

Impact of Natural and Synthetic Plant Stimulants on Moringa Seedlings Grown under Low-Temperature Conditions

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Abstract. Low-temperature results in various physiological and metabolic disturbances in cells of plants which are sensitive to low-temperatures. Moringa is getting popularity as a field crop because of its multipurpose usage. There is no information available about effects of low-temperature (14-18°C) on moringa seedlings and its mitigation. The present study was conducted to test the performance of moringa seedling grown in wire house under low-temperature conditions in response to foliar application of moringa leaf extract (3% solution), hydrogen peroxide (5 ml L⁻¹), ascorbic acid (50 mg L⁻¹) and salicylic acid (50 mg L⁻¹). Seeds of six moringa accessions [Local landrace grown at Agronomic Research Area, Z.A Hashmi Hall, Firdous Colony; Exotic landrace grown at Lalazaar Colony, Department of Agronomy and Agronomic Research Area] were collected and grown in polythene bags filled with equal ratio of compost, sand, silt and clay. All foliar treatments were applied twice; the first round at the seedling age one month and the second round at the seedling age two months. Foliar application of moringa leaf extract significantly enhanced number of branches (92%) and leaves (39%), leaf total chlorophyll contents (73%), leaf phenolic contents (53%) and membrane stability index (57%) of moringa seedlings compared to control. Healthy and vigorous growth of moringa seedlings with higher concentration of antioxidants ensured the defensive potential of moringa leaf extract against low-temperature condition.

1. Introduction

Moringa oleifera tree is referred to as a miracle tree because of rich source certain macro and micronutrients of great importance in human nutrition [1], agriculture [2] and pharmacology [3]. *Moringa oleifera* Lam., the main representative of the order *Moringa*, is native to Hamaylian track of Indo-Pak [4] with three landrace i.e. white seeded moringa, black seeded moringa and PKM1, present in Pakistan [5]. It belongs to family *Moringaceae* and it is also commonly known as horse radish tree or drum stick. Moringa has both medicinal and nutritional values and uses with essential minerals, amino acids and vitamins [6]. It is also commonly grown on plantations in Asia and Africa. Almost all plant part of this miracle tree have been found to be very useful and edible as they contain a multitude of nutrients such as high level of essential amino acids, iron calcium and carotenoids [7]. It can be cultivated in different ways, but more often the tree is cultivated intensively in plantations to harvest large volume of leaf material [8, 9].

Low-temperature is one of main important plants abiotic stress issues which curtail plant growth, development and yield. Accumulation or synthesis of compatible metabolites is a fundamental and useful strategy for the survival of plant in metabolic acclimation. A prominent object of metabolic analysis with respect to chilling stress of cold acclimation condition is to recognize the metabolites which are frequently involved in homeostasis and maintaining the optimum and normal cellular metabolism [10]. Exogenous application of plant growth regulators (PGRs) with specific mineral nutrients, organic and inorganic chemicals and antioxidants has been successfully used to encourage crop development and growth and inducing biotic and abiotic stresses tolerance that outcomes in more economic yield [11, 12]. Natural sources like microorganisms and/or organic compounds are also being used as biostimulant in agriculture. Most commonly used biostimulants are microbial inoculants, seaweed extracts, humic acids, fulvic acids, plant extracts, protein hydrolysates and amino acids [13]. Moringa leaf extract obtained from fresh leaves of moringa possesses high antioxidant activity as it is rich in some plant secondary metabolites and osmoprotectants [14]. Moringa leaves are natural and economical source of calcium, iron, vitamin A and C, beta-carotene, phenolics and riboflavin [15]. The potential of MLE when applied as either seed priming agent or plant foliage has been exposed to increase the plant tolerance to abiotic stresses [16].

