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Ameliorating potential of *Moringa oleifera* leaf extract (MLE) for drought stressed *Foeniculum vulgare* plants

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Ameliorative role of *Moringa oleifera* leaf extract (MLE) on stressed *Foeniculum vulgare* plant was studied. The experiment was conducted for seeking the ameliorative role of *Moringa oleifera* leaf extract (MLE) on growth, ions and non-enzymatic antioxidants of stressed *Foeniculum vulgare* plant. Experiment was performed by growing seeds in sandy loam soil filled in pots placed with complete randomization. Drought was imposed by withholding water quantity according to soil saturation percentage. After 20 days of germination, thinning of plants was done and three plants were left in each pots. Plants were foliarly sprayed twice with 2%, 4% and 6% MLE with an interval of 10 days. Data were collected after 45 days of germination taking three plants of each treatment as replicates. Statistical analysis was performed to compare means. Water Stressed resulted in significant reductions of shoot length, root lengths and plant proliferation as number of branches. When MLE were applied on stressed plants then a significant increase in growth attributes were found. Phosphorus, potassium and terpenoids concentrations were increased by MLE. Stress reduced the phosphorus contents in stem and leaves; Potassium and alkaloid contents of these were compensated by MLE applications. However, terpenoids contents were increased in stem and root. Stress was ameliorated more effectively by 4% MLE

Keywords: Alkaloids, Extract, Foeniculum, Growth, Moringa, Water stress

INTRODUCTION

Moringa oleifera L. is a tree commonly known also as miracle tree. The *Moringa* genus includes 14 species (Rani et al. 2018). *Moringa oleifera* is native tree of India, Pakistan, Africa, Asia and America. In addion to being used for medicinal purposes, it is also used as food and food supplements (Mainenti 2018; Divya et al. 2019; Meireles et al. 2020). Due to being a highly nutritious plant, *M. oleifera* is considered ideal for malnutrition treatments in under developed countries (Zongo 2013; Valdez-Solana et al. 2015; Gopalakrishnan 2016; Debajyoti et al. 2017). Dry leaves (100 g) of *M. oleifera* contain ten times more vitamin A than from carrots, nine times more protein than yoghurt, and twenty five times greater iron than spinach (Oduro et al. 2008; Rockwood 2013; Saini et al. 2016). The leaves, stem, root, flower and seed of *Moringa* are good sources minerals, vitamins, proteins, amino acids, β-carotene and phenolics (Anwar et al. 2007; Leone et al. 2015; Saini et al. 2016; Fahey 2017; Debajyoti et al. 2017; Divya et al. 2019). Inspite of many health importance of *M.*

oleifera phytochemicals, it contains harmful substances (Fahey et al. 2001; Annongu et al. 2014 including alkaloids and other phytotoxins which can affect human health adversely (Maizuwo et al. 2017). *Moringa oleifera* extract has anti-aging capacity and protective for plants injuries. (Siddhuraju et al. 2005). *Moringa oleifera* contain proteins, vitamins, ascorbic acid, β-carotene, phenolic compounds and sugars. It also has minerals such as Calcium, Sodium, Iron, Potassium and Phosphorus (Aanwar et al. 2011). Extract of *Moringa* leaves can regulate plant growth. It increases growth of root for more absorption of minerals (Fuglie 2000; Muhammad 2004).Therefore, it has the capacity to compensate the adverse water stress effects.

Antioxidants are useful for plant to tolerate environmental stresses. Fennel plant has much quantity of antioxidants and hence is used in cure of many diseases. The antioxidants are used to detoxify the reactive oxygen species (ROS) generated during stress. The leaves and fruits of funnel plant, due to having antioxidants, also detoxify the free radicals (Oktay et al. 2003). Aqueous or Ethanolic extracts of fennel have phenolic compounds which exhibit the strong antioxidant activity. Water stress have strong influence on phytochemistry and growth of this plant (Patel et al.2000). *Foeniculum vulgare* plant parts are used for various purposes such as fragrance,taste and medicines (Agarwal et al.2008). The informations about pharmacology and toxicology of *Foeniculum vulgare* is available in literature (Choi and Hwang 2004).

