Available Online at http://www.journalajst.com

ISSN: 0976-3376

ASIAN JOURNAL OF
SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 14, Issue, 12, pp. 12812-12816, December, 2023

RESEARCH ARTICLE

ABIOTIC STRESS MITIGATION: A CASE STUDY OF MORINGA LEAF EXTRACT AS A NATURAL BIOSTIMULANT ON CUCURBITA

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ARTICLE INFO

Article History:
Received 14th September, 2023
Received in revised form
28th October, 2023
Accepted 03rd November, 2023
Published online 30th December, 2023

Keywords:
Biostimulant, Abiotic stress, Moringa-leaf- extract, Cucurbita , Drought, Heat stress.

ABSTRACT

The two major problems: climate change and global population are now common in worldwide. According to UNDESA 2017, there will be 10 billion global populations within 2050 and more desiccation will be observed with rising temperatures. It will likely have negative consequences on crop growth and productivity worldwide. Chemical fertilizers are imperative resource of plant nutrition, but quite expensive, representing a source of environmental pollution. Increasing restrictions on pesticide application in agricultural food products indicate urgent need for safer alternatives to maintain the product quality and restrain pathogen infections. Recent alternatives to chemical fertilizers and pesticides improve crop growth, using a promising and eco-friendly plant biostimulants. Thus plant fitness enhances. The need of fertilizers will be reduced, also resistance to abiotic stresses. Our study aimed to apply Moringa leaf extract (MLE) on seedlings of *Cucurbita* plant to acclimate and tolerate further heat stress exposition as a pre-treatment. MLE was applied in 5 plants as foliar spray (FS). The results showed that all treatments significantly increased plant vegetative growth, recommending higher yield grown in MLE. Plant extract could potentially provide a safe alternative to chemical fertilizers and plant regulators. Our experimental study indicates that MLE significantly improve growth of the plant alleviating the abiotic stress.

Citation: ***Sumana Ghosal, Misba Kaji and Aparna Sadhu.* 2023.** "Abiotic stress mitigation: a case study of moring a leaf extract as a natural biostimulant on cucurbita", *Asian Journal of Science and Technology*, 14, (12), 12812-12816

INTRODUCTION

Alteration in global climate induced abiotic stresses (e.g., drought, salinity, extreme temperatures, heavy metals, and UV radiation) to destabilize the fragile agro-ecosystems and impaired plant performance, thereby reducing crop productivity and quality (Bibi *et al*. 2023). Abiotic and biotic stresses limit the growth and productivity of plants. In the current global scenario, in order to meet the requirements of the ever-increasing world population, chemical pesticides and synthetic fertilizers are used to boost agricultural production. These harmful chemicals pose a serious threat to the health of humans, animals, plants, and the entire biosphere aims to approach the use of biostimulants in plant growth according to the raw material used in their compositions as well as their effects on plants subjected to abiotic stresses (Shukla *et al*. 2019). Abiotic stresses may be prevented by optimizing plant growth conditions and through the provision of water and nutrients and plant growth regulators (PGRs—auxins, cytokinins, gibberellins, strigolactones, and brassinosteroids). In addition to these traditional approaches, biostimulants have been highlighted as a promoter of optimizing productivity by modifying physiological processes in plants. Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase yield. (Carolina *et al*. 2019).