Phenolic compounds present in moringa plant could prevent chronic non-communicable diseases. An increase in the secondary metabolites of *Moringa oleifera* could improve its functional properties [17]. Siddhuraju and Becker [18] observed antioxidant properties in the solvent extract of moringa leaves. Moreover, moringa leaf extract is supplemented with zeatin, a purine adenine derivative of plant hormone group cytokinin [19] known for stay green and stress tolerance competences. Salicylic acid, a plant phenolic PGR of ubiquitous nature, plays a key role in the plant response to unfavorable environment [20]. Ascorbic acid and related enzymes like ascorbate peroxidase (APX) play various roles in physiological process in plants, including differentiation, growth, metabolism and expansion [21]. Furthermore, ascorbate regenerate α -tocopherol which defend the plant from programmed cell death, oxidized by ROS [22]. Hydrogen peroxide (H_2O_2) has numerous roles as a plant signaling molecule, secondary messenger, mediating the acquisition of tolerance to abiotic stress [23].

The beneficial effects of hydrogen peroxide, salicylic acid and ascorbic acid in comparison with moringa leaf extract to enhance the growth of moringa seedling under-low temperature have not been reported. It is evident from earlier mentioned reports that moringa leaf extract preserves antioxidants in significant amounts but very slight published literature is available about effectiveness of moringa leaf extract under low-temperature stress regarding moringa seedlings. In view of all these reports, it is hypothesized that leaf extract from moringa, having a number of plant growth promoters, mineral nutrients and vitamins in a naturally balanced composition, may be beneficial for growth and development of moringa seedlings under low-temperature environment. Therefore, the present study was conducted to investigate the performance of moringa seedling under low temperature in response to foliar application of moringa leaf extract, salicylic acid, hydrogen peroxide and ascorbic acid.

2. Materials and Methods

Experiment particulars

The current study was planned to explore the defensive potential of moringa leaf extract (MLE), salicylic acid, hydrogen peroxide and ascorbic acid against low-temperature stress through the performance of moringa seedlings of different landraces. Two most prominent landraces i.e. local landrace (LL) (a white seeded moringa) and an exotic landrace (EL) (PKM1; Indian cultivar), were selected for current experimentation. Seeds of three accessions of local landrace were collected from Agronomic Research Area (LL1), Z.A Hashmi Hall (LL2) and Firdous Colony (LL3). Similarly, seeds of three accessions of PKM1 were collected from Lalazaar Colony (EL1),

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Seedlings growth and treatments

Seedlings were grown in polythene bags under wire house at Old Botanical Garden, Department of Botany, University of Agriculture, Faisalabad-Pakistan in winter season (November 2016 to February 2017) with average temperature of 14-18°C. Normally, moringa is cultivated during summer season because moringa is a plant of tropical and sub-tropical region with average temperature of 25-35°C [24]. Cultivation of moringa seedlings under natural winter season is considered as low temperature. Weather data are given in table 1. Five seeds from each accession were sown in polythene bags. Polythene bags (18 cm length and 10 cm width) were filled with growing media. Growing media consist of compost, sand, silt and clay with equal ratio. Completely randomized design (CRD) with factorial arrangements having three replications was used to conduct the experiment. Foliar spray of water, salicylic acid @ 50 mg L⁻¹, hydrogen peroxide @ 50 ml L⁻¹, ascorbic acid @ 50 mg L⁻¹ and moringa leaf extract @ 3% [5] was applied on the seedling which were later compared with control seedlings which received no exogenous treatment. Moringa leaf extract was prepared according to Khan [5]. The volume of spray for each plant was 40 ml. During course of experimentation, all foliar treatments were applied twice; first round at the seedling age one month and second round at the seedling age of two months.

Seedling growth and biochemical analysis

Data of growth parameters like number of branches per seedling, number of compound leaves per seedling and number of leaflets per compound leaf were recorded after one week of foliar treatment application of the second round. Chlorophyll of moringa seedlings were determined according to Arnon [25].

