Influence of foliage applied moringa leaf extract on growth and yield of sunflower (Helianthus annuus L.) underwater deficit conditions

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ABSTRACT

With increasing population, the demand for food is also increasing. So, with the aim to increase the productivity of crop a field experiment was conducted to determine the effectiveness of the foliar application of moringa leaf extract (MLE) at growth and yield of sunflower under water deficit conditions at Agronomy research area, Bahauddin Zakariya University, Multan. The treatments comprise of Factor A: Irrigation, (I0 = Normal irrigation, I1 = Skipped at knee height stage, I2 = Skipped at knee height + heading stage, I3 = Skipped at knee height + heading + seed development stage and Factor B: Foliar application of MLE (Subplot), T0 = No spray, T1 = Spray at knee height stage, T2 = Spray at knee height + heading stage, T3 = Spray at knee height + heading + seed development stage with randomized complete block design (RCBD) split-plot design in three replications. The interactive effect between moringa leaf extract (MLE) and irrigation was significant. Maximum head diameter (22.17 cm), number of achenes per head (1030.30), 1000-achenes weight (49.73 g), achene yield (3474 kg/ha), and biological yield (10729.83 kg/ha), oil content (41.734%), protein content (20.8%) was observed in F3 (K+H+S) and I0 (Normal) irrigation. While minimum head diameter (15.57 cm), number of achenes per head (681.67), 1000-achenes weight (42.001 g), achene yield (2536.33 kg/ha), and biological yield (8209.33 kg/ha), oil content (36.3%), protein content (16.27%) was observed in F0 (no spray) and I3 (K+H+S) irrigation. Results showed that foliar application of moringa leaf extract under normal conditions boost the crop yield and drought stress at (K+H+S) is detrimental for growth and development of sunflower.

INTRODUCTION

Sunflower (Helianthus annuus L.) is ranked 4th important oilseed crop in the world after soybean, rapeseed and mustard. Pakistan is producing only 7 percent edible oil of its total consumption. In Pakistan, the sunflower is also important oilseed crop because its seed contains 40-45% oil contents and cultivated in spring and autumn season which is helpful to overcome the annual import of edible oil. World population is increasing with the passage of time so food production must be increased to meet the need of growing population. Crops face different environmental stress during the growing season which affects its growth and development processes. Sunflower is highly sensitive to water at early flowering and achenes filling stage. It reported that drought stress reduces the achenes, oil quality and oil yield greatly. Underwater deficit condition sunflower growth is affected due to poor photosynthetic rate which is resulted by stomata closure. Low moisture in sunflower resulted declined in stomatal conductance and CO2 assimilation. Water shortage also caused overproduction of ROS species which damage cellular components.
To overcome this water shortage in field crop production different strategies like cultural practices and breeding strategies can be adopted. Exogenous applications of natural growth regulators like auxins and osmoprotectants can also make the crop resistant to drought stress. Different synthetic and natural growth regulators are being used but natural growth regulators is gaining popularity as they are environmentally safe and cheap.

Among them, Moringa leaf extracts (MLE) has a notable position. Moringa is considered a miracle tree because it is a source of protein, vitamin C, easily digestible iron (Fe) and Ca. Moringa leaves extract produced effective growth hormones. He also resulted that it increases crop yield up to 30%. reported that moringa leaf extract enhances the crop growth, improve the resistance against pests and diseases, accelerate the size and number of fruits, number of roots and whole plant defence system.

MATERIALS AND METHODS
Preparation of Moringa leaf extract
For moringa leaf extract (MLE), young moringa (Moringa oleifera L.) leaves were harvested from research area of Department of Forestry, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. In extraction machine young laves of moringa were ground with water at the rate of 1L per 10kg fresh material as described by. After grinding, the material was sieved via muslin cloth and was centrifuged for 15 mints at 8000×g. The dilution of (1:30) MLE-30 (1ml MLE diluted with 30ml distilled water) of the extract was prepared as described by and this solution was used in the experiment as a foliar spray.