MATERIALS AND METHODS

This experiment was designed to explore the effect of foliarly applied *Moringaolifera* leaf extract on the *Foeniculum vulgare* cultivar under drought stress. The seeds of fennel variety were obtained from research centre. The soil used was sandy loam in texture which is thought to be more appropriate for the growth of plant. Plastic pots of 30 cm diameter were filled with soil and were placed in completely randomized design. The pots were watered and kept to attain field capacity moisture contents. Sowing was done and 75 % seed germination took place after 5 days. After ten days of germination, thinning was carried to keep three plants in each pot. After fifteen days of emergence, half of the pots were exposed to water stress treatments twice by withholding water till the temporary wilting stage of plants reached. At the age of twenty days, plants of stressed and non stressed conditions were sprayed with 2%, 4% and 6% aquaous *Moringa olifera* leaf extract (MLE). For *Moringa* leaf extract preparation, fresh leves of were extracted in according to Foidle et al. (2001) and Yasmeen et al. (2012). The pure extract was then centrifuged for 10 minutes at 1000 rpm. The extract was diluted by distilled water to obtain concentrations of 2%, 4 % and 6%. and stored at 4^0 C for subsequent use.

The following treatments plan was experienced

T_1 = Normal irrigation (non stressed)+ distilled water foliar spray

T_2 = Water stress + distilled water foliar spray

T_3 = Water stress + 2% MLE foliar spray

T_4 = Water stress + 4% MLE foliar spray

T_5 = Water stress + 6% MLE foliar spray

Data collection

At the age of 45 days, three plants from each group were selected for data collection. For growth studies numbers of branches were counted. Plant height and root length were measured after careful harvesting of plant ensuring gentle removal to avoid root damage. After collection of data, the samples were oven dried 60˚C for 48 hours. Biochemical analysis was done for potassium and phosphorous contents in root, stem and leaves. The concentration of potassium was determined using flame photometer (Hitachi model 1996, Japan). Standards of 0.1, 0.2, 0.3, 0.4 meq/ L were used prepared by using KCl salt Absorbance of standard series plant samples were taken at 7,680 A.

Phosphorous contents determination

After digestion of samples, 2ml sample and 2ml Barton reagent were mixed and 50 ml volume was raised by distilled water. Colour was generated after keeping samples for half an hour. Phosphorus (P) was analyzed by spectrophotometer (Jackson, 1962). Barton reagent was prepared usingammonium molybedate, Ammonium metavandate and conc. HNO_3.

Alkaloid contents determination

Alkaloids were determined in root, stem and leaves using the method of Amine et al. (2016). Acetic acid and Ethanol were used for extraction. Ammonia solution was used for alkaloid precipitation..

Statistical Analysis

Analysis of variance (ANOVA) technique was used for data analysis by computer COSTAT package. Means were compared by using Duncan's Multiplier range test at 5 % level.

RESULTS

Shoot length (cm)

The data regarding increase in shoot length are presented in table 1. Shoot length growth showed significant differences for treatment of different extracts. Results showed that application of MLE increased shoot length.. The length of shoot was maximum increased (56.38%) by 4% extract spray. Foliar application of 2% MLE increased shoot upto 55.56%. By foliar spray of 6%, shoot length was increase 40.15 % in stressed plants. The MLE treatment nullified the stress effect by supporting shoot elongation in stressed plants.

Root length (cm)

Data for root length are shown in table 1. Root length root length was 7.21% more in stressed plants as compared to control. The length of roots was increased by 27.47% and 30.43% by 2% and 4% spray respectively. By 6% foliar spray of MLE, the root length was increased by 13.04%.

Number of branches/plant

Data regarding to increase in number of branches per plant are presented in Table 1. In stressed plants throughout, an increase of 12.0% was noted. Under the application of 2%MLE maximum increase (30.18%) in number of branches was noted. Foliar spray of 4% enhanced 9.09% branches while increase was 18.5% when 6% *Moringa oliefera* extract was applied to stressed plants.