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{)} = [\{(0.0127 \times A_{663}) - (0.00269 \times A_{645})\} \times 100] / 0.5,$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{)} = [\{(0.0229 \times A_{645}) - (0.00468 \times A_{663})\} \times 100] / 0.5.$$

Total phenolic contents in leaf samples were measured by using a method as described by Ainsworth and Gillespie [26]. Leaf Membrane Stability Index (MSI) was determined according to the method of by Sairam [27]. MSI was calculated using the following formula;

$$\text{MSI} = [1 - (C_1 / C_2)] \times 100.$$

Statistical analysis

The collected data were analyzed statistically by employing the Fisher's analysis of variance technique [28] and treatments' means were compared by using Tucky HSD test at 5% probability level. Computer package Statistix 8.1 was used for statistical analysis (Analytical Software, Tallahassee, TN, USA).

3. Results

Growth parameters of moringa seedlings

Foliar application of moringa leaf extract @ 3% solution, salicylic acid @ 50 mg L⁻¹, hydrogen peroxide @ 50 ml L⁻¹ and ascorbic acid @ 50 mg L⁻¹ significantly improved growth attributes (i.e. number of branches per seedling, number of compound leaves per seedling and number of leaflets per compound leaf) of moringa seedlings grown under low temperature conditions as compared to control (Table 2). The number of branches and compound leaves per seedling, and number of leaflets per compound leaf were not affected within landraces and accessions but only affected by foliar application (Table 2). All the foliar treatments enhanced the number of branches and compound leaves per seedling, and number of leaflets per compound leaf over control but maximum improvement was recorded by application of moringa leaf extract (Table 3). Foliar application of moringa leaf extract produced maximum branches, compounds leaves and leaflets per

seedling over control. The number of branches was also statistically at par with application of ascorbic acid and hydrogen per oxide. The number of leaves and leaflets were also statistically at par with ascorbic acid, hydrogen per oxide and salicylic acid.

Biochemical parameters of moringa seedlings

Foliar application of moringa leaf extract @ 3% solution, salicylic acid @ 50 mg L⁻¹, hydrogen peroxide @ 50 ml L⁻¹ and ascorbic acid @ 50 mg L⁻¹ significantly improved biochemical attributes (i.e. Chlorophyll *a*, *b* and total leaf chlorophyll contents, total leaf phenolic contents and membrane stability index) of moringa seedlings grown under low-temperature conditions as compared to control (Table 2). Leaf chlorophyll *a* and *b* contents and total leaf phenolics (Fig. 2a) were found similar in the landraces and accessions but total leaf chlorophyll contents were found significantly different (Table 2, Fig. 1a, b). Chlorophyll *a* contents were also statistically at par with ascorbic acid, hydrogen per oxide and salicylic acid. Cell membrane stability index was also recorded significant among the accessions (Table 3, Fig. 2b). Foliar application of moringa leaf extract produced more leaf chlorophyll and phenolic contents over control. Interaction among landraces, accession and treatments regarding chlorophyll *a* contents, total chlorophyll and total phenolics was also significant (Table 3) as expressed in Fig. 1a, b and 2a respectively. Maximum cell membrane stability index was recorded by application of moringa leaf extract as foliar spray over control (Table 3).

