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The Effect of *Moringa oleifera* Lam. Leaf Aqueous Extract on Seed Yield and Fibre Quality of Linseed under Water Deficit Stress

(Kesan Akueus Ekstrak Daun *Moringa oleifera* Lam. terhadap Hasil Biji Benih dan Kualiti Serat Biji Rami di bawah Tekanan Kekurangan Air)

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ABSTRACT

Moringa oleifera leaf extract contains active ingredients with stimulatory effects on natural processes of plants like uptake of nutrients, photosynthesis, biomass production and flowering. Therefore, we conducted field experiments to determine the effects of *M. oleifera* leaf aqueous extract (MLAE) on growth and fibre quality of three linseed varieties viz. Roshni, BL1 and Chandni under water deficit stress for two years. Water deficit stress was imposed during tillering growth phase (60 days after sowing) by skipping four irrigations, keeping 40% soil field capacity. The MLAE (5%) was applied to leaves once before the start of water deficit stress period and next 15 days after imposition of water deficit stress. Analysis of MLAE showed the presence of natural phenolics (150 mg gallic acid equivalents (GAE)/mL extract) and essential nutrients like Ca, Mg, K, Zn, Mn, and Fe. Positive impact of the MLAE was observed on plant height, tillers production, leaf chlorophyll pigments, phenolics content, sugars content, seed yield and fibre quality of linseed both under non-stress and water deficit stress. Our studies concluded that MLAE can be a probable approach for maintaining normal growth and fibre quality of linseed plants under short supply of water.

Keywords: Biostimulant; electrolyte leakage; fibre quality; phenolics; soluble sugars

ABSTRAK

Ekstrak daun *Moringa oleifera* mengandungi bahan aktif dengan kesan perangsang pada proses semula jadi tumbuhan seperti pengambilan nutrien, fotosintesis, pengeluaran biomas dan pembungaan. Oleh itu, kami menjalankan uji kaji lapangan untuk menentukan kesan ekstrak akueus daun *M. oleifera* (MLAE) terhadap pertumbuhan dan kualiti serat tiga varieti biji rami, iaitu Roshni, BL1 dan Chandni yang mengalami tekanan kekurangan air selama dua tahun. Tekanan kekurangan air dikenakan pada fasa pertumbuhan tiler (60 hari selepas semaian) dengan melangkau empat kali pengairan, mengekalkan kapasiti tanah ladang sehingga 40%. MLAE (5%) diaplikasikan pada daun sebanyak sekali sebelum bermulanya tempoh tekanan air dan 15 hari berikutnya setelah dikenakan tekanan. Analisis MLAE menunjukkan kehadiran fenol semula jadi (150 mg setara asid galik (GAE)/mL ekstrak) dan nutrien penting seperti Ca, Mg, K, Zn, Mn dan Fe. Kesan positif MLAE diperhatikan pada ketinggian tanaman, penghasilan tiler, pigmen klorofil daun, kandungan fenol, kandungan gula, hasil biji dan kualiti serat biji rami baik dalam keadaan tekanan dan kekurangan air. Kajian kami menyimpulkan bahawa MLAE berkemungkinan boleh menjadi pendekatan untuk mengekalkan pertumbuhan normal dan kualiti serat tanaman biji rami walaupun kekurangan bekalan air.

Kata kunci: Bioperangsang; bocoran elektrolit; fenol; gula larut; kualiti serat

INTRODUCTION

Abiotic stresses caused by soil salinity, drought, high and low temperatures and ultra violet rays severely decrease growth and yield of crop plants (Vaughan et al. 2018; Zafar et al. 2018). Farmers use various kinds of materials such as synthetic plant growth regulators, nutrients and inorganic chemicals to improve economic value of their crop products and to protect them from environmental stresses (Mohamed & Gomaa 2012). However, the continuous use of synthetic chemicals can create environmental pollution and deteriorate soil fertility (Atafar et al. 2010). The synthetic growth regulators are very effective in the production of crops; however, their extensive use has caused contamination of soil and water sources with toxic effects on human beings (Yasmeen et al. 2014).

The phytochemicals present in different parts of plants can be an option to replace synthetic growth regulators as they are reported for various growth regulating effects (Yasmeen et al. 2013). Biostimulants are materials which have biologically active ingredients with stimulatory effects on natural processes of plants like uptake of nutrients, photosynthesis, biomass production and flowering. They have potential to increase tolerance of plants to environmental stresses (Sharma et al. 2014). Biostimulants of biological significance and hormone-like activities have been extracted from plants like algae, *Moringa oleifera* and *Pongamia pinnata* (Bibi et al. 2018, 2016; Stirk & Van Staden 2006; Yasmeen et al. 2012). Biostimulants have diverse groups of compounds like phenols, flavonoids, minerals and natural phytohormones depending upon the plant species (Di Mola et al. 2019). The bioactive compounds present in plant extracts may function as growth stimulators or growth inhibitors (Popa et al. 2002, 1998). Due to their environmental friendly and effectiveness on growth of plants, global market for biostimulants has been increased recently (Anon 2013). Application of a commercially available biostimulant Megafol (Valagro, Atessa, Chieti, Italy) containing amino acids, vitamins, betains and proteins of plant and algal origin enhanced induction of drought stress-related genes such as *RAB18* and *RD29B* in tomato plants. Moreover, tomato plants treated with biostimulant had no variations in fresh weight and leaf relative water content after exposure to drought stress (de Vasconcelos et al. 2009; Petrozza et al. 2014).

Moringa oleifera belongs to family Moringaceae and ranges from 5 to 10 m in height. It is a drought resistant and fast growing tree species native to tropical and subtropical areas of South Asia (Parrotta 2004). It is used as a vegetable in Arabia, India, Africa and Pakistan (Sengupta & Gupta 1970). Moringa leaves are source of essential minerals namely iron, calcium and potassium, amino acids, ascorbic acid, phenolics and Zeatin (Foidle et al. 2001; Siddhuraju & Becker 2003). *M. oleifera* leaf extract was reported as a biostimulant for improving growth and yields of many plant species such as tea, melon, *Vigna anguiculata,* soybean and tomato (Fuglie 1999; Maishano et al. 2017; Yasmeen et al. 2014).

Linseed is an herbaceous plant species belonging to Linaceae. It is an industrial crop grown for its fibres and seed oil (Jhala & Hall 2010). Linseed oil is used in industry for making varnishes, printing inks, soap and paints (Woodfield & Harwood 2017). Linseed stem fibre is used for making ropes, clothes, and table linen (Jhala & Hall 2010). Linseed seed contains 30 to 40% high quality oil of medicinal value due to the presence of unsaturated fatty acids (Storlien et al. 1998). Studies have shown that low availability of soil moisture severely decreases growth and yield of linseed (Guo et al. 2012; Kariuki et al. 2016).

Pakistan is among the countries which are highly affected by climate change in recent years. In major parts of the country like Sindh and Baluchistan, drought spells are expected to occur in coming years as the intensity and pattern of rainfall has been changed (Chandio 2012). Moreover, rise in temperature and pollution of fresh water bodies due to human activities and global warming will further increase need of good quality water supply for irrigation of crops (Bibi et al. 2016; Farooq et al. 2009). Hence, it is needed to adopt sustainable and green technologies for the protection of crops from shortage of water supply (Pervez et al. 2017). Therefore, we have attempted to minimize water deficit stress effects on growth, yield and fibre quality of linseed by the application of *M. oleifera* leaf aqueous extract.

Materials and Methods

MATERIALS AND EXPERIMENTAL CONDITIONS

The *M. oleifera* leaves (1 kg) were collected from 15 healthy trees in District Bannu KP, Pakistan and dried in shade for 3 weeks and cut into small pieces (1-2 cm). The experiment was carried out at the University of Science and Technology Bannu KP Pakistan (altitude 380 m a.s.l., latitude 32°.59 N, longitude 70°.36 E) for two years (2018-2019 and 2019-2020). The physicochemical properties of field soil are shown in Table 1. The meteorological data of linseed growing season for the two years is shown in Figure 1. Two varieties of Linseed Roshni and Chandni were obtained from Ayub Agriculture Research Institute Faisalabad. Local variety (BL1) was obtained from Agriculture Research Station Bannu KP, Pakistan.

