DOI: http://doi.org/10.4038/jrrisl.v99i0.1885

*Journal of the Rubber Research Institute of Sri Lanka* (2019) **99**, 23-35

# *Moringa oleifera* leaf extract as a biostimulant on growth and other physio-chemical attributes of rubber (*Hevea brasiliesnsis*) under drought and heat stress conditions

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**Abstract**

*Drought and high temperature are the major impediments limiting growth of Hevea. We studied the efficacy of foliar applied Moringa (Moringa oleifera) leaf extract (MLE) as a biostimulant on the response of Hevea to sub-optimal climatic conditions. Rubber plants of clone RRISL 203 cultivated in the Intermediate Zone (IZ) and Dry Zone (DZ) were spray treated with water (mock treatment), MLE at 5, 10 and 15% concentrations for IZ and 3 and 5% for DZ. Physiological parameters viz. net photosynthesis (Pr), chlorophyll content (Cc), stomatal conductance (gs) and leaf water potential (Ψ) were recorded after three months from the commencement of treatments whilst girth measurements were recorded at three and twelve months after treatments in the IZ. Total phenolic content (TPC) and antioxidant activities, employing FRAP (ferric reducing assay power) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) techniques, were estimated after three months from first spraying in the DZ. Significantly higher Pr, Cc and gs values were recorded from plants treated with MLE at all three concentrations as compared to control. Significantly higher TPC values (17.17 and 15.90 mg of gallic acid equivalent/ g of leaf sample), and FRAP values (14.79 and 14.70 mg of trolox equivalent/g of leaf sample) and ABTS values (70.26 and 59.43 mg of TE/g of leaf sample) were recorded in plants treated with MLE at both concentrations (3 and 5%) as compared to control (12.50 mg of GAE/g of leaf sample, 10.68 mg of TE/g of leaf sample and 49.96 mg of TE/g of leaf sample), respectively. A significantly higher girth (11.5 cm) was recorded in plants treated with MLE at 5% as compared to control (9.66 cm) after 12 months of treatments. Improved growth and physio-chemical attributes of rubber plants could be attributed to the beneficial effect of MLE as a biostimulant and therefore, exogenous application of MLE could effectively be utilized as an environmental friendly and inexpensive strategy for growth improvement in Hevea under sub-optimal climatic conditions.*

***Key words***: abiotic stress, growth, Moringa leaf extract, rubber

## Introduction

Rubber has been grown traditionally in the Low and Mid Country Wet Zones covering the South–Western, Southern and Central parts of Sri Lanka. However, as the scope of further expansion of cultivation in traditional areas is being limited, there has been some effort to extend rubber plantation to non-traditional areas, such as Moneragala, Padiyathalawa, Ampara, Vavuniya, Hambanthota and Puttalam in the Intermediate and Dry Zones of the country (Rodrigo *et al.*, 2009, 2011 and 2014; Iqbal *et al.*, 2010). The mean annual rainfall in the Intermediate and Dry Zones are 1000-1500 mm and 1000 mm, respectively with four to seven month-long dry periods and one major monsoon rainy period (Lakmini *et al.*, 2006).

Crop growth and yields are negatively affected by sub-optimal water supply and high temperatures due to physical damages, physiological disruptions, and biochemical changes (Fahad *et al.*, 2017). Stress phenomena that occur simultaneously, such as drought and heat, have shown to be more detrimental to plant growth than each of these stresses individually (Perdomo *et al*., 2015). Plants which are exposed to stress, accumulate reactive oxygen species (ROS) like superoxide anion radical, hydroxyl radical, and hydrogen peroxide which are very lethal and cause extensive damage to protein, DNA and lipids and thereby affect normal cellular functioning (Foyer and Noctor, 2005). Redox homeostasis in plants during stressful conditions is maintained by enzymatic and non-enzymatic low molecular compounds, *i.e.* antioxidants, like ascorbic acid, reduced glutathione, α-tocopherol, carotenoids, phenolics, flavonoids, and proline (Gill and Tuteja, 2010).

Severe growth reduction and longer immaturity periods due to drought stress have been reported in rubber grown in non-traditional dry areas (Chandrashekar *et al.*, 1998; Sreelatha *et al.*, 2011). Under water stress conditions, very low stomatal conductance (Chandrashekar *et al*., 1998) and severe inhibition of photosynthesis and transpiration were reported in rubber (Krishna *et al*., 1991). Drought tolerance in plants is a complex phenomenon being controlled by a large number of minor genes and loci on chromosomes (Mohammadi *et al.*, 2005). The genetic improvements in combination with the proper cultural practices are considered important in managing the abiotic stresses in crop plants (Wahid *et al.*, 2007). Nevertheless, genetic variation in the existing *Hevea* germplasm being limited, drought tolerant rubber clones are yet to be produced in Sri Lanka through conventional breeding.