Experimental treatments, design and crop husbandry
Experimental treatments
This field experiment was conducted at the Research area of Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan. This site lies at 30.258° N and 71.517° E. The experimental treatments were comprised of two factors, Factor-A: Irrigation (Main plot), I0 = Normal irrigation, I1 = Skipped at knee height stage, I2 = Skipped at knee height + heading stage, I3 = Skipped at knee height + heading + seed development stage, Factor B: Foliar application of MLE (Subplot), T0 = No spray, T1 = Spray at knee height stage, T2 = Spray at knee height + heading stage, T3 = Spray at knee height + heading + seed development stage.

Experimental design
The experiment was laid out in Randomized Complete Block Design (RCBD) with the split-plot arrangement was laid out in three replicates. The net plot size was 15m².

Crop husbandry
For fine field bed preparation, the designed field was ploughed 2 times and tractor drew blade was used for eradication of weeds and other roots. For sowing purpose, fine structures of ridges were made at the distance of (R × R) 5 feet. A hybrid seed of (Hysun-33) was collected from the local market (Seraj Agro Services) of Multan. The crop was sown during the spring season on January 15, 2018. To attain the desired population, the optimum distance from the plant to plant was kept (P×P= 0.75 feet). A recommended dose of NPK was applied @ of 150, 100 and 60 kg/ha. The sources of artificial fertilizer include the DAP, Urea and SOP. The total amount of Nitrogen was applied into three splits, the 1/3 amount of nitrogen was applied at the time of sowing; the leftover amount of nitrogen was applied at 2nd and 4th irrigation. To attain the desired population, thinning was done at 3rd leaf stage. Due to sensitive to hormones, the use of herbicides or weedicides was avoided. To control weeds competition, manual weeding and hoeing was done. In irrigation scheduling, first irrigation was applied after 15 days of sowing, second irrigation was applied at knee height, 3rd irrigation was applied at 50 days after sowing (DAS), 4th irrigation was applied at 70 days after sowing (DAS), and last irrigation was applied at the seed development stage. In drought stress treatments, as per treatments, the irrigation was skipped at knee height stage, heading stage and seed developmental stage. Foliar spray of moringa leaf extract (MLE) was sprayed at knee height, heading stage and seed development as per treatments. Crop protection measurements were also done at every stage. The sprays of Carbofuran @ 8kg/acre and Lufenuran @ 250ml/acre were sprayed at leaf stage to protect from stem borer and attack of armyworm respectively. During experiment different morphological, physiological and yield-related attributes were observed.
Table 1. Pre experiment physicochemical analysis of soil.

| Determination          | Value | Unit  |
|------------------------|-------|-------|
| pH                     | 8.6   |       |
| EC                     | 2.5   | dsm⁻¹ |
| Organic matter (O.M)   | 0.67  | %     |
| N                      | 0.04  | %     |
| P                      | 5.51  | ppm   |
| K                      | 192   | ppm   |
| Sand                   | 27    | %     |
| Silt                   | 52    | %     |
| Clay                   | 21    | %     |
| Soil texture           | Silty clay loam |       |

Procedures

Leaf area index
Leaf area index was calculated by Watson (1947) formula. To calculate leaf area index, total plant in the area of 1m² were harvested and their leaves were weighed immediately.

\[ \text{LAI} = \frac{\text{Leaf area (cm)}}{\text{Ground area (cm)}} \]

To calculate leaf area, leaf length was multiplied by means of leaf width.

Crop growth rate (g m⁻² day⁻¹)
To determine crop growth rate, five plants from each plot were chopped after harvesting with an interval of thirty days. Chopped samples were oven dried at 70°C and constant their dry weight to determining crop growth rate as by Hunt (1978).

\[ \text{CGR} = \frac{w_2 - w_1}{t_2 - t_1} \]

Where \( w_1 \) and \( w_2 \) are final dry matter at \( t_1 \) and \( t_2 \) respectively.

