

## INDUCTION OF TERMINAL HEAT STRESS TOLERANCE IN CHICKPEA UNDER EXOGENOUS APPLICATION OF GROWTH PROMOTERS

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### ABSTRACT

High temperature stress has been measured as one of the crucial environmental features that adversely affect the plant growth and development. Therefore, a field experiment was carried out at Land Reclamation Research Station, 7/3-L Ahmad Pur Sial, Jhang, Pakistan to reduce the heat stress induced-losses to chickpea (*Cicer arietinum* L.) plants with the foliar spray of growth promoters during two consecutive years 2017-18 and 2018-19. Experimental treatments comprised of two chickpea cultivars (DHUST and Bhakkar 2011) cultivated on two different sowing dates (15<sup>th</sup> November and 1<sup>st</sup> December) and foliar spray of growth promoters (Benzyl-amino purine (BAP) and Moringa leaf extract (MLE30)) including tap water as a control. This study was laid out in a Randomized Complete Block Design (RCBD) with factorial arrangements having three repeats. Results suggested that high temperature stress severely reduced the chickpea growth and productivity. However, exogenous application of MLE had a tremendous effect on the studied attributes. Exogenous application of MLE improved the growth and yield contributing attributes, which ultimately increased the final productivity. Moreover, foliar spray of MLE produced significantly higher enzymatic antioxidants activities that modulated the adverse impacts of heat stress on yield contributing traits. These results suggest that foliar spray of growth promoters particularly MLE modulated the heat stress induced losses to chickpea plants by improving their antioxidant defense mechanism and produced significantly higher grain yield during 2017-18 and 2018-19.

**Keywords:** Benzyl-amino purine, Moringa leaf extract, Catalase, Peroxidase, Grain yield

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### INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of principal leguminous crop, plays an important role in nourishment for huge population of the undeveloped countries and is studied as a healthy food in several well-developed countries (Merga and Haji, 2019). It is also an essential source of forage and fixes atmospheric nitrogen, therefore plays a major role in improving the production of the present cropping systems and considered as an essential constituent of crop rotations in the Australia, Iran, Turkey, Iraq and Pakistan (Khaitov *et al.*, 2016). Although chickpea is a winter season crop, however, high temperatures often restricted its growth and productivity. Optimum temperature at different growth stages adjusting the crop growth and scheduling of blooming thus affecting the economic yield (Teixeira *et al.*, 2013). Though, environment changes increased the average temperature around 0.74 °C per 100 years (IPCC, 2007). However, during the last 50 years, the linear heating tendency has been approximately twofold the rate of the preceding 100 years. Forecasts to the expiration of the twenty first century appraise to increase in worldwide average temperature between 1.8 and 4 °C, depending on the greenhouse emissions and disparities in precipitation patterns (IPCC, 2007). These variations in temperature

will definitely influence crop growth and reduce productivity up to 30% (ICRISAT, 2009).

Global rise in temperature, variable rainfall and moisture deficit are the alarming indications can have erratic production patterns all over the world. Unfavorable influences of abiotic stresses on chickpea plants can be minimized with the foliar spray of bio-stimulants and synthetic growth promoters that brings significant variations in plant physiology and biochemistry (Verma *et al.*, 2016). Among various growth promoters, benzyl-amino purine (BAP) and moringa leaf extract (MLE) possess a definite role in improving the plant growth and development for inducing a-biotic stress tolerance by generating modulations in plant physiology and its biochemistry needed for stress tolerance (Abd El-Rahman and Mohamed, 2014). Foliar spray of these growth promoters is the one of efficient technique involved in encouraging the plant growth and productivity by diminishing or mitigating the negative impacts of heat stress (Sadak and Dawood, 2014). As these growth promoters could encourage the growth of plants and mitigate the abiotic stress by interfering in metabolic and photosynthetic processes via osmotic adjustment, scavenging Reactive Oxygen Species (ROS), improving enzymatic antioxidants activities and leaf soluble protein (Rashid *et al.*, 2018; Shafiq *et al.*, 2021).

Though lot of research has been conducted on distinctive crops to alleviate the adverse influences of high temperature stress by foliar spray of growth promoters. However, only a few number of studies exposing the prospective of benzyl-amino purine and morinag leaf extract to encourage the chickpea productivity under heat stress conditions are accessible. So, we hypothesized that the foliar spray of BAP and MLE contributes towards enhancing the physiological and antioxidant traits and improve the chickpea production in heat stress environments. Keeping in view the above-mentioned discussion, the presented two years study was planned to explore the influence of heat stress and role of natural and synthetic growth promoters in modulating its adverse effects on chickpea.