Water accounts for 80%–95% of the fresh biomass of plants and plays a vital role in physiological processes, including plant growth, development, and metabolism (Brodersen *et al*. 2019) Thus, water scarcity or osmotic stress is considered the main environmental constraint for crops that could destabilize world food security (Snyder *et al*. 2015). Drought stress typically leads to a reduction in leaf size, stem elongation, root growth, and water use efficiency (WUE). the reduction of soil water potential, ionic imbalance and disturbances in solute accumulation. MLE has been shown to be an effective plant growth modulator during drought stress events. Foliar or root application of MLE led to the enhancement of leaf area, plant height, biomass production (Zea mays L.) (Islam *et al*. 2022) Glycine max (soybean) and Cucurbita pepo (Squash) under drought stress (Sardar *et al*. 2021). In general, biostimulants are produced as a junction of natural or synthetic substances composed of hormones or precursors of plant hormones. The effects of biostimulants are still not clear. They can act on plant productivity as a direct response of plants or soils to the biostimulant application or an indirect response of the biostimulant on the soil and plant microbiome with subsequent effects on plant productivity. They contain a wide number of bioactive compounds. These compounds are able to improve various physiological processes that stimulate plant growth and development and increase nutrient use efficiency, reducing chemical fertilizers without adverse effects on yields and their qualities (Bulgari *et al*., 2015). Moringa oleifera (Lam.) is classified as one of the most current biostimulants (leaves of the trees) (Taia *et al*. 2017). Among PDBs, moringa (Moringa oleifera Lam., syn. M. pterygosperma Gaertn.) leaf extracts (MLEs) received more attention in recent years because of their positive effect on plant productivity (Zulfiqar *et al*. 2017). Indications of MLE's growth and yield enhancing effects along with an amelioration effect on plant physiology under abiotic stresses (e.g. drought and salinity), justify the consideration of MLE as alternative to synthetic agro-chemicals for improving crop yield. Moringa leaves are potential source of vitamins (A, B, C), essential minerals (K, Ca, Fe) and amino acids Gopalakrishnan *et al*. 2016. Moreover, moringa leaf extract is enriched with cytokinins, auxins and Abscisic Acid (ABA) like growth substances. Hence, its leaf extract in water contains growth enhancing substances and can be used as biostimulants. Moringa leaf extract, when applied to salt stressed plants, promoted seed emergence, improved proteins synthesis, induced antioxidant activities and finally increased grains yield of wheat (Basra *et al*. 2011). The presence of large amount of phenolic compounds, having an ability to combat ROS and high antioxidant activity, and polyphenols contents (Randhir *et al*. 2004) is important in stress tolerance. Moreover, antioxidants like glutathione and ascorbic acid are synthesized in the chloroplast in significant amounts, which can improve plant tolerance against oxidative stress (Foyer and Noctor *et al*. 2003) (Batool *et al*. 2016). Our study henceforth aims to unearth the possible role of aqueous extracts of moringa fresh leaves via medium supplementation in the alleviation of heat stress induced oxidative damage in Cucurbita, a commercially important crop.

MATERIALS AND METHODS

Study Area with Weather: Lowa Santoshpur, Dwarnari, Purba Bardhaman District, West Bengal, India, where the temperature was 40±5°C, relative humidity was 75±5%, humus soil, forming the 'Location-Wise an Ideal Place' for keeping-and-caring of the *Cucurbita* plants, with the average rainfall was 150 millimetres, and it was the locality as 'Sample Area'

Group Formation: Group was formed with 2 students from Lowa Dibakar Vidyamandir High School residing at the same locality; age group of 13-14 yrs with one team leader, one team member.

***Study Samples*:** The 'Study Samples' were the developed Cucurbita plants in mentioned place.

Duration of Study: The duration of study for physiological parameters of the plant was from May 2023 to July 2023.

Experiment method: Identification of the problem. The soil was divided into plots (2.5×2.5m). Amounted of cattle manure was added for each plot by mixing with the soil during the preparation process. Five seedlings were chosen in each plot and were tagged with numbers indicating control and Moringa Leaf Extract (MLE) treatment group under drought and heat stress condition. During the course of the experiment seedlings were irrigated regularly. The extract was diluted with distilled water to spray directly onto the plant leaves using hand sprayer at the second week from transplanting at a regular interval of day 1st, 7th, 15th, 30th and 45th. At the harvesting time, data were recorded concerting the vegetative growth presented in plant height cm, number of leaves, leaf area, and number of main branches. Mean values are depicted in tables and are represented in graphs.

Extracts Preparation: Fresh moringa leaves were collected from already planted moringa trees. The production of water extracts is one of the easiest methods and serves the purposes of the end-user - farmers Water extracts have many advantages such as are eco-friendly, easily degradable, are not persistent in the soil, and are not toxic to animals and humans. Before extraction process 100gm of mature, disease free and healthy leaves were rinsed with water. The crude aqueous extract of moringa leaves were prepared by mixer grinder and solution was prepared in 1 liter of water. Ten Cucurbita plants were selected randomly and cut off from the two outer rows of every experimental plot to estimate shoot length, branch nos, leaf nos and leaf area. Sprayed directly onto trees thoroughly sprayed till run off at a regular interval of day 1st, 7th, 15th, 30th and 45th

Collection and Recording of Data: The following parameters on vegetative growth were recorded using 5 fully developed plants from each plot:

i. Shoot height
ii. Number of leaves
iii. Number of branches
iv. Average leaf area of the largest leaf.