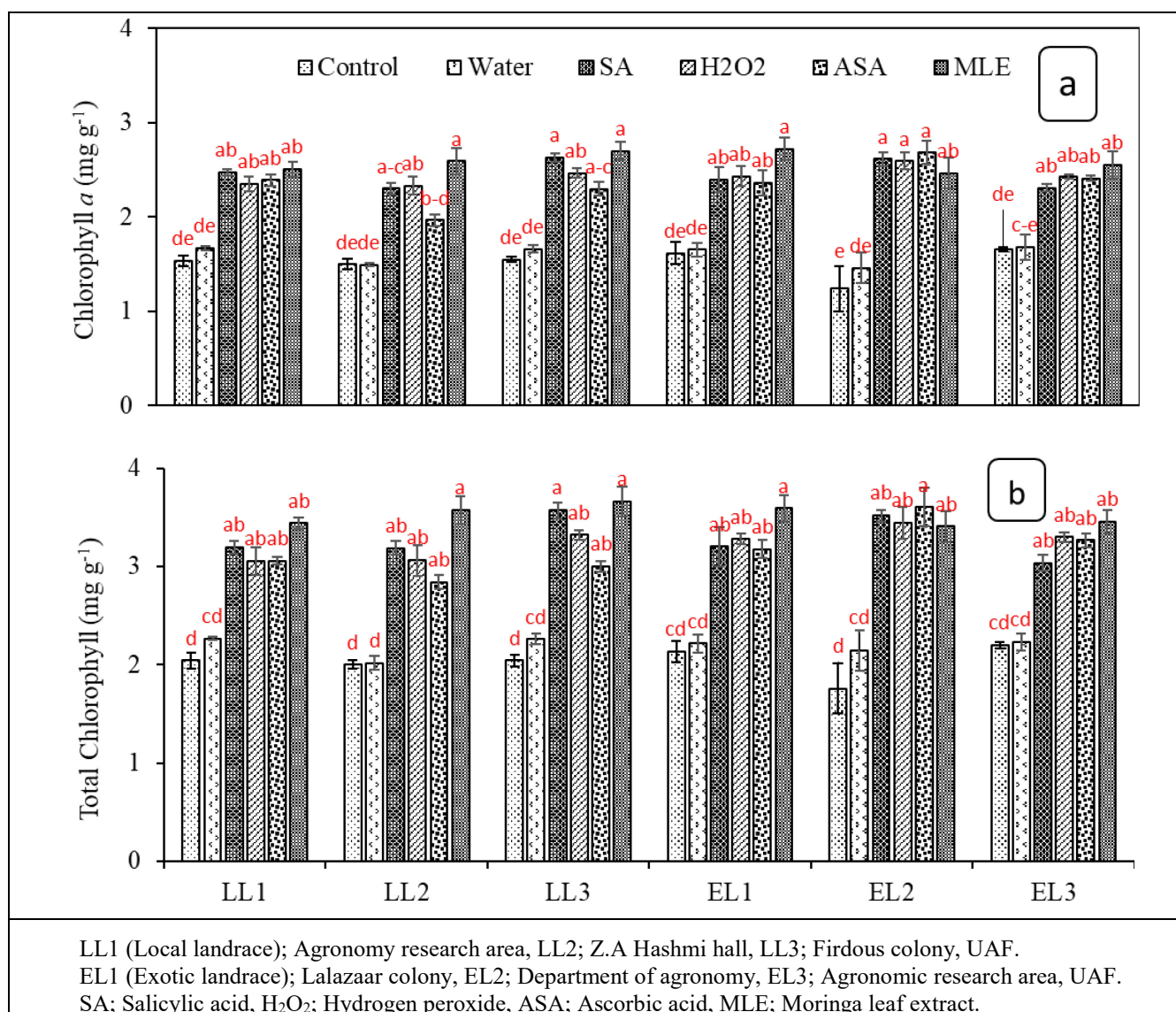


Figure 1. Leaf chlorophyll *a* (a) and total chlorophyll contents (b) of moringa seedlings under low-temperature stress in response to foliar application of growth promoting substance