As an alternative to long-term breeding approach, priming (preconditioning) has been widely adopted as a short-term strategy to induce resistance to drought and heat stress in various crop plants (Savvides *et al.*, 2016). Exogenous application of growth regulators and osmoprotectants at different growth stages can play an important role in inducing resistance against drought and heat (Fahad *et al.*, 2017). Cost effective exogenous use of plant-based extracts (biostimulants) containing plant growth regulators, hormones and antioxidants have also been reported for the improvement of crop performance with higher economic returns (Bakhtavar *et al.*, 2015). Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality (Van Oosten *et al.,* 2017).

In recent years, Moringa (*Moringa oleifera*) has gained enormous fame as having biostimulant potential (Bakhtavar *et al.,* 2015). Moringa is commonly known as 'drumstick' or 'horseradish' and grows in the tropical and subtropical regions of the world. Moringa leaves are well-known as a vegetable and are rich in vitamins, particularly A and C, iron, calcium, carotenes and phenolics (Foidl *et al*., 2001; Rady *et al.,* 2013; Yasmeen *et al*., 2013; Rady and Mohamed, 2015). Leaf extracts of Moringa show potential antioxidant properties (Siddhuraju and Becker, 2003). In addition, extracts of Moringa possess sufficient quantity of cytokinins (Rady *et al.,* 2013, Rady and Mohamed, 2015).

Exogenous MLE has shown to alleviate abiotic stresses *viz.,* drought, heat, salinity, and improve growth and physiological attributes in various crop plants such as Pear *(Pyrus communis),* Wheat, Maize, Quinona (*Chenopodium* spp.) and Sorghum (El-Hamied and El-Amary, 2015; Nawaz *et al.,* 2016; Pervez *et al.,* 2017; Maswada *et al.,* 2018; Rashid *et al.,* 2018; Ahmad *et al.,* 2016). In Sri Lanka, especially in the Intermediate and Dry Zones, Moringa is a predominant plant grown along fences and in some home gardens. Rubber x Moringa intercropping systems are being popularized in the North and East of Sri Lanka under a Special Capital Project No. 22-01.17 of the Rubber Research Institute of Sri Lanka. Therefore, Moringa leaves are readily available and can be collected free of cost or at a very low cost in the above areas at present.

Nayanakantha *et al.* (2018) demonstrated, for the first time, that exogenous MLE improved physiological parameters of *Hevea* in the Intermediate Zone. Nevertheless, growth and physio-chemical attributes of *Hevea* in response to MLE treatment under drought and heat stress conditions in both Intermediate and Dry Zones have not been reported. The current study, thus, aimed to investigate the effect of MLE as a biostimulant on growth and physio-chemical attributes of *Hevea* under sub-optimal climatic conditions.

## Materials and Methods

### *Experiment in the Intermediate Zone (IZ)*

A medium holder's rubber field, Nottinghill Private Estate in Mawathagama (IZ), of clone RRISL 203 (three months old) was selected for the study. Rainfall and the number of rainy days were recorded for a period of three months from the day of treatment imposition. Soil moisture content in the field was recorded using Theta Probe (ML3, Delta-T devices, UK) equipment. Day time temperature and relative humidity (RH) in the field were recorded on the same day of data collection using a pocket weather meter (Kestrel 3000 wind meter, USA). Fresh Moringa leaves (1 kg) were collected and crushed with two liters of water using a blender. The resultant juice extract was named as 50% solution, from which 5, 10 and 15%

solutions were made with appropriate dilutions. Plants were spray treated (primed) separately with aqueous Moringa leaf extract (MLE) at 5, 10 and 15% concentrations at monthly intervals for a period of six months from January to June in 2017. Control plants were devoid of priming treatments. In the mock treatment, plants were also sprayed with water at monthly intervals. There were three blocks and each block had 20 plants. Four plants were imposed with each treatment in each block, according to a randomized completed block design (RCBD), so that there were 12 plants for each treatment. Other agro management practices were done as recommended by RRISL.

**Measurements of growth and physiological parameters**

Girth was recorded before imposing the treatments and also at 3 and 12 months after the first spraying. Physiological parameters *viz.,* net photosynthesis (*Pr)* and stomatal conductance ($g_s$) on intact mature leaves were recorded using a portable photosynthesis system (LI-6400), LI-COR, U.S.A. A photosynthetic active radiation (PAR) of 1800 μmol $m^{-2}$ $s^{-1}$was supplied by a light unit mounted on the top of leaf chamber. Two leaves were selected from each plant for each treatment and three plants were used for each treatment. Measurements were taken between 09:00-11:00 hrs after three months from the first spraying. Leaf water potential (Ψ) was recorded using pressure chamber equipment (Model 1000, PMS Company, USA), after three months from first spraying. For each treatment, two plants were selected and one leaf was collected from each plant for recording water potential data. Chlorophyll content in intact mature leaves from three plants (2 leaves per plant) for each treatment was measured using a SPAD-502 plus Chlorophyll meter (Minolta Camera Co., Ltd., Japan).