The data were collected by student, recorded in the 'Logbook' and were authenticated by the guiding teacher Aparna Sadhu.

***Analysis of Data*:** The data were analyzed by comparison and referring to well-reputed published papers. Data were means of five variables from two independent experiments. ±SD determined using Microsoft excel, ver. 2010. The significant differences between treatment means were determined using analysis of variance and mean separation at a 5% significance level ($p \leq 0.05$).

Data Collection: Data represented here are the mean value of 5 samples from each plot. Height (cm) of the plants were measured from 10 plants 5 control and 5 treated with MLE under drought and heat stress condition. The total number of branches from ten randomly chosen plants was counted at harvest time and average number of branches plant was calculated. The leaf area of the largest leaf (cm^2) was measured from 5 plants from each group of treated and untreated plants.

Satistical Analysis: The compilation of data was done in Microsoft Excel and analyzed by using a simple bar diagram. Significant differences among groups were determined using Student's t test. All differences were determined to be significant at the $p<0.05$ level.

RESULT AND DISCUSSION

Plant height: Aloe leaf extract (ALE) especially at highest concentration (40 ml/L) significantly increased the plant height (Abbas *et al*. 2013). Taller plants were recorded by foliar application of MLE during first year under normal-sowing (Nabila Rashid *et al*., 2021). On applying 10% *Moringa oleifera* and *Moringa peregrena* extracts to salt-stressed basil, growth parameters like shoot length were increased by 32% and 38% (Hassanein *et al*. 2019). In this study it is shown that the treatments MLE had a more significant effect on shoot length as compared with the control under drought stress. A significant difference was observed between the MLE treated and control plants which signify their effectiveness in drought stress environments. It is reported in Figure 1. Mean comparison of control under drought stress vs treatments were also found significant for plant height (cm) of *Cucurbita. (p value< 0.05).* With increase in the interval of spraying Moringa leaf extract, plant height enhanced and thus taller plants (814.8cm) were recorded when MLE sprayed at 30th and 45th days after sowing in Figure 1. According to *Mehmood et al*. (2021) the use of moringa leaf extract during critical vegetative development phases increased the black cumin crop's plant height. Similar results were recorded by *Abbas et al*. (2013) that moringa leaf extract enhanced plant height and improved fresh and dried weight of wheat root when compared to control. Thus increases plant height proclaiming a morphological strategy of plants to tolerate drought with the help of Moringa leaf extract as a potent biostimulant.

Number of Branches: Foliar application of MLE contains an adequate amount of stimulating substances that promote cell division and enlargement at a faster rate. Zeatin, a growth hormone found in moringa leaf extract, encourages the growth of lateral buds, which leads to an increase in the number of branches (Mehmood *et al*., 2021).

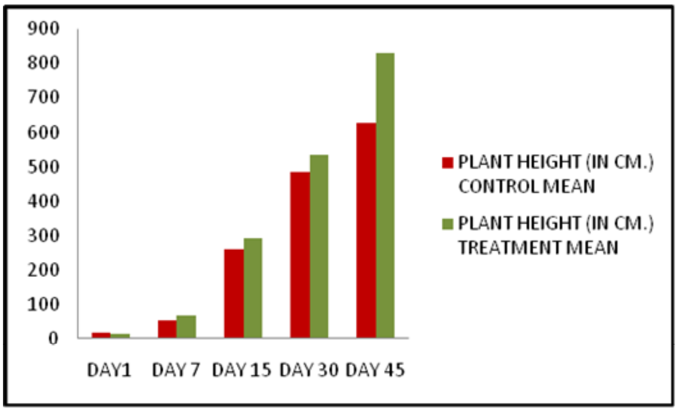

Figure 1. Height of Plant

Branches of Cucurbita were significantly influenced by moringa leaf extract concentrations, stages of application as well as their interaction is depicted in Figure 2. Number of branches per plant was reduced significantly under heat stress and drought condition. ($p<0.05$). The planned mean comparison of control under drought stress verses rest was also found significant for branches in plant. The unsprayed against sprayed treatments of moringa leaf extract showed that in unsprayed control under drought stress plots number of branches were 64.8 (mean value) which were less than plants sprayed with moringa leaf extract (73.8). The highest number of branches/plants (64.8) was observed in moringa leaf extract treated plots after 15th and 30th day of growth stage. Similar results were recorded by Jain *et al*. (2020), who reported MLE positively enhanced plant growth attributes of wheat. He also stated that with increasing MLE application intervals, the growth parameters such as branches were increased in arithmetic order. This increase in traits might be due to presence of antioxidants and vitamins in the MLE which induced production of antioxidants and vitamins in seedlings which ultimately improved growth of seedlings. MLE possesses high antioxidant activity as it is rich in some plant secondary metabolites and osmoprotectants (Rady *et al*., 2013).