Phosphorous contents (mg/g) in root

The data for phosphorus contents of stem are shown in table 2. Amount of phosphorus was observed to be increased under water stress condition upto 180.68%. Foliar spray of 2% extract on stressed plants promoted the increase in phosphorus contents to a level of 198.86%. Foliar spray of 4% extract increased 115.91% while by 6% MLE spray only 46.59% enhancement was observed. The most effective concentration in this regard was proven 2% MLE.

Phosphorous contents (mg/g) in stem

The data for phosphorus contents of stem are shown in table 2. In stressed plants amount of phosphorus in root was decreased to 23.43%. This reduction was lowered by 2% and 4% MLE upto 18.23% and 32.81% respectively. However the maximum effect of MLE was expressed when 4% spray was applied which increased phosphorus contents of stem as 2.60%..

Phosphorous contents (mg/g) in leaves

The data for phosphorus contents of leaves are shown in table 2. In stressed plants amount of phosphorus in leaves was lowered to 13.84%. This reduction was enhanced by 2% and 4% MLE to 17.85% and 1.73% respectively.. Among all of the treatment of *Moringaoliefera* leaf extracts, 6% extract was more effective in favour of phosphorus accumulation in leaves.

Potassium contents (mg/g) in the root

The data for potassium contents in root are given in table 3. Potassium quantity in roots decreased due to stress condition to a level of 33.96%. Results described that by applying MLE to stressed plants, potassium content were increased in root. The most promising concentration was 6% extract which increased the contents to 39.62%. By 2% and 4% spray amount of potassium ion in roots of stressed plants were 3.77% % and 5.66% more than control plants.

Potassium contents (mg/g) in stem

Potassium ion data amount in stem given in table 3. Data presented that potassium amount was increased under stress condition.in stem. This increase was strengthened by applying different treatments of MLE. *Moringaolifera* leaf extract foliar spray of 6% ontressed plants increased potassium concentration to a maximum level of 362.5%. *Moringa olifera* sprays of 2% and 4% increased potassium amount in stem by values of 331.33% and 320.83% respectively.

Potassium contents (mg/g) in leaves

The data for amount of potassium in stem are given in table 3. Potassium concentration (mg/g) in leaves was decreased by stress to a level of 16.93%. However this reduction was minimized by foliar spray of MLE. When plants were sprayed with 2% and 4% MLE, the reduction was only 3.06% and 10.48 % respectively. Application of *Moringa oliefera* leaf extract of 6% minimized this reduction to a level of 2.42% only. Therefore, MLE decreased the water stress effects regarding potassium accumulation in leaves.

Alkaloids contents (mg/g) in root

The data for amount of alkaloids in root are given in table 4. Alkaloids concentrations in root were increased by stress to a level of 5.25%. Enhancement in alkaloids was promoted by foliar spray of MLE. When plants were sprayed with 2% and 4% MLE, the increase was 26.31% and 15.79 % respectively. Application of *Moringa oliefera* leaf extract of 6% increased this level to 36.84%.

Alkaloids contents (mg/g) in stem

The data regarding the quantity of alkaloids in stem are given in table 4. Alkaloids concentrations in stem were increased by stress as well as MLE concentrations. The most effective concentration in this regard was 4% which enhanced the alkaloids by 44.0%. Increases in alkaloids were 12% and 32% when plants were sprayed with 2% and 6% MLE respectively.

Alkaloids contents (mg/g) in leaves

The data for amount of alkaloids of leaves are given in table 4. Alkaloids contents in leaves were increased by stress to a level of 2.65%. By the application of 2% and 4% MLE, the increases were 3.53% and 7.96 % respectively. Application of *Moringa oliefera* leaf extract of 6% enhanced the alkaloids contents in leaves to a level of 10.62%.

Table 1: Absolute growth studies of 45 days old Fennel [*Foeniculum vulgare*] plants grown under normal irrigation and water stressed conditions and exposed to foliar spray of 2%, 4% and 6% aqaeous extract of *Moringa oleifera* leaves extract (MLE) at 15 and 25 days of age.

