre protected the photosynthetic organ not by increasing heat dissipation but by maintaining photosynthetic organs to improve the activity of the photosystem. An encouraging effect of Tre was observed in increasing the activities of NR and CA enzymes in the present study (Table 2). Exogenous Tre increased concentration and assimilation of nitrogen and growth by modulating the related enzymes (Lin *et al*. 2017) [31].

Phenolic compounds play an important role as antioxidants in scavenging free radicals arising from their high reactivity as hydrogen or electron donors, to stabilize and delocalize the unpaired electron, and from their ability to chelate transition metal ions (Huang *et al*. 2005) [20]. Our study regarding Tre-mediated increase in leaf phenol content gets support with the findings of Aldesuquy and Ghanem (2015) [5], Ibrahim and Abdellatif (2016) [21] and Khater *et al*. (2018) [26], they reported that Tre application increased total phenols and flavonoids in case of wheat cultivars and *Vigna unguiculata* L., respectively. The beneficial effects of Tre may be due to it signaling function, through the induction of different metabolic pathways (Alam *et al*. 2014) [4]. Tsai and (2014) [50] also reported that trehalose 6-phosphate (T6P) acts as a global regulator of metabolism and transcription promoting plant growth and triggering developmental phase transitions in response to sugar metabolism.

Conclusion

The results indicated that Tre application at the rate of 20 mg L^{-1} (Tre 20) demonstrated the best results for most of the parameters studied. These parameters were increased owing to applied Tre 20. Noticeably, this concentration also enhanced the level of phenol content indicating that the plant increased its defense mechanism. The results concluded that Tre through foliar application significantly improved all physiological and biochemical attributes of *O. tenuiflorum*. Trehalose is cost effective, water soluble, non-toxic and eco-friendly, environmentally safe chemical, hence it can be successfully used for achieving the maximum performance of this medicinal herb. Further, this study is of new kind as no one has studied the effect of Tre on other medicinal plant.

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