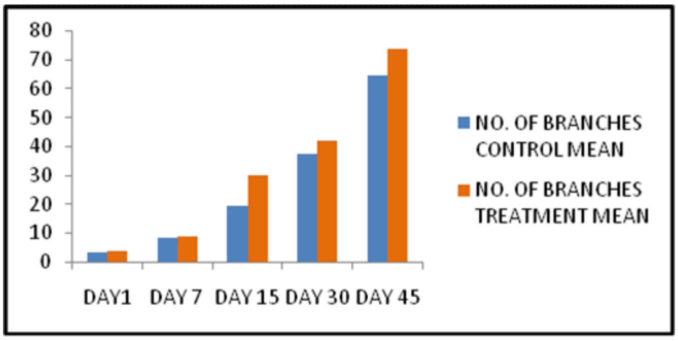

Figure 2. Number of Branches

Number of leaves: According to, Saa *et al*. (2016) the number of leaves per shoot was higher in the biostimulant treatments than in the control plants, irrespective of the soil K conditions . The presence of nutrients and cytokine in MLE may be responsible for the increase in leaf no. Moringa leaf is rich in cytokine and micro- and macronutrients (Taiz and Zeiger 2010). MLE is a plant growth promoter that contains a many essential nutrients, phenols, ascorbic acid, and antioxidants that are important for plant development and growth (Yasmeen *et al*. 2013). Alkuwayti *et al*. (2019) found that when basil plants were sprayed with MLE, they produced the maximum numbers of leaves as compared to control plants. In our experiment the data regarding the number of leaves $plant^{-1}$ are given in Figure 3. The statistical analysis in Figure 3 shows that there were significant differences between control under drought stress and MLE treated ($P \leq 0.05$) in terms of leaf number $plant^{-1}$. The mean data show that maximum no.of leaves in treated plants is 57.6 in comparison to untreated control under drought and heat stress plants as 47.4 (Table 3). As compared to control MLE treatment showed increased no of leaves in Cucurbita.

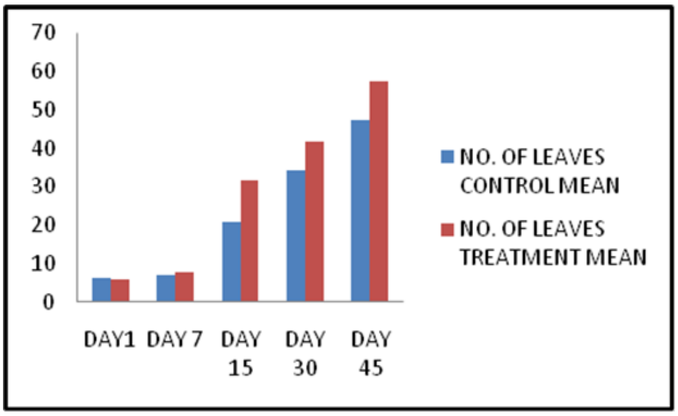

Figure 3. Number of leaves

Average leaf area: Leaf area of the MLE treated plants showed in Figure 4. Few plants showed increase in leaf area from 25.6 cm^2 to 210 cm^2 in treatment as compared to control under stress condition from 26.2 cm^2 to 242 cm^2(Table 4). Whereas some treated plants had shown 18.5 cm^2 to 169.5 cm^2 increase in leaf area. Data are represented in figure 4. This shows an insignificant result ($p>0.05$) of MLE treatment. The possible reason for such result is the increased no. of leaf in treated plants compared to control plants.