**Experiment in the Dry Zone (DZ)**

One year after the first study at Mawathagama, a smallholder's rubber field of clone RRISL 203 (three months old) at Malayalapuram in Kilinochchi was selected for the second study. Soil moisture content in the field was recorded using Theta Probe equipment. Day time temperature and relative humidity (RH%) in the field were recorded on the same day of data collection using a pocket weather meter. Plants were spray treated with MLE at 3 and 5% concentrations at monthly intervals for a period of three months. A lower concentration of MLE (3%) was selected on the basis of the results obtained from the first study and subsequent studies were conducted under the glass house conditions at RRISL. Control plants were devoid of priming treatments. For the mock treatment, plants were sprayed with water. There were three blocks and each block had 20 plants. Five plants were imposed with each treatment in each block according to a RCBD, so that there were 15 plants for each treatment. Other agro management practices were done as recommended by RRISL.

**Antioxidant analysis**

Three months after first spraying in the DZ, three plants from each treatment, representing each block, were selected and a leaf (from middle area of the stem) was collected from each plant so that three leaves were collected for each treatment. Cut ends of the petioles were wrapped with moistened cotton plugs, put into polythene bags, sealed, labeled and transported to the Herbal Technology Section, Industrial Technology Institute (ITI), Halbarawa Garden, Malambe, on the same day the samples were collected.

Leaf samples were subjected to various antioxidant assays. Free radical scavenging activity of leaf samples was determined by ABTS (2,2'-azino-bis-3-ethylbenzothiazoline -6-sulphonic acid) radical cation decolorization assay following the procedure of Re *et al.* (1999). The results were expressed as milligrams of Trolox equivalent (TE) per gram of leaf sample. FRAP (ferric reducing antioxidant power) assay was done following the procedure of Benzie and Strain (1996). The results were expressed as milligrams of TE per gram of leaf sample. The total phenolic contents (TPC) in leaf samples were determined spectrophotometrically according to Folin-Ciocalteu method (Singleton *et al.*, 1999) and the results were presented as milligrams of gallic acid equivalent (GAE) per gram of leaf sample. All the samples were analyzed in triplicates.

**Data analysis**

Significance of the observed treatment differences was tested by analysis of variance using proc ANOVA procedure of the SAS software package (version 9.1) and significant means were separated using the least significant difference (LSD).

**Results and Discussion**

Day time temperature and relative humidity (11:00-11:30 hrs) recorded at experimental sites in the IZ (for 2017) and in the DZ (for 2018) are shown in Figure 1. The amount of rainfall and the number of rainy days recorded for a period of four months from January-April 2017 for the IZ are shown in Figure 2. No rainy days were recorded during the study period (January-April 2018) in the DZ. The soil moisture content recorded in the IZ on 24$^{th}$ April 2017 and in the DZ on 21$^{st}$ April 2018 were 2.1% v/v and 2.0% v/v, respectively.

Results revealed that high temperature, low humidity and low soil moisture conditions prevailed in the experimental sites in both the zones. Therefore, it can be presumed that the plants had been subjected to abiotic stresses caused by drought and heat during the study period.

**Physiological parameters**

Under the above mentioned sub-optimal soil and weather conditions, significantly higher net photosynthetic rates were recorded in plants treated with MLE at all three concentrations (5, 10 and 15%) as compared to the control, three months after treatment. Nevertheless, no significant differences in photosynthetic rates between MLE treated and mock (water) treated plants were recorded (Table 1).

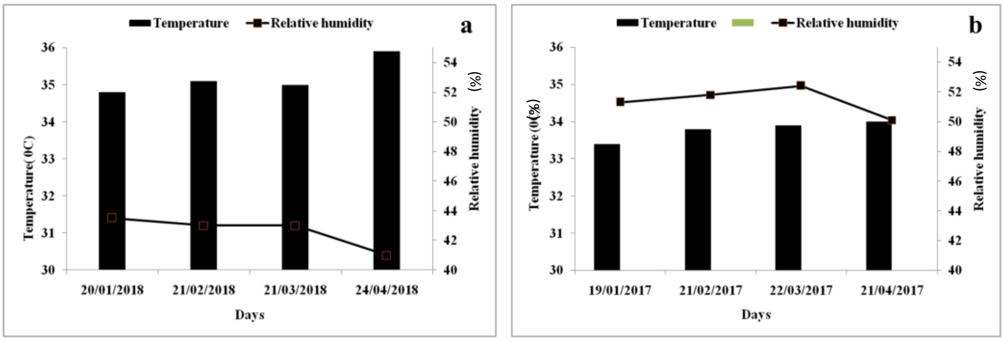


**Fig. 1.** Day time temperature and relative humidly recorded at the experimental site in the DZ on the same days of data collection from January to April 2018 (a) and in the IZ from January-April 2017 (b).