Net assimilation rate (gm⁻² day⁻¹)
Hunt (1978) formula was used to calculate net assimilation rate.

\[ \text{NAR} = \frac{\text{TDM}}{\text{LAD}} \]

Where TDM is total dry matter.

Achenes oil contents (%)
It was measured by Soxhlet Fat Extraction method (A.O.A.C., 1990) with the following equation.

\[ \text{Oil percentage} = \frac{\text{wt. of flask + oil - the weight of flask}}{\text{wt. of flask + achenes - weight of flask}} \times 100 \]

Achenes protein contents (%)
Kjeldahl method (Bremner, 1964) was used to calculate Achenes protein contents (%) by given formula.

\[ \text{Protein} = \frac{V_1 - V_2}{W} \times N \times 6.25 \times 14 \times 100 \]

Where, \( V_1 = \) titration sample (ml), \( V_2 = \) Blank titration (ml), \( N = \) Sulphuric acid Normality

\( W = \) Weight of sample

Weather data
Weather data was obtained from the observatory (agrometeorological department) of Central Cotton Research Institute (CCRI), Multan.

Statistical analysis
The data was analyzed to Fischer’s analysis of the variance by using the Statistix 8.1. By using the LSD test with \( P \leq 5\% \) multiple the comparisons to separate means of the treatments were performed.

![Figure 1. Meteorological data of air temperature sunshine and air temperature.](image-url)
RESULTS
Application of moringa leaf extracts foliar spray and irrigation levels significantly affect the growth, yield and quality attributes of sunflower (table 2). The interaction of moringa leaf extract with irrigation levels also significantly affected on plant height, head diameter, stem diameter, number of achenes per head, 100-achenes weight, oil yield, protein yield, achenes yield, straw yield and biological yield. The interaction of foliar application of moringa leaf extract and irrigation levels was also significant on quality parameters like oil contents and protein contents on crop growth rate (CGR) and leaf area duration (LAD).
Foliar application of moringa leaf extract (MLE) at F3 (K+H+S) stage with I0 (normal irrigation significantly (p≤0.05) increased the plant height (257.33), head diameter (22.17cm), stem diameter (4.43cm), number of achenes per head (1030.30) and 1000-achenes weight (49.7g) as compared to F0 (no spray) with I3 where minimum plant height (225.33), head diameter (19.57cm), stem diameter (4.03cm), number of achenes per head (1001.7) and 1000-achenes weight (47.8g) was observed.
Yield attributes were ranged from Oil yield (1449.9-1322.7kg/ha), achenes yield (3474-3306.7kg/ha), straw yield (7505-7171kg/ha) and biological yield (10979-10478.6kg/ha) at F3 with I0 and F0 with I3 respectively (table 2).
Like growth and yield parameters, quality and physiological parameters were also affected by foliar application of moringa leaf extract (MLE) and irrigation regimes. Maximum oil contents (41.7%), protein contents (20.8%), harvest index (31.64), leaf area duration (171.5) and crop growth rate (15.89) was observed at F3 (K+H+S) stage and I0 treatment. While, minimum oil contents (40%), protein contents (19.47%), leaf area duration (134.2) and crop growth rate (12.54) was observed in F0 (no spray) and minimum harvest index (31.26) was observed at F0 (K+H) and I3 treatment.

Figure 2. Meteorological data of relative humidity and rainfall recorded.
Figure 3. Effect of foliar application of moringa leaf extract (MLE) on Leaf area index of sunflower under different irrigation regimes.

K + H = knee height + heading; K + S= knee height + seed development; K + H +S = knee height + heading + seed development

Figure 4. Total dry matter of sunflower as the affected by foliar applied moringa leaf extract under various irrigation regimes

K + H = knee height + heading; K + S= knee height + seed development; K + H +S = knee height + heading + seed development