TABLE 1. Physical and chemical properties of field soil

Soil components	Value and unit
Clay	16%
Silt	44%
Sand	40%
Texture	Loam
pH	8.1
EC	230 µS/cm
Nitrogen	0.03%
Phosphorus	4.00%
Potassium	150.06 mg/kg
Organic matter	0.69%
Zinc	0.97 mg/kg
Iron	0.80 mg/kg
Carbonate	1.4 mEq/L
Bicarbonate	1.9 mEq/L
Calcium/magnesium	9.5 mEq/L

*mEq/L: milliequivalents per litre

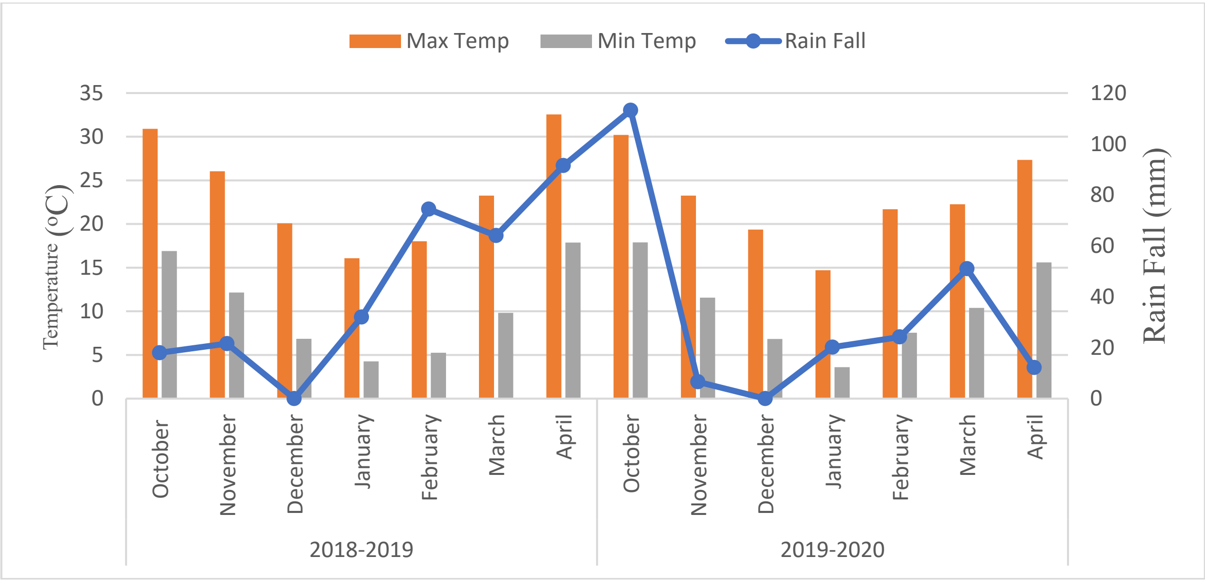


FIGURE 1. Meteorological data of two years

PREPARATION OF AQUEOUS LEAF EXTRACT OF *Moringa oleifera*

The leaf powder of *M. oleifera* (1 kg) was soaked in 4 L distilled water. The mixture was left for 48 h at 25 °C and filtered using Whatman No.1 filter paper. The extract obtained was designated as 25% Moringa leaf aqueous extract (MLAE) which was further diluted to make 5% extract and kept at 4 °C for future use.

DETERMINATION OF PHENOLICS CONTENT OF MLAE

The 1 mL of MLAE was mixed with 0.5 mL of Folin-Ciocalteu reagent and kept at room temperature for 5 min and then added with 7.5% sodium carbonate solution. The mixture volume was raised to 8 mL by adding autoclaved distilled water and kept for 2 h. Optical density of samples was measured by using spectrophotometer (SP-3 Tokyo Japan) at 765 nm. The optical density readings of samples were compared with a standard curve of gallic acid (GAE) solutions. Samples of MLAE were analyzed in triplicate for the content of phenolics. The total content of phenolics was expressed as mg gallic acid eq/mL extract (Chun et al. 2003; Singleton et al. 1965).

DETERMINATION OF MINERAL NUTRIENTS CONTENT OF MLAE

The MLAE was put in a silica crucible and heated in a muffle furnace at a constant temperature (400 °C) till there was no smoke evolution. The ash obtained was moistened with sulphuric acid (concentrated) and heated until there was no fume evolution of sulphuric acid. Then, 1 g of ash was dissolved in 5% HCl (100 mL) to make ready the samples for minerals analysis using atomic absorption spectrophotometer (Perkin-Elmer Germany). Calibration curved were generated for standard solutions of each element independently using atomic absorption spectrophotometer (Indrayan et al. 2005). Samples of MLAE were analyzed for mineral nutrients in triplicate.

EXPERIMENTAL DESIGN

Seeds of linseed were sterilized with mercuric chloride for 3 min and then washed with autoclaved distilled water and sown in the field at rate of 30 kg per hectare. Recommended doses of NPK were applied at the rate of 80:40:30 kg/ha in the field during seed sowing. Each of the plot size of 1 m^2 contained four rows, and the row-to-row distance was 20 cm in the field. Hand weeding was done at 30 days after sowing in both years. Water stress was started 60 DAS (days after sowing) for a period of one month at 40% soil field capacity by skipping four irrigations (four irrigations were not given for one month to create water stress). Soil field capacity was determined by gravimetric method. A small plastic pot perforated at the base having a fitted filter paper was filled with 100 g of soil and flooded with water until a saturated paste was produced and water started flowing out of the pot. The weight of saturated paste (wet weight) was recorded. Then, the soil was allowed to dry until last drop of water came out of the filter paper and dry weight was noted (Al-Shaheen et al. 2018; Aschonitis 2013). Field capacity was determined as percentage of water in 100 g.

$$\text{Soil moisture (\%)} = \frac{\text{Wet weight of soil} - \text{dry weight of soil}}{\text{Dry weight of soil}} \times 100$$

The experimental design was split-split plot design having three replicates for each and every treatment. The irrigation treatments were kept in main plots as control (irrigated maintaining 100% soil field capacity), water deficiency stress (non-irrigated maintaining 40% soil field capacity) and MLAE treatments (0 and 5%) as subplots and three linseed varieties (Roshni, BL1, Chandni) in subplots. The MLAE was applied as foliar spray once just before the start of water deficiency stress and next 15 days after imposition of water deficiency stress. Linseed plants in the field were sprayed with MLAE by using an automated sprayer having spraying ability of 250 dm^3 liquid per hectare.

GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL ANALYSES

After treating plants with water stress for a period of one month, leaf samples were randomly collected from 10 plants per plot for physiological and biochemical parameters determination. Plant height of 10 randomly selected plants per plot was measured using a measuring tape. Similarly, number of tillers per plant of 10 randomly selected plants per plot were counted. The plants in all plots were irrigated to achieve control soil field capacity (100% soil field capacity) and allowed to grow to maturity. When the capsules became mature and turned brown, the plants were harvested. For seed yield determination, seed yield per plot of each treatment was determined which was later converted into kilogram per hectare (kg/ha). For straw yield weight of air dried straw (g), all plants without capsules in area of each plot was determined before converted into kg/ha. For the extraction of fibres straw, the straw was put into stagnant water for four days and then allowed for drying and retting in the bright sun. By using scotching method, the fibres were separated (Dhirhi et al. 2015). Length of randomly selected 20 individual fibres per plot of each treatment was measured by using a steel measuring tape. Weight of 20 individual fibres per plot of every treatment was determined by using an electronic balance.