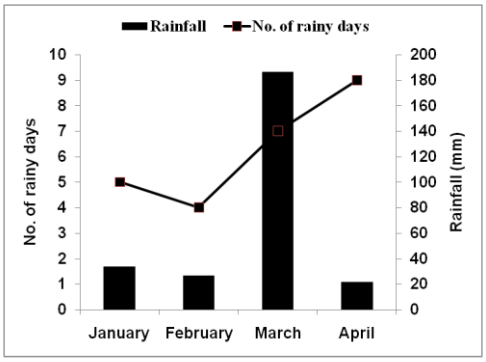


**Fig. 2.** Rainfall and the number of rainy days recorded at the experimental site in the IZ from January to April 2017

Significantly higher stomatal conductance values were recorded in plants treated at all levels of MLE as compared to the control. However, MLE at 10% had a significantly higher value as compared to both control and mock treatment (Table 1). But there were no significant differences in stomatal conductance values in plants treated with MLE at 5 or 15% as compared to mock treatment. Higher leaf water potential (more negative) values were recorded from plants treated with MLE, especially at 10 or 15%, as compared to control or mock treatment (Table 1).

**Table 1.** *Effect of MLE on physiological attributes of Hevea under sub-optimal climatic conditions in the IZ, three months after treatments imposition*

| Treatment | Net photosynthetic rate (µmol $m^{-2}$ $s^{-1}$) | Stomatal conductance (mmol $m^{-2}s^{-1}$) | Chlorophyll content (SPAD value) | Leaf water potential (Ψ) (Mpa) |
|---|---|---|---|---|
| Control | 14.00±2.01$^{b}$ | 0.185±0.02$^{c}$ | 51.66±0.17$^{b}$ | -1.45±0.05 |
| Water | 16.00±0.70$^{ab}$ | 0.237±0.02$^{bc}$ | 52.53±0.49$^{b}$ | -1.55±0.15 |
| 5% MLE | 18.53±0.31$^{a}$ | 0.297±0.02$^{ab}$ | 55.83±1.05$^{a}$ | -1.95±0.05 |
| 10% MLE | 19.33±0.33$^{a}$ | 0.300±0.02$^{a}$ | 56.10±1.51$^{a}$ | -2.15±0.05 |
| 15% MLE | 19.53±0.86$^{a}$ | 0.327±0.03$^{ab}$ | 58.36±0.67$^{a}$ | -2.25±0.05 |
| $LSD_{0.5}$ | 3.641 | 0.791 | 2.777 | |

Data presented are the means ± SEs. Values followed by the same letter in a column are not significantly different at $p<0.05$.

One of the key physiological phenomena affected by the drought and heat stress in plants is photosynthesis. It is mainly affected due to reduced leaf expansion, improper functioning of the photosynthetic machinery and leaf senescence (Fahad *et al.,* 2017). Stomatal closure under drought reduces the $CO_2$ availability which makes plant more susceptible to photo damage. The reduced moisture availability induces negative changes in photosynthetic pigments, damages the photosynthetic machinery and impairs the performance of important enzymes causing considerable losses in plant growth and yield (Lawlor and Cornic, 2002).

Rubber tree regulates the stomatal apertures to avoid excessive loss of water and stomatal closure occurred to maintain the water potential above a critical threshold, thus protecting against xylem cavitation (Kunjet *et al.,* 2013). Under drought stress, the water deficit causes a decrease in the leaf water potential and stomatal conductance which leads to a reduction of transpiration in the rubber trees (Kunjith *et al.,* 2013). Very low stomatal conductance (Chandrashekar *et al.,* 1998) and severe inhibition of photosynthesis and transpiration (Krishna *et al.,* 1991) have already been reported for *Hevea* under drought stress.