**DISCUSSION**

In the world, the sunflower is an important oilseed crop and ranked as 4th after soybean, rapeseed and peanut. In Pakistan, it is also cultivated during spring and autumn season as an oilseed crop. Sunflower achenes contain 40-45 % oil of high quality. In crop production, water stress is a great environmental limitation and it depends on some factors like rainfall distribution and occurrences, soil water storing capacity and evaporative demand. Sunflower is sensitive to heat and water stress at flowering and achenes filling stage due to inadequacy in regulating the transpiration rate and leaf expression.
under deficient availability of soil moisture. In minimum rainfall, region yield is eventually decreasing significantly due to a reduction in soil moisture which causes the leaf wilting. reported that oil quality and achenes yield of sunflower is reducing greatly due to moisture stress. Plant height reduces in case of drought stress at vegetative stage. reported that in sunflower severe drought stress reduces net assimilation rate (NAR) which results in stunted growth. In sunflower drought stress is responsible for the reduction in chlorophyll synthesis which results in less production of photoassimilates, and reported that when drought stress is subjected at the vegetative stage in sunflower, it affects on carbon capturing and physiological parameters. Leaf area duration (LAD) and crop growth rate (CGR) is reduced when drought stress is subjected at critical stages of sunflower-like knee height, seed development and at heading stage.

Plant growth can be enhanced by exogenous application of plant growth regulators (PGR) like auxins, zeatin, cytokinin and vitamin-c. But the artificial application of these compounds is very costly. The scientist start work on exploring natural plant extract that contains these plant growth regulators which can improve the crop yield. A substantial amount of zeatin is available in extract of moringa leaves. Moringa leaf extract (MLE) not only improve plant growth and development but it also improves crop yield. Culver reported that foliar application of moringa leaf extract (MLE) improves plant height. concluded that maximum height in sunflower is the result of zeatin present in the moringa leaf extract. Presence of cytokinins, acrobats, phenol and minerals like calcium and potassium in the extract of moringa leaf is responsible for the enhancement of crop growth rate in sunflower. Increase in leaf area index under drought or normal condition might be responsible due to presence of growth enhancer in moringa leaf extract.

CONCLUSION
It was concluded that drought stress in sunflower at knee height+ heading+ seed development is most detrimental for growth and development. Also, three foliar application of moringa leaf extract (MLE) at height+ heading+ seed development under normal irrigation boost up the crop yield and high quality of seed can be obtained. Furthermore, more research is recommended on the exogenous application of moringa leaf extract (MLE) to confirm the results.

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Table 2. Response of foliar application of moringa leaf extract (MLE) to growth, yield, quality and physiological parameters of sunflower under different irrigation regimes.

| Parameters                                | F0          | F1          | F2          | F3          |
|-------------------------------------------|-------------|-------------|-------------|-------------|
| Plant height (cm)                         | 225.33c     | 163.67gh    | 178.0e      | 136.67i     |
| Stem diameter (cm)                        | 4.03c       | 3.26gh      | 3.66e       | 2.6k        |
| Head diameter (cm)                        | 19.57de     | 17.23h      | 16.94hi     | 15.57j      |
| Number of achenes per head                | 1001.7a     | 803.3d      | 741.0fg     | 681.7h      |
| 1000 achene weight (g)                    | 47.80cd     | 44.0lj      | 43.50jk     | 42.0l       |
| Oil yield Kg/ha                           | 1322.7c     | 1031.9g     | 1036.3g     | 920.8i      |
| Achene yield Kg/ha                        | 3306.7b     | 2786.3f     | 2803.3f     | 2536.3h     |
| Straw yield Kg/ha                         | 7172        | 6179        | 6093.7      | 5653        |
| Biological yield Kg/ha                    | 10478.6c    | 8965.3g     | 8897g       | 8209.3j     |
| Harvest index                             | 31.56bc     | 31.23bc     | 31.51bc     | 30.84bc     |
| Oil contents (%)                          | 40c         | 37.0j       | 36.9j       | 36.3k       |
| Protein contents (%)                      | 19.47de     | 16.67jk     | 16.8j       | 16.27k      |
| CGR                                       | 12.54c      | 10.50de     | 7.40gh      | 8.47fg      |
| LAD                                       | 134.2d      | 96.6i       | 106.8h      | 81.1j       |

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