## MATERIALS AND METHODS

A field experiment was performed at Land Reclamation Research Station, 7/3-L Ahmad Pur Sial, Jhang, Pakistan to evaluate terminal heat and induce terminal heat stress tolerance in chickpea by means of growth promoters during two consecutive years 2017-18 and 2018-19. The experiment was designed in completely randomized blocks with factorial arrangements and had three repeats. Experimental treatments comprised of two chickpea cultivars (DHUSHT and Bhakkar 2011), two sowing dates (15<sup>th</sup> November and 1<sup>st</sup> December) and two foliar spray of growth promoters (moringa leaf extract (MLE30) (Arif *et al.*, 2019), and Benzyl-amino purine (BAP) 50 mg L<sup>-1</sup> (Ali *et al.*, 2011) including tap water as a control. Chickpea plants were sprayed twice at 50% flowering and at 50% pod formation stage by using hand sprayer. The water spray treatment was applied first and the volume of water to be used was determined. Then in other two treatments same volume of water with MLE30 1:29 and BAP 50 mg/L were sprayed.

The soil texture was silty clay, with pH 8.24 and 8.20, EC 4.93 and 5.02 dS m<sup>-1</sup>, total nitrogen 0.05 and 0.05%, available phosphorus 8.96 and 8.99 mg k g<sup>-1</sup>, exchangeable potassium 247.30 and 250.00 mg kg<sup>-1</sup> and organic matter 0.56 and 0.55% in 2017-18 and 2018-19, respectively.

**Crop husbandry:** A fine seedbed was prepared by two dry cultivations and after soaking irrigation at optimum moisture, experimental soil was cultivated twice each followed by planking. Chickpea was sown with hand drill by using 65 kg ha<sup>-1</sup> seed rate. To get optimum plant populations, chickpea plants were thinned out by keeping one plant per hill at 15 days after emergence. Crop was

once irrigated at flowering stage of crop to save the crop from moisture stress. Recommended dose of Urea @ 20 kg N ha<sup>-1</sup> and DAP @ 22 kg P ha<sup>-1</sup> was applied at the time of seedbed preparation. Earthing up and plant protection measures were carried out at 50 days after sowing immediately after hand weeding. All other practices were kept identical for all the experimental units. The weather statistics from chickpea sowing to final harvesting was collected from observatory of CCRI, Multan during the 2017-18 and 2018-19 (Figure 1a & b).

**Data collection:** After 20 days of emergence, 15 randomly selected plants were tagged from each experimental unit to record the final plant height, number of pods per plant, number of grains per pod, pod weight and 1000 grains weight. Two weeks after every foliar spray of growth promoters, ten leaves sample were taken from each experimental unit to observe the antioxidant enzyme activities. All the leaves samples freeze, dried and then 0.5 g powder taken from freeze-dried leaves sampled obtained previously selected tagged plants were homogenized with 50mM Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> buffer containing 0.2 mM EDTA and 2% insoluble polyvinyl pyrrolidone in a chilled pestle and mortar. The slurry was centrifuged at 12,000×g for 20 min and the supernatant was used for enzyme activities assay. Standard protocols were adopted to measure peroxidase (POD), catalase (CAT) (Chance and Maehly, 1955) and superoxide dismutase (SOD) (Giannopolitis and Reis, 1997). While leaf total soluble protein was determined by adopting the procedure and following formula proposed by Bradford (1976).

$$\text{Leaf total soluble protein} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{Dilution factor}}{\text{Weight of Sample} \times 100} \text{ (mg/g)}$$

The mature crop was harvested on the 3<sup>rd</sup> and 9<sup>th</sup> April 2018 and 2019, respectively and threshed manually to determine grain yield and biological yield. Harvest index was recorded by using the following equation.

$$\text{Harvest index (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

**Statistical analysis:** All the collected data was computed and analyzed statistically using Fisher's analysis of variance technique of RCBD in three-way ANOVA using computer-based software MSTAT-C. Moreover, means of different treatments were compared by LSD at 5% level of probability (Steel *et al.*, 1997).

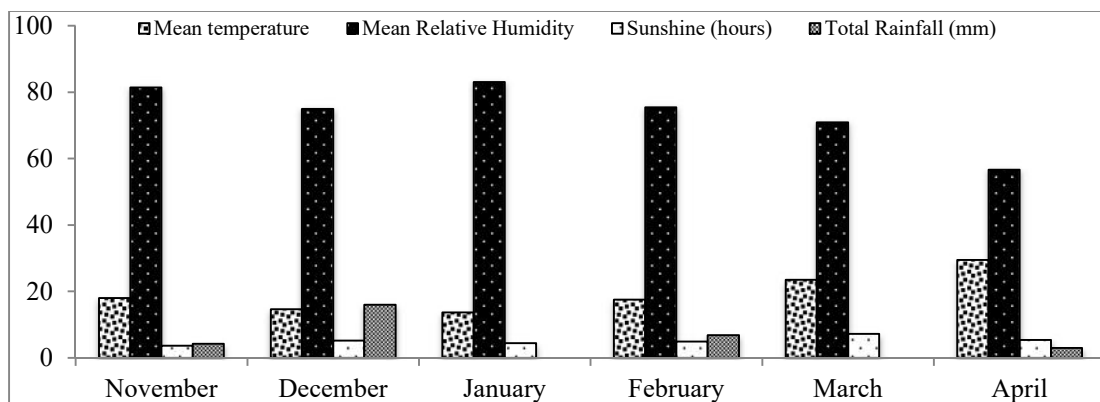


Figure 1a: Meteorological data for chickpea growth period during 2017-18.