Leaf relative water content was determined at the end of water stress period by collecting fully expended leaves of 10 random plants per plot. After measuring fresh weight of leaves (FWL) they were kept overnight in distilled water to get their turgid weight (TWL). The leaves were than dried in an oven at 72 °C to constant weight and their dry weight (DWL) was determined.

$$\text{Leaf relative water content } (\%) = \frac{\text{FWL} - \text{DWL}}{\text{TWL} - \text{DWL}} \times 100$$

Leaf chlorophyll *a*, *b* and carotenoids contents were determined at the end of water stress period by the methods of Arnon (1949). Leaf total soluble sugar contents were determined by the method of Dubois et al. (1956). Leaf pieces were ground in 10 mL distilled water and centrifuged for 5 min at 3000 rpm. The sample supernatant (0.1 mL) was mixed with 1 mL phenol and 5 mL concentrated sulphuric acid. The absorbance of sample was recorded at 420 nm by using spectrophotometer (SP-3 Tokyo, Japan). Standard curve of glucose was used as a reference for comparison of samples O.D values.

$$\text{Total soluble sugars (mg/g)} = \text{Absorbance value} \times \text{K value} \times \frac{\text{Dilution factor}}{\text{Weight of sample}}$$

K value = 20, dilution factor = 10, weight of sample = 500 mg

After completion of water stress period, leaf electrolytic leakage was determined by following the method of Blum and Ebercon (1981). Leaf tissue was cut into small pieces and placed in distilled water in a test tube for 12 h. Initial electrical conductivity (EC1) of the solution was recorded after 12 h with electrical conductivity (EC) meter. The sample was heated in water bath at 60 °C for 20 min. The sample was cooled at room temperature and again electrical conductivity (EC2) of sample was noted. The value of electrolyte leakage was calculated according to the following formula:

$$\text{Electrolyte leakage } (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100$$

STATISTICAL ANALYSIS

Two-way ANOVA test was performed for data analysis using least significant difference test for comparison of mean values. Coefficient of correlation (Pearson) was determined among various growth parameters using Statistix-10.

$$r = \frac{\Sigma^{XY} - ((\Sigma^{X})(\Sigma^{Y})/n)}{\sqrt{(\Sigma^{X^2} - ((\Sigma^{X})/n)^2)(\Sigma^{Y^2} - ((\Sigma^{Y})/n)^2)}}$$

X indicates values in the first data set and Y indicates values in the second data set. Value of n is 12.

RESULTS AND DISCUSSION

We characterized MLAE for phenolics content and nutrients composition (Table 2). It was found that MLAE contains natural phenolics (150 mg GAE/mL) and nutrients such as Ca (7.16 mg/g), Mg (0.812 mg/g), K (5.607 mg/g), Zn (0.051 mg/g), Fe (0.175 mg/g), and Mn (0.014 mg/g). Various studies can be found indicating the presence of phenolics in aqueous extracts of Moringa leaf (Bibi et al. 2016; Pervez et al. 2017). Phenolics play crucial role in the protection of plants from abiotic stresses (Naikoo et al. 2019; Sharma et al. 2019). Phenolic compounds are essential components of cell wall and play a crucial role in non-enzymatic antioxidant defense system of plants. In water stress conditions, phenolic compounds decrease reactive oxygen species pool by quenching them and thus prevents oxidative damage of cells (Šamec et al. 2021).

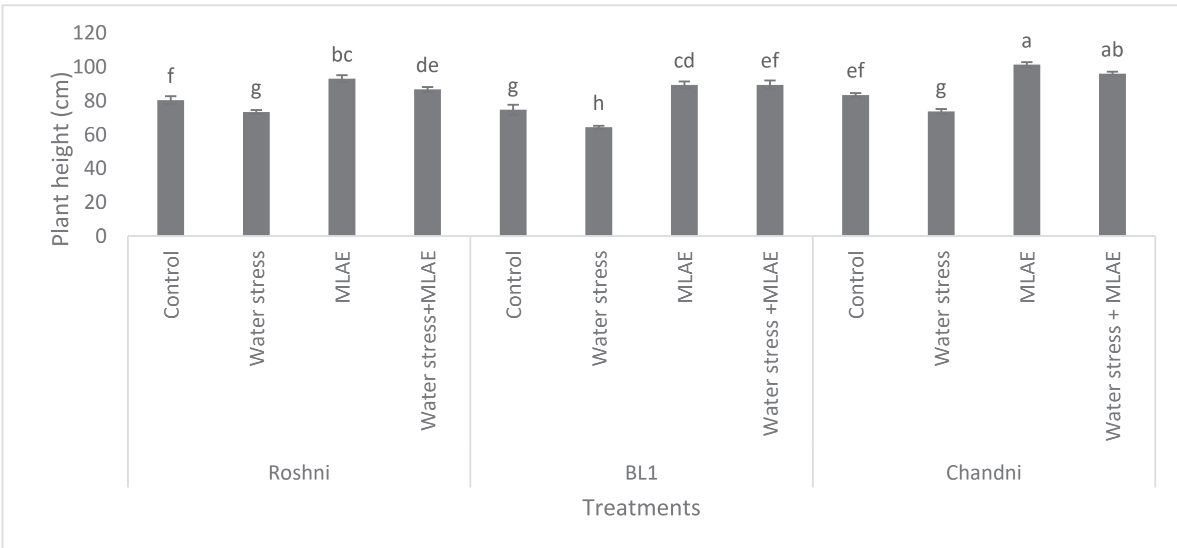
TABLE 2. Phenolics and minerals content of MLAE

Compositions of MLAE	Values
Phenolics	150 ± 8.00 mg GAE/mL
Ca	7.16 ± 1.34 mg/g
Mg	0.812 ± 0.47 mg/g
K	5.607 ± 0.97 mg/g
Fe	0.175 ± 0.035 mg/g
Zn	0.051± 0.002 mg/g
Mn	0.014 ± 0.008 mg/g

In our studies, water deficit stress decreased plant height in all three varieties of linseed (Figure 2(a)). However, maximum decrease in plant height due to water deficit stress was recorded in BL1 (13.84%) followed by Chandni (11.59%) and Roshni (8.71%) than their respective untreated and irrigated control. In a non-stress environment, foliar spray of MLAE resulted in taller plants over untreated and irrigated control. We noted that MLAE completely remediated water deficit stress effect on plant height of all the three linseed varieties. Water deficit stress inhibited production of tillers in all varieties of linseed (Figure 2(b)). However, maximum decrease in number of tillers due to water deficit stress was recorded in Roshni (54.49%), BL1 (44.33 %) and Chandni (40 %) than their respective untreated and irrigated control. The foliar spray of MLAE under non-stress conditions significantly improved tillers production per plant (Figure 2). Similarly, water deficit stress effect on tillers production was completely reversed by MLAE application.

Previous studies have shown that water deficit stress decreased plant height and number of tillers of linseed (Kariuki et al. 2016; Mohammad et al. 2012; Zhao et al. 2012). Stoyanov (2005) showed that water deficit stress decreased plant height and relative water content in bean plant. Water deficiency stress decreases turgidity of cells leading to a decreased mitotic activity in growing regions (Abdul Jaleel et al. 2009). The beneficial effects of MLAE on plant height and number of tillers per plant under water stress may be accredited

a



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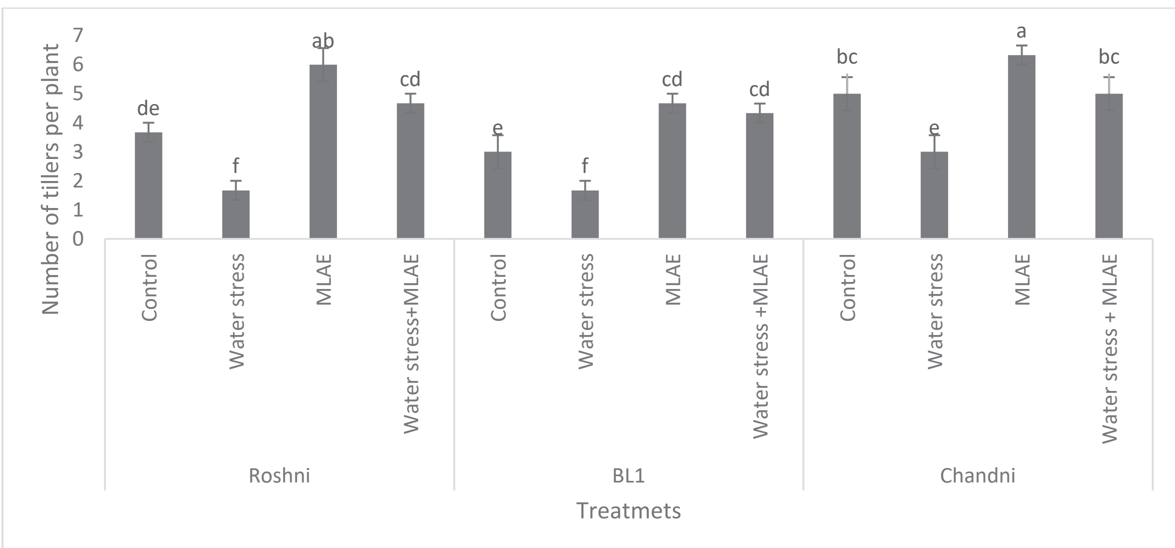


FIGURE 2. Effect of Moringa leaf aqueous extract on (a) plant height, and (b) number of tillers per plant of linseed under water stress. Data of two years was pooled together due to non-significant variations. Means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

to its phytochemicals composition. The MLAE contained nutrients like K, Mg, Ca, Fe, and Zn which are crucial for various physiological activities of plants. The Fe and Zn are required for the synthesis of chlorophyll molecules, Ca functions as a secondary messenger during hormonal signaling under abiotic stresses and K has significant role in stomatal conductance of plants (Aliniaeifard et al. 2020; Samreen et al. 2013; Sawan et al. 2008). Moreover, phenolic compounds present in MLAE might have contributed in improving antioxidant potential of linseed plants and thus have played a role in improving water stress resistance potential of linseed plants. Phenolic compounds lowered water stress effects on plants by retaining turgidity of cells via increasing cell wall thickness and as natural antioxidants to prevent oxidative stress (Šamec et al. 2021).