In the present study, improved physiological parameters *viz.* photosynthesis and stomatal conductance in plants treated with MLE as compared to control under sub-optimal climatic conditions could be directly attributed to the beneficial effect of MLE as a rich source of antioxidants such as phenolics, ascorbate, carotenoids (lutein, alpha-carotene, beta-carotene and xanthin), vitamins like (A, B, C), different essential minerals (K, Ca, Fe), proteins and zeatin which can increase the efficiency of PSII (Foidle *et al.,* 2001). Exogenous application of MLE has been shown to improve photosynthesis and stomatal conductance in Quinoa (*Chenopodium* spp.) (Rashid *et al.,* 2018), Maize (Bakhtavar *et al.,* 2015) and rocket (*Eruca vesicaria* sub sp. *sativa*) (Abdalla, 2015) under abiotic stress conditions. Nevertheless, treatment of rubber plants with water alone also improved photosynthesis in par with MLE treatments, suggesting the beneficial effect of foliar spraying of water on growth of rubber under sub-optimal climatic conditions. Higher water potential (more negative values) due to MLE treatment could be attributed to the accumulation of more osmolytes in *Hevea.* Accumulation of osmolytes, such as proline, glycine betaine, soluble proteins and soluble sugars, is a strategy to overcome osmotic stress provoked by drought (Ahmad *et al.,* 2016).

The leaf chlorophyll content increased significantly in rubber plants treated with MLE at all three concentrations (5, 10 and 15%) as compared to control and mock treatment after 3 months of treatment imposition (Table 1). Drought and heat stress trigger metabolic changes especially by excess ethylene production leading to early senescence and chlorophyll degradation. Thus, maintenance of stay green character is very important and considered as best indicator of thermo tolerance (Rashid *et al.,* 2018). Yasmeen *et al.* (2013)

demonstrated that exogenous MLE significantly increased the chlorophyll content in leaves of salt stressed wheat plants. Ali *et al.* (2011) reported that treatment of maize plants with MLE could increase the leaf chlorophyll contents through activation of cytokinin dependent isopentenyl transferase (ipt) biosynthesis thus avoiding premature senescence of leaves. In the present study, increased chlorophyll content in rubber plants treated with MLE could be attributed to the presence of cytokinin, such as zeatin in MLE extract. Besides zeatin, MLE contains high nutritional potentialities of several macro elements such as Mg (Yameogo *et al.,* 2011), a constituent of chlorophyll, which would account for the increase in chlorophyll content in rubber plants.

**Antioxidant analysis**

MLE at both concentrations (3 & 5%) significantly increased the TPC and FRCP in leaf samples as compared to control and the mock treatment in the DZ (Table 2). Nevertheless, a significantly higher ABTS value was recorded from plants treated with MLE at 5% as compared to control and other treatments. Further, significantly higher ABTS values were recorded from plants treated with MLE at 3% or water as compared to control.

To overcome oxidative damage under stressed conditions, plants develop an antioxidant defense system consisting of various antioxidant enzymes and nonenzymatic antioxidants *e.g.,* proline, tocopherols, glycine betaine, total soluble sugars, total free amino acids, phenolic compounds, ascorbic acid, and carotenoids (Mittler, 2002). This antioxidant system maintains ROS at a less toxic level by converting them into water and oxygen. Therefore, increased accumulation of non enzymatic antioxidants in MLE treated rubber plants under sub-optimal climatic conditions could be directly attributed to the beneficial effect of MLE as a biostimulant containing a rich source of antioxidants, hormones and essential macro and micro nutrients (Foidl *et al.,* 2001; Siddhuraju and Becker, 2003; Rady and Mohamed, 2015).

The ROS-scavenging systems play an essential role in maintaining redox homoeostasis. Activities of antioxidant enzymes; (superoxide dismutase (SOD), peroxidase, catalase (CAT) and glutathione reductase (GR)) and concentrations of antioxidant molecules (glutathione and ascorbate) are the most predominant functions in plants (Zhang *et al.,* 2018). The antioxidant elevation in rubber leaves in the present study could also be due to the stimulatory effect of MLE on activation of genes responsible for the above enzymes which scavenge free radicals.

TPC, FRAP and ABTS techniques have been employed for estimation of antioxidant activities in latex of *Hevea* (Siriwong *et al.,* 2018). MLE application has also been reported to increase leaf antioxidant contents and ameliorate the negative effects exerted due to drought and heat stresses in wheat and Quinoa plants (Nawaz *et al.,* 2016; Rashid *et al.,* 2018).

**Growth parameters**

A significantly higher girth was recorded in plants treated with MLE at 15% concentration as compared to control after three months from the first spraying (Table 3). However, there was no significant difference in plants treated with MLE at all three concentrations as compared to mock treatment after 3 months from the first spraying. Nevertheless, after 12 months from the first spraying, a significantly higher girth value was recorded in plants treated with MLE at 5% as compared to control and mock treatment. Girth values in plants imposed with MLE at 10% and 15% were on par with those in control and mock treatment after 12 months of first spraying. This suggests that application of MLE at low concentration (5%) at monthly intervals over a long period is beneficial than applying higher concentrations of MLE for growth improvement in *Hevea.* Nevertheless, application of MLE at high concentrations (10% and 15%) on *Hevea* over a long period, including months with extreme weather conditions, might exert more stress so that the beneficial effect of MLE could not be obtained.