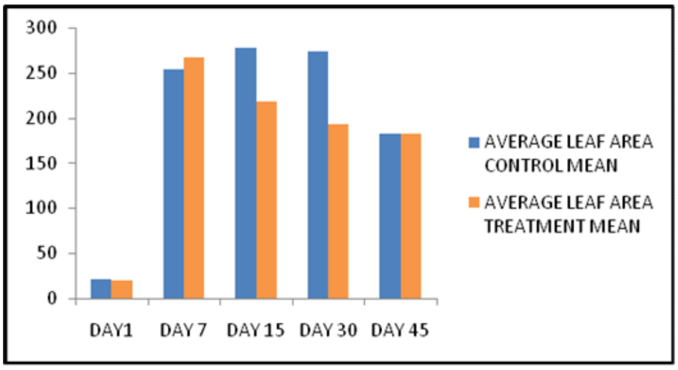

Figure 4. Average area of leaf (sq.cm)

Mitigating Abiotic stress: Results of this study show that exogenous application of MLE was effective in mitigating the physiological response of stress damages (i.e., drought) (picture1,2,3,4). Plant-derived biostimulants that trigger abiotic stress tolerance to ameliorate stress-induced yield reduction involve many different mechanisms and most of these involve phytohormones and upregulated antioxidant defense systems (Bulgari *et al*., 2017). However, foliar spraying of MLE significantly enhanced these attributes and also mitigated the negative impact of drought stress. Furthermore, a study by Rashid *et al*. (2018) confirmed the presence of a primary growth regulator, zeatin (a cytokinin derivative) that provided abiotic stress tolerance and also boost up the yield of field crops in range of 10–45%. Moringa extract constitutes considerable quantities of secondary metabolites, antioxidants, and osmoprotectants (Rehman *et al*., 2015). MLE's growth-promoting and abiotic stress tolerance inducing effects justify its use as a potential alternative to synthetic chemical fertilizers for improving *Cucurbita* productivity. Spraying MLE diluted solution on plant leaves seems to have a considerable positive impact, i.e., delay in senescence, higher sugar contents, increased plant height, and bigger fruits and seeds (Yasmeen *et al*., 2013a; Nasir *et al*., 2014). This validates the potential effects of MLE to be used to improve plant vigor specifically in suboptimal environmental situations like drought stress as a foliar application.

Picture 1 Picture 2 Picture 3 Picture 4

Picture 1 and 2: *Cucurbita* plant affected due to abiotic stress (drought and heat); untreated.; Picture 3 and 4: *Cucurbita* plant; treated with MLE mitigating drought and heat stress

The available studies have shown that moringa leaf extract has the potential to improve the plant growth and development.

CONCLUSIONS

The application type and application mode of extracts are of great importance in view of the effectiveness and availability in any medium. Previously moringa dry leaves extract has been used as seed priming strategy most of the times under normal conditions for promoting plant growth (Yasmeen *et al*. 2013; 2014), while potentiality of moringa extracts to combat heat stressed has been rarely studied. This project has confirmed that the applications of MLE may have a beneficial impact on plant growth, productivity, quality, and tolerance to various abiotic stresses. Due to their multifaceted properties, they have increasingly been considered as valuable advanced farming techniques used in worldwide agricultural production. Biostimulant represents a new generation of products and an eco-friendly complement to widely used agro-chemicals. In the coming few years, we can expect that plant biostimulants including both natural and synthetic substances, as well as microbial inoculants, will not only make a significant contribution to ecologically and economically sustainable crop production systems within more resilient agro-ecosystems but will also lay the cornerstone for a future large-scale sustainable agriculture catalysed by the biobased industry development of new products and/or upgrades, production, Marketing and distribution of commercial biostimulants, could implement a sustainable strategy to improve plant tolerance/ resistance against such environmental limitations, which is of great importance to secure and optimize globe. Our investigation provide that the foliar application with moringa leaf aqueous extract as a biostimulants is a cheap source of plant growth hormones and minerals especially with the trend of organic farming for improved yield in horticulture crops.

Future Plan: We understand the physiology of plants today better than ever, thanks to scientific and technical breakthroughs in many disciplines over the last decades. Most of these achievements have used a limited number of model organisms in controlled environments. A challenge is now to use this knowledge and these tools for the characterization of biostimulants and their effects on a wide range of cultivated plants.

ACKNOWLEDMENT

These authors express our sincere gratitude to Dr. Subrata Kumar Ghosh, Assistant teacher, Rathtala Manohar Das Vidyaniketan High School, Burdwan and Dr Sarmishtha Chatterjee, Assistant Professor, Belur Sramajibi Swasthya Prakalpa Samity, Belur, Howrah for their constant and tireless help in this project work. We will be forever indebted to local people for their cooperation and sharing of their knowledge.

Author's Contribution: The authors contributed equally in compiling this manuscript.

Funding: No external funding was received.

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