Water deficit stress decreased leaf relative water content (LRWC) in all three varieties of linseed; however, maximum decrease in LRWC due to water deficit stress was recorded in BL1(13.63) followed by Roshni (5.46%), and Chandni (4.90%) than their respective untreated and irrigated control (Figure 3(a)). In non-stressed environment, the foliar spray of MLAE increased LRWC content as compared to untreated and irrigated control. The foliar spray of MLAE maintained normal value of LRWC under water deficit stress. Water deficit

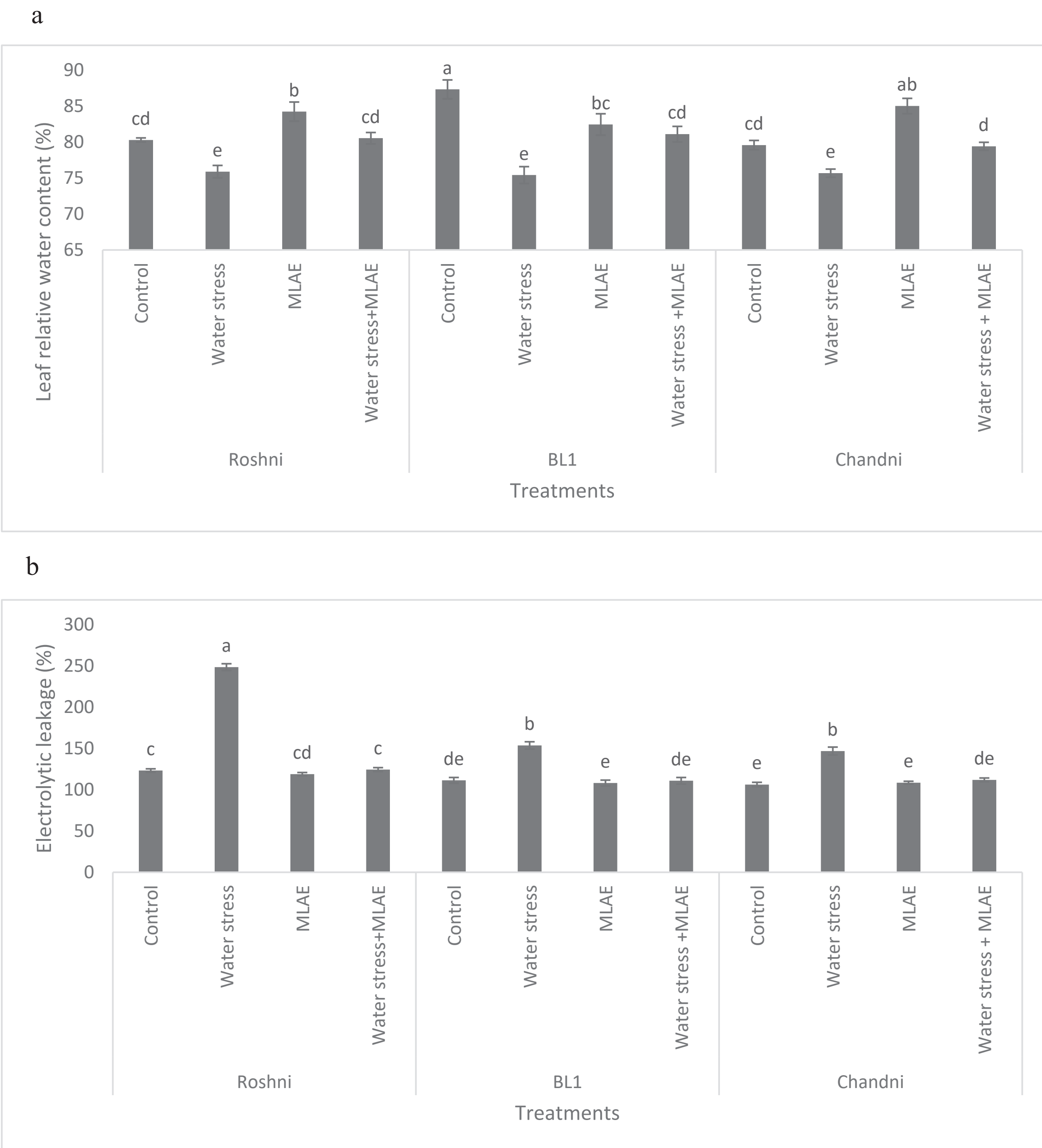
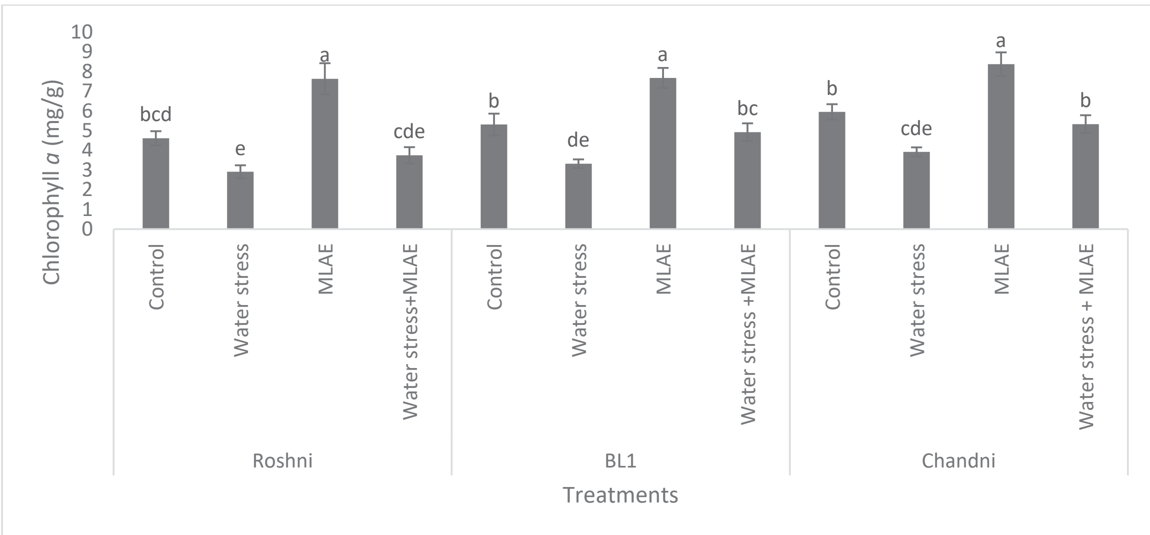


FIGURE 3. Effect of Moringa leaf aqueous extract on (a) leaf relative water content, and (b) electrolyte leakage of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

stress increased electrolyte leakage in all three varieties of linseed. However, maximum increase in electrolytic leakage due to water deficit stress was recorded in Roshni (50.37%) followed by Chandni (27.63%) and BL1 (27.53%) than their respective untreated and irrigated control. In a non-stress and stress environment, foliar spray of MLAE retained normal electrolyte leakage and statistically similar to control ($p<0.05$) (Figure 3(b)).