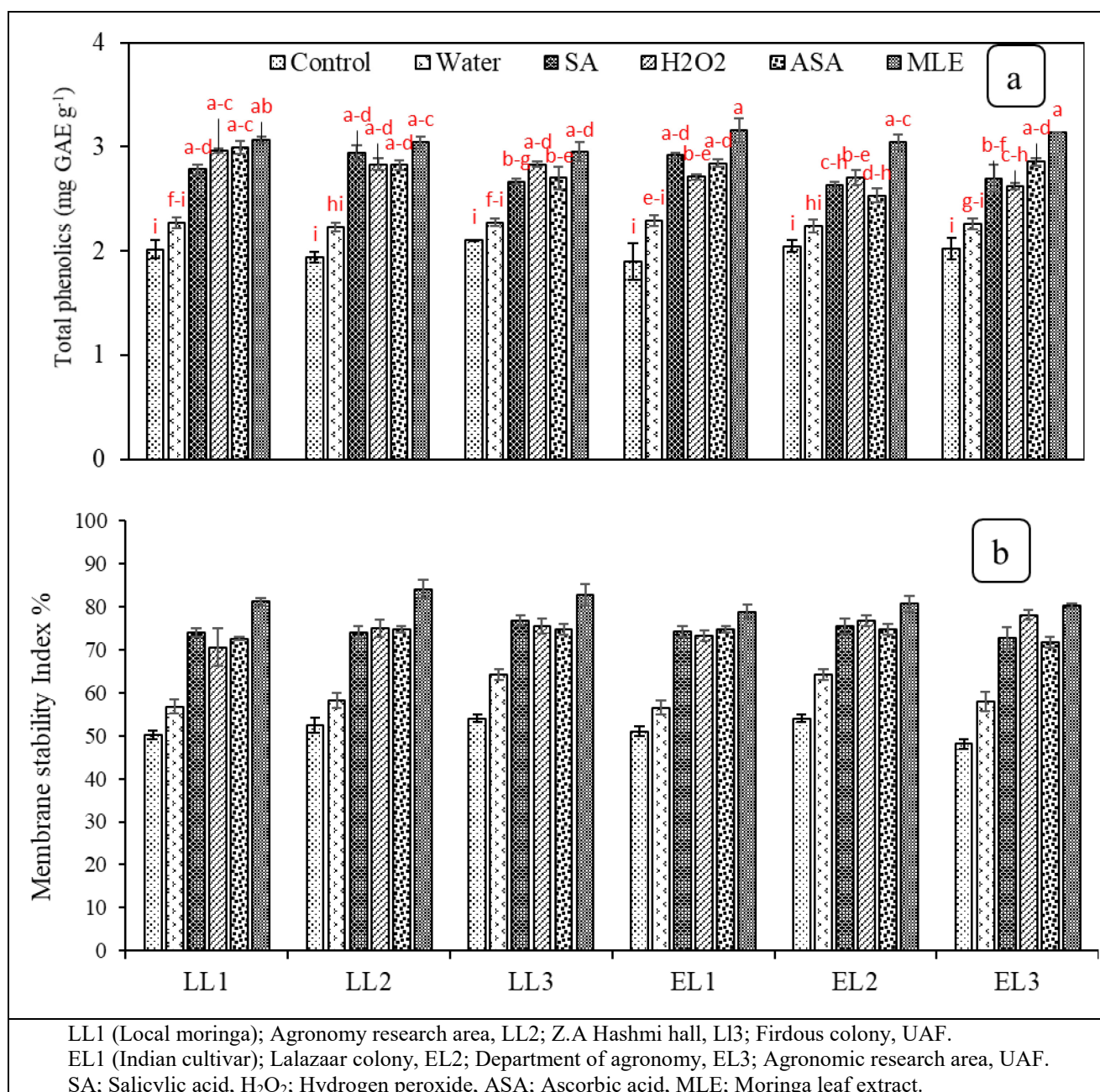


Figure 2. Total leaf phenolic contents (a) and membrane stability index (b) of moringa seedlings under low-temperature stress in response to foliar application of growth promoting substance

Table 1. Average monthly weather conditions during the seedling growth period

Weather conditions	Nov-2016	Dec-2016	Jan-2017	Feb-2017
Mean temperature (°C)	20.1	16.4	12.9	16.8
Mean relative humidity (%)	60.1	68.7	72.0	53.0
Total rainfall (mm)	0.00	0.00	11.5	4.10
Sun shine hours	6.40	6.70	1.30	6.60
Evapotranspiration (mm)	1.80	1.70	0.90	1.90
Wind speed (km hr ⁻¹)	2.60	2.80	3.50	4.00

Table 2. Mean sum of squares of growth and biochemical parameters of moringa seedlings in response to foliar application of moringa leaf extract, salicylic acid, hydrogen peroxide and ascorbic acid