Under stress conditions plants utilize most of their resources for improvement in defence mechanisms rather than growth and development (Yasmeen *et al.,* 2013). Nevertheless, growth enhancement of rubber plants could be attributed to the beneficial effect of MLE as a biostimulant containing macro and micro nutrients, growth promoting hormones, amino acids and antioxidants (Yasmeen *et al*., 2013; Rady and Mohamed, 2015). Foliar spray of MLE has been shown to boost the growth of crop plants by increasing leaf area and photosynthetic rate as mineral composition of MLE makes it an excellent natural growth promoting substance (foliar nutrients) influencing physiological processes in a positive way (Yasmeen *et al*., 2013).

**Table 2.** *Effect of MLE on total phenolic contents and antioxidant activities of Hevea after three months of treatments imposition at monthly intervals in Kilinochchi*

| Treatment | TPC (mg of GAE/g of leaf sample) | ABTS activity (mg of TE/g of leaf sample) | FRAP activity (mg of TE/g of leaf sample) |
|---|---|---|---|
| Control | 12.50±0.11$^{b}$ | 49.96±0.08$^{c}$ | 10.68±0.14$^{b}$ |
| Water | 13.55±0.07$^{b}$ | 55.08±0.48$^{b}$ | 11.59±0.23$^{b}$ |
| 3% MLE | 15.90±0.10$^{a}$ | 59.43±0.14$^{b}$ | 14.70±0.66$^{a}$ |
| 5% MLE | 17.17±0.19$^{a}$ | 70.26±0.10$^{a}$ | 14.79±0.17$^{a}$ |
| $LSD_{0.5}$ | 1.542 | 4.725 | 1.249 |

Data presented are the means ± SEs (n = 3), triplicate analysis from the pooled leaf samples for each treatment. Values followed by the same letter in a column are not significantly different at $p<0.05$

**Table 3.** *Effect of MLE on growth of Hevea before and after imposing treatments at monthly intervals for six months*

| Treatment | Girth (cm) at Day 0 | Girth (cm) after 3 months | Girth (cm) after 12 months |
|---|---|---|---|
| Control | 4.54±0.11[a] | 5.78±0.08[b] | 9.66±0.14[b] |
| Water | 4.44±0.07[a] | 6.43±0.48[ab] | 9.82±0.23[b] |
| 5% MLE | 4.27±0.10[a] | 6.66±0.14[ab] | 11.5±0.66[a] |
| 10% MLE | 4.54±0.19[a] | 6.83±0.10[ab] | 10.8±0.17[ab] |
| 15% MLE | 4.45±0.06[a] | 7.00±0.31[a] | 10.5±0.29[ab] |
| $LSD_{0.5}$ | 0.3205 | 1.051 | 1.249 |

Data presented are the means ± SEs (n = 15). Values followed by the same letter in a column are not significantly different at $p<0.05$

Among the plant growth regulators, zeatin, a naturally occurring form of cytokinins present in MLE, has a critical role in promoting cell division and modification in apical dominance in plants and thus enhancing growth of crop plants (Ahmad *et al.*, 2016). Spraying Sorghum plants with MLE at 3% increased growth parameters and yield (Ahmad *et al.*, 2016). Moreover, Abdalla (2015) reported that treatment of rocket (*Eruca vesicaria* subsp. *sativa*) plants with MLE at 2% increased the content of N, P and K in leaves.

## Conclusion

Our study demonstrates, for the first time, that exogenous application of MLE is effective in improving physio-chemical attributes and growth of *Hevea* under sub-optimal climatic conditions, possibly by antioxidant elevation. Therefore, application of MLE, preferably 5%, may effectively be utilized as an inexpensive and environment friendly biostimulant for enhanced performance of *Hevea* under drought and heat stress conditions as an adaptation measure for climate change.

## Acknowledgements

The authors wish to thank the owner and the Manager of the Nottinghill Private Estate, Mawathagama and Mr Parameshwaran, owner of the experimental field at Kilinochchi, for providing all the facilities and allowing us to conduct the field experiments. Antioxidant analysis done by Dr Kaushalya Abeysekera at ITI and statistical assistance given by Dr (Mrs) Wasana Wijesuriya, Principal Research Officer, Biometry Section, RRISL, are gratefully acknowledged.

## References

Abdalla, M M (2015). The potential of *Moringa oleifera* extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria* subsp. *sativa*) plants. *Afr. J. of Crop Science* **3**(2), 116-122.

Ahmad, W, Noor, M A, Afzal, I, Bakhtavar, M A, Nawaz, M M, Sun, X, Zhou, B, Ma, W and Zhao, M (2016). Improvement of Sorghum crop through exogenous application of natural growth-promoting substances under a changing climate. *Sustainability* **8**, 1330; doi:10.3390/ su8121330.