Tambussi et al. (2000) observed that water deficit stress decreased relative water content in different varieties of wheat. The MLAE contained a reasonable amount of potassium which has integral role in the maintenance of water budget of plants under conditions of low soil water availability (Cakmak 2005; Wang et al. 2013). Increase in leaf cells electrolyte leakage due to water stress is a common phenomenon (Masoumi et al. 2010). Water deficit stress results in the production of reactive oxygen species which causes lipid peroxidation destroying cell membrane (Sharma et al. 2012). The MLAE foliar spray significantly minimizes water deficit stress effect on leaf cells electrolyte leakage. The MLAE contained natural phenolics which are reported for antioxidative properties (Sankhalkar 2014). Phenolics function as antioxidants and protect cells from toxicity of reactive oxygen species produced under the biotic and abiotic stresses (Valentine et al. 2003).

a



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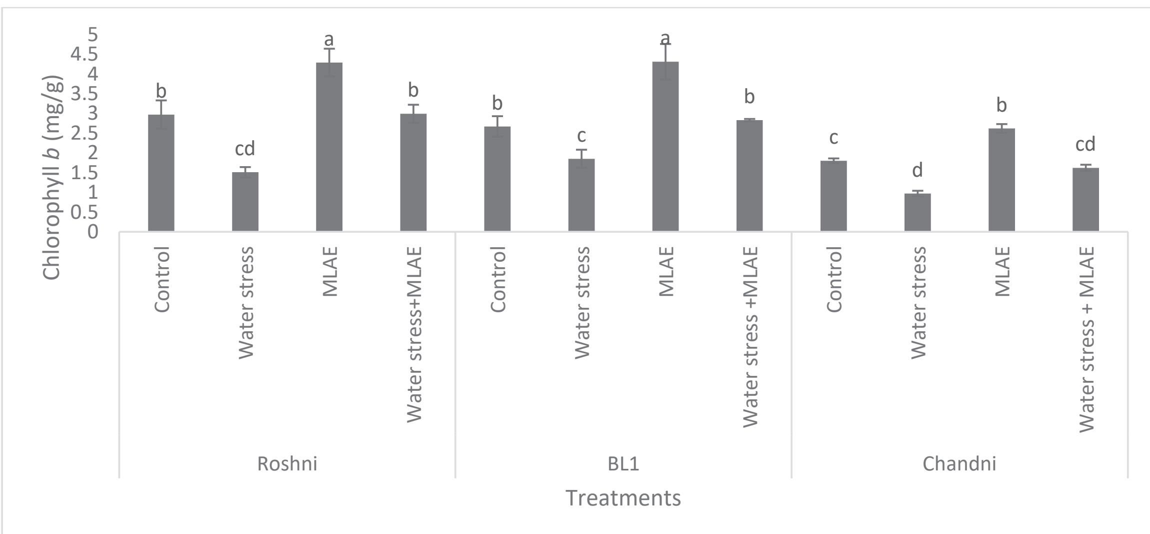


FIGURE 4. Effect of Moringa leaf aqueous extract on (a) chlorophyll *a*, and (b) chlorophyll *b* content of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

Water deficit stress decreased chlorophyll *a* content in all three varieties of linseed. However, maximum decrease in chlorophyll *a* due to water deficit stress was recorded in BL1 (37.47%) followed by Roshni (36.87%) and Chandni (34.11%) than their respective untreated and irrigated control. In a non-stress environment, foliar spray of MLAE significantly increased chlorophyll *a* content by over untreated and irrigated control ($p<0.05$). We observed that MLAE completely remediated water deficit stress effect on chlorophyll *a* content (Figure 4(a)). Similarly, water deficit stress decreased chlorophyll *b* in all three varieties of linseed. However, maximum decrease in chlorophyll *b* due to water deficit stress was recorded in Roshni (49.15%) followed by Chandni (46.11%) and BL1 (30.71%) than their respective untreated and irrigated control. The foliar spray of MLAE under non-stress conditions significantly improved chlorophyll *b* content in linseed. The foliar spray of MLAE under water deficit stress condition significantly retained chlorophyll *b* content (Figure 4(b)).

Water deficit stress also decreased carotenoids content in all three varieties of linseed. However, maximum decrease in carotenoids content due to water deficit stress was recorded in Roshni (35.5 %) followed by Chandni (29.03%) and BL1 (24.83 %) than their respective untreated and irrigated control. In a non-stress environment, foliar spray of MLAE significantly increased carotenoids content over untreated and irrigated control ($p<0.05$) (Figure 5(a)). We noted

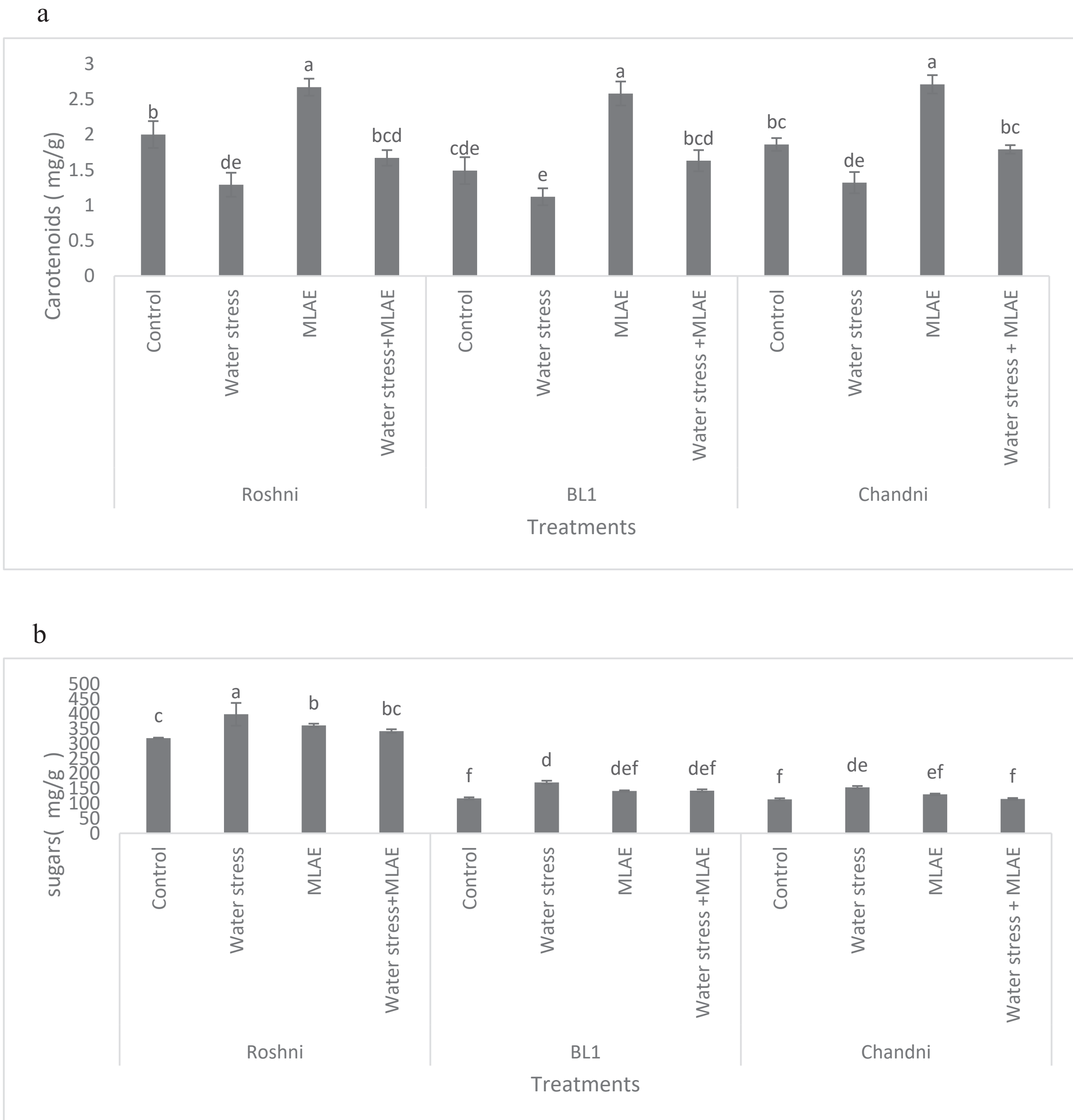


FIGURE 5. Effect of Moringa leaf aqueous extract on (a) leaf carotenoids, and (b) leaf soluble sugars content of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

that MLAE significantly alleviated water deficit stress effect on carotenoids content and retained normal value of carotenoids content as compared to untreated and irrigated control.