		Normal irrigation + Distilled water spray	Stressed + Distilled water spray	Stressed + 2% MLE spray	Stressed + 4% MLE spray	Stressed + 6% MLE spray
Shoot Length (cm)	Treatment Mean	23.66±5.15	26.5±9.18	36.83±8.40	37.00±8.11	33.16±7.03
	% diffrence		+12.00	+55.66	+56.38	+40.15
Root length (cm)	Treatment Mean	11.50±3.04	12.33±2.31	14.66±2.54	15.00±2.48	13.00±2.81
	% difference		+7.21	+27.47	+30.43	+13.04
Number of branches	Treatment Mean	5.5±3.3	6.16±0.801	7.16±1.39	6.00±0.63	6.5±0.52
	% difference		+12.00	+30.18	+9.09	+18.18

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control values

Table 2: Phosphorus contents (mg/g) of 45 days old Fennel [*Foeniculum vulgare*] plants grown under normal irrigation and water stressed conditions and exposed to foliar spray of 2%, 4% and 6% aqaeous extract of *Moringa oleifera* leaves extract (MLE) at 15 and 25 days of age.

		Normal irrigation + Distilled water spray	Stressed + Distilled water spray	Stressed + 2% MLE spray	Stressed + 4% MLE spray	Stressed + 6% MLE spray
Root	Treatment Mean	0.88±0.04	2.47±0.031	2.63±0.054	1.90±0.048	1.29±0.081
	% difference		+180.68	+198.86	+115.91	+46.59
Stem	Treatment Mean	1.92±0.04	1.47±0.033	1.57±0.045	1.97±0.043	1.29±0.098
	% difference		-23.43	-18.23	+2.60	-32.81
Leaves	Treatment Mean	2.24±0.054	1.93±0.067	1.84±0.059	1.91±0.051	2.21±0.055
	% diffrence		-13.84	-17.85	-14.73	-1.34

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control values

Table 3: Potassium contents (mg/g) of 45 days old Fennel [*Foeniculum vulgare*] plants grown under normal irrigation and water stressed conditions and exposed to foliar spray of 2%, 4% and 6% aqaeous extract of *Moringa oleifera* leaves extract (MLE) at 15 and 25 days of age.

		Normal irrigation + Distilled water spray	Stressed + Distilled water spray	Stressed + 2% MLE spray	Stressed + 4% MLE spray	Stressed + 6% MLE spray
Root	Treatment Mean	0.53±0.054	0.35±0.067	0.55±0.059	0.56±0.051	0.32±0.055
	% difference		-33.96	+3.77	+5.66	+39.62
Stem	Treatment Mean	0.24±0.044	0.43±0.054	1.04±0.048	1.01±0.047	1.11±0.051
	% difference		+79.16	+333.33	+320.83	+362.5
Leaves	Treatment Mean	1.24±0.063	1.03±0.051	1.14±0.049	1.11±0.038	1.21±0.039
	% diffrence		-16.93	-8.06	-10.48	-2.42

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control values

Table 4: Alkaloid contents (mg/g) of 45 days old Fennel [*Foeniculum vulgare*] plants grown under normal irrigation and water stressed conditions and exposed to foliar spray of 2%, 4% and 6% aqaeous extract of *Moringa oleifera* leaves extract (MLE) at 15 and 25 days of age.

		Normal irrigation + Distilled water spray	Stressed + Distilled water spray	Stressed + 2% MLE spray	Stressed + 4% MLE spray	Stressed + 6% MLE spray
Root	Treatment Mean	0.019±0.91	0.020±0.98	0.024±0.94	0.022±0.24	0.026±0.95
	% difference		+5.25	+26.31	+15.79	+36.84
Stem	Treatment Mean	0.025±0.21	0.031±0.18	0.028±0.20	0.036±0.24	0.033±0.23
	% difference		+24.00	+12.00	+44.00	+32.00
Leaves	Treatment Mean	1.13±22.1	1.16±2.56	1.17±2.634	1.22±2.47	1.25±2.641
	% diffrence		+2.65	+3.53	+7.96	+10.62