Ali, Z, Basra, S M A, Munir, H, Mahmood, A and Yousaf, S (2011). Mitigation of drought stress in maize by natural and synthetic growth promoters. *J. Agric. Soc. Sci.* **7,** 56-62.

Bakhtavar, M A, Afzal, I, Basra, S M A Ahmad, A U H and Noor, M A (2015). Physiological strategies to improve the performance of spring maize (*Zea mays* L.) planted under early and optimum sowing conditions. PloS One: 10: e0124441.

Benzie, I F and Strain, J J (1996). The ferric reducing ability of plasma (FRAP) as measurement of "antioxidant power" The FRAP assay. *Anal Biochem*. **239,** 70-76.

Chandrashekar, T R, Nazeer, M A, Marattukalam, J G, Prakash, G P, Annamalainathan, K and Thomas, J (1998). An analysis of growth and drought tolerance in rubber during the immature phase in a dry sub-humid climate. *Expt. Agri*. **34**, 287-300.

El–Hamied, S A and El-Amary, E I (2015). Improving growth and productivity of "Pear" trees using some natural plants extracts under North Sinai conditions. *IOSR J. of Agri. and Vet. Sci*. **8**(1). 1-9.

Fahad, S, Bajwa, A A, Nazir, U, Anjum, S A, Farooq, A, Zohaib, A Sadia, S, Nasim, W, Adkins, S, Aud, S, Ihsan, M Z, Alharby, H, Wu, C, Wang, D and Huang, J (2017). Crop production under drought and heat stress: Plant responses and management options. *Front. in Plant Sci*. doi: 10.3389/fpls.2017. 01147.

Foidl, N, Makkar, H P S and Becker, K (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. In: *Proceedings of the International Workshop What development potential for Moringa products?* Dar-es-Salaam, Tanzania. 47-67.

Foyer, C H and Noctor, V (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell.* **17**, 1866-1875. doi:10.1105/ tpc.105.033589.

Gill, S S and Tuteja, N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem***. 48,** 909-930. doi: 10.1016/j.plaphy. 2010.08.016.

Iqbal, S M M, Rodrigo, V H L and Karunathilake, P K W (2010). Feasibility of rubber (*Hevea brasiliensis* Muell. Arg.) cultivation in Eastern province of Sri Lanka with the peasant community. *Journal of the Rubber Research Institute of Sri Lanka* **90**,18-30.

Krishna, T M, Bhaskar, C V S, Rao, P S, Chandrashekar, T R, Sethuraj, M R and Vijayakumar, K R (1991). Effect of irrigation on physiological performance of immature plants of *Hevea brasiliensis* in North Konkan. *Indian Journal of Natural Rubber Research* **4**(1), 36-45.

Kunjet, S, Thaler, P, Gay, F, Kositsup, B, Chuntuma, P, Sangkhasila, K and Kasemsap, P (2013). Diurnal dynamics of stand transpiration and stomatal conductance in rubber (*Hevea brasilliensis* Muell. Arg.). *Kasetsart J.* (*Nat. Sci*.) **47,** 647-662.

Lakmini, W G D, Nainanayake, N P A D and Costa, W A J M (2006). Physiological responses for moisture stress and development of an index for screening coconut (*Cocos nucifera* L.) genotypes for drought tolerance. *Tropical Agriculture Research and Extension* **9,** 17-26.

Lawlor, D W and Cornic, G (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environment* **25,** 275–294. doi:10.1046/j.0016 -8025.2001.00814.x.

Maswada, H F, El-Razek, U A A, El-Sheshtawy, A N A and Elzaawely, A A (2018). Morpho-physiological and yield responses to exogenous moringa leaf extract and salicylic acid in maize (*Zea mays* L.) under water stress. *Arch. of Agro. and Soil Sci.* **64** (7), 994-1010, DOI: 10.1080/ 03650340. 2017. 1406079.

Mittler, R (2002). Oxidative stress, antioxidant and stress tolerance: a review. *Trends Plant Science* **7,** 405-410.

Mohammadi, M., Taleei, A., Zeinali, H., Naghavi, M.R., Ceccarelli, S. and Grando Baum, M. (2005). QTL analysis for phenologic traits in doubled haploid population of barley. *Int. J. Agric. Biol*. **7,** 820-823.

Nayanakantha, N.M.C., Panditharatne, B.M.M.S., Nakandala, S.A., Karunathilake, W and Seneviratne, P. (2018). Exogenous *Moringa oleifera* leaf extract as a biostimulant improves growth and physiological attributes of rubber (*Hevea brasiliensis*) under sub-optimal climatic conditions. *Proceedings of the Postgraduate Institute of Science Research Congress,* University of Peradeniya, Peradeniya. Abstract, p.58.