Previous studies of Nyachiro et al. (2001) have shown that water deficit stress decreased chlorophyll *a*, *b* and carotenoids content in six varieties of wheat. The MLAE significantly ameliorated the adverse effect of water stress on chlorophyll *a*, chlorophyll *b* and carotenoids contents. The MLAE has bioactive compounds of *M. oleifera* like phenolics, amino acids, zeatin, ascorbic acid and minerals which inhibit the synthesis and activity of enzyme chlorophyllase and thus, protect photosynthetic pigments from degradation by water stress (Latif & Mohamed 2016). The MLAE contained Mg which is an essential constituent of chlorophyll and may have contributed in improving concentration of chlorophyll pigments in leaves of linseed.

Water deficit stress increased leaf total soluble sugars in all three varieties of linseed. However, maximum increase in total soluble sugars content due to water deficit stress was recorded in BL1 (31.41%) followed by Chandni (26.11%) and Roshni (20.03%) than their respective untreated and irrigated control. The foliar spray of MLAE under non-stress conditions significantly improved leaf total soluble sugars content in linseed ($p<0.05$). However, MLAE foliar spray under water stress conditions reversed increased in soluble sugars content and such that the value was similar to untreated and irrigated control (Figure 5(b)). Previous study has shown that water stress increased total soluble sugars in different varieties of wheat, rice and canola (Nazarli & Faraji 2011; Nosrati et al. 2014; Zain et al. 2014). Increase in soluble sugars content indicates intensity and severity of drought stress (Prado et al. 2000). The decrease in sugars content due to MLAE application under water deficit stress indicates that it improved physiological and biochemical pathways of linseed plants leading to an improved resistance of this plant species to water deficit stress.

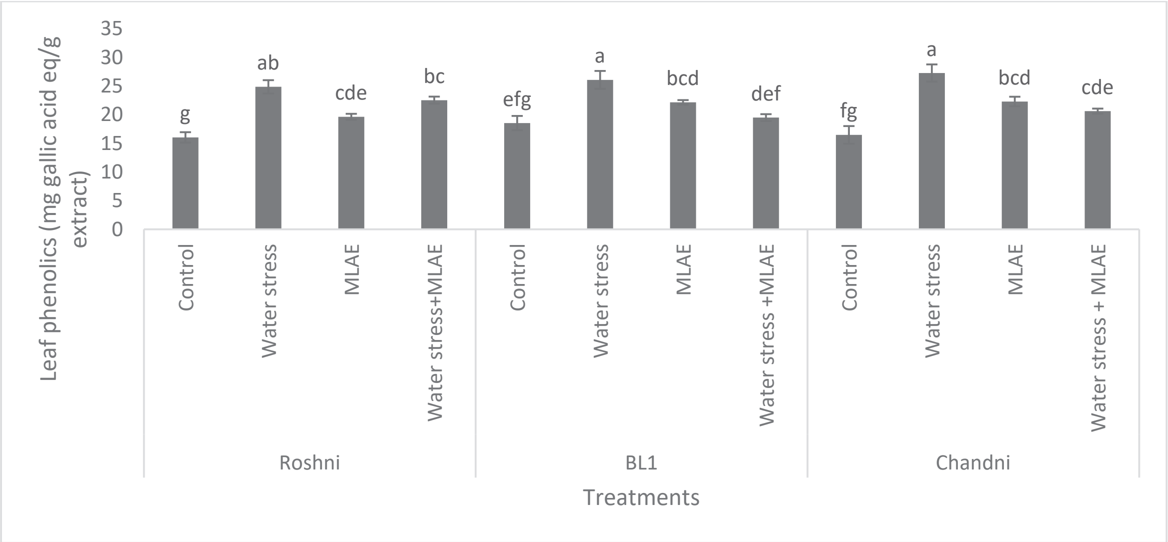
Water deficit stress increased leaf phenolics content in all the three varieties of linseed. However, maximum increase in leaf phenolic due to water deficit stress was recorded in Chandni (39.58%) followed by Roshni (35.51%) and BL1 (28.88%) than their respective untreated and irrigated control. Foliar spray of MLAE both in non-stress and stress condition increased (20.36%) leaf phenolics content over untreated and irrigated control ($p<0.05$) (Figure 6(a)). Water deficit also increased root phenolics content in all three varieties of linseed. However, maximum increase in root phenolics content due to water deficit stress was recorded in Roshni (43.82%) followed by Chandni (36.41%) and BL1 (30.48%) than their respective untreated and irrigated control. Foliar spray of MLAE both in non-stress and stress condition increased (31.74%) root phenolics over untreated and irrigated control ($p<0.05$) (Figure 6(b)).

Application of Moringa leaf extract on maize increased phenolics content which protected the plant from high temperature induced stress (Bakhtavar et al. 2015). The positive effect of MLAE on the phenolics content in leaves and roots of linseed under water deficit stress may be attributed to its phytochemical composition. We have found that MLAE was a rich source of natural phenolics which have improved the water deficit tolerance potential of linseed plants by regulating the metabolism of endogenous phenolics in leaves and roots. Studies of Pervez et al. (2017) have shown that bioregulator prepared from leaves of *M. oleifera* was stimulatory on production of phenolics in leaves of maize under drought stress.

Water deficit stress decreased seed yield in all the three varieties of linseed. However, maximum decreased in total seed yield due to water deficit stress was recorded in BL1 (19.51%) followed by Roshni (10.77%) and Chandni (8.30%) than their respective untreated and irrigated control. In non-stressed environment, MLAE increased total seed yield of linseed as compare to untreated and irrigated control ($p<0.05$). MLAE in water deficit stress condition increased total seed yield and ameliorate the adverse effect of water deficit stress (Figure 7(a)).

Water deficit stress reduced straw yield in all the three varieties of linseed. However, maximum decrease in straw yield due to water deficit stress was recorded in Chandni (37.02%) followed by Roshni (12.60%) and BL1 (10.55%) than their untreated and irrigated control. In a non-stress environment, foliar spray of MLAE resulted more straw yield over untreated and irrigated control ($p<0.05$). We observed that MLAE not only remediated water deficit stress effect on straw yield but also caused production of more straw yield over untreated and irrigated control (Figure 7(b)).

Previous studies of Khan et al. (2005) showed that water stress decreased total seed yield of wheat. However, MLAE reversed decrease in total seed yield caused by water stress. These results are in accordance with the previous finding of Mustapha et al. (2019) that showed the Moringa leaf aqueous extract application to maize plants increased seed yield of maize. Improvement a



b

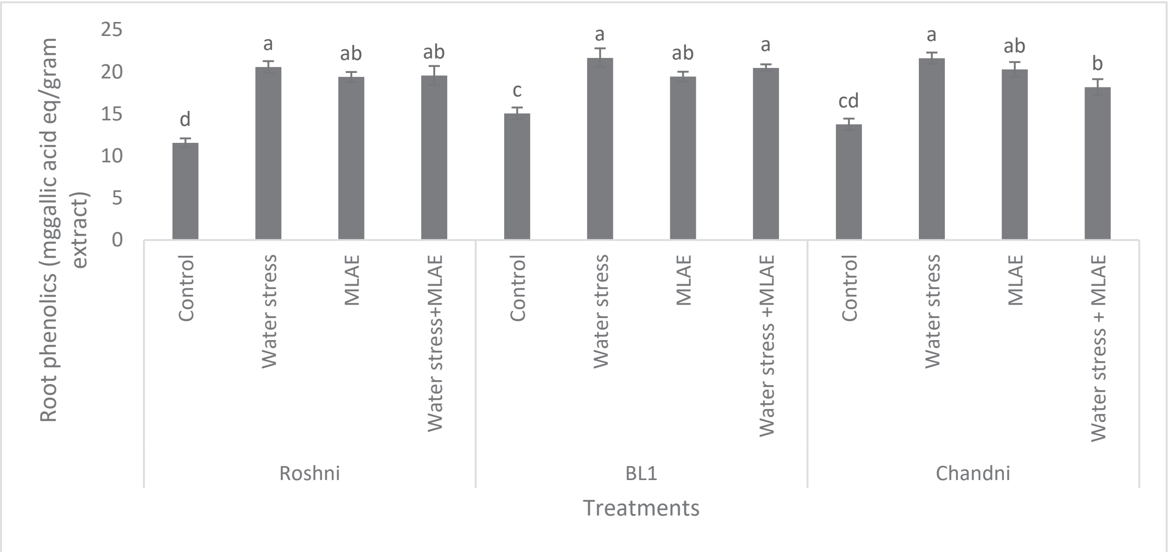
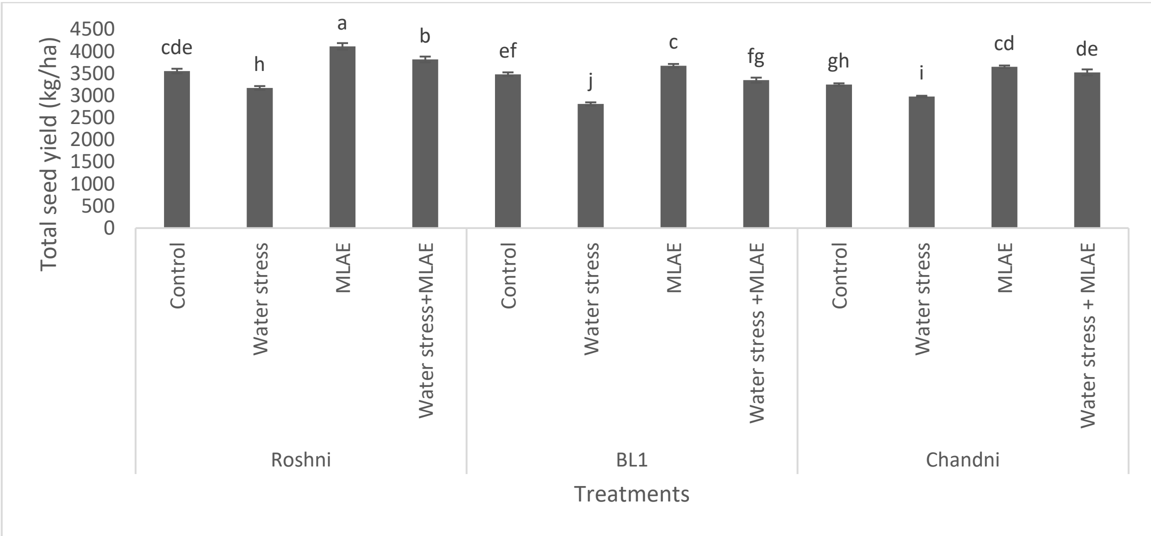