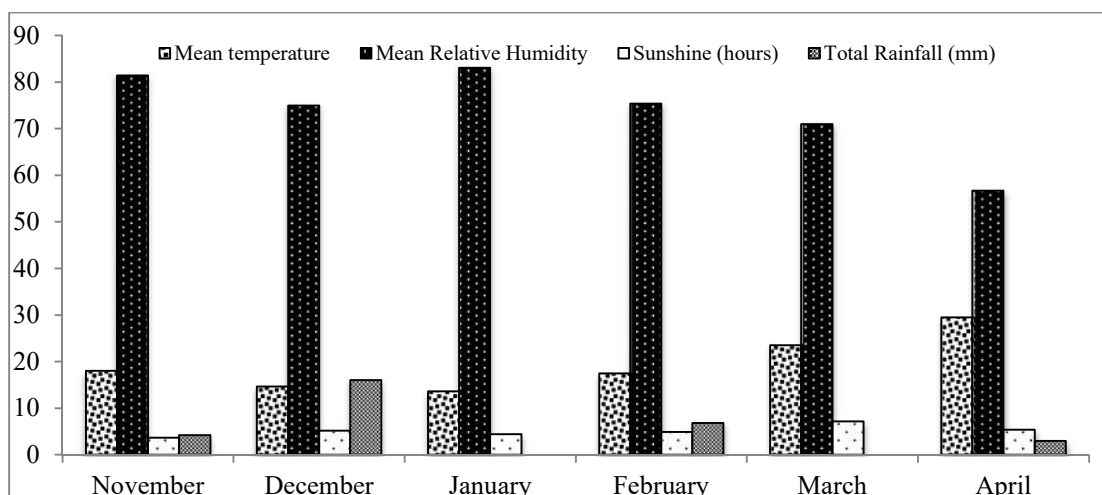


Figure 1a: Meteorological data for chickpea growth period during 2018-19.

## RESULTS

**Plant height (cm):** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the final plant height during both growing seasons (Table 1). Exogenous application of growth promoters under heat stress conditions significantly improved the plant height of chickpea cultivars during both growing years. However, foliar spray of MLE on chickpea cultivar DHUST cultivated on optimum sowing time (15<sup>th</sup> November) produced significantly taller plants during both growing seasons. Minimum plant height was perceived with the foliar application of tap water on chickpea cultivar Bhakkar 2011 cultivated on 1<sup>st</sup> December during both growing years (Table 1).

**Number of pods per plant:** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the pods per plant during 2017-18 and 2018-19 (Table 1). Exogenous application of growth promoters under heat stress conditions significantly

improved the number of pods of both chickpea cultivars. However, foliar spray of MLE on chickpea cultivar DHUST cultivated on optimum sowing time (15<sup>th</sup> November) produced maximum pods per plant. While, minimum pods per plant were detected with the foliar application of tap water on chickpea cultivar Bhakkar 2011 cultivated on 1<sup>st</sup> December during both growing years (Table 1).

**Number of grains per pod:** Statistically analysis of the data showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the number of grains per pod during both growing seasons (Table 1). Exogenous application of MLE on Bhakkar 2011 produced maximum grains per pod during 2017-18 and 2018-19. While, minimum number of grains per pod was perceived with the foliar application of tap water on both chickpea cultivars DHUST and Bhakkar 2011 cultivated on 1<sup>st</sup> December during 2017-18 and 2018-19, respectively (Table 1).

**Pod weight (g):** Statistically analysis of the data showed that interaction among foliar spray of growth promoters

on both chickpea cultivars and their sowing date significantly affected the pod weight during first growing season only (Table 1). Exogenous application of tap water and BAP on both chickpea cultivars DHUST and MLE on Bhakkar 2011 produced heavier pod weight, respectively. While, least pod weight was perceived with the foliar application of BAP on Bhakkar 2011 cultivated on 1<sup>st</sup> December during second growing season (Table 1).

**1000 grain weight (g):** The perusal of table 1 data further indicated that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the 1000 grain weight during both growing seasons (Table 1). Exogenous application of growth promoters on both chickpea cultivars produced significantly higher 1000 grain weight during both growing seasons. Experiment conducted during first growing season showed that foliar spray of MLE on chickpea cultivar Bhakkar 2011 and BAP on chickpea cultivar DHUST cultivated on 15<sup>th</sup> November and 1<sup>st</sup> December produced significantly higher 1000 grain weight, respectively. While foliar spray of tap water on chickpea cultivar Bhakkar 2011 cultivated on 15<sup>th</sup> November produced minimum 1000 grain weight.

Whereas, experiment conducted during second growing season showed that foliar spray of MLE on chickpea cultivar Bhakkar 2011 and BAP on DHUST cultivated on 1<sup>st</sup> December produced significantly higher 1000 grain weight. While foliar spray of tap water on chickpea cultivar DHUST cultivated on 15<sup>th</sup> November produced minimum 1000 grain weight.