SOV	DF	No of Branches	No of Leaves	No of Leaflets	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total Chlorophyll	Total Phenolics	Membrane Stability Index
Landraces (L)	1	0.6379 ^{NS}	0.9259 ^{NS}	2.725 ^{NS}	0.0618 ^{NS}	0.0218 ^{NS}	0.157**	0.0542 ^{NS}	5.15 ^{NS}
Accessions (A)	2	6.5348 ^{NS}	8.7870 ^{NS}	1.815 ^{NS}	0.0866 ^{NS}	0.0384 ^{NS}	0.0456 ^{NS}	0.0609 ^{NS}	62.24**
L×A	2	10.164 ^{NS}	14.45 ^{NS}	8.037 ^{NS}	0.0801 ^{NS}	0.0141 ^{NS}	0.1529 ^{NS}	0.029 ^{NS}	50.16**
Treatments (T)	5	9.873**	11.92**	17.49**	3.988**	0.465**	7.139**	2.823**	2255**
L×T	5	0.5931 ^{NS}	0.6815 ^{NS}	0.6981 ^{NS}	0.062 ^{NS}	0.021 ^{NS}	0.1433*	0.0432*	12.46 ^{NS}
A×T	10	0.6147 ^{NS}	0.8204 ^{NS}	0.837 ^{NS}	0.0234 ^{NS}	0.0097 ^{NS}	0.0411 ^{NS}	0.0235 ^{NS}	8.63 ^{NS}
L×A×T	10	0.3517 ^{NS}	0.4426 ^{NS}	0.5037 ^{NS}	0.093**	0.0114 ^{NS}	0.1017*	0.0344*	11.45 ^{NS}

SOV = Source of variance, DF = Degree of freedom, NS = non-significant, * = Significant at $P \leq 0.05$, ** = Significant at $P \leq 0.01$

Table 3. Growth and biochemical parameters of moringa seedlings under low-temperature stress in response to foliar application of growth promoting substance

Treatments	No of Branches	No of Leaves	No. of Leaflets	Chlorophyll <i>a</i> (mg g ⁻¹)	Chlorophyll <i>b</i> (mg g ⁻¹)	Total Chlorophyll	Total Phenolics	MSI
Control (No spray)	3.167 D	6.22 C	4.83 C	1.5147 C	0.5131 C	2.0278 C	2.0043 D	51.583 D
Water spray	3.72 CD	7.22 BC	5.17 BC	1.6021 C	0.5871 C	2.1891 C	2.2615 C	59.618 C
Salicylic acid	4.72 BC	7.72 AB	6.33 AB	2.4530 AB	0.8314 B	3.2844 B	2.7726 B	74.476 B
Hydrogen peroxide	4.83 ABC	7.67 AB	6.39 AB	2.4309 AB	0.8117 B	3.2425 B	2.7770 B	74.797 B
Ascorbic acid	5.056 AB	7.94 AB	6.78 A	2.3509 B	0.8067 B	3.1575 B	2.7916 B	73.783 B
Moringa leaf extract	6.11 A	8.67 A	7.44 A	2.5897 A	0.9315 A	3.5212 A	3.0669 A	81.203 A
Tukey HSD	1.333	1.3565	1.3883	0.1805	0.0940	0.2135	0.1234	3.2343

MSI = Membrane stability index

4. Discussion

Moringa oleifera tree, usually known as miracle tree, is a well-known source of micro and macronutrients, proteins, vitamins [29]. Each part of this plant is very palatable and considered “natural nutrition of tropics” because it possesses many important metabolites e.g. quercetin, campesterol, kaempferol, sitosterol and zeatin [29, 30]. *Moringa oleifera* L. is native to Hamaylian track of Indo-Pak [4] with two prominent landrace i.e. local landrace (LL) (a white seeded moringa) and an exotic landrace (EL) (PKM1; Indian cultivar) present in Pakistan [5]. Its landraces and/or accessions are characterized by phenotypic difference, such as shape of leaves, plant height, production of leaf biomass and secondary metabolites [8, 31]. Two landraces, with three accessions of each, studied in this experiment showed growth (Table 3) and biochemical (Fig. 1 and 2)

differences under similar environmental conditions in response to foliar application of salicylic acid, hydrogen peroxide, ascorbic acid and moringa leaf extract.