FIGURE 6. Effect of Moringa leaf aqueous extract on (a) leaf phenolics, and (b) root phenolics of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

in seed yield of linseed varieties due to MLAE application may be mainly due to its chemical makeup and beneficial effects on plant height, tillers production, photosynthetic pigments, leaf relative water content and in minimizing leaf electrolytes leakage under water stress. Moringa leaf extract has been characterized for the presence of zeatin which is a cytokinin responsible for greater plant growth and yield (Biswas et al. 2016). Reduction in straw yield by water deficit stress can be due to decrease in the content of photosynthetic pigments under low soil availability. Drought condition leads to the inhibition in photosynthetic activity leading to decrease in biomass production (Ullah et al. 2012).

a



b

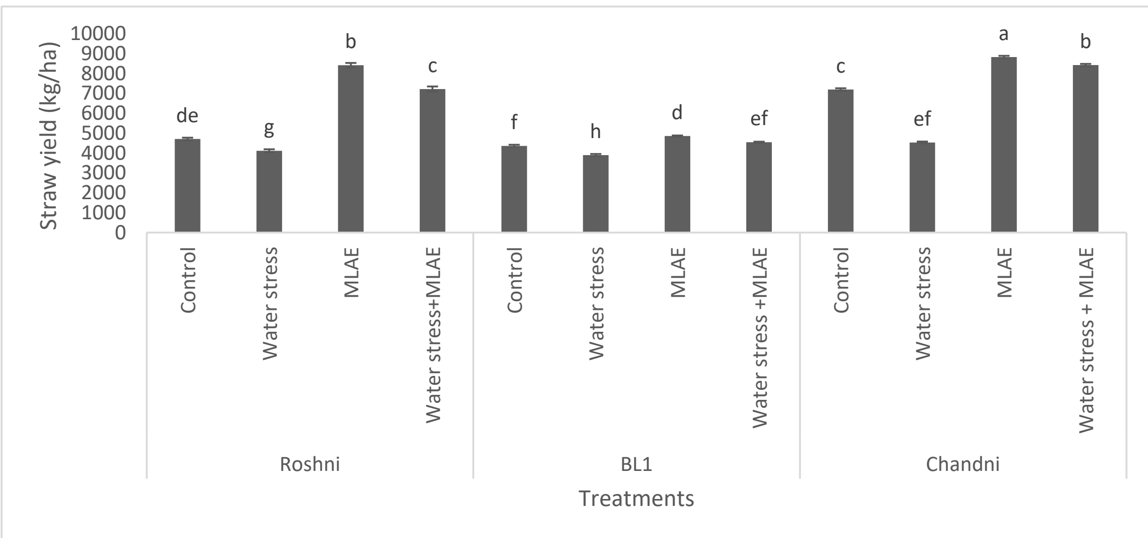


FIGURE 7. Effect of Moringa leaf aqueous extract on (a) tot, and (b) straw yield of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at $p<0.05$

Water deficit stress decreased fibre length in all varieties of linseed. However, maximum decrease in fibre length due to water deficit stress was recorded in Roshni (24.05%) followed by BL1 (13.95%) and Chandni (8.60%) than their respective untreated and irrigated control. In non-stress condition MLAE increased fibre length of linseed as compared to untreated and irrigated control (p<0.05) (Figure 8(a)). MLAE in water deficit stress condition retained normal fibre length.

Water deficit stress decreased fibre weight in all the varieties of linseed. However, maximum reduction in fibre weight due to water deficit stress was recorded in BL1 (33.00%) followed by Chandni (5.76%) and Roshni (4.34%) than their respective untreated and irrigated control. Foliar spray of MLAE in non-stress condition increased the fibre weight of linseed as compared to untreated and irrigated control ($p<0.05$) (Figure 8(b)).

MLAE in water deficit stress condition increased fibre weight as compared to untreated and irrigated control. The beneficial effects of MLAE on fibre quality can be due to its presence of various kinds of phytochemicals like essential macro and micronutrients and phenolics. The MLAE contained K which is a major solute in single cells of fibres necessary for maintaining turgor pressure crucial for fibre elongation. It has been found that limited K supply during fibre elongation period resulted in shorter fibres (Oosterhuis 1995; Sawan et al. 2008). Moreover, K has been credited for its role in the rapid translocation of photoassimilates from source to sink (Sangakkara et al. 2000). The application of nutrients like Mg, Zn and Fe have been reported for their beneficial effects on the photosynthetic outcomes of plants (Liu et al. 2016; Ye et al. 2019) and therefore, as MLAE was rich in these nutrients which ultimately assisted in improving fibre length and weight of linseed. Results of this study provided evidence that MLAE not only improved vegetative growth but also reproductive growth of linseed plants, including seed yield and fibre quality.