**Biological yield (Kg ha<sup>-1</sup>):** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the biological yield during both growing years (Table 1). Exogenous application of growth promoters under heat stress conditions significantly improved the biological yield of both chickpea cultivars. However, foliar spray of MLE on both chickpea cultivars DHUST and Bhakkar 2011 cultivated on 15<sup>th</sup> November produced significantly higher biological yield during 2017-18 and 2018-19, respectively. While, minimum biological yield was observed with the foliar spray of tap water on chickpea cultivar Bhakkar 2011 cultivated on 1<sup>st</sup> December during both growing years (Table 1).

**Table 1: Effect of growth promoters on plant height (cm) and yield contributing attributes of chickpea.**

|   | Foliar spray            | 2017-18                           |                      |                       |                      | 2018-19                           |                      |                       |                      |
|---|-------------------------|-----------------------------------|----------------------|-----------------------|----------------------|-----------------------------------|----------------------|-----------------------|----------------------|
|   |                         | Sowing date of chickpea cultivars |                      |                       |                      | Sowing date of chickpea cultivars |                      |                       |                      |
|   |                         | DHUST                             |                      | Bhakkar-2011          |                      | DHUST                             |                      | Bhakkar-2011          |                      |
| Plant height (cm)                       |                         | 15 <sup>th</sup> Nov.             | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov. | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov.             | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov. | 1 <sup>st</sup> Dec. |
|   | Tap water               | 65.41 <sup>ab</sup>               | 65.04 <sup>ab</sup>  | 62.92 <sup>a,c</sup>  | 59.00 <sup>c</sup>   | 64.33 <sup>ab</sup>               | 63.28 <sup>a,c</sup> | 64.00 <sup>ab</sup>   | 58.97 <sup>c</sup>   |
| No. of pods per plant                   | MLE                     | 66.27 <sup>a</sup>                | 61.33 <sup>a,c</sup> | 60.89 <sup>a,c</sup>  | 60.47 <sup>bc</sup>  | 65.27 <sup>a</sup>                | 60.89 <sup>bc</sup>  | 62.00 <sup>a,c</sup>  | 62.65 <sup>a,c</sup> |
|   | BAP                     | 63.96 <sup>a,c</sup>              | 63.88 <sup>a,c</sup> | 64.09 <sup>a,c</sup>  | 60.34 <sup>bc</sup>  | 62.65 <sup>a,c</sup>              | 64.09 <sup>ab</sup>  | 63.66 <sup>ab</sup>   | 60.32 <sup>bc</sup>  |
| No. of grains per pod                   | LSD 0.05 <sub>p</sub> = | 5.57                              |                      |                       |                      | 4.35                              |                      |                       |                      |
|   | Tap water               | 60.89 <sup>b</sup>                | 62.33 <sup>ab</sup>  | 63.51 <sup>ab</sup>   | 61.15 <sup>b</sup>   | 62.00 <sup>ab</sup>               | 63.85 <sup>ab</sup>  | 62.67 <sup>ab</sup>   | 60.79 <sup>b</sup>   |
| Pod weight (g)                          | MLE                     | 65.84 <sup>a</sup>                | 63.57 <sup>ab</sup>  | 63.95 <sup>ab</sup>   | 61.48 <sup>b</sup>   | 65.67 <sup>a</sup>                | 64.28 <sup>ab</sup>  | 63.22 <sup>ab</sup>   | 62.18 <sup>ab</sup>  |
|   | BAP                     | 64.00 <sup>ab</sup>               | 62.43 <sup>ab</sup>  | 62.89 <sup>ab</sup>   | 62.02 <sup>ab</sup>  | 64.33 <sup>ab</sup>               | 62.02 <sup>ab</sup>  | 63.04 <sup>ab</sup>   | 61.78 <sup>b</sup>   |
| 1000 grains weight (g)                  | LSD 0.05 <sub>p</sub> = | 3.91                              |                      |                       |                      | 3.71                              |                      |                       |                      |
|   | Tap water               | 1.95 <sup>a</sup>                 | 1.77 <sup>b</sup>    | 1.91 <sup>ab</sup>    | 1.90 <sup>ab</sup>   | 1.93 <sup>cd</sup>                | 1.98 <sup>a,d</sup>  | 1.99 <sup>a,d</sup>   | 1.92 <sup>d</sup>    |
| Biological yield (Kg ha <sup>-1</sup> ) | MLE                     | 2.04 <sup>a</sup>                 | 1.90 <sup>ab</sup>   | 2.06 <sup>a</sup>     | 1.96 <sup>a</sup>    | 1.97 <sup>a,d</sup>               | 2.05 <sup>a</sup>    | 2.05 <sup>a</sup>     | 1.99 <sup>a,d</sup>  |
|   | BAP                     | 2.02 <sup>a</sup>                 | 1.90 <sup>ab</sup>   | 1.99 <sup>a</sup>     | 1.92 <sup>ab</sup>   | 2.02 <sup>a,c</sup>               | 2.03 <sup>ab</sup>   | 1.94 <sup>b,d</sup>   | 1.99 <sup>a,d</sup>  |
| Pod weight (g)                          | LSD 0.05 <sub>p</sub> = | 0.16                              |                      |                       |                      | 0.09                              |                      |                       |                      |
|   | Tap water               | 0.31 <sup>NS</sup>                | 0.33                 | 0.32                  | 0.33                 | 0.31 <sup>a</sup>                 | 0.28 <sup>a,d</sup>  | 0.30 <sup>ab</sup>    | 0.26 <sup>cd</sup>   |
| 1000 grains weight (g)                  | MLE                     | 0.32                              | 0.35                 | 0.31                  | 0.34                 | 0.29 <sup>a,c</sup>               | 0.29 <sup>a,c</sup>  | 0.31 <sup>a</sup>     | 0.27 <sup>b,d</sup>  |
|   | BAP                     | 0.35                              | 0.30                 | 0.32                  | 0.31                 | 0.31 <sup>a</sup>                 | 0.27 <sup>b,d</sup>  | 0.30 <sup>ab</sup>    | 0.25 <sup>d</sup>    |
| Biological yield (Kg ha <sup>-1</sup> ) | LSD 0.05 <sub>p</sub> = | NS                                |                      |                       |                      | 0.03                              |                      |                       |                      |
|   | Tap water               | 230 <sup>bc</sup>                 | 238 <sup>ab</sup>    | 225 <sup>c</sup>      | 229 <sup>bc</sup>    | 210 <sup>d</sup>                  | 215 <sup>cd</sup>    | 214 <sup>cd</sup>     | 222 <sup>ab</sup>    |
| 1000 grains weight (g)                  | MLE                     | 237 <sup>ab</sup>                 | 238 <sup>ab</sup>    | 240 <sup>a</sup>      | 235 <sup>ab</sup>    | 223 <sup>a</sup>                  | 222 <sup>ab</sup>    | 221 <sup>a,c</sup>    | 224 <sup>a</sup>     |
|   | BAP                     | 239 <sup>ab</sup>                 | 240 <sup>a</sup>     | 238 <sup>ab</sup>     | 225 <sup>c</sup>     | 223 <sup>a</sup>                  | 224 <sup>a</sup>     | 216 <sup>b,d</sup>    | 223 <sup>a</sup>     |
| Biological yield (Kg ha <sup>-1</sup> ) | LSD 0.05 <sub>p</sub> = | 9                                 |                      |                       |                      | 7                                 |                      |                       |                      |
|   | Tap water               | 6103 <sup>a,c</sup>               | 5977 <sup>bc</sup>   | 5990 <sup>bc</sup>    | 5793 <sup>c</sup>    | 6211 <sup>ab</sup>                | 6224 <sup>ab</sup>   | 6165 <sup>ab</sup>    | 6027 <sup>b</sup>    |
| Biological yield (Kg ha <sup>-1</sup> ) | MLE                     | 6416 <sup>a</sup>                 | 6177 <sup>ab</sup>   | 5957 <sup>bc</sup>    | 6005 <sup>bc</sup>   | 6192 <sup>ab</sup>                | 6233 <sup>ab</sup>   | 6410 <sup>a</sup>     | 6205 <sup>ab</sup>   |
|   | BAP                     | 6040 <sup>bc</sup>                | 5964 <sup>bc</sup>   | 5999 <sup>bc</sup>    | 6176 <sup>ab</sup>   | 6304 <sup>a</sup>                 | 6273 <sup>ab</sup>   | 6229 <sup>ab</sup>    | 6239 <sup>ab</sup>   |
| Biological yield (Kg ha <sup>-1</sup> ) | LSD 0.05 <sub>p</sub> = | 366                               |                      |                       |                      | 264                               |                      |                       |                      |