Nawaz, H., Yasmeen, A., Anjum, M.A. and Hussain, N. (2016). Exogenous application of growth enhancers mitigate water stress in wheat by antioxidant elevation. *Front. Plant Sci*. **7,** 597. doi: 10.3389/fpls.2016.00597.

Perdomo, J A, Conesa, M A, Medrano, H, Ribas-Carbó, M and Galmés, J (2015). Effects of long-term individual and combined water and temperature stress on the growth of rice, wheat and maize: relationship with morphological and physiological acclimation. *Physiol. Plant.* **155** (2), 149–165.

Pervez, K, Ullah, F, Mehmood, S and Khattak, A (2017). Effect of *Moringa oleifera* Lam. leaf aqueous extract on growth attributes and cell wall bound phenolics accumulation in maize (*Zea mays* L.) under drought stress. *Kuwait J. Sci.* **44** (4), 110-118.

Rady, M M, Varma, C B and Howladar, S M (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. *Scientia Hort.* **162,** 63-70.

Rady, M M and Mohamed, G F (2015). Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. *Scientia Hort*. **193,** 105-113.

Rashid, N, Basra, S M A, Shahbaz, M, Iqbal, S M M and Hafeez, M B (2018). Foliar applied Moringa leaf extract induces terminal heat tolerance in Quinoa. *Int. J. of Agri. & Biol*. **20**, 157-164. DOI:10.17957/IJAB/15. 0469.

Re, R, Pellegrini, N, Proteggente, A, Yang, N and Rice-Evans, C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. *Biol. Med*. **26**, 1231-7.

Rodrigo, V H L and Iqbal, S M M (2009). Rubber reaches east; Hand in hand with regaining Sri Lanka. *Bulletin of the Rubber Research Institute of Sri Lanka* **50,** 111-117.

Rodrigo, V H L, Iqbal, S M M and Dharmakeerthi, R S (2011). Potential for rubber (*Hevea brasiliensis* Muell. Arg.) cultivation in the Eastern Province of Sri

Lanka. *Journal of National Science Foundation of Sri Lanka* **39** (4), 403-411.

Rodrigo, V H L, Iqbal, S M M, Munasinghe, E S, Balasooriya, B M D C and Jayathilake, P M M (2014). Rubber in East assures the perceived benefits: a case study showing increased rubber production, amelioration of the climate and improved rural livelihood. *Journal of the Rubber Research Institute of Sri Lanka* **94**, 33-42.

Savvides, A, Ali, S Tester, M and Fotopoulos, V (2016). Chemical priming of plants against multiple abiotic stresses: Mission possible? *Trends in Plant Sci*. **21**(4), 329-340.

Siddhuraju, P and Becker, K (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J Agric. Food Chem*. **51,** 2144-2155.

Singleton, V L, Orthofer, R. and Lamuela-Raventos, R M (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods Enzymol.* **299**, 152-78.

Siriwong, S, Rungvichaniwat, A, Klinpituksa, P, Musa, K H and Abdullah, A (2018). Estimation of antioxidant activity in natural rubber (*H. brasiliensis*) using TPC, FRAP, CUPRAC, ABTS, DPPH, ORAC assays, individual phenolic and individual flavonoid quantity. *Glob. Adv. Res. J. of Agri. Sci.* **7**(2), 053-063.

Sreelatha, S, Mydin, K K, Simon, S P, Krishnakumar, R, Jacob, J and Annamalainathan, K (2011). Seasonal variations in yield and associated biochemical changes in RRII 400 series clones of *Hevea brasiliensis*. *Natural Rubber Research* **24,** 117–123.

Van Oosten, M J, Pepe, O, Pascale, S De, Silletti, S and Maggio, A (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and biological technologies in Agriculture* **4,** 5 DOI 10.1186/s40538-017-0089-5.

Wahid, A, Gelani, S, Ashraf, M and Foolad, M R (2007). Heat tolerance in plants: an overview. *Environ. Exp. Bot*. **61**, 199-223. doi: 10.1016/j.envexpbot.2007.05.011.

Yameogo, C W, Bengaly, M D, Savadogo, A, Nikiema, P A and Traore, S A (2011). Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. *Pak. J. Nutr.* **10**(3), 264-268.

Yasmeen, A, Basra, S M A, Farooq, M, Rehman, H, Hussain, N and Athar, H R (2013). Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Grow. Reg.* **69**, 225-233.

Zhang, Y, Leclercq, J and Montoro, P (2018). Reactive oxygen species in *Hevea brasiliensis* latex and relevance to tapping panel dryness. *Tree Physiology* **37**(2), 261-269.

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