In the present study, application of moringa leaf extract improved growth (Table 3) and biochemical traits (Fig. 1 and 2) of moringa seedlings. This increase in traits might be due to presence of antioxidants and vitamins in the moringa leaf extract which enhanced the growth of seedlings. Similar results were reported by Taha *et al.* [32] that when moringa leaf extract is applied @ 10%, it causes an increase in plant height and number of branches as compared to control in jojoba plant. Moringa leaf extract possesses high antioxidant activity as it is rich in some plant secondary metabolites and osmoprotectants [14]. Moringa leaves are natural and economical source of calcium, iron, vitamin A and C, beta-carotene, phenolics and riboflavin [15]. Current results were also supported by Nouman *et al.* [33] who stated that application of moringa leaf extract enhanced the number of tillers, leaf numbers and shoot vigor in *Echinochloa crusgalli* when applied as a priming agent.

Siddhuraju and Becker [18] observed antioxidant properties in the solvent extract of moringa leaves. On the basis of their results they reported that, moringa leaves are a potential source of natural antioxidants. According to Arabshahi *et al.* [34], extracts from drumstick and carrot had a higher antioxidant activity (83% and 80%) than α -tocopherol (72%). Moreover, moringa leaf extract (MLE) is supplemented with zeatin, a purine adenine derivative of plant hormone group cytokinin [19] known for stay green and stress tolerance competences. Juice of moringa leaves is so rich with a lot of plant growth hormones, especially zeatin that is reported to boost up the yield of field crops in range of 10-45%. It also contains many micronutrients in sufficient amounts and an appropriate concentration that enhance the growth and development, components of yield and yield of a vast range of field crop from oil seed to cereal crops, from tuber to forages crops and from sugar to fibrous crops [35]. Khan *et al.* [36] also reported that moringa leaf extract showed growth promoting potential due to presence of phenolics and ascorbic acid. They also stated that application moringa leaf extract was more effective regarding the growth, yield and biochemical attributes of wheat.

According to Taha *et al.* [32], maximum chlorophyll *a* and *b* contents were measured in the plants treated with higher concentration of moringa leaf extract @ 10% in Jojoba plants as compared with control treatments. They also stated that same response was observed regarding total chlorophyll contents as in the present study (Fig. 1). Abdalla [37] investigated that foliar spray of MLE at moderate concentration (2L) exhibited the maximum quantity of photosynthetic pigments (i.e. chlorophyll *a* contents and chlorophyll *b* contents, and carotenoids) as compared to untreated plants which were considered as control. Furthermore, moringa leaf extract spray also positively adjust membrane stability or structure under chilling stress, that is why plants treated with moringa leaf extract in both the conditions either in the absence and presence of stress conditions had more membrane stability index. Application of MLE is responsible for the healthy metabolic condition of stressed plants which resulted in vigorous plant growth in term of enhanced total chlorophyll and total phenolic contents. It may be endorsed to that moringa leaf extract is an excellent source of amino acids, minerals, antioxidants and soluble sugars. Howladar [38] reported that twice application of moringa leaf extract, at 21 and 28 days after sowing of bean seeds, increase the membrane stability index 69.4%. Reliable findings were also found by Prabhu *et al.* [39] and Balakumbahan and Rajamani [40] who reported that application of moringa leaf extracts @ 2% and 4% on sacred basil and senna plants respectively. They stated that foliar spray of MLE @ 2% was more effective as compared to control. Foliar application of MLE @ 2% increased various growth attributes like numbers of leaves, plant height, leaf area and LAI, dry and fresh weights, number of branches, number of pods, dry pod yield and dry leaf yield as compared to control.

Conclusion

Foliar application of moringa leaf extract, when applied @ 3% solution, is the most effective approach to maintain the growth of moringa seedlings under low-temperature conditions as it is a rich source of mineral nutrients, antioxidants, vitamins and growth hormones. The vigorous growth

of local landrace ensures tolerant phenomenon against low-temperature conditions as compared to exotic landrace (PKM1).

Conflict of Interest

The authors declare that there is no conflict of interest.

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