**Grain yield (Kg ha<sup>-1</sup>):** Statistically analysis of the data showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing

date significantly affected the grain yield during both growing seasons (Table 2). Exogenous application of growth promoters under heat stress conditions

significantly improved the grain yield of both chickpea cultivars. However, exogenous application of MLE on both chickpea cultivars Bhakkar 2011 and DHUST produced significantly higher grain yield during 2017-18 and 2018-19, respectively. While, minimum grain yield was perceived with the foliar spray of tap water on chickpea cultivar Bhakkar 2011 cultivated on 1<sup>st</sup> December during both growing years (Table 2).

**Harvest index (%):** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the harvest index during both growing seasons (Table 2). Foliar spray of MLE on Bhakkar 2011 and DHUST cultivated on optimum sowing time (15<sup>th</sup> November) produced maximum harvest index during 2017-18 and 2018-19, respectively. While, least harvest index was detected with the foliar application of tap water on early and late sown chickpea cultivar DHUST during 2017-18 and 2018-19 (Table 2).

**Leaf total soluble protein (mg g<sup>-1</sup>):** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the leaf total soluble protein enzyme during both growing years (Table 2). Foliar spray of BAP on chickpea cultivar DHUST and MLE on chickpea cultivar Bhakkar 2011 cultivated on 15<sup>th</sup> November and 1<sup>st</sup> December produced significantly higher leaf total soluble protein enzyme during 2017-18 and 2018-19, respectively. While, least leaf total soluble protein enzyme was observed with the foliar spray of MLE and BAP on chickpea cultivar Bhakkar 2011 cultivated on 15<sup>th</sup> November during 2017-18 and 2018-19 (Table 2).