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control values

DISCUSSION

Water Stress induced reduction in growth attributes while *Moringa* Leaf extract (MLE) alleviated this effects (Table 1). Stress induced decreased in plant growth might be due to its stimulating role for loss of chlorophyll contents (Shoresh et al. 2011). Foliar spray of MLE containing growth stimulators can alleviate the reduction in chlorophyll contents under stress (Saad 2014). Many researchers reported the role of MLE in improving plant growth and development (Henry et al. 2017; Ali et al. 2018; Nihayati and Najah, 2021; Nisar et al. 2021). Growth reduction under stress might be due to reactive oxygen species (ROS) production. For avoiding the oxidative damage by ROS, plants utilize internal metabolites for its defense mechanisms rather than growth phenomenon (Kolbert et al. 2012). For this purpose enzymatic and non enzymatic antioxidants are enhanced during the stress (Schutzendubel and Polle 2002). Both enzymatic and non-enzymatic antioxidants (AsA, phenols) are of prime importance in scavenging ROS (Sgherri et al. 2004). Foliar application of *Moringa* leaf extract (MLE) under stressed and non-stressed conditions enhance antioxidant enzymes as well as non-enzymatic antioxidants like carotenoids and proline (Saad 2014). Foliar spray of MLE alters antioxidant

enzymes activities (Yasmeen et al. 2013; Rady et al. 2013). The increase in antioxidant enzymes concentration is reported to be the result of over expression of DET2 gene, which can promote oxidative stress resistance mechanism (Cao et al. 2005).

Improvement in growth attributes i.e. shoot length, root length number of branches by MLE spray might be due to increased mobilization of growth promoting metabolites such as ascorbic acid, zeatin, K and Ca presented in MLE (Saad 2014) to the growing region and increase in reducing sugars participating in plant growth promotion (Foidle et al. 2001; Afzal et al. 2012). Furthermore, the hormones present in MLE like IAA, GAs and zeatin might be the source of plant growth promotion under stressed environment. Foliar application with MLE might provide strong bases for energetic completion of phenological events before (Rehman et al. 2014). Growth promotion might be attributed to enhanced photosynthesis or chlorophyll contents as MLE application stops leaf senescence thereby leaving greater leaf area and pigments (Rehman and Basra 2010). Foliar spray of MLE is reported to improve the chlorophyll contents in leaf of plant under stress (Hanaa et al. 2008).

Foliar spray of MLE increased the contents of potassium and phosphorus in root, stem and leaves (Table 2-3). MLE is reported to contain much potassium, calcium and phosphorus (Foidle et al. 2001; Oduro et al. 2008; Rockwood 2013; Saini et al. 2016). The Ca^{2+} appeared to be involved in growth promotion (Farooq et al. 2010). Exogenous application of MLE increased alkaloid contents (Table 4). MLE contains many secondary metabolites (Madukwe et al. 2013; Latif and Mohamed, 2016; Singh et al. 2020). Secondary metabolites are reported to be increased by application of MLE under stress conditions (Saad 2014). Where and when there was found results contrary to the general ongoing trends or where there was no response to *Moringa* extract the environmental conditions might play role. Fuglie (2000) reported that the better results of foliar spray of *Moringa* leaf extract can be obtained if soil nutrient supply, watering and other agricultural practices are experienced properly. The natural resources for antioxidants like algal extract (Hanaa et al. 2008), humic acid (HA), Seaweed Extract (Zhang *and* Ervin 2008) and extract of *Moringa* leaves (Foidle et al. 2001) can used for this purpose. Water Stress induced reduction in growth attributes while *Moringa* Leaf extract (MLE) alleviated this effects (Table 1). Stress induced decreased in plant growth might be due to its stimulating role for loss of chlorophyll contents (Shoresh et al. 2011). Foliar spray of MLE containing growth stimulators can alleviate the reduction in chlorophyll contents under stress (Saad 2014). Many researchers reported the role of MLE in improving plant growth and development (Henry et al. 2017; Ali et al. 2018; Nihayati and Najah, 2021; Nisar et al. 2021).

CONCLUSION

Water Stressed resulted in significant reductions of shoot length, root lengths and number of branches. When MLE were applied on stressed plants then a significant increase in growth attributes were found. Phosphorus, potassium and terpenoids concentrations were increased by MLE. Stress reduced the phosphorus contents in stem and leaves; Potassium and alkaloid contents of these were compensated by MLE applications.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GY designed the experiments SK perfoemed the experiment and collected data. AA analysed data. AA and IH helped in designing the experiment and reviewed the manuscript. All authors read and approved the final version.

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