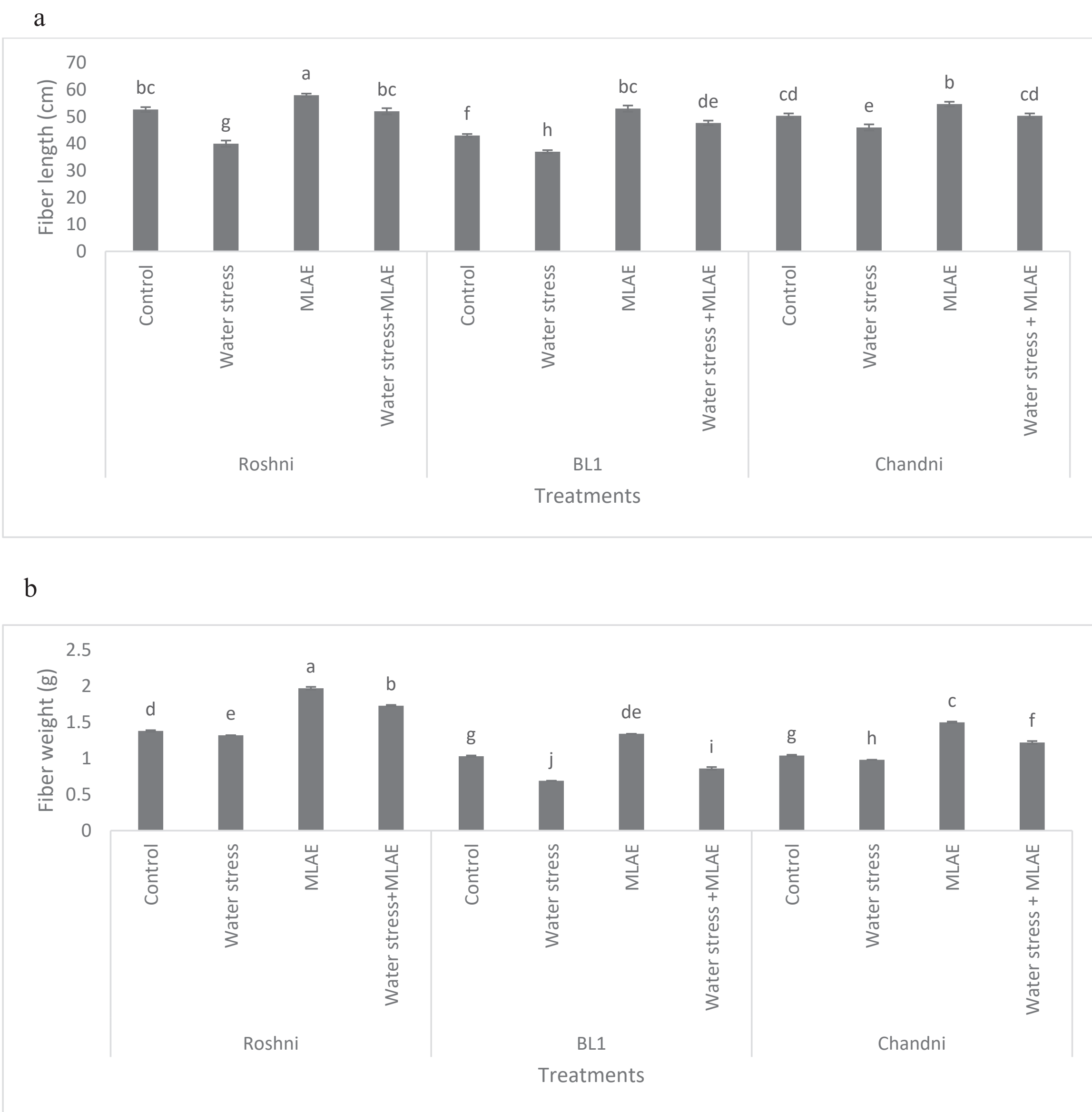


FIGURE 8. Effect of Moringa leaf aqueous extract on (a) fibre length, and (b) fibre weight of linseed under water stress. Data of two years was pooled together due to non-significant variations. In every figure means with similar letter (s) are identical on the basis of LSD test at *p*<0.05

CORRELATION OF GROWTH, YIELD AND FIBRE QUALITY PARAMETERS

Electrolytic leakage had significantly negative correlation with fibre length (r = -0.5839), number of tillers (r = -0.6122), plant height (r = -0.5156), seed yield (r = -0.3969), straw yield (r = -0.3894), chlorophyll *a* (r = -0.5451), chlorophyll *b* (r = -0.4021) and relative water content (r = -0.5473). Electrolytic leakage had significantly positive correlation with leaf phenolics (r = 0.4356) and leaf soluble sugars (r = 0.4969). Fibre length was positively associated with plant height (r = 0.8270), straw yield (r= 0.6920), chlorophyll *a* (r = 0.6909), chlorophyll *b* (r = 0.6284), fibre weight (r = 0.7464) and leaf relative water content (r = 0.4863). Plant height exhibited significantly positive correlation with seed yield (r = 0.6948), straw yield (r = 0.8003), chlorophyll *a* (r = 0.7418) and chlorophyll *b* (r = 0.4613). Root phenolics was significantly positive correlated with leaf phenolics (r = 0.7241). However, leaf phenolics is negatively correlated with relative water content (r = -0.3700) (Table 3).

TABLE 3. Correlation of growth and yield parameters

	ECL	FL	NT	PH	RPHL	SDY	LPHL	STRY	Chl a	Chl b	FW	RWC
FL	-0.5839 0.0002*											
NT	-0.6122 0.0001*	0.8000 0.0000*										
PH	-0.5156 0.0015*	0.8270 0.0000*	0.8419 0.0000*									
RPHL	0.2651 0.1238	-0.1238 0.4785	0.0551 0.7531	0.1347 0.4406								
SDY	-0.3969 0.0183*	0.8320 0.0000*	0.6439 0.0000*	0.6948 0.0000*	-0.0509 0.7714							
LPHL	0.4356 0.0089	-0.3517 .0383*	-0.2873 0.0942	-0.1490 0.3928	0.7241 0.0000*	-0.3055 0.0743						
STRY	-0.3894 0.0208*	0.6920 0.0000*	0.7877 0.0000*	0.8003 0.0000*	0.0781 0.6558*	0.5784 0.0003*	-0.1527 0.3811					
Chl a	-0.5451 0.0007*	0.6909 0.0000*	0.6988 0.0000*	0.7418 0.0000*	0.0533 0.7610	0.6244 0.0001*	-0.1645 0.3451	0.5463 0.0007*				
Chl b	-0.4021 0.0166*	0.6284 0.0001*	0.4323 0.0095*	0.4613 0.0053*	0.0341 0.8458	0.7913 0.0000*	-0.2271 0.1896	0.1649 0.3440	0.6257 0.0001*			
FW	-0.0978 0.5762	0.7464 0.0000*	0.5546 0.0005*	0.6107 0.0001*	0.0400 0.8195	0.8818 0.0000*	-0.1192 0.4952	0.6481 0.0000*	0.4321 0.0095*	0.5798 0.0003*		
RWC	-0.5473 0.0007*	0.4863 0.0031*	0.5128 0.0016*	0.5789 0.0003*	-0.0990 0.5714	0.6604 0.0000*	-0.3700 0.0287*	0.4019 0.0167*	0.7025 0.0000*	0.5883 0.0002*	0.4139 0.0134*	
Sugar	0.4969 0.0024*	0.1625 0.3511	0.0766 0.6618	-0.0858 0.6243	-0.0176 0.9199	0.4168 0.0127*	-0.0229 0.8962	0.0456 0.7946	-0.2289 0.1860	0.2631 0.1268	0.6372 0.0000*	-0.1319 0.4499

ECL: electrolytic leakage, FL: Fibre length, NT: number of tillers per plant, PH: plant height, RPHL: root phenolics, SDY: seed yield, LPHL: leaf phenolics STRY: straw yield, Chl a: chlorophyll *a*, Chl b: chlorophyll *b*, FW: Fibre weight, RWC: leaf relative water content, sugar: Leaf soluble sugars. Asterisks indicate the significant values at $p<0.05$

This study showed that increase of leaf electrolyte leakage was negatively correlated with growth related traits of linseed during water stress. Major portion of electrolyte leakage contains K^+ ions which are lost via roots under water stress conditions. Potassium is an important element for phloem transport, enzyme activity, proteins synthesis, stomatal adjustments, osmoregulation and energy transfer. Therefore, loss of K^+ ions via electrolyte leakage results in the loss of normal functions of cells (Wang et al. 2013). Moreover, electrolyte leakage has been linked with senescence of leaves during stress conditions (Kirnak & Demirtas 2006). This may be the reason that higher electrolyte leakage was negatively associated with growth related traits of linseed. The MLAE application may prevent the increase of leaf electrolyte leakage due to water stress.

The concentration of chlorophyll in leaves was positively correlated with plant height, seed yield and fibre quality of linseed. This may be attributed to the fact that MLAE improved concentration of green pigments in leaves which ultimately resulted in higher photosynthetic activity and dry matter production. The MLAE retained normal value of leaf relative water content which had positive correlation with fibre length and weight. The MLAE was rich in K which is a major solute in single cells of fibres necessary for maintaining turgor pressure crucial for fibre elongation (Sawan et al. 2008).

Conclusion

Water deficit stress had reducing effect on plant height, tillers production, leaf pigments, and leaf relative water content. In response to water deficit stress linseed plants have higher production of phenolics and soluble sugars. In non-stress environment, foliar spray of MLAE improved growth indicators, increased level of photosynthetic pigments in leaves and thus improving fibre quality of linseed. Moreover, in water deficit stress environment MLAE was highly effective and alleviated harmful results of water deficit stress on seed yield and fibre quality of linseed. All the three linseed varieties Chandni and Roshni performed better by producing highest seed yield after exposure to water stress. Moreover, these two varieties of linseed were highly responsive to foliar spray of MLAE. Therefore, linseed varieties Roshni and Chandni are recommended for cultivation under arid and semiarid regions for gaining maximum seed yield and fibre quality.

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