**Superoxide dismutase (IU min<sup>-1</sup>mg<sup>-1</sup> Protein):** Results showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the superoxide dismutase

enzyme during both growing seasons (Table 2). Foliar spray of MLE on Bhakkar 2011 and DHUST cultivated under late sown condition produced significantly higher superoxide dismutase enzyme in both years under study. While, least superoxide dismutase enzyme was detected with the foliar application of BAP on chickpea cultivar DHUST cultivated on 15<sup>th</sup> November and 1<sup>st</sup> December during 2017-18 and 2018-19 (Table 2).

**Peroxidase (POD) (IU min<sup>-1</sup>mg<sup>-1</sup> protein):** Statistically analysis of the data showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the peroxidase enzyme during both growing seasons (Table 2). Exogenous application of growth promoters under heat stress conditions significantly improved the peroxidase of both chickpea cultivars. However, exogenous application of MLE on Bhakkar 2011 cultivated on 15<sup>th</sup> November produced significantly higher peroxidase enzyme. While, minimum peroxidase enzyme was observed from both chickpea cultivars DHUST and Bhakkar 2011 sown on 15<sup>th</sup> November and 1<sup>st</sup> December received foliar spray of BAP and tap water during 2017-18 and 2018-19, respectively (Table 2).

**Catalase (CAT) (IU min<sup>-1</sup>mg<sup>-1</sup> protein):** Statistically analysis of the data showed that interaction among foliar spray of growth promoters on both chickpea cultivars and their sowing date significantly affected the catalase enzyme during both growing years (Table 2). Exogenous application of growth promoters under heat stress conditions significantly improved the catalase enzyme of both chickpea cultivars. However, exogenous application of MLE on chickpea cultivar DHUST cultivated on 1<sup>st</sup> December produced significantly higher catalase contents. While, minimum catalase enzyme was observed with the foliar spray of MLE on chickpea cultivar Bhakkar 2011 cultivated on optimum sowing time (15<sup>th</sup> November) during 2017-18 and 2018-19 (Table 2).

**Table 2: Effect of growth promoters on economic yield (kg ha<sup>-1</sup>), leaf soluble protein (mg g<sup>-1</sup>) and enzymatic antioxidants (IU min<sup>-1</sup>mg<sup>-1</sup> protein) of chickpea.**

|  | Foliar spray                                     | 2017-18                           |                      |                       |                      | 2018-19               |                      |                       |                      |
|--|--|-----------------------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|
|  |  | Sowing date of chickpea cultivars |                      |                       |                      |                       |                      |                       |                      |
|  |  | DHUST                             |                      | Bhakkar-2011          |                      | DHUST                 |                      | Bhakkar-2011          |                      |
|  |  | 15 <sup>th</sup> Nov.             | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov. | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov. | 1 <sup>st</sup> Dec. | 15 <sup>th</sup> Nov. | 1 <sup>st</sup> Dec. |
| Leaf total soluble protein (mg g <sup>-1</sup> ) | Grain yield (Kg ha <sup>-1</sup> )               | 1860 <sup>ab</sup>                | 1788 <sup>b</sup>    | 1867 <sup>ab</sup>    | 1735 <sup>b</sup>    | 1880 <sup>c</sup>     | 1913 <sup>bc</sup>   | 1956 <sup>bc</sup>    | 1877 <sup>c</sup>    |
|  | MLE  | 2119 <sup>a</sup>                 | 1969 <sup>ab</sup>   | 2081 <sup>a</sup>     | 1929 <sup>ab</sup>   | 2106 <sup>a</sup>     | 2028 <sup>ab</sup>   | 1982 <sup>a,c</sup>   | 1951 <sup>bc</sup>   |
|  | BAP  | 2118 <sup>a</sup>                 | 1918 <sup>ab</sup>   | 2068 <sup>a</sup>     | 1982 <sup>ab</sup>   | 2010 <sup>a,c</sup>   | 1978 <sup>a,c</sup>  | 1976 <sup>a,c</sup>   | 1961 <sup>bc</sup>   |
|  | LSD 0.05p=                                       | 278                               |                      |                       |                      | 138                   |                      |                       |                      |
|  | Harvest index (%)                                | 30.48 <sup>bc</sup>               | 29.90 <sup>c</sup>   | 31.16 <sup>a,c</sup>  | 30.00 <sup>c</sup>   | 30.29 <sup>c</sup>    | 30.74 <sup>bc</sup>  | 30.44 <sup>c</sup>    | 32.42 <sup>a,c</sup> |
|  | MLE  | 32.99 <sup>a,c</sup>              | 31.83 <sup>a,c</sup> | 35.03 <sup>a</sup>    | 32.11 <sup>a,c</sup> | 33.83 <sup>a</sup>    | 32.75 <sup>ab</sup>  | 31.45 <sup>bc</sup>   | 30.92 <sup>bc</sup>  |
|  | BAP  | 35.03 <sup>a</sup>                | 32.14 <sup>a,c</sup> | 34.46 <sup>ab</sup>   | 32.08 <sup>a,c</sup> | 31.90 <sup>a,c</sup>  | 31.58 <sup>bc</sup>  | 31.72 <sup>a,c</sup>  | 31.41 <sup>bc</sup>  |
|  | LSD 0.05p=                                       | 4.15                              |                      |                       |                      | 2.23                  |                      |                       |                      |
|  | Leaf total soluble protein (mg g <sup>-1</sup> ) | 0.95 <sup>bc</sup>                | 0.98 <sup>ab</sup>   | 1.01 <sup>a</sup>     | 1.00 <sup>a</sup>    | 0.99 <sup>bc</sup>    | 0.98 <sup>bc</sup>   | 1.04 <sup>b</sup>     | 1.11 <sup>a</sup>    |
|  | MLE  | 0.98 <sup>ab</sup>                | 1.01 <sup>a</sup>    | 1.01 <sup>a</sup>     | 1.01 <sup>a</sup>    | 1.01 <sup>bc</sup>    | 1.02 <sup>b</sup>    | 0.95 <sup>c</sup>     | 1.13 <sup>a</sup>    |
| BAP  | 1.02 <sup>a</sup>                                | 1.01 <sup>a</sup>                 | 0.93 <sup>c</sup>    | 1.01 <sup>a</sup>     | 1.05 <sup>b</sup>    | 1.00 <sup>bc</sup>    | 0.99 <sup>bc</sup>   | 0.98 <sup>bc</sup>    |                      |

|  |                         | LSD 0.05 <sub>p</sub> = |                     |                     | 0.04                |                      |                      | 0.07                 |                      |  |
|--|-------------------------|-------------------------|---------------------|---------------------|---------------------|----------------------|----------------------|----------------------|----------------------|--|
| Superoxide dismutase (IU min <sup>-1</sup> mg <sup>-1</sup> protein) | Tap water               | 83.67 <sup>b</sup>      | 82.00 <sup>bc</sup> | 84.33 <sup>b</sup>  | 85.00 <sup>b</sup>  | 87.00 <sup>c,f</sup> | 83.33 <sup>d,f</sup> | 90.00 <sup>a,d</sup> | 83.00 <sup>ef</sup>  |  |
|  | MLE                     | 85.00 <sup>b</sup>      | 92.67 <sup>a</sup>  | 93.67 <sup>a</sup>  | 94.67 <sup>a</sup>  | 88.67 <sup>b,e</sup> | 95.67 <sup>a</sup>   | 93.33 <sup>a,c</sup> | 95.00 <sup>ab</sup>  |  |
|  | BAP                     | 80.67 <sup>b,d</sup>    | 76.33 <sup>d</sup>  | 93.33 <sup>a</sup>  | 78.00 <sup>cd</sup> | 80.67 <sup>f</sup>   | 81.33 <sup>f</sup>   | 91.33 <sup>a,c</sup> | 83.33 <sup>d,f</sup> |  |
|  | LSD 0.05 <sub>p</sub> = | 4.84                    |                     |                     | 6.71                |                      |                      |                      |                      |  |
| Peroxidase (IU min <sup>-1</sup> mg <sup>-1</sup> protein)           | Tap water               | 7.12 <sup>e</sup>       | 7.18 <sup>e</sup>   | 8.81 <sup>ab</sup>  | 7.41 <sup>de</sup>  | 7.06 <sup>f</sup>    | 7.04 <sup>f</sup>    | 8.67 <sup>b</sup>    | 6.95 <sup>f</sup>    |  |
|  | MLE                     | 7.34 <sup>e</sup>       | 8.17 <sup>bc</sup>  | 9.07 <sup>a</sup>   | 8.11 <sup>c</sup>   | 7.00 <sup>f</sup>    | 8.17 <sup>cd</sup>   | 9.52 <sup>a</sup>    | 8.34 <sup>c</sup>    |  |
|  | BAP                     | 6.17 <sup>f</sup>       | 6.94 <sup>e</sup>   | 8.03 <sup>cd</sup>  | 8.81 <sup>ab</sup>  | 7.04 <sup>f</sup>    | 7.96 <sup>de</sup>   | 7.89 <sup>e</sup>    | 8.27 <sup>c</sup>    |  |
|  | LSD 0.05 <sub>p</sub> = | 0.68                    |                     |                     | 0.27                |                      |                      |                      |                      |  |
| Catalase (IU min <sup>-1</sup> mg <sup>-1</sup> protein)             | Tap water               | 41.00 <sup>b</sup>      | 44.33 <sup>ab</sup> | 43.67 <sup>ab</sup> | 43.67 <sup>ab</sup> | 43.33 <sup>a</sup>   | 43.67 <sup>a</sup>   | 41.67 <sup>ab</sup>  | 43.00 <sup>a</sup>   |  |
|  | MLE                     | 35.33 <sup>c</sup>      | 45.67 <sup>a</sup>  | 35.00 <sup>c</sup>  | 41.00 <sup>b</sup>  | 38.00 <sup>bc</sup>  | 44.67 <sup>a</sup>   | 37.00 <sup>c</sup>   | 42.00 <sup>ab</sup>  |  |
|  | BAP                     | 44.00 <sup>ab</sup>     | 45.33 <sup>a</sup>  | 44.33 <sup>b</sup>  | 45.00 <sup>ab</sup> | 43.00 <sup>a</sup>   | 44.33 <sup>a</sup>   | 41.00 <sup>a,c</sup> | 41.67 <sup>ab</sup>  |  |
|  | LSD 0.05 <sub>p</sub> = | 4.26                    |                     |                     | 4.26                |                      |                      |                      |                      |  |

## DISCUSSION

Heat stress has been measured one of the essential environmental features that impact the plant growth and development. Increasing overall temperature from global warming resultant in heat stress for several crops in conventional farming systems restricting the growth and metabolism that consequent in considerable harm to potential productivity worldwide (Kaushal *et al.*, 2016). One of the ways to deal with negative impacts high temperature stress may involve discovering some natural or synthetic molecules that have the potential to defend the plants from the harmful influences of heat stress. Growth promoters such as Benzyl-amino purine (BAP) and moringa leaf extract (MLE), which are elevated in response to diverse types of abiotic stresses (Yasmeen *et al.*, 2013). Therefore present study was designed to use growth promoters to alleviate the adverse effects of high temperature stress and improved the growth and productivity of chickpea. These results showed that high temperature stress adversely affected the plant height of both chickpea cultivars. However, exogenous application of growth promoters especially moringa leaf extract on chickpea significantly improved the plant height that might be due to the fact that MLE functions as a plant growth promoter due to the presence of macro and micronutrients, carotenoids, ascorbates, zeatin, phenols, antioxidants and it is proposed that exogenously applied MLE might have improve the plants endogenous hormonal levels thus ensuring in higher seedling growth even under abiotic stress conditions (Yasmeen *et al.*, 2013). Among growth enhancers exposed in MLE, cytokinins have an important role for the promotion of cell division, cell elongation, and chlorophyll biosynthesis that significantly improved the plant height (Taiz and Zeiger, 2006).

High temperature stress during the reproductive stage such as flowering, pod formation and grain filling extensively affected several reproductive parameters (Snider *et al.*, 2010). The present results indicated that

high temperature negatively affected the number of pods per plant, grains per pod, pod weight, thousand-grain weight that ultimately reduced the biological and economic yield of both chickpea cultivars. It might be due to the fact that high temperature could interrupt the process of pollination and succeeding fertilization leading to reduce fruit set. Higher temperature during day and then at night might exacerbate destructive influence and proposed a crucial reason of yield inconsistency (Oosterhuis, 2002). It is because the temperature surpasses the above optimal range throughout the day, it diminishes the rate of photosynthesis and synthesis of carbohydrates (Snider *et al.*, 2009). High temperature at nighttime improves the respiration rate and reduces the availability of carbohydrate that resultant in the reduction of yield and yield contributing attributes (Loka *et al.*, 2011). Under such conditions exogenous application of growth promoters mitigate these adverse affects of heat stress and improved the productivity of both chickpea cultivars. Although both (BAP and MLE) alleviate the adverse affects of heat stress and improved the productivity, however, exogenously applied MLE results were superior. Improved chickpea productivity with improved yield contributing traits in existing experiment might be attributed to alleviate the detrimental impacts of high temperature at reproductive stage in late sown chickpea cultivar due to exogenously applied MLE. It was earlier observed that foliar spray of MLE was found promising to enhance yield and yield contributing attributes (Arif *et al.*, 2019). It might be due to the fact that foliar spray of MLE improved the photosynthesis rate, contributing to photosynthates flow toward pods to increase carbohydrate delivery during grain filling stage (Blaise *et al.*, 2009). Moreover, this could also be due to existence of zeatin in MLE that improves the antioxidant properties of different enzymes and protects the cell under adverse environmental conditions (Zhang and Ervin, 2004).

In present study, exogenously applied MLE improved the enzymatic antioxidants activities in

chickpea plants even under heat stressed conditions. The improvement in antioxidant activities under abiotic stress was also observed in wheat plants with the foliar spray of MLE (Afzal *et al.*, 2020). Moreover, Hussain *et al.* (2020) established that exogenously applied MLE on plant encourages the production of endogenous antioxidants. This initiation of self-protection system might be related with superior minerals ( $K^+$  and  $Ca^{2+}$ ) and growth enhancers (zeatin and gibberellins) existed in moringa leaves because mineral elements are activator and cofactor of numerous antioxidant enzymes (Arif *et al.*, 2019). Likewise, this higher antioxidants activity was observed more obvious in leaves during reproductive phase. Amid distinctive physiological processes, photosynthesis is most sensitive to high temperature and its diminution adversely affects the growth and productivity (Wahid *et al.*, 2007). Exogenously applied MLE was found supportive to enhanced and maintained chlorophyll contents improved the process of photosynthesis along with enhanced leaf antioxidant activities that plays a vital role in detoxifying or scavenging toxic ROS (Sharma *et al.*, 2012).

**Conclusion:** High temperature stress considerably reduced the chickpea productivity. Under such conditions, foliar spray of growth promoters especially MLE mitigated the heat stress induced losses by improving their antioxidant defense mechanism and increased the chickpea productivity.

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